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NOTE

Mangromicin C, a new analog of mangromicin

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Mangromicin analogs were discovered in a cultured broth of a rare actinomycete, *Lechevalieria aerocolonigenes* K10-0216.[1,2] The mangromicin analogs showed antitrypanosomal and reactive oxygen species (ROS) scavenging bioactivities. Mangromicin analogs have unique structures, including common partial structures, notably a cyclopentadecane skeleton with a tetrahydrofuran unit and a 5,6-dihydro-4-hydroxy-2-pyrone moiety. In a previous report, we described eight new mangromicin analogs, which possessed anti-oxidative properties. At that time, we discovered mangromicin C (**1**) but were unable to determine its structure, which was clearly different from all the other analogs. We have now found that **1** has a structure which includes a tetrahydropyran ring (Figure 1). In this paper, we report the fermentation, isolation, structural elucidation and biological activity of **1**.

A loop of spores of the strain K10-0216 was inoculated into 100 ml of the seed medium, consisting of 2.4% starch (Wako Pure Chemical Industries, Osaka, Japan), 0.1% glucose (Wako), 0.3% peptone (Kyokuto Pharmaceutical Industrial, Tokyo, Japan), 0.3% meat extract (Kyokuto), 0.5% yeast extract (Oriental Yeast, Tokyo, Japan) and 0.4% $CaCO_3$ (Wako) (adjusted to pH 7.0 before sterilization) in a 500 ml Erlenmeyer flask. The flask was incubated on a rotary shaker (210 r.p.m.) at 27 °C for 3 days. A 1 ml portion of the seed culture was transferred to 500 ml Erlenmeyer flasks (total 150) containing 100 ml of starch medium, consisting of 2% soluble starch (Wako), 0.5% glycerol (Wako), 1.0% defatted wheat germ (Nisshin Pharma, Tokyo, Japan), 0.3% meat extract, 0.3% dry yeast (JT Foods, Tokyo, Japan) and 0.3% $CaCO_3$ (adjusted to pH 7.0 before sterilization) and fermentation was carried out on a rotary shaker (210 rpm) at 27 °C for eight days.

The cultured broth of K10-0216 strain (15 l) was centrifuged to separate the mycelium and supernatant. The supernatant was extracted three times with ethyl acetate (15 l). The organic layer was concentrated to dryness *in vacuo* to afford a crude material (6 g). The ethyl acetate extract was subjected to silica gel column chromatography FL100D (60 i.d. × 200 mm, Fuji Silysia, Tokyo, Japan), which was sequentially eluted with 1 l of a mixture of $CHCl_3$-CH_3OH (100:0, 100:1, 50:1 and 10:1) in that order. The eluate fraction (100:1) was concentrated to yield 550 mg and applied to an ODS column (40 i.d. × 150 mm; Senshu Scientific, Tokyo, Japan). After washing with 50% MeOH, the fractions containing **1** were eluted with 60% MeOH, followed by concentration *in vacuo*. The eluate fractions (278 mg) were purified by high performance liquid chromatography on an Inertsil ODS-4 column (14 i.d. × 250 mm, GL Sciences, Tokyo, Japan) with 40% MeOH at 9.3 ml min^{-1} and subsequently detected under UV light of 254 nm. The yield of **1** was 45.0 mg.

Compound **1** was obtained as a white powder ($[\alpha]_D^{25.3} = +23.2$; $c = 0.1$ in MeOH); UV (MeOH) λ_{max} (ε): 251 nm (5595), and its infrared spectrum showed the characteristic absorptions of hydroxyl and carbonyl groups (ν_{max}, 3430 and 1657 cm^{-1}). The HR-ESIMS of **1** produced the $[M+H]^+$ ion at *m/z* 395.2413 indicating the molecular formula was $C_{22}H_{34}O_6$ (calculated value for $C_{22}H_{35}O_6$, 395.2434). Since some broadening signals were observed in CD_3OD, the 1D and 2D nuclear magnetic resonance (NMR) spectra of **1** were obtained in DMSO-d_6. The sharpest NMR signals of **1** were observed at 70°C among various temperatures (see Supplementary Information).

