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NOTE

JBIR-58, a new salicylamide derivative, isolated from a marine sponge-derived *Streptomyces* sp. SpD081030ME-02

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Marine microorganisms, particularly marine actinomycetes, have attracted considerable attention as one of the most important resources for new biologically active metabolites.[1] For example, new compounds have been isolated from actinomycetes of sponge origin.[2–4] Our group was engaged in the isolation of actinobacteria from marine sponges. We have recently discovered novel compounds, namely the anthracyclines tetracenoquinocin and 5-imino aranciamycin,[5] a teleocidin JBIR-31,[6] the tetrapeptides JBIR-34 and -35,[7] and the isoprenoids JBIR-46–48.[8,9] Our intention was to support the idea that new species are capable of producing unique metabolites. For this purpose, we isolated new species of *Streptomyces* from marine sponges and then searched for secondary metabolites in the cultures of isolated strains. In this study, we isolated a new species (SpD081030ME-02) of *Streptomyces* from a Demospongiae class of marine sponge, and purified a new compound termed JBIR-58 (**1**, Figure 1a) from the fermentation broth of *Streptomyces* sp. SpD081030ME-02. We report herein the fermentation, isolation, structure elucidation and, in brief, the biological activity of **1**.

Streptomyces sp. SpD081030ME-02 was isolated from a Demospongiae class of marine sponge, collected offshore of Ishigaki City, Okinawa Prefecture, Japan. To identify the species of the strain SpD081030ME-02, we compared the 16S rRNA gene sequences (accession no. AB539864) with those available in the DNA Data Bank of Japan using the basic local alignment search tool.[10] The strain was identified as a new species of the genus *Streptomyces*, as the 16S rRNA gene sequence comparison exhibited a low sequence similarity of 98% with *Streptomyces chromofuscus*. SpD081030ME-02 was cultured at 27 °C for 5 days in 500-ml Erlenmeyer flasks containing 100 ml of a medium consisting 2.5% starch (Kosokagaku, Tokyo, Japan), 1.5% soybean meal (Nisshin Oillio, Tokyo, Japan), 0.2% dry yeast (Mitsubishi Tanabe Pharma, Osaka, Japan), 0.4% $CaCO_3$ (Kozaki Pharmaceutical, Tokyo, Japan), and Diaion HP-20 (Mitsubishi Chemical, Tokyo, Japan), with pH adjusted to 6.2 before sterilization. The supernatant of whole broth (2 l) collected by centrifugation was acidified to pH 2–3 and extracted with EtOAc. The EtOAc layer was dried over anhydrous Na_2SO_4 and evaporated to dryness *in vacuo*. The residue (2.03 g) was dissolved with *n*-hexane–$CHCl_3$ (6:1), $CHCl_3$, $CHCl_3$–MeOH (1:1) and MeOH, successively. The $CHCl_3$-soluble fraction (487 mg) was purified using normal-phase medium-pressure liquid chromatography (Purif-Pack SI 30 μm, Moritex, Tokyo, Japan; $CHCl_3$–MeOH, 99:1) and preparative TLC (SiO_2; $CHCl_3$–MeOH, 19:1) to yield **1** (9.8 mg, Rf=0.43).

Compound **1** was obtained as a yellow powder ($[\alpha]^{24}_D$ +72.3, c. 0.1 ($CHCl_3$), UV (MeOH) λ_{max} (log ε) 226 (4.23), 286 (4.18), 297 (4.10, sh) and 412 (3.57) nm). The IR spectrum ($CHCl_3$) of **1** revealed the characteristic absorptions of a ketone (ν_{max} 1662 cm^{-1}) and an amide (1662, 1624 and 1431 cm^{-1}) groups. The HR-ESI-MS data of **1** gave the sodium-adduct ion at *m/z* 332.0746 (calcd. for $C_{14}H_{15}NO_7Na$, 332.0753). The direct connectivity between protons and carbons was established by the heteronuclear single-quantum coherence spectrum, and the ^{13}C and ^{1}H NMR spectral data for **1** are shown in Table 1. The analyses of double-quantum filtered-COSY and constant time-HMBC[11] spectra revealed the structure of **1** as follows.

