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JBIR-37 and -38, Novel Glycosyl Benzenediols, Isolated from the Sponge-Derived Fungus, *Acremonium* sp. SpF080624G1f01

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In the course of our chemical screening program for new secondary metabolites, we isolated new compounds JBIR-37 (1) and -38 (2) from a culture broth of the marine sponge-derived fungus, *Acremonium* sp. SpF080624G1f01. The structures of 1 and 2 were determined to be di- and mono-*O*-*β*-D-glucopyranosyloxy-4-(1,1-dimethyl-2-propenyl)benzene on the basis of extensive NMR and MS spectroscopic data, respectively.

Key words: glycosyl benzenediol; marine sponge; Demospongiae; *Acremonium*

Many bioactive substances have been isolated from marine organisms such as marine microbes, phytoplankton, algae, sponges and tunicates.[1)] In particular, marine-derived fungi are emerging as an attractive source of new bioactive compounds.[2)] Indeed, cytotoxic metabolites such as diketopiperazine alkaloids,[3)] trichodermatides[4)] and carbonarones[5)] have been isolated from marine-derived fungi. We have also reported the new sesquiterpenes, JBIR-27 and -28, from a tunicate-derived fungus, *Penicillium* sp. SS080624SCf1,[6)] and the new aspochracin derivative, JBIR-15, from a marine sponge-derived fungus, *Aspergillus sclerotiorum* Huber Sp080903f04.[7)] We therefore attempted to isolate fungi from the marine sponge, Demospongiae, to obtain novel substances from the fungal culture broths of marine origin. We isolated two novel compounds designated as JBIR-37 (**1**) and -38 (**2**) from the culture broth of *Acremonium* sp. SpF080624G1f01. This paper describes the fermentation, isolation and structural elucidation of **1** and **2**.

The producing fungus, *Acremonium* sp. SpF080624G1f01, was isolated from the marine sponge, Demospongiae, collected from Ishigaki Island, Okinawa Prefecture, Japan. The SpF080624G1f01 strain was cultured in a 500-ml Erlenmeyer flask containing 15 g of brown rice (Hitomebore), 30 mg of a Bacto-Yeast extract (BD Biosciences), 15 mg of sodium tartrate (Kanto Chemical), 15 mg of K_2HPO_4 (Wako Pure Chemical) and 45 ml of H_2O at 27 °C for 14 d in static culture.

The culture was extracted with 80% acetone (100 ml). The resulting extract was evaporated *in vacuo*, and the residual aqueous concentrate was partitioned with EtOAc (100 ml × 3) before *n*-BuOH extraction (100 ml × 2). The *n*-BuOH layer was evaporated to dryness. The dried residue (80.2 mg) was chromatographed by reversed-phase MPLC (Purif-Pack ODS-100, Moritex) with an H_2O–MeOH linear gradient system (0–100% MeOH). Compounds **1** (5.4 mg) and **2** (0.5 mg) were respectively isolated as colorless amorphous solids from the 0–5% MeOH fractions by preparative reversed-phase HPLC, using a PEGASIL ODS column (Senshu Pak, 20 i.d. × 150 mm) with 20% aqueous MeOH containing 0.4% formic acid (t_R 17.3 min) and 30% aqueous MeOH containing 0.35% formic acid (t_R 21.4 min) at a flow rate of 10 ml/min.

The physico-chemical properties of **1** and **2** are summarized in Table 1. The molecular formulas of **1** and **2** were respectively established as $C_{23}H_{34}O_{12}$ and $C_{17}H_{24}O_7$ on the basis of an HR-ESI-MS analysis.

