Available online at www.sciencedirect.com
SCIENCE DIRECT®
Tetrahedron Letters 47 (2006) 1505–1508
Tetrahedron Letters

Kehokorins A–C, novel cytotoxic dibenzofurans isolated from the myxomycete *Trichia favoginea* var. *persimilis*

Kouken Kaniwa,[a] Takashi Ohtsuki,[a] Yukinori Yamamoto[b] and Masami Ishibashi[a,*]

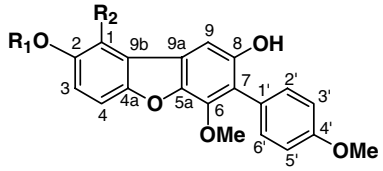
[a]*Graduate School of Pharmaceutical Sciences, Chiba University, Chiba 263-8522, Japan*
[b]*Graduate School of Pharmaceutical Sciences, Chiba University, 1010-53 Ohtsu-ko, Kochi 781-5102, Japan*

Received 6 December 2005; revised 27 December 2005; accepted 5 January 2006
Available online 23 January 2006

Abstract—Kehokorins A (**1**)–C (**3**), three novel dibenzofurans, have been isolated from field-collected fruit bodies of the myxomycete, *Trichia favoginea* var. *persimilis*, and their structures were elucidated by spectral data. Kehokorin A (**1**) was a α-L-rhamnopyranoside of kehokorin B (**2**), while kehokorin C (**3**) was a 1-demethoxy analog of **2**. Kehokorin A (**1**) was cytotoxic against HeLa cells with an IC_{50} value of 1.5 μg/mL.

1. Introduction

Myxomycetes (true slime molds) are an unusual group of primitive organisms that may be assigned to one of the lowest classes of eukaryotes, and chemical studies on the secondary metabolites of the myxomycetes are limited so far.[1] During our search for bioactive natural products from myxomycetes,[2,3] we recently investigated a field-collected sample of fruit bodies of *Trichia favoginea* var. *persimilis* (Trichiaceae). Here we describe the isolation and structure elucidation of three novel dibenzofurans, kehokorins A (**1**)–C (**3**). Kehokorins A (**1**) was revealed to possess cytotoxic activity against the HeLa human epithelial carcinoma cell line, and the presence of a rhamnose unit proved to be important for the cytotoxicity.



1 R_1=α-L-Rha, R_2=OMe
2 R_1=H, R_2=OMe
3 R_1=R_2=H

2. Results and discussion

The fruit bodies of the myxomycetes, *T. favoginea* var. *persimilis*,[4] collected in Kochi Prefecture, Japan, were extracted with 90% MeOH and 90% acetone. The combined extracts were subjected to flash chromatography on silica gel, followed by gel filtration chromatography using Sephadex LH-20 to give kehokorins A (**1**), B (**2**), and C (**3**) in 0.06%, 0.06%, and 0.007% yield, respectively.[5]

Kehokorin A (**1**),[6] yellow amorphous solid, $[\alpha]_D^{25}$ −49 (*c* 0.5, MeOH), showed a quasi-molecular ion peak at *m/z* 513 $(M+H)^+$ in its positive FAB mass spectrum. The molecular formula of **1** was revealed as $C_{27}H_{28}O_{10}$ by the HRFABMS data [*m/z* 513.1759, $(M+H)^+$, Δ −0.2 mmu]. The UV spectrum of **1** showed absorption maxima at 298, 274, and 226 nm, indicating the presence of conjugated or aromatic system(s). The ^{1}H NMR spectrum of **1** in acetone-d_6 (Table 1) showed signals for three methoxy groups at δ_H 4.09 (3H, s), 3.92 (3H, s), and 3.85 (3H, s) and aromatic hydrogens at δ_H 7.39 (1H, d, $J = 7.5$ Hz), 7.30 (1H, d, $J = 7.5$ Hz), 7.43 (1H, s), and 7.37 and 7.00 (each 2H, d, $J = 7.0$ Hz; a *p*-substituted benzene ring). The ^{1}H NMR also showed signals assignable to a sugar moiety at δ_H 5.48 (1H, s), 4.20

