

HETEROCYCLES, Vol. 81, No. 8, 2010, pp. 1931 - 1935. © The Japan Institute of Heterocyclic Chemistry
Received, 8th June, 2010, Accepted, 23rd June, 2010, Published online, 24th June, 2010
DOI: 10.3987/COM-10-11989

NAPHTHOPYRONES FROM CULTURED LICHEN MYCOBIONTS OF *PYRENULA SP.*

Yukiko Takenaka,^a Naotaka Nagakura,^a Nobuo Hamada,^b and Takao Tanahashi^{a*}

^aKobe Pharmaceutical University, 4-19-1 Motoyamakita-machi, Higashinada-ku, Kobe 658-8558, Japan. E-mail: tanahash@kobepharm-u.ac.jp; ^bOsaka City Institute of Public Health and Environmental Sciences, 8-34 Tojo-cho, Tennouji-ku, Osaka 543-0026, Japan

Abstract – Spore-derived mycobionts of the lichen *Pyrenula* sp. were cultivated on a malt-yeast extract medium supplemented with 10% sucrose and their metabolites were investigated. A new naphthopyrone derivative, pyrenulin, as well as toralactone were isolated. Their structures were determined by spectroscopic methods. This is the first instance of the isolation of naphthalene derivatives from lichen mycobionts.

Lichens, which exhibit symbiotic association, produce a variety of characteristic secondary metabolites, some of which have been found to exhibit a wide range of potentially useful biological activities.^{1,2} Recent studies demonstrated that cultures of spore-derived lichen mycobionts have the ability to produce certain lichen substances³⁻⁵ or novel metabolites that are structurally related to fungal metabolites⁶⁻¹⁰ under osmotically stressed conditions. These findings suggested that cultures of lichen mycobionts could be a new source of bioactive compounds. In the course of our studies on cultured lichen mycobionts, we cultivated the spore-derived mycobiont *Pyrenula* sp. and isolated a novel naphthopyrone derivative along with toralactone from its cultures. In this paper, we report the isolation and characterization of the new compound.

Specimens of *Pyrenula* sp. were collected from the bark of trees in Miyazaki. The polyspore-derived mycobionts were cultured on conventional malt-yeast extract medium supplemented with 10% sucrose at 18°C in the dark. After 14 months, the cultivated colonies were harvested and extracted with ether and then with acetone. Subsequent purification of the extracts by preparative TLC afforded two compounds **1** and **2**.

Compound **1**, obtained as an unstable substance, had the molecular formula of C₁₅H₁₂O₅ as established by

hydrogen-bonded hydroxy groups were located from their HMBC interactions. Furthermore, the HMBC correlations from the signal due to a hydroxy group at δ_{H} 7.08 to an acetal carbon and from the acetal proton at δ_{H} 6.98 to a carbonyl carbon at δ_{C} 169.7 suggested the presence of a lactol bridge. Significant HMBC correlations from both aromatic protons to an aromatic quaternary carbon (C-6a) at δ_{C} 114.5, and NOESY cross peak between the acetal proton and the methyl group, together with the presence of a hydrogen-bonded hydroxy group adjacent to a carbonyl group, demonstrated the substitution of functional groups on the naphthalene ring as shown in Figure 2.

Table 1. ^1H and ^{13}C NMR Spectral Data of **2** (acetone- d_6) and **2a** (CDCl_3)

C	2			2a		
	δ_{H}		δ_{C}	δ_{H}		δ_{C}
1			169.7			159.1
3	6.98	d (7.0)	95.4	7.80	br s	89.2
3a			126.3			123.7
4			137.5			136.6
5	7.26	br s	126.8	7.36	br s	128.3
6			132.1			131.4
6a			114.5			117.0
7			163.1			160.9
8	6.70	s	98.7	6.71	s	103.5
9			166.9			155.4
9a			93.8			102.8
9b			129.8			130.5
4-CH ₃	2.58	br s	18.0	2.47	br s	18.2
6-COOCH ₃	3.91	s	52.6	3.96	s	52.7
6-COOCH ₃			170.7			170.0
7-OCH ₃	4.09	s	57.4	4.04	s	57.0
3-OH	7.08	d (7.0)				
9-OH	12.37	br s				
3-OCOCH ₃				2.06	s	20.8
3-OCOCH ₃						168.8
9-OCOCH ₃				2.46	s	21.2
9-OCOCH ₃						169.0

Values in parentheses are coupling constants in Hz.

Acetylation of **2** gave a diacetate **2a**, $\text{C}_{20}\text{H}_{18}\text{O}_9$. The results of detailed 2D-NMR experiments with **2a** were fully coincident with the proposed structure. Thus, the structure of the new compound was elucidated as **2** and designated pyrenulin. Metabolites with a naphthalene skeleton are rarely found as lichen substances, although simonyelline (**5**)¹⁵ and euplectin¹⁶ have so far been isolated from thalli of lichens. This is the first instance of isolation of this type of metabolite from cultured mycobionts of

lichen.