The ^{13}C- and ^{1}H-NMR spectral data for **1** and **2** are shown in Table 2. The direct connectivity of these protons and carbons was established by HSQC spectra. Since the ^{1}H-NMR signals of **1** assignable to sugar moieties were highly overlapped, the structure was elucidated by using **2** which possessed a single sugar moiety. The structure of **2** was elucidated by a series of NMR analyses involving DQF-COSY and CT-HMBC[8)] spectra. *Ortho*-coupling between aromatic protons 5-H and 6-H, which were *meta*-coupled to 2-H, indicated the presence of a 1,3,4-trisubstituted benzene ring moiety. By taking into consideration the low-field ^{13}C chemical

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Abbreviations: HPLC, high-performance liquid chromatography; MPLC, medium-performance liquid chromatography; HR-ESI-MS, high-resolution-electrospray ionization-mass spectroscopy; DQF-COSY, double quantum filtered-correlation spectroscopy; HSQC, heteronuclear single quantum coherence; CT-HMBC, constant time heteronuclear multiple bond connectivity

Table 1. Physico-Chemical Properties of **1** and **2**

	1	**2**
Appearance	colorless amorphous solid	colorless amorphous solid
$[\alpha]^{25}_{D}$[a]	-38.1° (*c* 0.27, MeOH)	-42.0° (*c* 0.05, MeOH)
Molecular formula	$C_{23}H_{34}O_{12}$	$C_{17}H_{24}O_7$
HR-ESI-MS[b] (*m/z*)		
	found: 525.1937 $[M + Na]^+$	found: 363.1396 $[M + Na]^+$
	calcd.: 525.1948	calcd.: 363.1420
UV[c] λ_{max} (MeOH) nm (ε)	224 (9,650), 280 (2,680)	224 (6,010), 280 (2,540)
IR[d] ν_{max} (KBr) cm^{-1}	1631, 1596 (benzene)	1635, 1596 (benzene)

[a]Optical rotation was measured with a Horiba SEPA-300 polarimeter.
[b]HR-ESI-MS data were recorded on a Waters LCT-Premier XE.
[c]The UV spectrum was measured by a Beckman Coulter DU730 UV/Vis spectrophotometer.
[d]The IR spectrum was obtained with a Horiba FT-720 spectrophotometer.

Table 2. ^{13}C- and ^{1}H-NMR Data for **1** and **2**

Position	**1** δ_C	δ_H (integral, mult, *J* in Hz)	**2** δ_C	δ_H (integral, mult, *J* in Hz)
1	153.7		152.1	
2	117.8	7.12 (1H, d, 2.5)	115.4	6.83 (1H, d, 2.6)
3	152.6		150.2	
4	138.6		138.3	
5	116.1	7.10 (1H, d, 9.0)	116.2	7.03 (1H, d, 8.8)
6	116.0	6.98 (1H, dd, 9.0, 2.5)	114.0	6.67 (1H, dd, 8.8, 2.6)
7	41.9		41.5	
8	149.8	6.34 (1H, dd, 17.6, 10.8)	149.8	6.32 (1H, dd, 17.4, 10.7)
9	110.5	5.03 (1H, d, 17.6)	110.3	5.04 (1H, d, 17.4)
		5.00 (1H, d, 10.8)		5.01 (1H, d, 10.7)
10	28.0	1.53 (3H, s)	28.1	1.50 (3H, s)
11	27.7	1.52 (3H, s)	27.5	1.51 (3H, s)
1′	101.7	4.94 (1H, m)	101.5	4.94 (1H, d, 7.8)
2′	75.1	3.54 (1H, dd, 8.8, 7.9)	74.8	3.55 (1H, t, 8.2)
3′	78.6	3.49 (1H, t, 8.8)	78.0	3.52 (1H, t, 8.6)
4′	71.4	3.44 (1H, m)	71.0	3.44 (1H, t, 9.0)
5′	78.2	3.47 (1H, m)	77.5	3.47 (1H, ddd, 9.0, 5.4, 1.6)
6′	62.6	3.93 (1H, m)	62.3	3.95 (1H, dd, 12.0, 1.6)
		3.73 (1H, m)		3.77 (1H, dd, 12.0, 5.4)
1″	103.3	4.81 (1H, d, 7.3)		
2″	75.0	3.47 (1H, m)		
3″	78.0	3.48 (1H, m)		
4″	71.4	3.41 (1H, m)		
5″	78.1	3.42 (1H, m)		
6″	62.5	3.93 (1H, m)		
		3.74 (1H, m)		

The ^{13}C- (150 MHz) and ^{1}H- (600 MHz) NMR spectra were recorded on a Varian NMR System 600 NB CL. The samples were measured in CD_3OD, and the solvent peak was used as an internal standard (δ_H 3.35 and δ_C 49.0).