Keywords: Myxomycete; *Trichia favoginea* var. *persimilis*; Dibenzofuran; Fruit body; Cytotoxicity.
* Corresponding author. Tel./fax: +81 43 290 2913; e-mail: mish@p.chiba-u.ac.jp

0040-4039/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2006.01.012

(1H, br s), 3.95 (1H, dd, $J = 7.8$ and 3.0 Hz), 3.55 (1H, t, $J = 7.8$ Hz), 3.90 (1H, m), and one doublet methyl group at δ_H 1.25 (3H, d, $J = 4.5$ Hz). The ^{13}C NMR spectrum of **1** (Table 1) gave signals due to 18 sp^2 carbons, three *O*-methyl carbons, and a sugar moiety. Interpretation of the ^1H–^1H COSY spectrum of **1** implied the presence of one 6-deoxyhexose unit from the connectivities observed from H-1″ (δ_H 5.48, 1H, s) to H_3-6″ (δ_H 1.25, 3H, d) as shown in Figure 1. The HMBC spectrum of **1** indicated the presence of three benzene rings, which were shown to be di-, tetra-, and penta-substituted, as shown in Figure 1. The HMBC spectrum also showed connectivities from the methoxy protons on δ_H 4.09, 3.92, and 3.85 to sp^2 quaternary carbons at δ_C 146.5, 144.0, and 160.1, respectively, which were assigned to the C-1, C-6, and C-4′ positions, respectively, by the HMBC correlations shown in Figure 1. NOE correlations were observed from the 1-OMe (δ_H 4.09) to H-9 (δ_H 7.43) and from 4′-OMe (δ_H 3.85) to H-3′(5′) (δ_H 7.00), supporting that the methoxy groups are located on the C-1 and C-4′ positions. The presence of a 6-deoxyhexopyranose unit was corroborated by the HMBC correlations for H-1″/C-3″, H-1″/C-5″, H-2″/C-3″, H-2″/C-4″, H-3″/C-4″, H-4″/C-3″, H-4″/C-5″, H-4″/C-6″, H-5″/C-4″, H_3-6″/C-4″, and H_3-6″/C-5″, and this sugar unit was attached on the C-2 position (δ_C 145.9), revealed by the HMBC correlations observed from H-1″ (δ_H 5.48) to C-2 (δ_C 145.9). The *p*-substituted benzene ring was shown to be located on the C-7 position by the HMBC correlations from H-2′(6′) (δ_H 7.37) to C-7 (δ_C 122.5). Three carbons resonating at δ_C 154.0, 143.5, and 152.6 were implied to bear an oxygen atom from their ^{13}C chemical shifts, and these carbons were suggested to be C-4a, C-5a, and C-8, respectively, from the HMBC spectrum (Fig. 1). Since 13 out of 14 unsaturation equivalents were accounted for by the presence of three benzene rings and one hexose unit, compound **1** was inferred to possess another ring, which was likely to be an ether ring located between the C-4a and C-5a positions, constructing a dibenzofuran nucleus for the basic skeleton of compound **1**. Low-field resonance of the remaining C-8 carbon (δ_C 152.6) suggested that this carbon bore a hydroxyl group, and therefore the whole planar structure of kehokorin A was revealed as **1**. The 6-deoxyhexopyranose unit was identified as rhamnose on the basis of the ^1H–^1H coupling constants[7] and the ^{13}C NMR chemical shift data (Table 1), which was confirmed by the HPLC analysis after enzymatic hydrolysis.[8] The anomeric configuration for the rhamnose unit was deduced as α from the one-bond ^{13}C–^1H coupling constant between C-1″ and H-1″ ($^1J_{C-H} = 170.9$ Hz; literature values:[9] α-anomer, 169 Hz; β-anomer, 160 Hz), while the absolute configuration of rhamnose was revealed as L on the basis of HPLC analysis of the enzymatic hydrolysis product[8] by comparison with the authentic samples using an optical rotation detector.