The proton spin coupling between an oxymethine proton 5-H (δ_H 4.57) and a methyl proton 11-H (δ_H 1.57), and the sequence from an oxymethine proton 6a-H (δ_H 5.09) through methylene proton 7-H (δ_H 2.41, 2.03) to an acetal proton 8-H (δ_H 5.42) were observed in the double-quantum filtered-COSY spectrum (Figure 1b). A methoxyl

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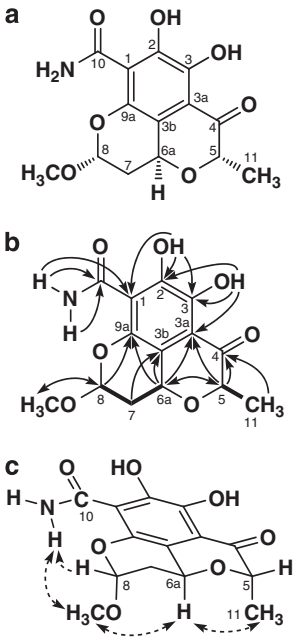

Figure 1 (**a**) Structure of JBIR-58 (**1**) and (**b**) key correlations in double-quantum filtered-COSY (bold lines) and constant time-HMBC (arrows) spectra of **1.** (**c**) NOE observation for **1** (dashed arrows).

Table 1 ^{13}C and ^{1}H NMR data for 1

	δ_C	δ_H
1	108.0	
2	153.7	
3	146.8	
3a	112.6	
3b	112.0	
4	202.9	
5	76.0	4.57 (q, 7.5)
6a	57.6	5.09 (dd, 12.5, 6.0)
7	31.6	2.41 (ddd, 12.5, 6.0, 2.5); 2.03 (td, 12.5, 2.5)
8	100.2	5.42 (t, 2.5)
9a	139.0	
10	171.6	
11	14.6	1.57 (d, 7.5)
8-*O*-CH_3	56.5	3.52 (s)
2-OH		13.80 (br s)
3-OH		11.20 (br s)
10-NH_2		8.21 (br s); 6.48 (br s)

^{13}C (150 MHz) and ^{1}H (600 MHz) NMR spectra were measured using a NMR System 600 NB CL (Varian, Palo Alto, CA, USA) in $CDCl_3$, and the solvent peak was used as an internal standard (δ_C 77.0, δ_H 7.26 p.p.m.).

proton (δ_H 3.52) and 5-H were long-range coupled to C-8 (δ_C 100.2) and C-6a (δ_C 57.6), respectively, indicating the 3,7-dioxaoctane structure. The HMBCs from 5-H to a carbonyl carbon C-4 (δ_C 202.9) and an aromatic carbon C-3a (δ_C 112.6), from 6a-H to three aromatic carbons C-3a, C-3b (δ_C 112.0) and C-9a (δ_C 139.0), and from 8-H to C-9a established a pyranopyran structure.

The long-range coupling from an amide proton 1-NH (δ_H 6.48) to a carbonyl carbon C-10 (δ_C 171.6) and an aromatic proton C-1 (δ_C 108.0), from a hydroxyl proton 2-OH (δ_H 13.80) to three aromatic carbons C-1, C-2 (δ_C 153.7) and C-3 (δ_C 146.8), and from a hydroxyl proton 3-OH (δ_H 11.20) to three aromatic carbons C-2, C-3 and C-3a indicated that a hydroxysalicylamide unit conjugated to the pyranopyran unit (Figure 1b). Although direct connectivity between C-1 and C-9a was not observed in COSY and HMBC, the NOEs among 8-H,8-*O*-CH_3 and an amide proton 1-NH (δ_H 8.21) suggested the conjugation between C-1 and C-9a (Figure 1c). Thus, the planar structure of **1** was determined as shown in Figure 1a.

The relative configurations were assigned on the basis of coupling constants and the analysis of NOE experiments. The large coupling constant (J=12.5 Hz) between 6a-H and 7-H (axial, δ_H 2.03) indicated that 6a-H is in axial location. The NOEs between 6a-H and 11-H, and between 6a-H and 8-*O*-CH_3, also revealed that both the methyl group on C-5 and the methoxyl group on C-8 are in the same location as 6a-H (Figure 1c).

To evaluate the cytotoxic activity of **1** against human cervical carcinoma HeLa cells, the activity was tested by WST-8 [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2*H*-tetrazolium, monosodium salt] colorimetric assay (Cell Counting Kit, Dojindo, Kumamoto, Japan). As a result, **1** showed cytotoxic effect against HeLa cells with an IC_{50} value of 28 μM for 48 h.

We have isolated a new salicylamide derivative having pyranoisochromone structure from the culture of a new species of *Streptomyces*. The pyranoisochromone derivatives have been reported to have been isolated as berkelic acid[12] from extremophile *Penicillium* sp. and as perinadine A[13] from marine-derived *Penicillium citrinum*. The compounds show cytotoxicity against several cancer cell lines. However, this is the first report of the pyranoisochromone derivative isolated from *Streptomyces*. These results confirm that this sponge contains undiscovered microorganisms that possess the ability to produce new substances. We anticipate that this study will convince chemists that new species of *Streptomyces* can produce compounds containing unique skeletal structures and also encourage them to investigate such species.

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