shifts of C-1 and C-3 and the high-field shift of C-2, the oxygen atoms should be substituted at the C-1 and C-3 positions (Fig. 1, right). Singlet methyl protons 10-H and 11-H were ^{1}H-^{13}C long-range coupled to each other and commonly coupled to aliphatic quaternary carbon C-7 and olefinic methine carbon C-8, which in turn were ^{1}H spin coupled to olefinic exomethylene protons 9-H. These results established a 3-methyl-butenyl moiety (Fig. 1, right). The ^{1}H-^{13}C long-range correlations in the HMBC spectrum from methyl protons 10-H and 11-H to aromatic carbon C-4, and from aromatic proton 5-H to quaternary carbon C-7 determined the 3-methyl-butenyl moiety to be substituted at the C-4 position in the benzene ring.

The remaining unit was deduced to be a sugar residue by establishing the sequence from anomeric proton 1′-H to hydroxymethyl protons 6′-H through oxymethine protons 2′-H, 3′-H, 4′-H and 5′-H. In addition to these coupling constants, ^{1}H-^{13}C long-range coupling from 1′-H to C-5′ elucidated a β-glucoside moiety (Fig. 1, right). The direct ^{13}C-^{1}H coupling constant at C-1′ ($^{1}J_{C-H} = 156.5$ Hz)[9] also supported the β-glucopyranoside moiety. The location of this glucopyranoside was determined by ^{1}H-^{13}C long-range coupling from 1′-H to C-3. Therefore, the structure of **2** was determined as 1-hydroxy-3-*O*-β-glucopyranosyloxy-4-(1,1-dimethyl-2-propenyl)benzene (Fig. 1, right).

The structure of **1** was also deduced to be 1,3-di-*O*-β-glucopyranosyloxy-4-(1,1-dimethyl-2-propenyl)benzene after confirming the aglycon of **1** to be the same as that of **2** by analyses of the DQF-COSY and CT-HMBC spectra. Although it was not possible to analyze the coupling constants among proton signals due to the high degree of overlapping, two sugar moieties were recog-

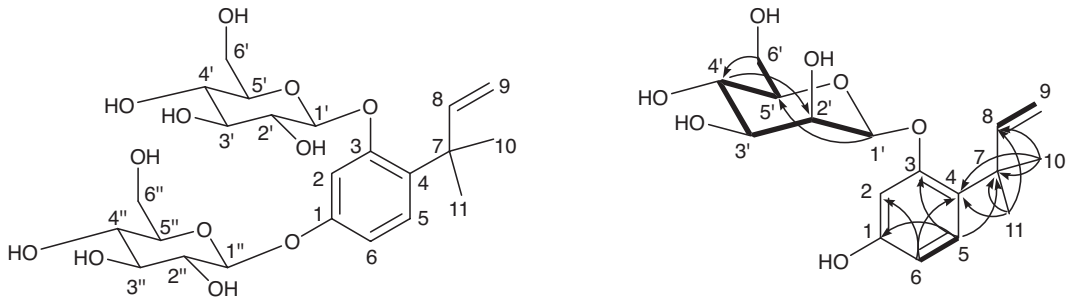

Fig. 1. Structures of **1** and **2**, and Key Correlations of ^{1}H-^{1}H (bold line) and ^{1}H-^{13}C (arrows) of **2**.

