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Monomeric and dimeric dibenzofurans from cultured mycobionts of *Lecanora iseana*

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Abstract

Spore-derived mycobionts of the lichen *Lecanora iseana* were cultivated on a malt-yeast extract medium supplemented with 10% sucrose and their metabolites were investigated. Four 3,7-dihydroxy-1,9-dimethyldibenzofuran derivatives along with the known 3,7-dihydroxy-1,9-dimethyldibenzofuran and five norlichexanthone derivatives were isolated. Their structures were determined by spectroscopic methods.

Keywords: *Lecanora iseana*; Lichen; Mycobiont; Dibenzofurans; Xanthones; Lecanorafurans A and B

1. Introduction

Lichens, a symbiotic association of mycobiont and photobiont partners, produce a variety of characteristic secondary metabolites. On the other hand, it has been found that cultures of spore-derived lichen mycobionts have an ability to produce novel metabolites structurally related to fungal metabolites under osmotically stressed conditions (Tanahashi et al., 1997). These findings suggested the possibility that the cultures of lichen mycobionts could be sources of new bioactive compounds. We have recently isolated diverse compounds from the cultured mycobionts of *Graphis* (Tanahashi et al., 1997, 2000; Takenaka et al., 2000) and *Pyrenula* species (Tanahashi et al., 1999). In the course of our studies on cultured lichen mycobionts, we cultivated the spore-derived mycobionts of *Lecanora iseana* and isolated diverse dibenzofurans and xanthones from their cultures. We report herein the isolation and characterization of these compounds.

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2. Results and discussion

The polyspore-derived mycobionts of *L. iseana* Räs collected in Japan were cultivated on conventional malt-yeast extract medium supplemented with 10% sucrose at 18 °C in the dark. After cultivation over 6 months, the colonies were harvested and extracted with acetone. The extract was separated by a combination of preparative TLC and preparative HPLC to afford four new (**1**–**4**) and six known (**5**–**11**) compounds along with fatty acids and triacylglycerols. Compounds **5**–**11** were determined to be 3,7-dihydroxy-1,9-dimethyldibenzofuran (Tanahashi et al., 2001), norlichexanthone (Broadbent et al., 1975), 4-chloronorlichexanthone (Sundholm, 1978), 2,4-dichloronorlichexanthone (Huneck and Höfle, 1978), 4,5-dichloronorlichexanthone (Fitzpatrick et al., 1980), arthothelin (Huneck and Höfle, 1978), and thiophanic acid (Huneck, 1966), respectively.

Compound **1**, obtained as a colorless crystalline solid, had a molecular formula of $C_{14}H_{11}ClO_3$ as established by HR-EIMS. It showed UV maxima at 217.5, 229,

241.5sh, 257.5sh, 264.5, 298.5sh, and 306.5 nm, and IR bands at 3337 (OH) and 1616, 1558 (substituted aromatic system) cm^{-1}. Its ^1H and ^{13}C NMR spectroscopic data were similar to those of 3,7-dihydroxy-1,9-dimethyldibenzofuran (**5**), suggesting it was a chlorinated derivative of **5** (Tanahashi et al., 2001). A significant HMBC correlation between the methyl protons at δ 2.79 and an aromatic carbon bearing a hydrogen at δ 115.4, and a COSY correlation of the methyl protons with an aromatic proton at δ 6.79 (*d*, $J = 2.5$ Hz) revealed that the chlorine atom was substituted at C-4 rather than C-2. This was supported by the downfield shift of C-4 as well as the upfield shifts of C-1, C-3 and C-4a of **1**, when compared with the corresponding ^{13}C NMR signals of **5**. Thus, the structure of **1** was determined as 4-chloro-3,7-dihydroxy-1,9-dimethyldibenzofuran.

The ^1H and ^{13}C NMR spectral features of **2**, together with its molecular formula of $C_{14}H_{10}Cl_2O_3$, suggested a symmetrical structure. The ^{13}C NMR spectral data of **2** were in good agreement with those of chlorinated aromatic ring of **1**. The substitution pattern was further confirmed by HMBC experiments with **2** (Fig. 1). Thus, the structure of **2** was determined as 4,6-dichloro-3,7-dihydroxy-1,9-dimethyldibenzofuran.

The HR-EIMS spectrum of **3** established the composition of $C_{28}H_{22}O_6$. Its ^1H NMR spectrum exhibited signals for two methyl groups and three aromatic protons. The ^{13}C NMR spectrum of **3** showed two methyl carbons, three aromatic CH carbons and nine quaternary carbons, four of which were oxygenated (Table 1). The ^{13}C NMR spectral features of **3** resembled those of **1** except for the chemical shifts of C-1, C-3, C-4, and C-4a. These findings, together with its structural formula, suggested that the isolated compound was a symmetrical dimer in which two 3,7-dihydroxy-1,9-dimethyldibenzofuran (**5**) units were linked through a C–C bond between C-4 and C-4′. The linkage of two dibenzofuran units was further supported by significant HMBC correlations from the aromatic proton at δ 6.77 (*d*, $J = 0.5$ Hz) to the methyl carbon, C-3, C-4 and C-9b. Accordingly, the structure of **3** was established as shown and designated lecanorafuran A.

