ORGANIC LETTERS

2009
Vol. 11, No. 2
285−288

JBIR-23 and -24, Novel Anticancer Agents from *Streptomyces* sp. AK-AB27

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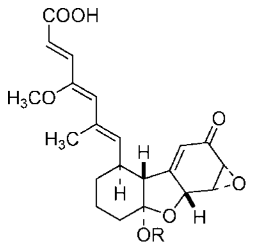
Received October 16, 2008

ABSTRACT



R = CH$_3$ JBIR-23
R = H JBIR-24

The screening for active compounds against malignant pleural mesothelioma (MPM) cells produced by *Streptomyces* sp. AK-AB27 resulted in the isolation of two compounds with a dodecahydrodibenzo[*b*,*d*]furan skeleton named JBIR-23 (1) and -24 (2). Their structures were established on the basis of extensive NMR and MS analyses. Compounds 1 and 2 exhibited cytotoxic effects against several MPM cell lines.

Malignant pleural mesothelioma (MPM), which is associated with exposure to asbestos fiber, is an aggressive neoplasm developing from the pleura that is highly invasive to surrounding tissues.[1,2] In particular, MPM has been demonstrated to be resistant to all conventional therapies, including chemotherapy, radiotherapy, and surgery, and the prognosis of patients remains very poor. Consequently, it is a tumor that continues to be a difficult clinical problem.[3,4] Therefore, developing novel therapeutic agents against MPM are strongly required. In the course of our screening program for cytotoxic compounds against MPM from microbial metabolites, we isolated novel anti-MPM compounds designated as JBIR-23 (**1**) and -24 (**2**) from *Streptomyces* sp. AK-AB27 (Figure 1).

Purification of JBIR-23 (1) and -24 (2). *Streptomyces* sp. AK-AB27 was cultured at 27 °C for 5 days by rotary shaking in 500-mL baffled Erlenmeyer flasks each containing 100 mL of culture medium. The broth was partitioned between EtOAc and H_2O. The EtOAc layer was collected and dried over Na_2SO_4. The organic layer was concentrated in vacuo followed by sequential silica gel flash column chromatography. The pure compounds of **1** and **2** were obtained by reversed-phase HPLC (see Supporting Information).

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10.1021/ol802611w CCC: $40.75

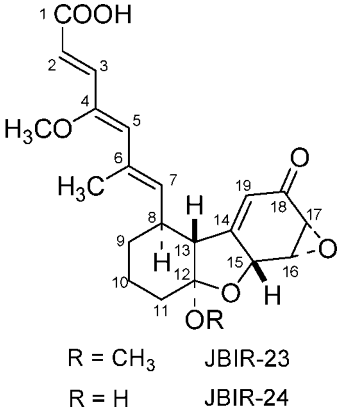

Figure 1. Structures of JBIR-23 (**1**) and -24 (**2**) (relative stereochemistry).

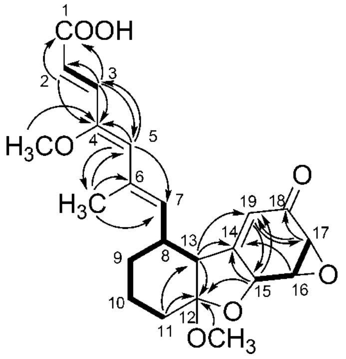

Figure 2. ^{1}H−^{1}H (bold line) and main ^{1}H−^{13}C (arrows) correlations in the 2D NMR of **1**.

Structure Elucidation. Compound **1** was isolated as a yellow powder (mp 130−140 °C; $[\alpha]_D$ +46.0, *c* 0.8, in MeOH; UV (MeOH) λ_{max} (ε) 309 (26,200), 242 (12,000, sh) that gave an $[M + H]^+$ ion at *m/z* 403.1772 in the HRESIMS consistent with a molecular formula of $C_{22}H_{26}O_7$ (calcd for $C_{22}H_{27}O_7$, 403.1752). Compound **1** displayed an IR spectrum [(KBr) υ_{max}] at 1687 and 1623 cm^{-1}, which indicated the presences of an α,β-unsaturated carboxylic acid and an α,β-unsaturated ketone, respectively. The ^{13}C NMR spectrum recorded for **1** in $CDCl_3$ showed 22 resonances (3 × CH_3; 3 × CH_2; 10 × CH; 6 × C), in agreement with the HRESIMS measurement. The direct connectivity between protons and carbons was established by the HSQC spectrum, and the tabulated ^{13}C and ^{1}H NMR spectral data for **1** are shown in Table 1. Further NMR studies including DQF-COSY and HMBC spectra established the planar structure of **1** as follows (Figure 2).