The molecular formula of kehokorin B (**2**)[10] was revealed as $C_{21}H_{18}O_6$ by the HRFABMS data [*m*/*z*

Table 1. ^1H and ^{13}C NMR data of kehokorins A (**1**), B (**2**), and C (**3**)

Position	**1** (Acetone-d_6)[a]				**2** (Acetone-d_6)[b]				**3** ($CDCl_3$)[b]			
	δ_H	*J* (Hz)		δ_C	δ_H	*J* (Hz)		δ_C	δ_H	*J* (Hz)		δ_C
1				146.5				142.8	7.31	d	2.8	106.2
2				145.9				146.3				151.5
3	7.39	d	8.8	119.6	7.06	d	8.5	117.6	7.39	dd	8.3, 2.8	115.5
4	7.30	d	8.8	107.8	7.20	d	8.5	108.0	7.41	d	8.3	112.2
4a				154.0				152.2				151.3
5a				143.5				143.5				142.9
6				144.0				144.0				142.6
7				122.5				122.3				119.9
8				152.6				152.4				149.4
9	7.43	s		103.6	7.39	s		103.5	7.17	s		99.9
9a				124.8				124.8				125.5
9b				119.7				119.3				125.1
1′				127.4				127.4				124.2
2′	7.37	d	8.5	133.3	7.36	d	8.8	133.3	7.36	d	8.5	131.9
3′	7.00	d	8.5	114.4	6.98	d	8.8	114.4	7.07	d	8.5	114.8
4′				160.1				160.0				159.6
5′	7.00	d	8.5	114.4	6.98	d	8.8	114.4	7.07	d	8.5	114.8
6′	7.37	d	8.5	133.3	7.36	d	8.8	133.3	7.36	d	8.5	131.9
1″	5.48	s		102.2								
2″	4.20	br s		72.2								
3″	3.95	dd	7.8, 3.0	72.7								
4″	3.55	t	7.8	73.8								
5″	3.90	m		70.8								
6″	1.25	d	4.5	18.5								
1-OMe	4.09	s		61.7	4.02	s		61.2				
6-OMe	3.92	s		61.1	3.90	s		61.1	4.01	s		60.8
4′-OMe	3.85	s		55.8	3.84	s		55.8	3.89	s		55.3
8-OH									4.92	s		

[a] 600 MHz for ^1H and 150 MHz for ^{13}C NMR.
[b] 500 MHz for ^1H and 125 MHz for ^{13}C NMR.

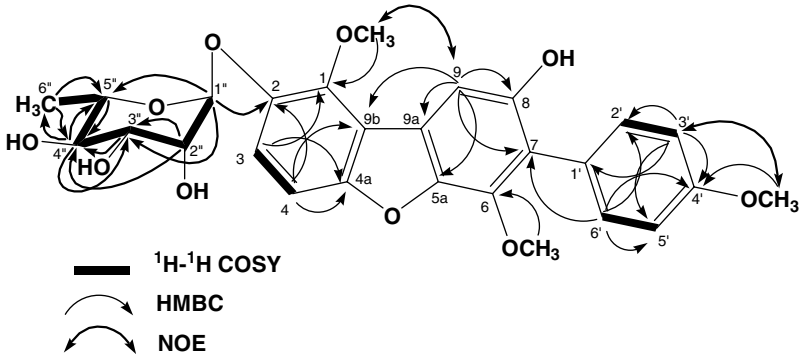


Figure 1. Key ^{1}H–^{1}H COSY, HMBC, and NOE data of **1**.

366.1096, (M^{+}), Δ −0.7 mmu], having a $C_6H_{10}O_4$ unit less than that of kehokorin A (**1**). The ^{1}H and ^{13}C NMR spectral data of **2** (Table 1) as well as its UV absorption were almost parallel to those of compound **1**, except for the fact that the NMR signals due to a rhamnose unit were not observed for compound **2**. The ^{1}H–^{1}H COSY, HMQC, and HMBC data suggested that kehokorin B (**2**) was an aglycone of kehokorin A (**1**); this was further confirmed by the enzymatic hydrolysis of **1** to afford an aglycon, which was identical with kehokorin B (**2**) on the basis of the TLC examination and the ^{1}H and ^{13}C NMR spectral data.

