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Novel fluorescent isoquinoline pigments, panaefluorolines A–C from the cultured mycobiont of a lichen, *Amygdalaria panaeola*

Kaoru Kinoshita,[a] Yoshikazu Yamamoto,[b] Kiyotaka Koyama,[a] Kunio Takahashi[a,*] and Isao Yoshimura[c]

[a]*Department of Pharmacognosy and Phytochemistry, Meiji Pharmaceutical University, Noshio 2-522-1, Kiyose-shi, Tokyo 204-8588, Japan*

[b]*Department of Biological Production Science, Faculty of Bioresource Sciences, Akita Prefectural University, 241-7, Kaidobata-nishi, Shimoshinjo-nakano, Akita 010-0195, Japan*

[c]*Hattori Botanical Laboratory, Kochi Branch, Edagawa 2576-27, Ino-cho, Agawagun, Kochi, Japan*

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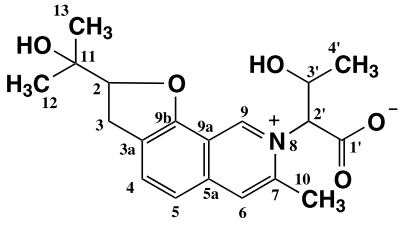
Abstract—Novel fluorescent substances, named panaefluorolines A–C (**1**–**3**), were isolated from the cultured mycobiont of a lichen, *Amygdalaria panaeola*. These structures were elucidated on the basis of spectroscopic data, especially 2D NMR.

Lichens are symbiotic associations of algal and fungal partners and produce many characteristic phenolics such as depsides, depsidones and dibenzofurans. These compounds were generally considered to be biosynthesized by their fungal partners.[1] Novel metabolites, however, were found recently from cultured mycobionts of lichens under stressed conditions.[2–7] In the course of our search for new bioactive compounds from cultured mycobionts, we have already reported two isofurano-naphtoquinone derivatives with bostrycoidin and 8-*O*-methylbostrycoidin from the mycobiont, *Arthonia cinnabarina* (DC.) Wallr.[8]



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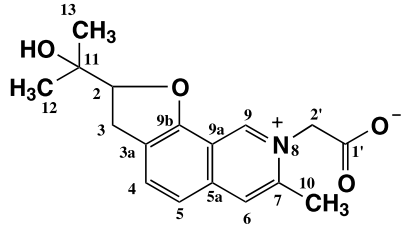

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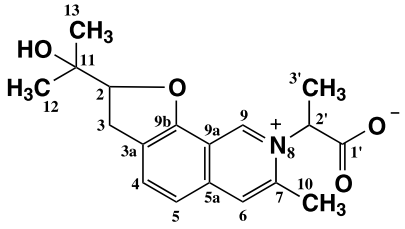

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Keywords: lichen; *Amygdalaria panaeola*; fluorescence; isoquinoline; amino acid.
* Corresponding author. Tel./fax: +81-424-95-8912; e-mail: diamonds@my-pharm.ac.jp

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Table 1. ^{13}C and ^{1}H NMR spectral data of **1**, **2** and **3** in CD_3OD

Position	**1**		**2**		**3**	
	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	93.9	5.03 (t, 8.8)	94.1	5.05 (t, 8.8)	94.0	5.07 (t, 8.8)
3	31.5	3.47 (d, 8.8)	31.6	3.48 (d, 8.8)	31.7	3.47 (m)
3a	127.9		128.1		127.9	
4	136.2	7.98 (d, 8.1)	136.1	7.99 (d, 8.1)	136.1	8.00 (d, 8.1)
5	118.6	7.53 (d, 8.1)	119.0	7.56 (d, 8.1)	118.7	7.56 (d, 8.1)
5a	138.0		138.7		138.2	
6	126.1	8.12 (s)	126.8	8.15 (s)	126.7	8.15 (s)
7	144.3		144.8		144.5	
9	144.7	10.00 (s)	147.8	9.63 (s)	144.4	9.53 (s)
9a	114.3		115.0		114.8	
9b	159.7		159.5		159.3	
10	20.5*	2.82 (s)	19.4	2.77 (s)	19.9	2.83 (s)
11	72.3		72.4		72.5	
12	25.2	1.27 (s)	25.2	1.28 (s)	24.6	1.27 (s)
13	25.6	1.39 (s)	25.6	1.40 (s)	25.8	1.41 (s)
1′	170.5		170.5		173.1	
2′	75.7	5.28 (d, 6.1)	62.3	5.24 (s)	67.7	5.49 (q, 7.1)
3′	69.2	4.80 (brt, 6.1)			19.1	2.01 (d, 7.1)
4′	20.9*	1.27 (brs)				

* May be interchanged.

