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ORIGINAL ARTICLE

Quadoctomycin, a 48-membered macrolide antibiotic from *Streptomyces* sp. MM168-141F8

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Drug-resistant bacteria are still emerging, and screening of new skeletal antibiotics is important. During our continuous screening for antimicrobial agents, we discovered a new antimicrobial, named quadoctomycin, from solid culture of *Streptomyces* sp. MM168-141F8. The substance was purified by solvent extraction, silica gel chromatography and HPLC. Structural elucidation of quadoctomycin was performed by MS and NMR analyses and chemical degradation. Quadoctomycin possesses a 48-membered polyol macrolide skeleton in which an α-D-mannoside is connected to C-22 by an *O*-glycosidic linkage. The structure of quadoctomycin was found to be related to that of monazomycin A based on the analyses of NMR spectra in the same solvent (pyridine-d_5). Quadoctomycin showed potent antibacterial activity against *Staphylococcus aureus*, including methicillin-resistant *S. aureus*, and other Gram-positive pathogenic bacteria such as *Enterococcus faecalis* and *E. faecium* (including drug-resistant strains), but did not show activity toward Gram-negative bacteria or *Candida albicans*.
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INTRODUCTION

Antimicrobial resistance has become a global problem that crosses national borders. In 2011, the Director General of the World Health Organization issued an important warning about antimicrobial resistance and a coming global crisis: "no action today means no cure tomorrow".[1] In 2013, the US Centers for Disease Control and Prevention enumerated several pathogens that are considered a serious threat and for which effective antibiotic development is urgently needed. These bacteria include *Clostridium difficile*, carbapenem-resistant Enterobacteriaceae, extended spectrum β-lactamase-producing Enterobacteriaceae, drug-resistant *Neisseria gonorrhoeae*, multidrug-resistant *Acinetobacter*, drug-resistant *Campylobacter*, vancomycin-resistant *Enterococcus*, multidrug-resistant (MDR) *Pseudomonas aeruginosa*, drug-resistant non-typhoidal *Salmonella*, drug-resistant *Salmonella typhi*, drug-resistant *Shigella*, methicillin-resistant *Staphylococcus aureus*, drug-resistant *Streptococcus pneumoniae* and multidrug-resistant *Mycobacterium tuberculosis*.[2]

Conventionally, to overcome drug-resistant bacteria, development of next-generation semi-synthetic antibiotics has been carried by limited modification of existing antibiotic skeletons. However, as these superbugs spread more quickly than anticipated, it is becoming difficult to overcome resistance by this method. Compounds with new skeletons and mechanisms of action are becoming the scaffolds for the drug discovery process and they bring about changes to current chemotherapy. Natural products have the potential to present novel chemical scaffolds and provide new leads to organic chemists. The search for antibiotics with new structures from natural products is expected to be one approach to developing useful and innovative new drugs for overcoming antimicrobial resistance.[3,4] Indeed, the development and launch of several such antimicrobials effective against multidrug-resistant *M. tuberculosis* will be a change in the current treatment regimen.[5,6]

During our screening for antibiotics with a new skeleton and/or new mechanism(s) of action against antimicrobial resistance, we found a novel 48-membered macrolide, named quadoctomycin (Figure 1), in the solid culture material of a *Streptomyces* strain. In this paper, we describe the taxonomy and fermentation of the producing strain, and the isolation, structure elucidation and biological activities of quadoctomycin.

RESULTS AND DISCUSSION

Screening

In continuous screening for new antimicrobial substances against various test organisms, we found antibacterial activity against Gram-positive bacteria including methicillin-resistant *S. aureus* in the solid culture material of strain MM168-141F8, isolated from a soil sample collected in Shinagawa-ku, Tokyo, Japan. Small-scale culture of the strain was extracted with *n*-butanol and the dried extract was purified by semi-preparative HPLC. It was suggested that the active fractions gave a protonated molecule with *m/z* 1436.9961 by HPLC-high resolution ESI/MS (HRESI/MS). This molecule is not described in