Kehokorin C (**3**)[11] was obtained as a yellow amorphous solid, and its molecular formula was determined as $C_{20}H_{16}O_5$ by its HRFABMS data (*m/z* 336.0984, M^{+}, Δ −1.4 mmu), possessing a CH_2O unit less than that of kehokorin B (**2**). Although kehokorin B (**2**) possessed three methoxy groups, the ^{1}H NMR spectrum of **3** showed signals for only two methoxy groups [δ_H 4.01 (3H, s) and 3.89 (3H, s)]. In the ^{1}H NMR spectrum of **3**, a doublet signal was observed at δ_H 7.31 assignable to an aromatic hydrogen with *meta*-coupling ($J = 2.8$ Hz), which was not observed in that of B (**2**). This aromatic hydrogen was shown to be located on C-1 (δ_C 106.2) from the HMBC correlation data (H-1/C-2, H-1/C-3, H-1/C-9a, and H-3/C-1). Thus, it was revealed that the methoxy group on C-1 of kehokorin B (**2**) was replaced by a hydrogen for kehokorin C (**3**), being consistent with the molecular formula of **3** losing a CH_2O unit.

Kehokorins A (**1**)–C (**3**) are the first example of a *p*-terphenyl class of natural products isolated from myxomycetes, although these compounds (**1**–**3**) had a kind of structural resemblance with dictyomedins A and B,[12] a *m*-terphenyl class of natural products isolated from *Dictyostelium* cellular slime molds, having a carboxyl group on C-2 and another benzene ring attached on a different position (C-4) in the dibenzofuran nucleus. Although several types of *p*-terphenyl compounds had been isolated from fungi and mushrooms,[13,14] dibenzofuran glycoside derivatives such as kehokorin A (**1**) are rare to the best of our knowledge. Cytotoxic activity of kehokorins A (**1**)–C (**3**) against the HeLa human epithelial carcinoma cell line was examined and their IC_{50} values were revealed to be 1.5, 7.2, and >8.4 μg/mL, respectively, implying that the presence of a rhamnose unit is important for the cytotoxicity. Kehokorins A (**1**) exhibited antimicrobial activity weakly against *Staphylococcus aureus* (a diameter of inhibition zone 10.1 mm at 50 μg per paper disc (8 mm in diameter) but was inactive against *Bacillus subtilis* at that concentration, while kehokorins B (**2**) and C (**3**) were inactive against both *S. aureus* and *B. subtilis* at 50 μg/disc.

Acknowledgments

We thank Professor H. Takayama (Chiba University) for generous use of the optical rotation detector. We also thank Ms. Atsumi Osuga for technical assistances. This work was supported by a Grant-in-Aid for Scientific Research on Priority Areas (17035017) from the Ministry of Education, Culture, Sports, Science, and Technology (MEXT) of Japan.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2006.01.012.

References and notes

1. Steglich, W. *Pure Appl. Chem.* **1989**, *61*, 281–288.
2. Ishibashi, M.. In *Studies in Natural Products Chemistry*; Atta-ur-Rahman, Ed.; Elsevier: Amsterdam, 2003; Vol. 29, pp 223–262.
3. Ishibashi, M. *Med. Chem.* **2005**, *1*, 575–590, and references cited therein.
4. The fruit bodies of *Trichia favoginea* var. *persimilis* were collected at Tosayamada-shi in Kochi Prefecture, Japan, in January 2004. A voucher specimen (#25634) is maintained by Y.Y. (Ohtsu-ko, Kochi).
5. The wild fruit bodies (17.6 g) were extracted with 90% MeOH (200 mL × 3) and 90% acetone (200 mL × 1). The combined MeOH and acetone extracts (1.0 g) were subjected to silica gel column chromatography (30 × 180 mm) eluted with 0–100% methanol in chloroform, and the fraction (17 mg) eluting with 10% MeOH in $CHCl_3$ was separated by Sephadex LH-20 column (20 × 600 mm; MeOH) to afford kehokorin A (**1**, 10.0 mg). The fraction of the first silica gel column (49 mg) eluting with 2% MeOH in $CHCl_3$ was separated by the second silica gel

column eluted with 1% MeOH in $CHCl_3$ followed by Sephadex LH-20 column ($CHCl_3$/MeOH, 1:1) to afford kehokorin B (**2**, 10.0 mg) and kehokorin C (**3**, 1.2 mg).

