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NOTE

A new 16-membered tetraene macrolide JBIR-100 from a newly identified *Streptomyces* species

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In recent years, the malfunction of vacuolar-type ATPases (V-ATPases), which are ubiquitous proton pumps present in the endomembrane system of all eukaryotic cells and in the plasma membranes of many animal cells, has been correlated with an increasing number of diseases such as osteopetrosis, male infertility and renal acidosis.[1] In addition, V-ATPase has come into the focus of biomedical research and has even been considered to be an attractive target for cancer chemotherapy and osteoporosis treatment.[1] To understand the development of these diseases and to design efficient drugs for their therapy, it is necessary to identify substances that satisfy specific therapeutic requirements. In the course of our screening for V-ATPase inhibitors by using acridine orange staining of V-ATPase-dependent acidic organelles,[2] we isolated a new bafilomycin analogue termed JBIR-100 (**1**) from a culture of *Streptomyces* sp. IR027-SDHV6 (Figure 1a). In this article, we describe the fermentation, isolation, structure elucidation, and in brief, the biological activity of **1**.

Streptomyces sp. IR027-SDHV6 was isolated from a soil sample collected at Iriomote Island, Okinawa Prefecture, Japan, by the sodium dodecyl sulfate-yeast extract method.[3] Using the basic local alignment search tool (BLAST), we compared the 16S rRNA gene sequence (AB572859) of IR027-SDHV6 with 16S rRNA gene sequences available in the EzTaxon server to identify the species of strain IR027-SDHV6. *Streptomyces* sp. IR027-SDHV6 was expected as a new species of the genus *Streptomyces*; the 16S rRNA gene sequence comparison showed a low sequence similarity of 98.6% with *S. capoamus* JCM 4734 (AB045877). The detailed identification of this strain will be reported elsewhere.

The strain was cultured on a rotary shaker (220 r.p.m.) at 27 °C for 5 days in 500-ml Erlenmeyer flasks (20 flasks) containing 100 ml of a production medium consisting of 2.5% starch (Kosokagaku, Tokyo, Japan), 1.5% soybean meal (Nisshin Oillio Group, Tokyo, Japan), 0.2% dry yeast (Mitsubishi Tanabe Pharma, Osaka, Japan), 0.4% $CaCO_3$ (Kozakai Pharmaceutical, Tokyo, Japan) and 1% Diaion HP-20 (Mitsubishi Chemical, Tokyo, Japan), pH 7.0 (before sterilization). The mycelia from the culture broth (100 ml×20 flasks) were treated with Me_2CO (400 ml). After *in vacuo* concentration, the residue was extracted with EtOAc. The organic layer was dried over anhydrous Na_2SO_4 and evaporated to dryness *in vacuo*. The residue (312 mg) was purified using normal-phase medium-pressure liquid chromatography (Purif-Pack, SI 30 μm; Shoko Scientific, Yokohama, Japan) and developed with hexane–EtOAc (1:1). The active fraction was further purified by preparative reversed-phase HPLC using a CAPCELL PAK C_{18} MG II column (5.0 μm, 20 i.d. ×150 mm; Shiseido, Tokyo, Japan) and developed with 85% MeOH–H_2O containing 0.4% formic acid (flow rate, 10 ml min^{-1}) to yield **1** (20.0 mg, retention time=32.5 min).

Compound **1** was obtained as a colorless amorphous solid ($[\alpha]_D^{25}$–15.7, *c* 0.1, UV $\lambda_{max}(\varepsilon)$ 244 (28700), 272 (sh, 13400) nm, in MeOH). The IR spectrum ($CHCl_3$) of **1** revealed the characteristic absorptions of carbonyl groups (ν_{max}: 1720, 1700, 1680 cm^{-1}), suggesting the presence of conjugated esters and/or carboxylic acids. The molecular formula of **1** was determined to be $C_{39}H_{60}O_{11}$ (found: *m/z* 703.4082 $[M-H]^-$, calcd: *m/z* 703.4057 for $C_{39}H_{59}O_{11}$) by HR-electrospray ionization MS. The direct bonds between each proton and carbon were established by the heteronuclear single quantum coherence spectrum. The ^{13}C and ^1H NMR spectral data for **1** are shown in Table 1. Analysis of double-quantum filtered (DQF)-COSY and constant time heteronuclear multiple bond correlation[4] spectra revealed the structure of **1** as follows.