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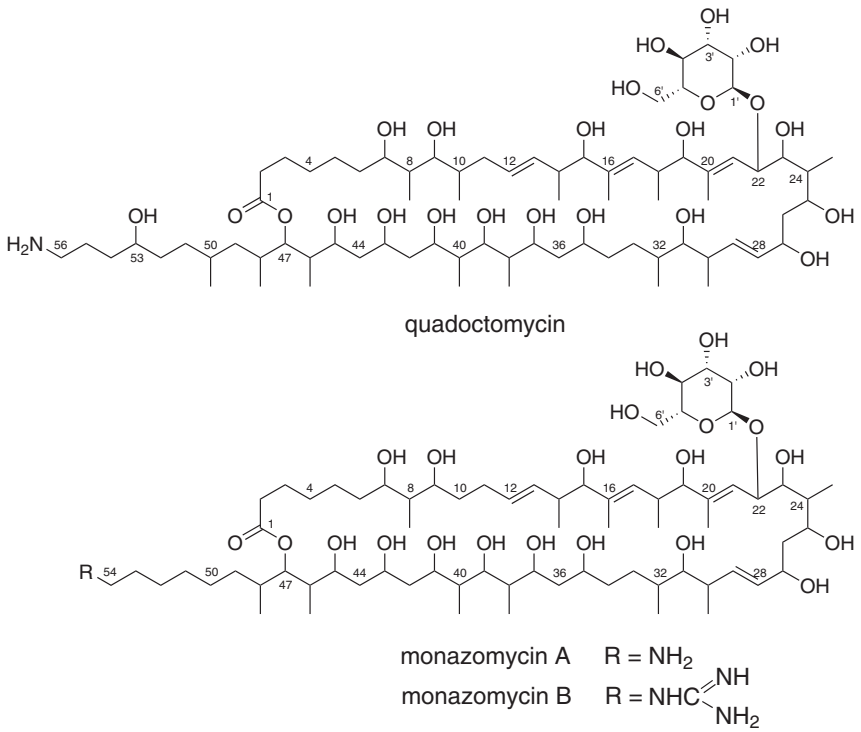

Figure 1 Structures of quadoctomycin and monazomycins.

Figure 2 Scanning electron micrograph of strain MM168-141F8 after growth on oatmeal agar (ISP medium No. 3) for 8 days at 30 °C. Bar, 1 μm.

the Dictionary of Natural Products database. Therefore, we decided to isolate this active substance.

Taxonomy of the antibiotics-producing strain

On oatmeal agar (ISP medium No. 3), strain MM168-141F8 formed well-branched substrate mycelia and straight to flexuous aerial mycelia. The substrate mycelia were light beige, the aerial mycelia were bayberry gray. Mature spore chains consisted of 5–≥20 spores. The spores were cylindrical to oval with a spiny surface and 0.7 × 1.0–1.0 × 1.2 μm in size (Figure 2). The diaminopimelic acid isomers in whole-cell hydrolysates of strain MM168-141F8 were determined to be the LL-form. A partial 16S ribosomal RNA gene sequence (1425 bp) was determined. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain MM168-141F8 is LC270914. The strain showed high similarity with those of the genus *Streptomyces*, such as *S. djakartensis* NBRC 15409^{T} (1417/1419 bp, 99.86%; ^{T}: Type strain) and *Streptomyces tuirus* NBRC 15617^{T} (1413/1420 bp, 99.51%). These phenotypic and genotypic data suggested that strain MM168-141F8 belongs to the genus *Streptomyces*. Therefore, the strain was tentatively designated *Streptomyces* sp. MM168-141F8.

Fermentation and isolation

Quadoctomycin was monitored using antimicrobial activity against *S. aureus* Smith. The fermentation of quadoctomycin was carried out in solid culture. A total of 7 ml of seed culture was transferred into 14 K-1 flasks each containing 40 g of production medium. The fermentation was carried out at 30 °C for 14 days in the dark. The producing culture (560 g) was extracted with acetone and the extract was concentrated. Water was added and the mixture was extracted with *n*-butanol. The organic layer was concentrated and the residue was partitioned with *n*-hexane/methanol. As the antimicrobial activity was in the methanol layer, the methanol solution was concentrated to dryness to obtain a brown solid (1 g). The residue was applied to a silica gel column and eluted with chloroform/methanol/H_2O to yield 339 mg of light-brown solid. The solid, containing quadoctomycin, was further purified by HPLC, which was developed with 35% acetonitrile aqueous solution containing 0.01% trifluoroacetic acid to give 55 mg of quadoctomycin as a white powder.