6. Kehokorin A (**1**): Yellow amorphous solid; $[\alpha]_D^{25}$ −49 (*c* 0.5, MeOH); UV (MeOH) λ_{max} 298 (ε 18,000), 274 (13,000), and 226 nm (33,000); IR (film) ν_{max} 3416, 2065, and 1637 cm^{-1}; ^1H and ^{13}C NMR (Table 1); FABMS *m*/*z* 513 $(M+H)^+$; HRFABMS calcd for $C_{27}H_{29}O_{10}$ $(M+H)^+$ 513.1761, found *m*/*z* 513.1759.
7. The ^1H–^1H vicinal coupling constants for the sugar unit of **1** in CD_3OD were $J_{1'',2''} = 1.5$ Hz, $J_{2'',3''} = 3.5$ Hz, $J_{3'',4''} = 10.0$ Hz, $J_{4'',5''} = 9.5$ Hz, and $J_{5'',6''} = 6.5$ Hz, indicating that H-2″ was equatorial and H-3″, H-4″, and H-5″ were axial.
8. Kehokorin A (**1**, 4.1 mg) in HOAc/KOAc buffer (pH 4.3, 6.0 mL) was treated with naringinase (8.0 mg, Wako) at 37 °C for 15 d. After cooling to room temperature, water was added to the reaction mixture, and the mixture was partitioned with $CHCl_3$. The aqueous layer was neutralized by passage through an Amberlite IRA-96SB column, then analyzed by HPLC [Capcell Pak NH_2 UG80, 4.6 × 250 mm; eluent, 85% CH_3CN; flow rate, 0.8 mL/min; column temperature, 40 °C; detection, RI and optical rotation (JASCO OR-1590)], to identify L-rhamnose (t_R 10.3 min, negative peak in optical rotation detector). The $CHCl_3$ layer afforded an aglycon (2.1 mg), which was identical with kehokorin B (**2**) on the basis of comparison of TLC [silica gel TLC, $CHCl_3$/MeOH (9:1), R_f 0.59] and ^1H and ^{13}C NMR data.
9. Bock, K.; Lundt, I.; Pedersen, C. *Tetrahedron Lett.* **1973**, *13*, 1037–1040.
10. Kehokorin B (**2**): Yellow amorphous solid; UV (MeOH) λ_{max} 302 (ε 21,000), 274 (15,000), and 222 nm (34,000); IR (film) ν_{max} 3417, 2090, and 1637 cm^{-1}; ^1H and ^{13}C NMR (Table 1); FABMS *m*/*z* 366 (M^+); HRFABMS calcd for $C_{21}H_{18}O_6$ (M^+) 366.1103, found *m*/*z* 366.1096.
11. Kehokorin C (**3**): Yellow amorphous solid; UV (MeOH) λ_{max} 307 (ε 16,000), 266 (9000), and 203 nm (28,000); IR (film) ν_{max} 3418, 2064, and 1646 cm^{-1}; ^1H and ^{13}C NMR (Table 1); FABMS *m*/*z* 336 (M^+); HRFABMS calcd for $C_{20}H_{16}O_5$ (M^+) 336.0998, found *m*/*z* 366.0984.
12. Takaya, Y.; Kikuchi, H.; Terui, Y.; Komiya, J.; Maeda, Y.; Ito, A.; Oshima, Y. *Tetrahedron Lett.* **2001**, *42*, 61–63.
13. Calí, V.; Spatafora, C.; Tringali, C. In *Studies in Natural Products Chemistry*; Atta-ur-Rahman, Ed.; Elsevier: Amsterdam, 2003; Vol. 29, pp 263–307.
14. (a) Radulovic, N.; Quang, D. N.; Hashimoto, T.; Nukada, M.; Asakawa, Y. *Phytochemistry* **2005**, *66*, 1052–1059; (b) Liu, J.-K.; Hu, L.; Dong, Z.-J.; Hu, Q. *Chem. Biodiversity* **2004**, *1*, 601–605, and references cited therein.