We isolated formerly the mycobiont from spores discharged from apothecia of *Amygdalaria panaeola* (Ach.) Hertel & Brodo, collected in Finland in 1990. Now the mycobiont was cultured on malt-yeast liquid medium for 4 weeks. The medium showed fluorescent yellowish green color after a week cultivation. The filtrated medium (1.36 L) was lyophilized and extracted by MeOH three times. The MeOH extract (10.83 g) was subjected to Diaion HP20 column to adsorb the fluorescent compounds. The MeOH eluate (1.23 g) was chromatographed on a silica gel column by using $CHCl_3$–MeOH–H_2O solvent system to yield crude fluorescent yellowish green pigments, **1**, **2**, and **3**. Each pigment was further purified by HPLC (a silica gel column, $CHCl_3$–MeOH–H_2O solvent system, and detection 365 nm) to obtain **1** (20.6 mg), **2** (38.0 mg) and **3** (25.4 mg), respectively.

Panaefluoroline A (**1**) was obtained as a yellowish green amorphous solid, $[\alpha]_D^{22} = +265.6°$ (c 0.40, MeOH), and was suggested to have the molecular formula $C_{19}H_{23}NO_5$ by its positive HRFABMS data m/z 346.1643 $[M+H]^+$, (calcd 346.1654, $C_{19}H_{24}NO_5$, $[M+H]^+$). The UV spectrum (MeOH) of **1** showed absorption at λ_{max} (log ε): 222 (4.08), 255 (4.14), 304 (3.46), 315 (3.38) and 412 (3.71). The IR spectrum exhibited a hydroxyl (3300 cm^{-1}) and a carboxyl (1635 cm^{-1}). The ^{1}H NMR spectrum[9] (Table 1) of **1** showed signals due to three singlet methyls at δ 1.27 (6H, brs), 1.39 (3H, s) and 2.82 (3H, s). The third methyl was considered to attach on sp^2 carbon, from the chemical shift. The ^{13}C NMR spectrum (Table 1) revealed 19 carbon signals, which were sorted by DEPT as four methyls, one methylene, seven methines and seven quaternary carbons including one carboxyl. A combination of the HMQC, ^{2}J and ^{3}J HMBC and ^{1}H–^{1}H COSY experiments, the structure was determined and allowed assignments of all protons and carbons. The methyl protons at δ 1.27 and 1.39 had a cross peak with δ 25.2 and 25.6 methyl carbon on HMQC, and each methyl protons showed long-range correlation with the other methyl carbon on HMBC, respectively. These methyl protons and the methylene protons at δ 3.47 (2H, d, J = 8.8 Hz), showing HMQC correlation at δ 31.5, showed HMBC correlation with quaternary carbon at δ 72.3 and methine carbon at δ 93.9. The quaternary carbon at δ 72.3 and methine carbon at δ 93.9 were assumed to attach to an oxygen atom from the chemical shift value. From these data, isoprene moiety was determined. Two aromatic methine protons at δ 7.53 and 7.98 (each 1H, d, J = 8.1 Hz) were coupling each other from their coupling constant and the cross peak on ^{1}H–^{1}H COSY. The HMBC spectrum of these aromatic protons afforded long-range correlations as shown in Figure 1, which suggested that **1** consisted of isoquinoline skeleton. The methyl protons at δ 2.82, which showed HMQC correlation with δ 20.5, had HMBC cross peak with quaternary carbon at δ 144.3 on the isoquinoline skeleton, and the aromatic methine proton at δ 8.12 showed the correlation on HMBC with the methyl carbon at δ 20.5. Then, the methyl was attached on C-2 of the isoquinoline skeleton. The methylene protons at δ 3.47, showed HMBC correlation at δ 159.7 and 127.9 quaternary carbons on

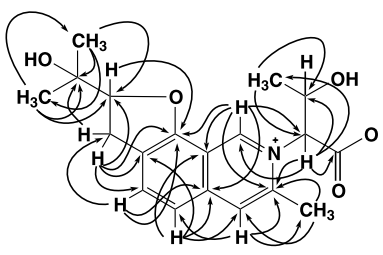

Figure 1. HMBC correlations for **1**.