Structure determination

The physicochemical properties of quadoctomycin are summarized in the Materials and Methods section. The optical rotation of the compound was +15° (*c* 0.5, methanol). It did not show a characteristic UV spectrum. The molecular formula of quadoctomycin was determined to be $C_{76}H_{141}NO_{23}$ by HRESI/MS. Although the ^{1}H and ^{13}C NMR spectra of quadoctomycin in methanol-d_4 initially showed a

Table 1 NMR data for quadoctomycin in pyridine-d_5

No.	^{13}C	*Multi*	^{1}H *(multiplicity, J in Hz)*	*HMBC*	*No.*	^{13}C	Multi	^{1}H *(multiplicity, J in Hz)*	*HMBC*
1	173.6	s			39	77.5	d	4.34 (dd, 9.0, 3.0)	
2	34.6	t	2.45 (m)	25.6, 29.9, 173.6,	40	41.3	d	2.07 (dqd, 9.0, 7.0, 2.3)	11.3, 72.2, 77.5,
3	25.6	t	1.77 (m)		41	72.2	d	4.77 (dt, 9.6, 2.3)	41.6, 72.6, 77.5,
4	29.9	t	1.42 (m), 1.50 (m)		42	41.6	t	1.89 (dt,14.0, 2.3), 2.18 (dt, 14.0, 9.6)	
5	26.5	t	1.47 (m), 1.76 (m)		43	72.6	d	4.59 (m)	
6	36.0	t	1.60 (m), 1.78 (m)		44	39.1	t	1.95 (ddd, 13.6, 10.3, 7.6), 2.09 (ddd, 13.6, 3.6, 1.3)	41.6, 70.9, 72.6,
7	76.5	d	4.07 (ddd, 8.7, 4.3, 2.5)	6.1, 26.5,	45	70.9	d	4.31 (ddd, 1.3, 3.3, 10.3)	10.9, 72.6,
8	39.2	d	1.85 (qt, 7.0, 2.5)	6.1, 76.5, 80.4,	46	41.1	d	2.44 (dqd, 9.4, 6.6, 3.3)	10.9, 39.1, 70.9, 79.4,
9	80.4	d	3.74 (dd, 9.1, 2.5)	6.1, 16.0, 36.8, 37.4, 39.2, 76.5,	47	79.4	d	5.09 (dd, 9.4, 3.4)	17.67, 32.4, 38.0, 41.1, 70.9, 173.6,
10	37.4	d	1.92 (m)	16.0,	48	32.4	d	2.02 (m)	
11	36.8	t	2.01 (ddd, 13.8, 9.6, 7.6), 2.91 (brd, 13.8)	16.0, 37.4, 128.5, 136.1,	49	38.0	t	1.19 (m), 1.55 (m)	
12	128.5	d	5.67 (dddd, 15.4, 7.6, 5.7, 0.8)	36.8, 41.3, 136.1,	50	30.7	d	1.57 (m)	
13	136.1	d	5.79 (ddt, 15.4, 7.7, 1.0)	16.4, 36.8, 41.3, 128.5,	51	31.9	t	1.13 (m), 1.84 (m)	
14	41.3	d	2.61 (m)	16.4, 80.7, 128.5, 136.1,	52	35.6	t	1.58 (m), 1.68 (m)	
15	80.7	d	4.16 (d, 6.8)	13.1, 16.4, 41.3, 130.0, 136.4,	53	71.0	d	3.82 (m)	25.2, 31.9,
16	136.4	s			54	35.1	t	1.75 (m)	
17	130.0	d	5.83 (d, 9.6)	13.1, 80.7,	55	25.2	t	2.20 (m), 2.27 (m)	35.1, 40.2, 71.0,