A ^{1}H−^{1}H spin correlation was observed between doublet olefinic protons H-2 (δ_H 6.02) and H-3 (δ_H 7.08). In the HMBC spectrum, ^{1}H−^{13}C long-range couplings were from the olefinic protons H-2 and H-3 to a carboxylic carbon C-1 (δ_C 172.1) and a quaternary olefinic carbon C-4 (δ_C 152.3). The singlet allylic methyl proton 6-Me (δ_H 2.01) was long-range coupled to olefinic carbons C-5 (δ_C 130.7), C-6 (δ_C 133.0), and C-7 (δ_C 138.0). In addition to these correlations, the long-range coupling between H-3 and C-5 established the side chain substructure from C-1 to C-7. The ^{1}H−^{13}C long-range couplings from an olefinic methine proton H-5 (δ_H 5.73) to olefinic carbons C-3 (δ_C 144.3), C-4, C-7 and an allylic methyl carbon 6-Me (δ_C 15.6) also supported this substructure. A methoxyl group [(δ_H 3.61, δ_C 60.4 (4-OMe)] was deduced to be attached to C-4 by the HMBC correlation between 4-OMe and C-4. These 2D NMR correlations and the absorption of an α,β-unsaturated carboxylic acid at 1687 cm^{-1} in the IR spectrum revealed a 4-methoxy-6-methylhepta-2,4,6-trienoic acid moiety in **1** as shown in Figure 2.

In addition to these resonances ascribed to the triene moiety, **1** showed 13 extra ^{13}C resonances. The sequence from H-7 (δ_H 5.52) to H-11 (δ_H 2.33, 1.49) through H-8 (δ_H 2.94), which was in turn coupled to H-13 (δ_H 2.45), H-9 (δ_H 1.68, 1.10), and H-10 (δ_H 1.71, 1.49), was established by the DQF-COSY spectrum. The ^{1}H−^{13}C long-range couplings from H-11 to an acetal carbon C-12 (δ_C 106.9) and C-13 (δ_C 56.2) deduced that these carbons constructed a six-membered ring system. The long-range coupling between a methoxyl proton 12-OMe (δ_H 3.25) and C-12 revealed that this methoxyl group was substituted at the position of C-12. The spin coupling system from an oxymethine proton H-15 (δ_H 4.86) to H-17 (δ_H 3.42) through H-16 (δ_H 3.88) were observed. According to the typical ^{13}C chemical shifts, C-16 (δ_C 50.8) and C-17 (δ_C 53.4) built an epoxyl functional group.[5] The long-range couplings from H-17 to an α,β-unsaturated carbonyl carbon C-18 (δ_C 194.8) and an olefinic carbon C-19 (δ_C 116.6), from H-15 to C-14 (δ_C 158.7) and C-19, and from an olefinic proton H-19 (δ_H 5.72) to C-15 (δ_C 75.0) and C-17 (δ_C 53.4) also established a six-membered ring structure consisting of the α,β-unsaturated carbonyl and the epoxyl functional groups. The long-range coupling from H-15 to C-12 determined that these carbons were connected through an oxygen atom. Finally, the ^{1}H−^{13}C long-range couplings from H-13 to C-14 and C-19 established the connectivity between these two partial structures, and a dodecahydrodibenzo[*b*,*d*]furan moiety was established as shown in Figure 2.