Compound **4**, named lecanorafuran B, was isomeric with **3**. The ^1H and ^{13}C NMR spectra of **4** demonstrated two sets of signals corresponding to 3,7-dihydroxy-1,9-dimethyldibenzofuran (**5**), implying an unsymmetrical dimer of **5**. Structure **4** with the linkage between C-2 and C-4′ was proposed as a sole possible unsymmetrical dimer. Significant HMBC correlations between the aromatic proton at δ 6.92 (*br s*) and oxygenated carbons at δ 155.5 and 158.5, and between the aromatic proton at δ 6.74 (*br s*) and carbons at δ 25.1 and 107.9, confirmed that two dibenzofuran units were linked between C-2 and C-4′. All of 2D NMR experiments with the isolated compound were consistent with the proposed structure **4** (Fig. 1). Thus, the structure of lecanorafuran B was elucidated as shown.

We cultivated the mycobionts of the lichen *L. iseana* and have isolated five dibenzofurans (**1**–**5**) as well as five known norlichexanthone derivatives (**6**–**11**) from the cultures. Norlichexanthone derivatives **6**–**11** have been isolated from natural lichens, although 4-chloronorlichexanthone (**7**) and 4,5-dichloronorlichexanthone (**9**)

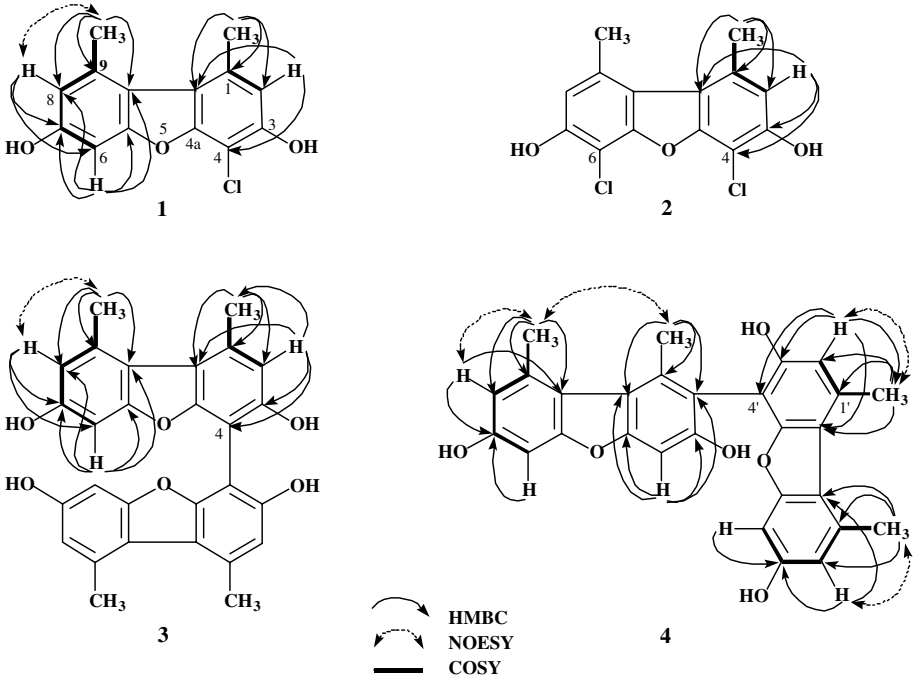


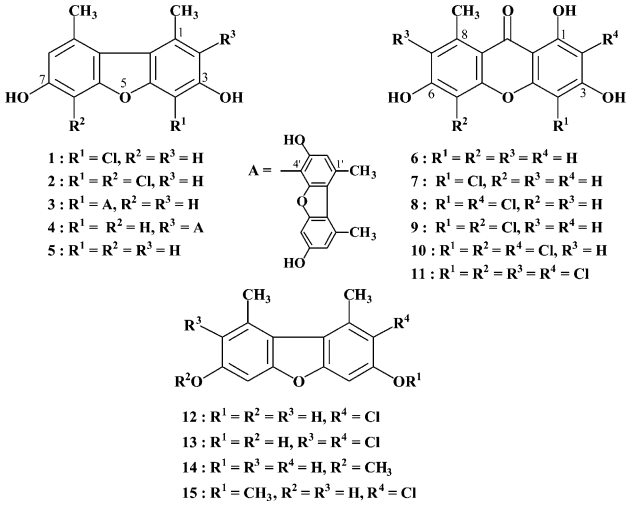
Fig. 1. H–^1H COSY, HMBC and NOESY correlations observed for **1**–**4**.