18	36.23	d	2.90 (dq, 9.6, 6.7)	17.0, 81.3, 130.0, 136.4, 146.3,	56	40.2	t	3.41 (m)	25.2, 35.1,
19	81.3	d	4.24 (d, 6.7)	13.4, 17.0, 36.23, 122.4, 130.0, 146.3,	8-Me	6.1	q	1.20 (d, 7.0)	39.2, 76.5, 80.4,
20	146.3	s			10-Me	16.0	q	0.96 (d, 6.6)	36.8, 37.4, 80.4,
21	122.4	d	6.03 (d, 9.5)	13.4, 78.3, 81.3,	14-Me	16.4	q	1.28 (d, 6.8)	41.3, 80.7, 136.1,
22	72.3	d	5.21 (dd, 9.5, 4.8)	39.4, 78.3, 97.7	16-Me	13.1	q	1.81 (brs)	80.7, 130.0, 136.4,
23	78.3	d	4.27 (dd, 6.7, 4.8)	12.5, 39.4, 69.2,	18-Me	17.0	q	1.29 (d, 6.7)	36.23, 81.3, 130.0,
24	39.4	d	2.27 (qdd, 7.0, 6.7, 1.5)	12.5, 69.2, 78.3,	20-Me	13.4	q	2.05 (brs)	81.3, 122.4, 146.3,
25	69.2	d	5.03 (dt, 10.3,1.5)	12.5, 43.2, 69.7, 78.3,	24-Me	12.5	q	1.24 (d, 7.0)	39.4, 69.2, 78.3,
26	43.2	t	1.91 (ddd, 13.1, 10.0, 1.5), 2.15 (ddd, 13.1, 10.3, 2.7)		30-Me	17.1	q	1.27 (d, 7.2)	40.9, 79.5, 133.7,
27	69.7	d	4.91 (ddd, 10.0, 5.8, 2.7)	43.2, 69.2, 133.7, 135.0,	32-Me	17.65	q	1.08 (d, 6.8)	26.7, 36.17, 79.5,
28	135.0	d	5.94 (dd, 15.4, 5.8)	40.9, 69.7,	38-Me	6.9	q	1.31 (d, 7.0)	40.5, 73.2, 77.5,
29	133.7	d	5.90 (dd, 15.4, 7.2)	17.1, 40.9, 69.7, 135.0,	40-Me	11.3	q	1.03 (d, 7.0)	41.3, 72.2, 77.5,
30	40.9	d	2.63 (sextet, 7.2)	17.1, 36.17, 79.5, 133.7, 135.0,	46-Me	10.9	q	1.10 (d, 6.6)	41.1, 70.9, 79.4,
31	79.5	d	3.44 (dd, 7.2, 4.2)	17.1, 17.65, 26.7, 40.9, 133.7,	48-Me	17.67	q	0.97 (d, 6.7)	32.4, 38.0, 79.4,
32	36.17	d	1.92 (m)		50-Me	21.4	q	0.95 (d, 6.2)	30.7, 31.9, 38.0,
33	26.7	t	1.77 (m), 2.10 (m)		1'	97.7	d	5.60 (brd, 1.5)	72.3, 73.1, 75.4,
34	36.4	t	1.68 (m), 1.92 (m)		2'	72.3	d	4.40 (dd, 3.3, 1.5)	69.7, 73.1,
35	68.7	d	4.33 (m)		3'	73.1	d	4.48 (dd, 9.2, 3.3)	69.7,
36	43.8	t	1.89 (ddd, 14.0, 9.1, 3.0), 2.13 (ddd, 14.0, 9.5, 2.4)		4'	69.7	d	4.56 (t, 9.2)	73.1, 75.4,
37	73.2	d	4.70 (dt, 9.5, 3.0)	6.9, 40.5, 43.8, 68.7, 77.5,	5'	75.4	d	4.35 (m)	
38	40.5	d	1.95 (qt, 7.0, 3.0)	6.9,	6'	63.3	t	4.35 (m), 4.56 (m)	69.7,

Chemical shifts in ppm adjusted with the pyridine signal at δ 7.19 (^{1}H) and δ 123.5 (^{13}C).