The relative configuration of **1** was determined by the proton coupling constants and NOEs observed in NOESY and 1D ROE spectra. The absolute configuration of the triene side chain moiety in **1** was established to be 2*E*, 4*Z*, and 6*E* by the proton spin coupling constant between H-2 and H-3 (J = 15.3 Hz), and the strong NOESY between H-3/H-5, H-5/H-7 and 4-OMe/6-Me, respectively (Figure 3A). In the tricyclic residue of **1**, NOESY correlations were observed between H-13/H-15, H-15/H-16, and H-16/H-17 as shown in Figure 3A. To determine the relative configuration of the remaining 1,1,2,3-tetrasubstituted cyclohexane ring, the deuterium solvent of **1** was exchanged from $CDCl_3$ to benzene-d_6 to distinguish proton signals. As a result, these proton signals in this ring system showed independent chemical shifts except for H-10ax and H-10eq protons in

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Table 1. ^{13}C (125 MHz) and ^{1}H (500 MHz) NMR Data for **1** and **2** in $CDCl_3$

	JBIR-23 (**1**)		JBIR-24 (**2**)	
position	δ_C	δ_H (multi, J in Hz)	δ_C	δ_H (multi, J in Hz)
1	172.1		171.3	
2	116.8	6.02 (d, 15.3)	116.3	6.02 (d, 15.3)
3	144.3	7.08 (d, 15.3)	144.1	7.09 (d, 15.3)
4	152.3		152.2	
5	130.7	5.73 (br s)	130.3	5.77 (br s)
6	133.0		133.7	
7	138.0	5.52 (d, 11.0)	138.1	5.55 (d, 10.0)
8	34.6	2.94 (dq, 11.0, 3.7)	40.5	2.35 (dq, 10.0, 3.9)
9	31.9	1.68, 1.10 (m)	30.2	1.62, 1.24 (m)
10	22.3	1.71, 1.49 (m)	21.2	1.75, 1.59 (m)
11	29.6	2.33, 1.49 (m)	35.2	2.20 (dt, 13.3, 3.9), 1.79 (m)
12	106.9		105.1	
13	56.2	2.45 (d, 11.0)	51.9	2.52 (d, 10.0)
14	158.7		162.1	
15	75.0	4.86 (q, 2.0)	75.2	5.11 (q, 2.0)
16	50.8	3.88 (dd, 4.2, 2.0)	48.9	3.94 (dd, 4.3, 2.0)
17	53.4	3.42 (dd, 4.2, 2.0)	53.1	3.44 (dd, 4.3, 2.0)
18	194.8		194.8	
19	116.6	5.72 (q, 2.0)	116.2	5.72 (q, 2.0)
4-OMe	60.4	3.61 (3H, s)	60.2	3.57 (3H, s)
6-Me	15.6	2.01 (3H, s)	15.2	1.92 (3H, s)
12-OMe	48.0	3.25 (3H, s)		

the ^{1}H NMR spectrum of **1** measured in benzene-d_6. All assignments of **1** in benzene-d_6 were confirmed by 2D NMR experiments (see Supporting Information). The selective 1D ROE correlations were observed between H-9ax/H-13ax, H-9ax/H-11ax, H-11ax/H-13ax, H-8ax/H-10, 12-OMe/H-11eq, 12-OMe/H-10, H-8ax/H-9eq, and H-9ax/H-10 (Figure 3B). These results indicated that of the 1,1,2,3-tetrasubstituted cyclohexane ring possessed a chair conformation.

Compound **2** was obtained as a yellow powder (mp 120−130 °C; $[\alpha]_D$ +43.6, c 0.1, MeOH). Compound **2** displayed the following UV and IR spectra: UV (MeOH) λ_{max} (ε) 306 (27,000), 250 (11,000, sh); IR (KBr) ν_{max} 1687, 1623 cm^{-1}. The molecular formula of **2** was determined to be $C_{21}H_{24}O_7$ by HRESIMS (m/z 389.1579 $[M + H]^+$, calcd 389.1595), which showed the lack of the methyl residue from **1**. Most of the NMR data for **2** were similar to those of **1** (Table 1). The ^{1}H and ^{13}C NMR spectra of **2** showed the disappearance of the methyl group at 12-OMe observed in **1**. These collective spectroscopic data proved **2** to be 12-*O*-demethyl JBIR-23.