nized in **1**. We thus carried out an acid hydrolysis of **1** to determine the absolute configuration of these sugar moieties. As a result, we obtained only a glucopyranoside as a sugar moiety (α-form, 1-H (δ_H 5.02, t, $J = 4.0$ Hz), 2-H (δ_H 3.32, dd, $J = 9.0$, 4.0 Hz), 3-H (δ_H 3.50, t, $J = 9.0$ Hz), 4-H (δ_H 3.20, t, $J = 9.0$ Hz), 5-H (δ_H 3.62, m), 6-H (δ_H 3.63, dd, $J = 12.0$, 3.0 Hz, δ_H 3.55, dd, $J = 12.0$, 6.0 Hz); β-form, 1-H (δ_H 4.44, d, $J = 8.0$ Hz), 2-H (δ_H 3.04, t, $J = 8.5$ Hz), 3-H (δ_H 3.28, t, $J = 9.0$ Hz), 4-H (δ_H 3.18, t, $J = 9.0$ Hz), 5-H (δ_H 3.25, ddd, $J = 9.0$, 6.0, 2.0 Hz) and 6-H (δ_H 3.69, dd, $J = 12.0$, 2.0 Hz, δ_H 3.51, m); in D_2O). The optical rotation ($[\alpha]^{25}_D$ +56.0° (*c* 0.05, H_2O)) of this sugar residue confirmed it to be a D-glucopyranoside.[10] Long-range couplings from anomeric proton 1′-H to aromatic carbon C-3, and from anomeric proton 1″-H to aromatic carbon C-1, together with direct ^{13}C-^{1}H coupling constants at C-1′ ($^{1}J_{C-H} = 156.2$ Hz) and C-1″ ($^{1}J_{C-H} = 156.7$ Hz) enable these glucopyranoside moieties to be deduced as substituted at C-3 and C-1, respectively, both with β orientation. The structures of **1** and **2** were therefore determined as 1,3-di-*O*-β-D-glucopyranosyloxy-4-(1,1-dimethyl-2-propenyl)benzene and 1-hydroxy-3-*O*-β-D-glucopyranosyloxy-4-(1,1-dimethyl-2-propenyl)benzene, repectively (Fig. 1). Although the aglycon[11] of these compounds and 1,5-di-*O*-β-D-glucopyranosyloxy-2-(3′,3′-dimethylallyl)benzene[12] have been isolated as plant secondary metabolites, this is the first report of the glycosyl benzene products isolated from fungi. Compounds **1** and **2** did not show any cytotoxic activities against human cervical carcinoma HeLa cells and human malignant pleural mesothelioma ACC-MESO-1 cells.[13]

Acknowledgments

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References

1) Blunt JW, Copp BR, Hu WP, Munro MHG, Northcote PT, and Prinsep MR, *Nat. Prod. Rep.*, **26**, 170–244 (2009).
2) Saleem M, Ali MS, Hussain S, Jabbar A, Ashraf M, and Lee YS, *Nat. Prod. Rep.*, **24**, 1142–1152 (2007).
3) Zhang M, Wang WL, Fang YC, Zhu TJ, Gu QQ, and Zhu WM, *J. Nat. Prod.*, **71**, 985–989 (2008).
4) Sun Y, Tian L, Huang J, Ma HY, Zheng Z, Lv AL, Yasukawa K, Pei YH, and Trichodermatides AD, *Org. Lett.*, **10**, 393–396 (2008).
5) Zhang Y, Zhu T, Fang Y, Liu H, Gu Q, and Zhu W, *J. Antibiot.*, **60**, 153–157 (2007).
6) Motohashi K, Hashimoto J, Inaba S, Khan ST, Komaki H, Nagai A, Takagi M, and Shin-ya K, *J. Antibiot.*, **62**, 247–250 (2009).
7) Motohashi K, Hashimoto J, Inaba S, Khan ST, Komaki H, Nagai A, Takagi M, and Shin-ya K, *Biosci. Biotechnol. Biochem.*, **73**, 1898–1900 (2009).
8) Furihata K and Seto H, *Tetrahedron Lett.*, **39**, 7337–7340 (1998).
9) Kasai R, Okihara M, Asakawa J, Mizutani K, and Tanaka O, *Tetrahedron*, **35**, 1427–1432 (1979).
10) Windholz M, "The Merck Index" 13th ed., Merck & Co., Inc., Rahway, pp. 638–639 (1983).
11) Zeng JF, Li GL, Xu X, and Zhu DY, *Phytochemistry*, **43**, 893–896 (1996).
12) Braca A, Bader A, Siciliano T, Morelli I, and Tommasi ND, *Planta Med.*, **69**, 835–841 (2003).
13) Usami N, Fukui T, Kondo M, Taniguchi T, Yokoyama T, Mori S, Yokoi K, Horio Y, Shimokata K, Sekido Y, and Hida T, *Cancer Sci.*, **97**, 387–394 (2006).