Table 1
^{13}C NMR spectroscopic data of compounds **1**–**5** in CD_3OD

C	1	2	3	4	5
1 (1′)	130.9	131.4	132.3	133.3 131.8	132.9
2 (2′)	115.4	115.9	115.2	118.5[a] 115.0	114.8
3 (3′)	152.6	153.1	154.8	155.5 154.4	156.9
4 (4′)	102.3	102.5	103.4	96.70[b] 107.9	96.6
4a (4′a)	154.6	154.7	157.6	158.5 157.5	159.2
5a (5′a)	159.3	154.7	159.3	159.4[c] 160.0[c]	159.2
6 (6′)	96.8	102.5	96.7	96.72[b] 96.77[b]	96.6
7 (7′)	157.5	153.1	156.8	157.0 157.0	156.9
8 (8′)	115.5	115.9	114.8	115.1 114.8	114.8
9 (9′)	133.5	131.4	132.9	133.2 132.9	132.9
9a (9′a)	117.5	118.8	118.0	118.1 118.1	117.6
9b (9′b)	118.8	118.8	117.8	117.3[a] 117.8	117.6
1 (1′)-CH_3	24.8	24.7	25.2	21.6 25.1	25.1
9 (9′)-CH_3	25.0	24.7	25.2	25.8 25.2	25.1

[a–c] Assignments with the same superscript may be interchanged.

had been erroneously characterized (Santesson, 1969a,b) and their structures were revised by chemical synthesis (Sundholm, 1978). This is the first reported isolation of diverse norlichexanthone derivatives from cultured lichen mycobionts. It is also noteworthy that the isolated dibenzofurans from the cultured mycobionts have no carboxyl group in contrast to the natural lichen dibenzofurans such as didymic acid. This structural characteristic has been recognized previously with 3,7-dihydroxy-1,9-dimethyldibenzofuran (**5**) and its derivatives **12**–**15** from the cultured mycobionts of *L. cinereocarnea* (=*L. leprosa*) (Tanahashi et al., 2001).



3. Experimental

3.1. General

Melting points were measured on a Yanaco micro melting point apparatus and are reported uncorrected. The UV spectra were recorded on a Shimadzu UV-240 spectrophotometer and the IR spectra on a Shimadzu FTIR-8200 infrared spectrophotometer. HR-EIMS were obtained with a Hitachi M-4100 mass spectrometer. The NMR experiments were performed with Varian VXR-500, Varian Gemini-300 and Varian Gemini-200 spectrometers, with tetramethylsilane as internal standard. HPLC was performed using a Waters system (600E Multisolvent Delivery System, 486 Tunable Absorbance Detector). Thin-layer chromatography was performed on precoated Kieselgel $60F_{254}$ plates (Merck) and spots were visualized under UV light.

3.2. Plant material

Specimens of *L. iseana* Räs. were collected from the bark of trees at Hachijo Island in Tokyo Prefecture, Japan (ca. 500 m alt.). The voucher specimens were identified by Prof. H. Miyawaki, Saga University, Japan and were deposited at Osaka City Institute of Public Health and Environmental Sciences with the Registration No. NH0013060. The mycobionts were obtained from spores discharged from apothecia of the thallus, and were cultivated in test tubes containing modified MY10 medium (malt extract 10 g, yeast extract 4 g, sucrose 100 g, agar 15 g, H_2O 1 l, pH 7) at 18 °C in the dark for 6 months.

3.3. Extraction and isolation

The harvested colonies (117 test tubes, freeze-dried weight 15.11 g) were extracted with acetone at room temperature, and the combined extracts were concentrated under reduced pressure to give a residue (1.04 g). The extract was repeatedly subjected to preparative TLC (toluene–acetone, 7:3 or toluene–AcOH, 7:3) and preparative HPLC (μBondasphere 5μC18-100 Å, MeCN–H_2O, 1:1), to afford to **1** (1.0 mg), **2** (3.8 mg), **3** (3.5 mg), **4** (2.0 mg), **5** (10.8 mg), **6** (55.0 mg), **7** (7.5 mg), **8** (30.4 mg), **9** (5.4 mg), **10**

(25.3 mg), **11** (9.9 mg), long-chain fatty acids (157.2 mg), and triacylglycerols (405.3 mg).