In conclusion, we isolated two compounds with the dodecahydrodibenzo[*b*,*d*]furan skeleton, **1** and **2**, from the culture broth of *Streptomyces* sp. AK-AB27. The olefinic side chain of **1** and **2** are identical to that of cuevaene A.[6] The structures of **1** and **2**, however, differ from that of cuevaene A in the presence of a 4,5-disubstituted 7-oxabicyclo[4.1.0]hept-3-ene-2-one moiety and the substituted position of the side chain on the tricyclic ring (corresponding to C-11 position in **1**). From a biosynthetic point of view, the structures of **1** and **2** seem to be quite reasonable, because they presumably biosynthesized through the polyketide pathway utilizing a benzoic acid or a cyclohexane carboxylic acid as the starter unit. Therefore, we would like to propose a revised structure of cuevaene A in which the side chain moiety is substituted at the same position as that of **1**.

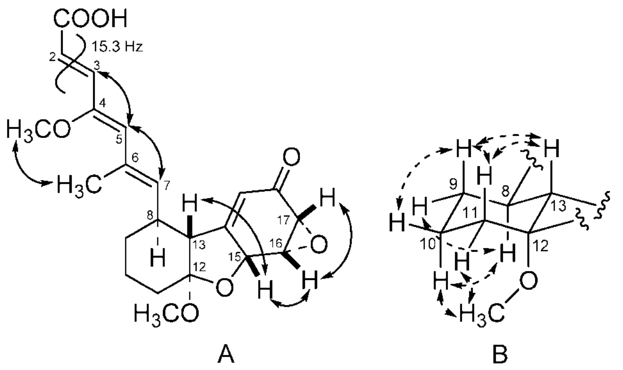

Figure 3. (A) Key NOESY (arrows) correlations in 2D NMR of **1** ($CDCl_3$). (B) The selective 1D ROE (arrows) correlations of **1** (benzene-d_6).

Biological Activity. The cytotoxic effects of **1** and **2** against various MPM cell lines were determined by the WST-8 colorimetric assay (see Supporting Information). Compound **1** exhibited cytotoxic effects against four MPM cell lines, ACC-MESO-1, MSTO-211H, NCI-H290, and NCI-H2052, with IC_{50} values of 25, 50, 10, and 20 μM, respectively (Table 2). The cytotoxic effects of **1** were about 8-fold higher than that of **2**. Thus, the acetal portion at C-12 in **1** might play an important role for the cytotoxic effects against MPM cells. Among these MPM cell lines, NCI-H2052 was reported to show cisplatin- and gemcitabine-resistance, and MSTO-211H also possessed gemcitabine-resistance.[7] In contrast to these antitumor agents, **1** exhibited almost the same cytotoxic effects against these MPM cell lines. Since **1** showed highly cytotoxic effects for MPM cell lines that demonstrate resistance to clinical anticancer drugs, it is expected to be a precious lead compound for developing anti-MPM drugs. Studies on detailed biological activities including the mechanism of action and the effect on normal cells are now underway.

Table 2. Cytotoxic Effects of **1** and **2** on Human MPM Cell Lines

	IC_{50} (μM)	
MPM cell lines	**1**	**2**
ACC-MESO-1	25	190
MSTO-211H	50	<200
NCI-H290	10	75
NCI-H2052	20	150

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Acknowledgment. This work was supported in part by the grant from the New Energy and Industrial Technology Development Organization (NEDO) of Japan, a Grant-in-Aid for Scientific Reserch, The Ministry of Education, Science, Sports and Culture, Japan, and The Japan Society for the Promotion of Science (JSPS) of Japan. J.H. was also supported by a Postdoctoral Fellowship (19.07627) from JSPS.

Supporting Information Available: ^{1}H NMR, ^{13}C NMR, DQF-COSY, HSQC, HMBC, and NOESY spectra in $CDCl_3$ and HRESIMS spectra of **1** and **2**; ^{1}H NMR, ^{13}C NMR, DQF-COSY, HSQC, HMBC, 1D ROE spectra and tabulated ^{13}C and ^{1}H NMR spectral data in benzene-d_6 of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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