Mangromicin C (**1**) structure was elucidated by comparison of the ^1H and ^{13}C NMR of other mangromicin analogs. The ^1H and ^{13}C NMR spectral data of **1** measured in DMSO-d_6 at 70 °C are listed in Table 1. The ^1H NMR data indicated the presence of three oxygenated sp^3 methines, three sp^3 methines, containing two methines coupled to methyl groups, seven methylenes, one primary methyl, two secondary methyls and one tertiary methyl. The ^{13}C NMR spectrum showed the resonances of 22 carbons, which were classified into two olefinic carbons, two carbonyl carbons at δ_c 164.6 and 209.3, one oxygenated sp^3 quaternary carbon, three oxygenated sp^3 methine carbons, three sp^3 methine carbons, seven sp^3 methylene carbons and four methyl carbons by heteronuclear-single-quantum coherence spectra.

The ^1H-^1H COSY indicated the presence of three partial structures (a) C-8/C-12, C-8/C-8-Me and C-12/C-12-Me, (b) C-15/C-16 (c) C-4/C-3′ (Me), as shown in Figure 2. Analysis of HMBC data confirmed the presence of a 5,6-dihydro-4-hydroxy-2-pyrone moiety (a partial structure in mangromicins), based on correlations from H-4 to C-2, C-3 and C-6; from H-5 to C-1, C-3 and C-1′; from H_2-15 to C-2; and from H_2-16 to C-1, C-2 and C-14. The HMBC correlations

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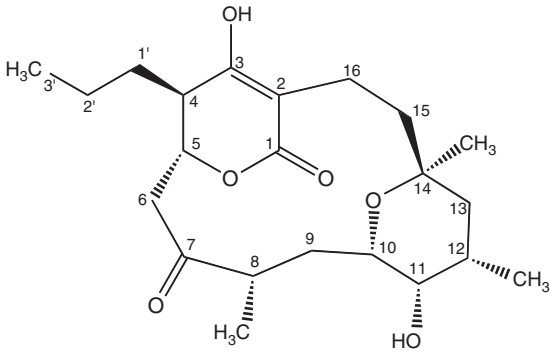

Figure 1 Relative configurations of mangromicin C (**1**).

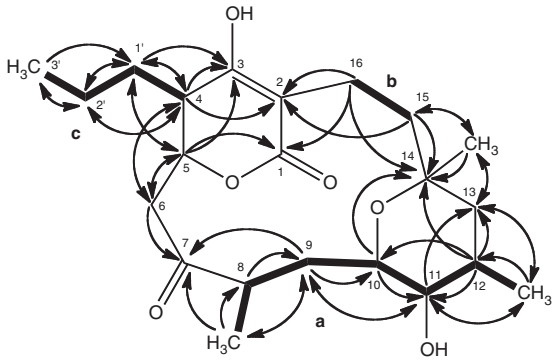

Figure 2 ^{1}H-^{1}H COSY (bold) and selected HMBC (arrow) correlations of mangromicin C (**1**).

Table 1 ^{1}H and ^{13}C NMR spectral data for mangromicin C (1)

Position	*δ_C, mult*	*δ_H (int., mult., J in Hz)*	*HMBC*
1	164.6, C		
2	102.1, C		
3	163.4, C		
4	41.5, CH	2.10 (1H, dd, 0.4, 4.8)	C-2, C-3, C-6, C-1′, C-2′
5	71.3, CH	4.56 (1H, dd, 0.4, 9.2)	C-1, C-3, C-6, C-1′
6a	42.5, CH_2	2.32 (1H, m)	C-4, C-7
6b		4.28 (1H, dd, 9.2, 15.2)	C-4, C-5, C-7
7	209.3, C		
8	42.4, CH	2.63 (1H, m)	C-9
9a	36.1, CH_2	1.60 (1H, m)	C-7, C-10, C-11, C-8-Me
9b		2.28 (1H, m)	C-7, C-10, C-11, C-8-Me
10	66.6, CH	3.41 (1H, m)	C-11
11	80.0. CH	3.24 (1H, dd, 2.4, 5.6)	C-9, C-13, C-12-Me
12	34.9, CH	2.27 (1H, m)	C-10, C-11, C-13, C-14
13	47.5, CH_2	1.59 (2H, m)	C-12-Me, C-14-Me
14	81.3, C		
15a	35.0, CH_2	1.16 (1H, ddd, 3.2, 3.2, 11.2)	C-2,
15b		2.32 (1H, m)	C-2, C-14
16a	18.2, CH_2	1.91 (1H, ddd, 3.2, 3.2, 12.0)	C-2, C-14
16b		2.72 (1H, ddd, 3.2, 3.2, 11.2)	C-1, C-2
1′	33.1, CH_2	1.50 (2H, m)	C-3, C-4, C-5, C-2′
2′a	19.2, CH_2	1.31 (1H, m)	C-1′
2′b		1.43 (1H, m)	C-4, C-1′, C-3′
3′ (Me)	13.4, CH_3	0.88 (3H, t, 8.0)	C-1′, C-2′
8-Me	14.4, CH_3	0.96 (3H, d, 6.4)	C-7, C-8, C-9
12-Me	14.4, CH_3	0.98 (3H, d, 5.6)	C-11, C-12, C-13
14-Me	24.4, CH_3	1.25 (3H, s)	C-13, C-14, C-15