3.3.1. 4-Chloro-3,7-dihydroxy-1,9-dimethyldibenzofuran (*1*)

Colorless crystalline solid, m.p. 158–159 °C (MeOH). UV λ_{max}^{MeOH} nm (log ε): 217.5 (4.26), 229 (4.31), 241.5 sh (4.19), 257.5 sh (3.90), 264.5 (3.93), 298.5 sh (4.00), 306.5 (4.02). IR ν_{max}^{KBr} cm^{-1}: 3337, 1616, 1558. ^{1}H NMR (CD_3OD): δ 2.79 (3H, *d*, *J* = 0.5 Hz, 1-CH_3), 2.80 (3H, *br s*, 9-CH_3), 6.60 (1H, *dd*, *J* = 2.5, 0.5 Hz, H-8), 6.68 (1H, *d*, *J* = 0.5 Hz, H-2), 6.79 (1H, *d*, *J* = 2.5 Hz, H-6). For ^{13}C NMR spectra, see Table 1. HR-EIMS *m*/*z*: Calc. for $C_{14}H_{11}{}^{35}ClO_3$ $[M]^+$: 262.0397. Found: 262.0378. Calc. for $C_{14}H_{11}{}^{37}ClO_3$ $[M]^+$: 264.0368. Found: 264.0377.

3.3.2. 4,6-Dichloro-3,7-dihydroxy-1,9-dimethyldibenzofuran (*2*)

Colorless crystalline solid, m.p. 197–198 °C (MeOH). UV λ_{max}^{MeOH} nm (log ε): 220 (4.41), 232.5 (4.47), 243 sh (4.37), 259 sh (4.01), 267 (4.08), 296 sh (4.12), 302 (4.13). IR ν_{max}^{KBr} cm^{-1}: 3367, 1609, 1447. ^{1}H NMR (CD_3OD): δ 2.76 (6H, *br s*, 1-CH_3, 9-CH_3), 6.70 (2H, *br s*, H-2, H-8). For ^{13}C NMR spectra, see Table 1. HR-EIMS *m*/*z*: Calc. for $C_{14}H_{10}{}^{35}Cl_2O_3$ $[M]^+$: 296.0007. Found: 296.0017. Calc. for $C_{14}H_{10}{}^{35}Cl^{37}ClO_3$ $[M]^+$: 297.9978. Found: 297.9935. Calc. for $C_{14}H_{10}{}^{37}Cl_2O_3$ $[M]^+$: 299.9948. Found: 299.9931.

3.3.3. Lecanorafuran A (*3*)

Colorless crystalline solid, m.p. 198–200 °C (MeOH). UV λ_{max}^{MeOH} nm (log ε): 214.5 (4.51), 228.5 (4.48), 244.5 (4.41), 257 (4.41), 264 sh (4.39), 299.5 sh (4.28), 308 (4.31). IR ν_{max}^{KBr} cm^{-1}: 3370, 1618, 1576. ^{1}H NMR (CD_3OD): δ 2.85 (6H, *br s*, 9-CH_3, 9′-CH_3), 2.90 (6H, *d*, *J* = 0.5 Hz, 1-CH_3, 1′-CH_3), 6.55 (2H, *br d*, *J* = 2.0 Hz, H-8, H-8′), 6.57 (2H, *br d*, *J* = 2.0 Hz, H-6, H-6′), 6.77 (2H, *d*, *J* = 0.5 Hz, H-2, H-2′). For ^{13}C NMR spectra, see Table 1. HR-EIMS *m*/*z*: Calc. for $C_{28}H_{22}O_6$ $[M]^+$: 454.1417. Found: 454.1414.

3.3.4. Lecanorafuran B (*4*)

Colorless crystalline solid, m.p. 170–171 °C (MeOH). UV λ_{max}^{MeOH} nm (log ε): 217.5 (4.52), 229.5 (4.54), 242 (4.54), 256 sh (4.41), 263.5 sh (4.29), 300.5 sh (4.35), 310.5 (4.40). IR ν_{max}^{KBr} cm^{-1}: 3367, 1609, 1584. ^{1}H NMR (CD_3OD): δ 2.54 (3H, *s*, 1-CH_3), 2.80 (3H, *br s*, 9-CH_3), 2.85 (3H, *br s*, 9′-CH_3), 2.89 (3H, *d*, *J* = 1.0 Hz, 1′-CH_3), 6.56 (1H, *dd*, *J* = 2.0, 1.0 Hz, H-8′), 6.58 (1H, *dd*, *J* = 2.0, 1.0 Hz, H-8), 6.62 (1H, *br d*, *J* = 2.0 Hz, H-6′), 6.74 (1H, *d*, *J* = 1.0 Hz, H-2′), 6.76 (1H, *br d*, *J* = 2.0 Hz, H-6), 6.92 (1H, *br s*, H-4). For ^{13}C NMR spectra, see Table 1. HR-EIMS *m*/*z*: Calc. for $C_{28}H_{22}O_6$ $[M]^+$: 454.1417. Found: 454.1420.

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