the isoquinoline skeleton. The aromatic proton at δ 7.98 correlated with the methylene carbon at δ 31.5 on the isoprene moiety. Moreover, the methine proton at δ 5.03 (1H, t, $J=8.8$ Hz) on the isoprene moiety showed HMBC correlation with quaternary carbons at δ 159.7 on the isoquinoline skeleton. These data suggested that the isoprene moiety made a five-membered ether ring at C-7 and C-8 of the isoquinoline skeleton. The compound, TMC-120A, having an isoquinoline skeleton with a five-membered ether ring like **1**, has already been isolated from *Aspergillus ustus* TC 1118.[10] The ^{1}H and ^{13}C NMR data of TMC-120A were similar to the data of **1**. The methine carbon at δ 75.7, showing HMQC correlation at δ 5.28 (1H, d, $J=6.1$ Hz), showed HMBC correlation with alcoholic methine carbon at δ 69.2 and carbonyl carbon at δ 170.5. The methine proton at δ 5.28, showing HMBC correlation with aromatic methine carbon at δ 144.3 on the isoquinoline skeleton, and the chemical shift value of this carbon (δ 75.7) suggested that the methine carbon was assumed to be attached to a nitrogen atom. These data suggested that the nitrogen atom was quaternary ammonium and the carboxylic acid was ionized. This structure of **1** was supported by the molecular formula, $C_{19}H_{23}NO_5$, by positive HRFABMS, and by the data of positive FABMS m/z 346 $[M+H]^+$, 368 $[M+Na]^+$, and negative FABMS m/z 345 (M^-). The structure of **1** was also supported by the ^{1}H–^{1}H COSY and NOESY. Therefore, the structure of panaefluoroline A was determined as shown in **1**.

Panaefluoroline B (**2**) was obtained as a yellowish green amorphous solid, $[\alpha]_D^{22}=+52.3°$ (c 0.57, MeOH), and was suggested to have the molecular formula $C_{17}H_{20}NO_4$ by its positive HRFABMS data m/z 302.1376 $[M+H]^+$ (calcd 302.1392). The UV spectrum (MeOH) of **2** showed absorption at λ_{max} (log ε): 218 (4.06), 256 (4.15), 305 (3.29), 317 (3.33) and 412 (3.65). The IR spectrum exhibited a hydroxyl (3310 cm^{-1}) and a carboxyl (1625 cm^{-1}). The ^{1}H NMR spectrum was similar to that of **1**, except for the N-side chain moiety, and additional protons and methylene carbon were δ 5.24 (2H, s) and δ 62.3. These protons showed HMBC correlation with carbonyl carbon at δ 170.5 and quaternary carbons at δ 147.8 and 144.8 on the isoquinoline moiety. From these data, N-side chain was determined to be CH_2COO^-. The structure of **2** was supported by the ^{1}H–^{1}H COSY and NOESY. Thus, the structure of panaefluoroline B was established as shown in **2**.

Panaefluoroline C (**3**) was obtained as a yellowish green amorphous solid (9.0 mg), $[\alpha]_D^{22}=+264.7$ (c 1.0, MeOH), and was suggested to have the molecular formula $C_{17}H_{19}NO_4$ by its positive HRFABMS data m/z 316.1563 $[M+H]^+$ (calcd 346.1549, $C_{17}H_{20}NO_4$, $[M+H]^+$). The UV spectrum (MeOH) of **3** showed absorption at λ_{max} (log ε): 220 (4.19), 257 (4.27), 305 (3.51), 316 (3.48) and 412 (3.79). The IR spectrum exhibited a hydroxyl (3400 cm^{-1}) and a carboxyl (1630 cm^{-1}). The ^{1}H NMR spectrum was similar to that of **1**, except for the N-side chain moiety. Comparing with the ^{1}H and ^{13}C NMR data of **2**, the methylen protons and carbon signals at δ 5.24 (2H, s) and δ 62.3 did not appear and the methyl protons at δ 2.01 (3H, d, $J=7.1$ Hz), carbon at d 67.7 newly appeared in the spectra of **3**. In the ^{1}H ^{1}H COSY, the methyl protons at δ 2.01 were coupling with the methine proton at δ 5.49. The methyl protons at δ 2.01 showed HMBC correlation with the methine carbon at δ 67.7 and the quaternary carbon at δ 173.1. The methine proton at δ 5.49 showed HMBC correlation with the methyl carbon at δ 19.1, the quaternary carbon at δ 173.1, and the aromatic carbons at δ 144.4 and 144.5 on the isoquinoline moiety. Thus, the structure of panaefluorolin C was established as shown in **3**.

These fluorescent pigments could not be detected in the lichen thallus by HPLC analysis.

Isoquinoline alkaloids in higher plants were biosynthesized from tyrosine. Although the isolated compounds in this paper have an isoquinoline skeleton, the nitrogen atom of **1**, **2** and **3** may be derived from different amino acids such as threonine, glycine and alanine, respectively. On the other hand TMC-120A, furo[2,3-*h*]-isoquinoline type alkaloids, isolated from *Aspergillus ustus* TC1118, has a similar structure to **1**–**3**, but it does not have an N-linked side chain. This is the first report for the new type of isoquinoline alkaloids from natural source.

These compounds are very interesting on both their structures and fluorescent characters, and they might be useful for analytical chemistry and other things in the future.

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