NOTES

Two New Macrolides Produced by *Streptomyces* sp. CS

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A strong antifungal and antitumor agent, 24-demethyl-bafilomycin C₁ (**1**) has been isolated from *Streptomyces* sp. CS¹⁾. The structure of **1** is different from those of bafilomycins at the replacement of the isopropyl group with ethyl group at C-23, and different from hygrolidins at C-2 substitution and the orientations of the substituents at C-21, C-22 and C-23 in the pyranose ring^{2~5)}. Therefore it belongs to a new subfamily of 16-membered macrolides. The isolation of **1** from this strain in previous work encouraged us to search for this type of metabolites further, which led to the isolation of compound **1** and two new bafilomycin derivatives **2** and **3**. Their structures were elucidated based on the NMR experiments.

Experimental

Fermentation and Extraction

A stock of *Streptomyces* sp. CS was cultured on ISP2 agar plates at 28°C for 7 days and a single colony was inoculated to a 500 ml Erlenmeyer flask containing 50 ml ISP2 broth as a seed medium. The flask was incubated at 28°C for 2 days on a rotary shaker (180 rpm). The seed culture was inoculated onto Petri dishes containing ISP3 agar media (ca. 20 ml/dish). The fermentation was cultivated at 28°C for 7 days.

The cultured agar media (14 liters, 700 dishes) was chopped, diced and extracted with EtOAc - MeOH - AcOH (80:15:5, 14 liters) at room temperature for over night.

The organic solution was collected through filtration with filter paper, and the remaining agar residue was extracted several times more as described above until the filtrate colourless. The combined filtrates were concentrated under vacuum to remove organic solvents. The aqueous solution was extracted five times with chloroform to afford the No. 1 CHCl₃ extract (11.9 g) after the removal of solvents under vacuum. After further concentration under vacuum, the aqueous phase was filtrated through filter paper to produce some crystal-like residue, which was dissolved in chloroform. After being washed with distilled water and the removal of solvents under vacuum, this chloroform solution afforded the No. 2 CHCl₃ extract (6.58 g) after the removal of solvents under vacuum. Those two portions of aqueous solutions were combined and further extracted exhaustively with chloroform to produce the No. 3 CHCl₃ extract (1.09 g) and H₂O extract (30.5 g) after the removal of solvents under vacuum, respectively.

Isolation

The No. 2 CHCl₃ extract (6.58 g) was first chromatographed over Sephadex LH-20 (120 g) and eluted with methanol. The fractions were combined according to the antifungal activities against *Penicillium avellaneum* UC-4376 with paper disc diffusion assay and TLC results over Si gel developed by petroleum ether - acetone (5:1, v/v) and chloroform - methanol (10:1, v/v). The combined fractions (No. 8~11, 2.0 g) were subjected to column chromatography over Si gel (40 g) and eluted with petroleum ether - acetone (10:1) and 12 fractions were collected. Fractions 7~12 showed strong antifungal activities. Fraction 1 (633 mg) was subjected to column chromatography over Si gel (8 g) eluted with petroleum ether - acetone (10:1), and the combination was monitored by TLC and followed by MPLC over reversed-phase C₁₈ Si gel (24 g) eluted with gradient acetone - water (7:3; 8:2, v/v) to afford compound **2** (15 mg). Fractions 4~5 (124 mg) were isolated with repeated MPLC over reversed-phase C₁₈ Si gel (24 g) eluted with acetone - water (7:3, v/v), and followed with column chromatography over Si gel (6.6 g) eluted with gradient chloroform - methanol

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This paper is dedicated to my mentor, Professor Dr. HEINZ G. FLOSS, at the University of Washington on the occasion of his 70th birthday.

