NOTE

Correction of the structure of cuevaene A and its identity with MBJ-0020 from *Streptomyces* sp. 30503

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Malignant pleural mesothelioma (MPM), which is associated with exposure to asbestos fibers, is an aggressive neoplasm that develops in the pleura, and is highly invasive to surrounding tissues.^{1–3} In particular, MPM has been shown to be resistant to all conventional therapies, including chemotherapy, radiotherapy and surgery, leading to a very poor prognosis of MPM patients. Consequently, it is a tumor that continues to be a difficult clinical problem.^{1–3} Therefore, development of novel therapeutic agents against MPM is highly desirable.

In the course of our screening program for cytotoxic compounds against MPM derived from microbial metabolites, we isolated novel anti-MPM compounds designated as JBIR-23 and -24 from *Streptomyces* sp. AK-AB27 (Figure 1).^{4,5} Further screening resulted in the discovery of a compound, MBJ-0020 (compound **1**), from the culture of *Streptomyces* sp. 30503 residing in the MBJ's special collection of microbial strains. Compound **1** was speculated to be cuevaene A⁶ according to its molecular formula, when analyzed with ultra-performance liquid chromatography coupled with time-of-flight MS. To confirm the structure of **1**, we carried out the structure determination of **1** by a series of NMR analyses. The result showed us that the reported structure of cuevaene A was incorrect. Here, we report the isolation, structural elucidation and brief biological activity of **1**, and discuss the structural revision of cuevaene A.

The strain *Streptomyces* sp. 30503 was isolated from sponges collected in Okinawa Prefecture, Japan, according to the published method.⁷ The strain was cultivated in 250-ml Erlenmeyer flasks, each containing 25 ml of a seed medium consisting of 2% potato starch (Tobu Tokachi Nosan Kako Agricultural Cooperative Assoc., Hokkaido, Japan), 2% glucose (Junsei Chemical, Tokyo, Japan), 2% soy bean powder (Honen SoyPro, J-Oil Mills, Tokyo, Japan), 0.5% yeast extract powder (Oriental Yeast, Tokyo, Japan), 0.25% NaCl (Junsei Chemical), 0.32% CaCO₃ (Wako Pure Chemical Industries, Osaka, Japan), 0.0005% CuSO₄·5H₂O (Wako Pure

Chemical Industries), 0.0005% ZnSO₄·7H₂O (Wako Pure Chemical Industries) and 0.0005% MnCl₂·4H₂O (Junsei Chemical). The flasks were placed on a rotary shaker (220 r.p.m.) at 28 °C and incubated for 3 days. Measured aliquots (0.5 ml) of the broth were then transferred to 500-ml Erlenmeyer flasks containing 50 ml of production medium of the same composition, and were cultured on a rotary shaker (220 r.p.m.) at 28 °C for 4 days. The whole culture broth (21) was then extracted with *n*-butanol (21).

After concentrating the extract *in vacuo*, the residual aqueous concentrate (15.8 g) was extracted with ethyl acetate (EtOAc) (100 ml × 3). The separated organic layer was dried over Na₂SO₄ and concentrated *in vacuo*. The dried residue (1.16 g) was subjected to normal-phase medium-pressure liquid chromatography (Purif-Pack SI-30; Shoko Scientific, Yokohama, Japan), and was eluted stepwise by using a hexane–EtOAc solvent system (0, 5, 10, 20 and 25% EtOAc) followed by a CHCl₃–MeOH solvent system (0, 2, 5, 10 and 20% MeOH). The active eluate (5% MeOH, 380 mg) was subjected to reversed-phase medium-pressure liquid chromatography (Purif-Pack ODS-100), and was eluted stepwise by using a H₂O–MeOH solvent system (40, 60, 80 and 100% MeOH). The active eluate (80% MeOH, 79 mg) was further purified by preparative reversed-phase HPLC by means of a Capcell Pak C₁₈ MGII column (5.0 μm, 20 i.d. × 150 mm; Shiseido, Tokyo, Japan) with a Model 2996 photodiode array detector (Waters, Milford, MA, USA) and a Model 3100 mass detector (Waters), eluting with 75% aqueous MeOH containing 0.1% formic acid (flow rate, 10 ml min^{−1}) to yield compound **1** (2.4 mg, retention time 37.9 min).