In DMSO-d_6 (70 °C)

from H-4 to C-2′; from H-5 to C-1′; from H_2-1′ to C-3, C-4, C-5, and C-2′; from H_2-2′ to C-4, C-1′ and C-3′; and from H_3-3′ to C-1′ and C-2′ confirmed an *n*-propyl group linked to the C-4 position. A tetrahydropyran unit was identified, based on HMBC correlations from H_2-9 to C-10 and C-11; from H-10 to C-11 and C-14; from H-11 to C-9 and C-13; from H-12 to C-10, C-11, C-13 and C-14. Moreover, the correlations from H-11 and H_2-13 to C-12-Me; from H_2-13 and H_2-15 to C-14-Me; from H_3-12-Me to C-11, C-12 and C-13; and from H_3-14-Me to C-13, C-14 and C-15 confirmed a secondary methyl and a tertiary methyl linked to the C-12 and C-14 positions, respectively. The HMBC correlations from H_2-15 to C-2 and C-14, and from H_2-16 to C-1, C-2 and C-14 also showed that the 5,6-dihydro-4-hydroxy-2-pyrone moiety and tetrahydropyran ring were connected by an ethylene bond. Finally, the cyclopentadecane ring was confirmed by the HMBC correlations from H_2-6 to C-4, C-5 and C-7; from H-8 to C-9; from H_2-9 to C-7, C-10, C-11, and C-8-Me; from H_3-8-Me to C-7, C-8 and C-9. Therefore, the planar structure of **1** was elucidated as shown in Figure 2, and it was designated as mangromicin C. Compound **1** was structurally different from all other mangromicin analogs, which have a tetrahydropyran ring instead of a tetrahydrofuran ring.

The relative configuration of **1** was estimated by the ^{1}H-^{1}H coupling constant analysis, differential NOE and ROESY experiments compared with mangromicin A.[1] The ROESY correlations, the same as mangromicin A, were observed between H-4/H-5, H-4/H-6a, H-5/H-6a, H_3-8-Me/H-10, H_3-12-Me/H_2-13, H_2-13/H_2-15, H_2-13/H_3-14-Me and H_3-14-Me/H_2-16 (Supplementary Figure S7). In addition, the relative configuration of the tetrahydropyran ring was determined by small coupling constant between H-11 and H-10 or H-12 (Table 1), and ROESY correlations between H-11/H_3-12-Me and H_2-13/H_3-12-Me. Moreover, ROESY correlations of H-10/H_3-8-Me suggested that H_3-8-Me is α-oriented. The coupling constants were observed between H-4 and H-5 (0.4 Hz) and H-5 and H-6b (9.2 Hz). The relative configuration of **1** was proposed as shown in Figure 1.

Compound **1** had more potent scavenging activity against 1,1-diphenyl-2-picrylhydrazyl free radicals ($IC_{50} = 3.8$ μM) than α-tocopherol ($IC_{50} = 11.4$ μM). In addition, **1** had scavenging activity against nitric oxide generated by lipopolysaccharide-stimulated RAW264.7 cells, a murine-macrophage cell line, at final concentration of 100 μM, without cytotoxic activity. Compared to the eight other analogs of mangromicin found, mangromicin C showed moderate anti-oxidant activity against ROS.[2] ROS are known to be potentially injurious to living organisms because, in excess, they cause oxidative stress and can damage lipids, proteins and nucleic acids.[3–5] Our results indicate that mangromicin analogs offer potential as ROS scavengers.

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Supplementary Information accompanies the paper on The Journal of Antibiotics website (http://www.nature.com/ja)