(100:1.5, 100:2) to afford **3** (5 mg). Fractions 7~12 (570 mg) were chromatographed over reversed-phase C₁₈ Si gel (140 g) and eluted with methanol-water (7:3) to produce compound **1** (80 mg). Compounds **1** (10 mg) and **2** (5 mg) were isolated from the No. 1 CHCl₃ extract through repeated column chromatography over reversed-phase C₁₈ Si gel (140 g) eluted with gradient acetone-water (7:3; 8:2) and normal phase Si gel eluted with chloroform-methanol (10:1, 5:1, v/v) and Sephadex LH-20 eluted with methanol as well. The R_f values for compounds **1**, **2** and **3** on TLC plates precoated with Si gel F₂₅₄ (Qingdao Marine Chemical Ltd., People's Republic of China) were

0.3 (developed twice with chloroform-methanol, 10:1), 0.4 (developed twice with petroleum ether-acetone, 3:1) and 0.3 (developed with chloroform-methanol, 20:1), respectively.

Biological Assays

The paper disc diffusion assay⁶⁾ was used for measuring the antifungal activity against *Penicillium avellaneum* UC-4376 and the protein-binding sulforhodamine (SRB) and microculture tetrazolium (MTT) method for the cytotoxicity against cancer cell^{7,8)}.

Table 1. The NMR data for compounds **2** and **3**.

Position	2^a			3^a	
	¹³ C ^b	¹³ C	¹ H ^c	¹³ C	¹ H ^c
1	167.7s	164.4s	/	164.2s	/
2	142.5s	141.7s	/	141.7s	/
3	134.3d	132.5d	6.73 (s)	132.4d	6.74 (s)
4	133.1s	131.7s	/	131.6s	/
5	144.8d	140.6d	5.92 (dd, 11.2, 17.2)	140.6d	5.87 (d, 9.1)
6	38.6d	38.5d	2.47 (t, 7.1)	38.5d	2.47 (df, 7.3)
7	81.0d	81.3d	3.49 (m)	81.3d	3.45 (br q, 1.4)
8	40.7d	38.5d	1.95 (m)	37.2d	1.86 (m)
9	42.7t	41.7t	2.32 (dd, 11.0, 14.9) 1.94 (m)	41.7t	2.35 (dd, 11.0, 14.8) 1.96 (m)
10	142.5s	141.8s	/	140.4s	/
11	125.7d	125.2d	6.02 (d, 9.8)	124.9d	5.92 (d, 11.0)
12	134.3d	130.3d	6.44 (d, 10.8)	130.3d	6.44 (dd, 11.1, 15.0)
13	127.0d	125.1d	5.36 (dd, 5.6, 15.9)	125.2d	5.35 (dd, 5.5, 15.4)
14	81.3d	82.5d	3.76 (m)	82.6d	3.77 (br dd, 2.4, 5.6)
15	76.3d	77.0d	4.94 (d, 9.2)	78.8d	4.94 (dd, 3.0, 9.3)
16	38.7d	37.1d	2.32 (dd,)	35.2d	2.83 (m)
17	71.3d	78.2d	4.40 (dd, 3.1, 10.1)	128.3d	6.03 (d, 10.2)
18	41.5d	38.4d	1.88 (m)	129.6s	/
19	104.5s	99.5s	/	149.7s	/
20	38.7t	38.5t	2.47 (t, 7.1)	99.4d	5.32 (d, 5.9)
21	75.7d	77.3d	1.50 (m)	117.6d	5.69 (dd, 1.5, 5.9)
22	40.9d	41.7	5.35 (dd, 5.5, 15.9)	129.0s	/
23	73.5d	73.5d	3.76 (m)	80.1d	4.40 (dd, 3.0, 9.8)
24	25.4t	24.3t	1.63 (m)	23.8t	1.49 (ddd, 3.3, 7.4)
25	9.6q	9.6q	1.00 (t, 3H)	9.6q	1.00 (t, 7.4, 3H)
CH ₃ O-2	60.6q	60.0q	3.67 (s, 3H)	60.1q	3.68 (s, 3H)
4a	14.1q	13.8q	1.95 (s, 3H)	13.5q	1.94 (s, 3H)
6a	18.0q	17.8q	1.01 (d, 6.5)	18.9q	1.05 (d, 7.1, 3H)
8a	22.8q	23.0q	0.99 (d, 6.9)	24.3q	1.01 (d, 6.5, 3H)
10a	19.9q	19.2q	1.79 (s, 3H)	17.5q	1.63 (s, 3H)
CH ₃ O-14	56.7q	56.2q	3.20 (s, 3H)	56.2q	3.21 (s, 3H)
16a	9.6q	9.6q	0.85 (m)	17.4q	1.00 (d, 6.5, 3H)
18a	7.7q	7.7q	1.05 (d, 7.0)	12.7q	1.79 (s, 3H)
22a	13.1q	12.7q	0.92 (m)	19.2q	1.73 (s, 3H)
CH ₃ O-19	56.1q	55.8q	3.46 (s, 3H)		