Compound **1** acquired this way was a colorless amorphous solid: $[\alpha]_D^{26} + 84.3$ (c 0.1, MeOH); UV $\lambda_{\max}$ nm (log ϵ): 303 (4.33), 258 (4.13) in MeOH. The molecular formula was determined to be C₂₁H₂₂O₅ by high-resolution ESI-MS (found: 353.1390 [M-H][−], calculated: 353.1389 [M-H][−]). The presence of hydroxy and α,β -unsaturated carboxylic acid functions was deduced from the

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Figure 1 (a) Structures of MBJ-0020 (compound **1**), originally proposed cuevaene A⁶, JBIR-23 and -24. (b) Correlations in DQF-COSY, TOCSY (bold lines) and CT-HMBC (arrows) spectra of compound **1** (revised structure of cuevaene A).

Table 1 ¹³C (150 MHz) and ¹H (600 MHz) NMR spectroscopic data for compound **1**

No.	δ _C	δ _H (multiplicity, J, in Hz)
1	170.6	—
2	115.8	6.03, d (15.5)
3	144.2	7.10, d (15.5)
4	151.7	—
5	131.0	5.82, s
6	131.8	—
7	140.1	5.76, d (10.0)
8	32.3	3.81, dt (10.0, 3.2)
9	29.8	2.00, m
10	21.4	1.54, m
11	—	2.05, m
12	—	1.85, m
13	23.4	2.72, m
14	155.3	—
15	114.5	—
16	149.3	—
17	111.1	7.22, d (8.5)
18	111.2	6.66, dd (8.5, 1.7)
19	151.1	—
4-OMe	104.5	6.68, d (1.7)
6-Me	60.2	3.66, s
	14.9	2.21, s

NMR spectra were obtained in CDCl₃ using the Varian NMR system 600 NB CL (Palo Alto, CA, USA). The solvent peak was used as an internal standard (δ_H 7.25 p.p.m. and δ_C 77.0 p.p.m.).

compound's IR spectrum ($\nu_{\max}$ 3355, 1681 cm⁻¹). The direct connectivity between protons and carbons was established by a HSQC spectrum. The ¹³C and ¹H NMR spectroscopic data for compound **1** is shown in Table 1. The structure elucidation of compound **1** by means of DQF-COSY and constant time-HMBC⁸ spectra is described in the following section.

Three substructures were recognized by analyzing DQF-COSY and CT-HMBC. The ¹H–¹H spin correlation was observed between doublet olefinic protons H-2 (δ_H 6.03) and H-3 (δ_H 7.10). In the CT-HMBC spectrum, ¹H–¹³C long-range couplings were observed from the olefinic protons H-2 and H-3 to a carboxylic carbon C-1 (δ_C 170.6) and a quaternary olefinic carbon C-4 (δ_C 151.7), which in turn coupled to a methoxy proton 4-OCH₃ (δ_C 60.2, δ_H 3.66) and an olefinic proton H-5 (δ_H 5.82). The singlet allylic methyl proton 6-Me (δ_H 2.21) was long-range-coupled to olefinic carbons C-5 (δ_C 131.0), C-6 (δ_C 131.8) and C-7 (δ_C 140.1). These 2D NMR correlations and the absorption of an α,β-unsaturated carboxylic acid at 1681 cm⁻¹ in the IR spectrum revealed a 4-methoxy-6-methyl-hepta-2,4,6-trienoic acid moiety in compound **1** as shown in Figure 1b.

In addition to these resonances ascribed to the triene moiety, compound **1** showed 12 extra ¹³C resonances. The sequence from an olefinic proton H-7 (δ_H 5.76) to methylene protons H₂-11 (δ_H 2.72) through a methine proton H-8 (δ_H 3.81), methylene protons H₂-9 (δ_H 2.00, 1.54) and H₂-10 (δ_H 2.05, 1.85) was established with the help of the DQF-COSY spectrum. The ¹H–¹³C long-range couplings from both the methine proton H-8 and the methylene protons H₂-11 to an oxygenated olefinic quaternary carbon C-12 (δ_C 155.3) and an olefinic quaternary carbon C-13 (δ_C 114.5) showed that these carbons existed in a six-membered ring system. The ¹H–¹H correlation,