In the DQF-COSY spectrum, we observed four allylic couplings between a methyl proton 2-CH_3 (δ_H 2.03) and an olefinic proton 3-H (δ_H 7.22), between a methyl proton 4-CH_3 (δ_H 1.99) and an olefinic

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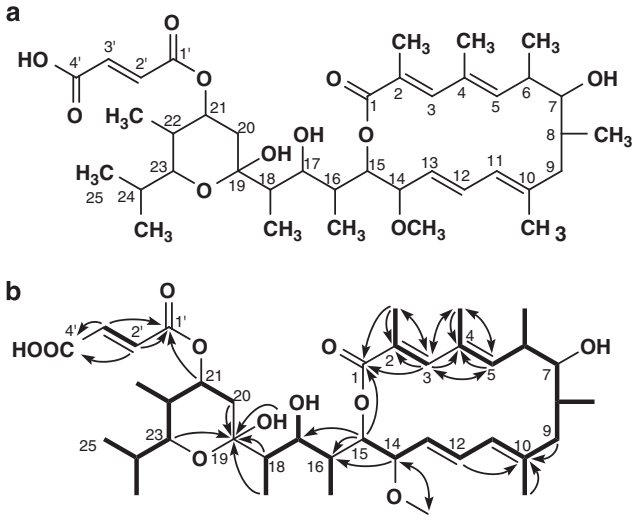

Figure 1 (**a**) Structure of **1**. (**b**) Key correlations of ^{1}H-^{1}H DQF-COSY (bold lines) and HMBC (solid arrows).

proton 5-H (δ_H 5.85), between methylene protons 9-H (δ_H 2.16, 1.97) and an olefinic proton 11-H (δ_H 5.85) and between a methyl proton 10-CH_3 (δ_H 1.94) and the olefinic proton 11-H (Figure 1b). In addition, we determined the sequences from 5-H to 9-H through a methine proton 6-H (δ_H 2.56), which was in turn coupled to a methyl proton 6-CH_3 (δ_H 1.08), an oxymethine proton 7-H (δ_H 3.30, δ_C 81.3) and a methine proton 8-H (δ_H 1.95), which showed ^{1}H spin coupling with a methyl proton 8-CH_3 (δ_H 0.94). The sequences from an olefinic proton 11-H to an oxymethine proton 15-H (δ_H 4.96) through olefinic protons 12-H (δ_H 6.50) and 13-H (δ_H 5.19) and an oxymethine proton 14-H (δ_H 3.91) were also determined. Furthermore, on the basis of the HMBC correlations from 2-CH_3 and 15-H to a carbonyl carbon C-1 (δ_C 172.0), from 4-CH_3 to C-3 (δ_C 146.4) and from a methoxyl proton (δ_H 3.25) to C-14 (δ_C 82.6), we found that the structure of **1** was that of a 16-membered macrolide.

Three other partial structures were elucidated as follows. The sequences from a methyl proton 16-CH_3 (δ_H 0.81) to 18-CH_3 (δ_H 1.02) through methine protons 16-H (δ_H 2.08), 17-H (δ_H 4.08), which was in turn coupled to a hydroxyl proton 17-OH (δ_H 4.85), and 18-H (δ_H 1.74) indicated a pentan-3-ol substructure. The sequence from methylene protons 20-H (δ_H 2.37, 1.24) to 25-H (δ_H 0.91) through an oxymethine proton 21-H (δ_H 5.10), 22-H (δ_H 1.61), which was also coupled to a methyl proton 22-CH_3 (δ_H 0.83) and oxymethine proton 23-H (δ_H 3.62) and 24-H (δ_H 1.90), was established. Additional spin couplings between 22-H and a methyl proton 22-CH_3 (δ_H 0.83) and between 24-H and a methyl proton 26-H (δ_H 0.78) determined the substituted positions of these methyl residues. In addition, ^{1}H-^{13}C long-range couplings from 18-H, 18-Me, a hydroxyl proton 19-OH (δ_H 5.67), 20-H and 23-H to an acetal carbon C-19 (δ_C 98.8) determined a pyran ring substructure. Finally, ^{1}H-^{13}C long-range couplings from 15-H to C-16 (δ_C 37.6) indicated that this side chain substructure was substituted at the position of C-15 in the macrolide unit.

The remaining fumaric acid unit was deduced on the basis of proton coupling between two olefinic protons 2′-H (δ_H 6.84) and 3′-H (δ_H 6.92), and long-range couplings of 2′-H and 3′-H to two carbonyl carbons C-1′ (δ_C 164.1) and C-4′ (δ_C 169.0). A long-range coupling from oxymethine proton 21-H to C-1′ proved that the substitution position of the fumaric acid unit was at the C-21 position, as shown in Figure 1b.