^a¹H, ¹³C NMR, HMB and ¹H-¹H COSY spectra were obtained at 400 MHz, 100 MHz and 500 MHz, respectively, and recorded in CDCl₃ at room temperature. Deuterated solvents were used as internal standards for measuring NMR spectral data.

^brecorded in CD₃OD.

^cCoupling constants are presented in Hertz. Unless otherwise indicated, all proton signals integrate to 1H.

Result and Discussions

Compound **1** was determined to be 24-demethyl-bafilomycin C₁ by comparing with the authentic sample on TLC, and the literature spectroscopic data¹⁾.

Compound **2**, colorless crystal, was determined to have the molecular formula of C₃₅H₅₈O₉ based on the negative HRFABMS data [*m/z*, 622.4070, calcd 622.4081]. Inspection of the NMR data (proton, carbon, DEPT, HMQC and HMBC) revealed a bafilomycin-type 16-membered macrolide (Table 1)³⁾. The ¹³C-NMR and DEPT spectra of **2** showed thirty-five carbon signals for eight methyl, three methoxy, three methylene, sixteen methine, and five quarternary carbon atoms. According to the ¹H-¹³C long-range correlations, particularly, those of eight methyl protons with the corresponding carbons and ¹H-¹H COSY spectra, a bafilomycin A₂ derivative was elucidated³⁾. The absolute configuration was determined by comparing the coupling constant with those of bafilomycin C₁⁹⁾. Therefore, compound **2** was determined to be 24-demethyl-bafilomycin A₂. In contrast to the ¹H NMR data in MeOD (methanol-*d*₄) as ill-defined, compound **2** gave crisp, clean ¹H NMR spectra in CDCl₃ at ambient temperature. But compound **2** has weak stability in CDCl₃, and after 18 hours the ¹³C NMR spectra showed signals in pairs. HMQC and HMBC experiments unambiguously assigned ¹³C NMR data for C-18 to C-25 of **2a** and **2b** (Figure 1), determining the structures of two open chain ketones **2a** and **2b** which probably produced through free-radical reactions.

Compound **3** was determined to have the molecular formula C₃₄H₅₀O₆ based on HRESIMS data (C₃₄H₅₀O₆Na, *m/z* 577.3484, calcd 577.3505), and showed similar pattern as compound **2** in the ¹H and ¹³C NMR spectra except for the signals for C-16, C-17, C-18, C-18a and the pyranose ring (Table 1). The two protons at δ 5.32 (H-20) and 5.69 (H-21) indicated the presence of carbon-carbon double bond in the pyranose ring and determined the ¹³C NMR assignments for this moiety with the aid of HMBC and HMQC experiments. Thus the structure of **3** was deduced to be dehydrated form of compound **2** (Figure 1), and fully supported by extensive interpretation of 2D NMR experiments. The orientation of the substituent at C-23 was determined to be equatorial by comparing with compound **1** and **2**.

Compound **1** showed strong antifungal and potent cytotoxicity against human cell lines¹⁾. Compounds **2** and **3** showed no inhibitory activity against *Penicillium avellaneum* UC-4376 at 200 μg/disc in paper disc diffusion assays. Compound **2** showed strong antitumor activities against P388 and A-549 cell lines with IC₅₀ 1.13 and

Fig. 1. The structures of compounds **1**, **2**, **2a**, **2b** and **3**, and the selected ¹³C NMR spectral data for **2a** and **2b**.

0.01 μM, respectively.

Previous studies showed that the substitution change at C-21-OH in bafilomycins and hygrolidins was the main effect on their activity. But compound **2** showed almost the same cytotoxic activities as **1** and no antifungal activities, which suggested that the substitute of fumaric acid at C-21 does not affect the anticancer activity. Structural modification at C-21 would give valuable structure-activity information.

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