together with the spin-coupling constants among aromatic protons H-16 (δ_{H} 7.22), H-17 (δ_{H} 6.66) and H-19 (δ_{H} 6.68) revealed a 1,3,4-trisubstituted benzene ring moiety. In addition, strong *m*-couplings from H-17 and H-19 to an aromatic quaternary carbon C-15 (δ_{C} 149.3), and from H-16 to an aromatic quaternary carbon C-14 (δ_{C} 129.1) and C-18 (δ_{C} 151.1) established the assignments of the benzene ring moiety. According to the typical ^{13}C chemical shifts of C-15 (δ_{C} 149.3) and C-18 (δ_{C} 151.1), these carbons were assigned to be oxygenated. Finally, the ^1H – ^{13}C long-range coupling from the aromatic proton H-19 to the olefinic quaternary carbon C-13 established the link between these two partial structures. By taking into consideration an unsaturated number obtained from the molecular formula of compound **1**, a 6,7,8,9-tetrahydrodibenzo [*b,d*]furan-2-ol moiety was established as shown in Figure 1b. In addition, treatment with TMS-diazomethane resulted in the production of a 1-COOMe derivative, which also confirms that C-1 consists of a carboxylic acid group.

The configurations of the triene side chain moiety in compound **1** were established to be 2*E*, 4*Z* and 6*E* by the means of analyzing the proton spin-coupling constant between H-2 and H-3 ($J = 15.5\text{ Hz}$), and the strong NOESY correlations between H-3/H5 (2*E*), H-5/H-7 (4*Z*) and 4-OMe/6-Me (6*E*). We observed that the high-field-shifted ^{13}C chemical shift value at 6-Me (δ_{C} 14.9) due to the γ -effect also supported the stereochemistry at C-6. Thus, the planar structure of compound **1** was determined as shown in Figure 1a.

The cytotoxic effects of compound **1** against various MPM cell lines were determined by a colorimetric assay with 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulphophenyl)-2*H*-tetrazolium monosodium salt (WST-8, Cell Counting Kit; Dojindo, Kumamoto, Japan) for 48 h. Compound **1** exhibited cytotoxic activities against three MPM cell lines, Meso-1, Meso-8A and Meso-8D with IC_{50} values of 32, 40 and 78 μM , respectively. These cytotoxic effects were similar to those of JBIR-23 (IC_{50} values of 31, 39 and 111 μM , respectively). As compound **1** showed considerable effects on the MPM cell lines that demonstrate resistance to clinical anticancer drugs, it is expected to be an invaluable lead compound for developing anti-MPM drugs.

The structure of compound **1** differs from that reported for cuevaene A in the substitution position of the side chain on the tricyclic ring system of cuevaene A (corresponding to C-11 position in compound **1**). Cuevaene A, which displayed moderate antibacterial activity against Gram-positive bacteria, was isolated from *Streptomyces* sp. HKI 0180.⁶ We have reported two structurally related compounds, named JBIR-23 and -24, isolated from the same species, which showed moderate cytotoxic effects against several MPM cell lines.^{4,5} All of these natural products embrace the tricyclic core and the triene side chain with an enol methyl ether. Although the structure

of cuevaene A was originally proposed as shown in Figure 1a, we suggest that it is revised to that of compound **1** by altering the connective position of the triene side chain to the same site on the cyclohexane ring as that in JBIR-23 and -24, based on the extensive NMR correlations and the possible biosynthetic relationship among the compounds.

Recently, Liu *et al.*⁹ reported the syntheses of these two structures. The NMR spectra of the synthetic isomers are very similar to each other, and the published data for original cuevaene A do not match either isomer very well. On the other hand, Taylor *et al.*¹⁰ have just reported the total synthesis of cuevaene A with alternative structure. The NMR spectroscopic data of synthesized cuevaene A with revised structure corresponded with those of compound **1**. Therefore, we revised the structure of cuevaene A as compound **1**.

From a biosynthetic point of view, the structure of compound **1** seems to be quite plausible, because it presumably arises through the polyketide pathway utilizing a benzoic acid as a starter unit, in which the side chain moiety is substituted at the same position corresponding to JBIR-23 and JBIR-24. Studies on detailed biological activities including the mechanism of action and the biosynthesis of compound **1** are now under way.

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