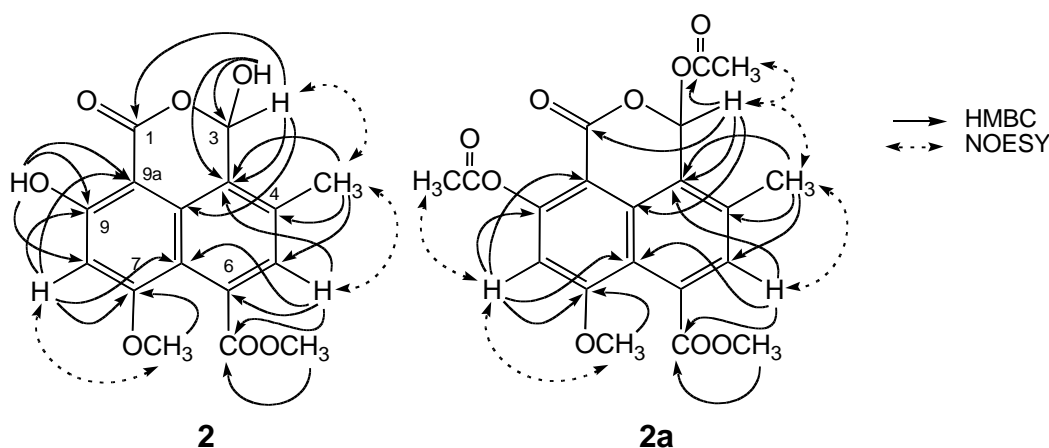


Figure 2. HMBC and NOESY correlations of **2** and **2a**

EXPERIMENTAL

General Procedures. Melting points were measured on a Yanaco micro melting point apparatus and are not corrected. The UV spectra were recorded on a Shimadzu UV-240 spectrophotometer and the IR spectra on a Shimadzu FTIR-8200 infrared spectrophotometer. HREIMS were obtained with a Hitachi M-4100 mass spectrometer. The NMR experiments were performed with a Varian VXR-500 spectrometer with tetramethylsilane as an internal standard. Thin-layer chromatography was performed on pre-coated Kieselgel 60F₂₅₄ plates (Merck), and spots were visualized under UV light.

Plant Material and Isolation of Compounds. Specimens of *Pyrenula* sp. were collected from the bark of trees in Hongo-cho, Miyazaki Pref., Japan (100m alt.) by N. Hamada. The voucher specimen (NH 96101336) was identified by Prof. H. Miyawaki, Saga University, Japan, and was deposited at Saga University. Mycobionts of *Pyrenula* sp. were obtained from the spores discharged from apothecia of a thallus, and were cultivated in 7 test tubes containing modified MY10 medium (malt extract 10 g, yeast extract 4 g, sucrose 100 g, agar 15 g, H₂O 1 L, pH 7) at 18 °C in the dark. After cultivation for 14 months, the harvested colonies (dry weight 2.05 g) were continuously extracted with ether and then with acetone at rt, and the combined extracts were concentrated under reduced pressure to give residues (ether ext., 23.3 mg; acetone ext. 234.3 mg). The respective residues were repeatedly subjected to preparative TLC with toluene-acetone (19:1 or 9:1), giving rise to toralactone (**1**) (6.7 mg) and pyrenulin (**2**) (7.8 mg).

Pyrenulin (2): Colorless needles, mp 163-164 °C (MeOH). UV (EtOH) λ_{\max} nm (log ϵ): 225sh (4.26), 243.5 (4.56), 301 (3.88), 313 (3.98), 342 (3.87), 354 (3.85), 378sh (3.23). IR (KBr) ν_{\max} cm⁻¹: 3320, 1736, 1664, 1643, 1614, 1508. HREIMS m/z : Calcd for C₁₆H₁₄O₇ [M]⁺: 318.0740. Found: 318.0718.

Acetylation of 2. Compound **2** (5.6 mg) was acetylated with Ac₂O-pyridine (each 0.1 mL) and the crude acetate was purified by preparative TLC (toluene-acetone, 19:1) to yield acetate (**2a**) (4.6 mg). **2a**: Pale yellow crystalline solid, mp 205 °C (MeOH). UV (EtOH) λ_{\max} nm (log ϵ): 218 (3.87), 245.5 (4.30), 317sh

(3.65), 329.5 (3.71), 343sh (3.57). IR (KBr) ν_{\max} cm^{-1} : 1771, 1740, 1719, 1601, 1522. HREIMS m/z : Calcd for $\text{C}_{20}\text{H}_{18}\text{O}_9$ $[\text{M}]^+$: 402.0951. Found: 402.0964.

ACKNOWLEDGEMENTS

We are grateful to Prof. H. Miyawaki for identification of the voucher specimen. Thanks are also due to Dr. M. Sugiura (Kobe Pharmaceutical University) for ^1H and ^{13}C NMR spectra, and to Dr. K. Saiki (Kobe Pharmaceutical University) for MS spectra measurements. This research was financially supported in part by a Grant-in-Aid for Scientific Research (C) (No. 11672129) from the Ministry of Education, Sciences, Sports, and Culture of Japan and Kobe Pharmaceutical University Collaboration Fund.

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