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

Position	δ_C	δ_H
1	172.0	
2	122.3	
2-CH_3	13.7	2.03 (s)
3	146.4	7.22 (br s)
4	134.5	
4-CH_3	15.2	1.99 (s)
5	144.6	5.85 (m)
6	36.7	2.56 (qui, 7.0)
6-CH_3	17.5	1.08 (d, 7.0)
7	81.3	3.30 (br d, 5.5)
8	39.8	1.95 (m)
8-CH_3	21.5	0.94 (d, 6.0)
9	41.2	2.16 (br d, 12.5); 1.97 (m)
10	142.6	
10-CH_3	20.1	1.94 (s)
11	125.4	5.85 (m)
12	132.5	6.50 (dd, 15.0, 10.5)
13	127.5	5.19 (dd, 15.0, 9.5)
14	82.6	3.91 (t, 9.0)
14-*O*-CH_3	55.6	3.25 (s)
15	76.0	4.96 (d, 8.5)
16	37.6	2.08 (m)
16-CH_3	9.8	0.81 (d, 7.0)
17	70.4	4.08 (br d, 10.5)
17-OH		4.85 (br s)
18	41.7	1.74 (qd, 7.0, 1.5)
18-CH_3	6.9	1.02 (d, 7.0)
19	98.8	
19-OH		5.67 (br s)
20	39.9	2.37 (dd, 11.5, 5.0); 1.24 (br t, 11.5)
21	75.7	5.10 (td, 11.0, 5.0)
22	38.2	1.61 (tq, 11.0, 6.5)
22-CH_3	12.3	0.83 (d, 6.5)
23	75.4	3.62 (dd, 10.5, 2.0)
24	27.9	1.90 (br sept-d, 7.0, 2.0)
25	20.7	0.91 (d, 7.0)
26	14.3	0.78 (d, 6.5)
1′	164.1	
2′	132.1	6.84 (d, 15.5)
3′	136.0	6.92 (d, 15.5)
4′	169.0	

^{13}C (125 MHz) and ^{1}H (500 MHz) NMR spectra were recorded on a Varian NMR System 500 NB CL (Palo Alto, CA, USA) in $CDCl_3$, and the solvent peak was used as an internal standard (δ_C 77.0 and δ_H 7.26).

The four olefins at C-2, C-4, C-12 and C-2′ were all determined to be *E*-configuration on the basis of their high-field ^{13}C chemical shifts of the methyl residues 2-CH_3 (δ_C 13.7) and 4-CH_3 (δ_C 15.2), and the coupling constants between 12-H/13-H ($J_{12\text{-H}-13\text{-H}}$,-15.0 Hz) and 2′-H/3′-H ($J_{2'\text{-H}-3'\text{-H}}$,-15.5 Hz). Another olefin at C-10 also appeared to possess the *E*-configuration when the ^{13}C chemical shifts at 10-CH_3 (δ_C 20.1) were compared with those of known analogues.[5] Thus, the structure of **1** was determined to be that of a new 16-membered macrolide, as shown in Figure 1a. The structure of **1** was found to be structurally related to bafilomycin C_1, which shows V-ATPase inhibitory activity, and differs from that of bafilomycin C_1 in the presence of the methyl group at the C-2 position.

The cytotoxic activity of **1** against human cervical carcinoma HeLa cells was tested by the WST-8 (2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2*H*-tetrazolium, monosodium

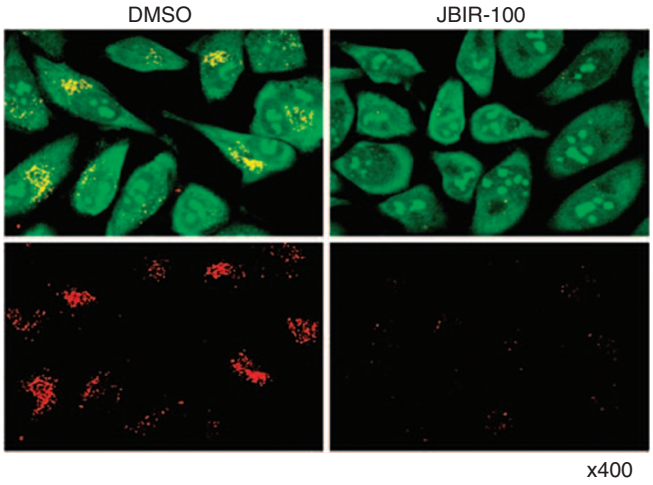

Figure 2 Inhibition of V-ATPase activity by **1** in HeLa cells. Cells were treated with 100 nM of **1** for an hour. Then, the cells were stained with acridine orange (Sigma, St Louis, MO, USA) at a concentration of 0.5 μg ml^{-1}, incubated for 10 min, and washed twice with phosphate-buffered saline. The images were visualized under an LSM 510 Confocal Laser Scanning microscope (Carl Zeiss MicroImaging, Thornwood, NY, USA). Acridine orange emits red fluorescence in acidic vesicular organelles and green fluorescence in the cytoplasm and nucleus.

salt) colorimetric assay (Cell Counting Kit; Dojindo, Kumamoto, Japan). Compound **1** exhibited cytotoxic effect against HeLa cells (IC_{50}=72.6 nM) at 48 h after administration. Next, the V-ATPase inhibitory activity of **1** against the accumulation of intracellular acidic organelles, which is strongly linked to V-ATPase inhibition in HeLa cells, was evaluated by acridine orange staining.[2] Dye accumulation (orange dots) was found to be inhibited by the administration of **1** (100 nM) (Figure 2), indicating the inhibitory property of **1** against V-ATPase.

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