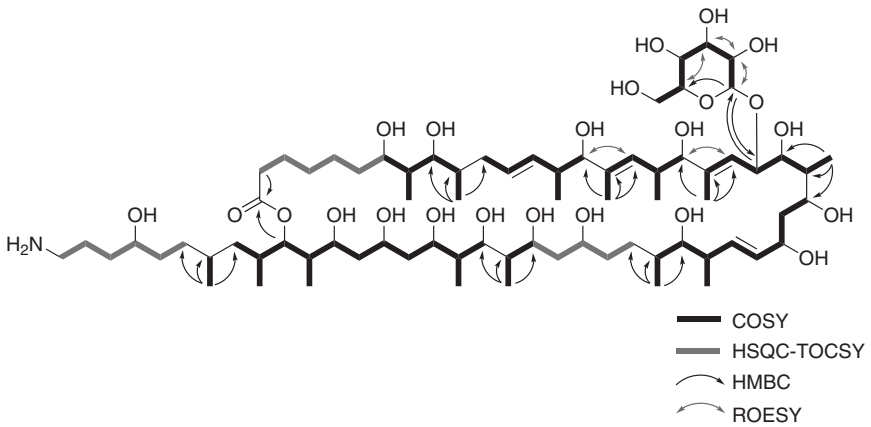

Figure 3 NMR analyses of quadoctomycin. A full color version of this figure is available at *The Journal of Antibiotics* journal online.

simple signal, minor signals appeared gradually, giving complicated spectra as shown in Supplementary Figure S2. The reason is not clear, but this finding was also supported by LC-HRESI/MS, which gave peaks of the same *m/z* with different retention times in an extracted ion chromatogram (see, Supplementary Figure S3). Pyridine-d_5 at 35 °C was suitable for NMR analysis. Table 1 summarizes ^{1}H and ^{13}C NMR data for quadoctomycin. The ^{13}C NMR and DEPT-135 spectra revealed that quadoctomycin contains 76 carbon atoms, including 14 methyl-, 19 methylene-, 34 sp^3 methine-, six sp^2 methine-, two fully substituted sp^2- and one carbonyl-carbon(s).

The structure of quadoctomycin was determined by ^{1}H-^{1}H COSY, HSQC-TOCSY and HMBC. ^{1}H-^{1}H COSY showed correlations from isolated olefin, hydroxy methine or methyl signals to adjacent protons from H-7 to H-10-Me, H-11 to H-15, H-16-Me to H-19, H-20-Me to H-24-Me, H-25 to H-32-Me, H-37 to H-38-Me, H-39 to H-49 and from H-50 to H-50-Me. Other correlations from characteristic hydroxy methine signals from H-1' to H-6' suggested the presence of a sugar moiety. The HSQC-TOCSY spectra confirmed these connectivities and other correlations from H-2 to H-8-Me, H-33 to H-37 and H-51 to H-56, establishing the partial structures shown in bold in Figure 3. The connectivities of these partial structures were analyzed by HMBC. The cross-peaks observed from H-10-Me to C-9-11, H-16-Me to C-15-17, H-20-Me to C-19-C-21, H-21 to C-19, H-24-Me to C-23-25, H-32-Me to C31-33, H-38-Me to C-37-39, H-50-Me to C-49-51 and the methylene proton of H-2 and the oxymethine of H-47 to C-1, established a 48-membered macrolide skeleton. The sugar moiety was established by the correlation from H-1' to C-5' and the moiety was connected to C-22 by the cross-peaks between H-22/C-22 and C-1'/H-1' in the HMBC spectrum. The planar structure of quadoctomycin determined from these results as shown in Figure 3.

Quadoctomycin has four double bonds and their geometries were determined to be 12*E*, 16*E*, 20*E* and 28*E* from the large proton–proton coupling constants ($^{3}J_{H12H13}$ = 15.4 Hz, $^{3}J_{H28H29}$ = 15.4 Hz), ^{13}C chemical shifts ($\delta_{C\text{-}16Me}$ 13.1 p.p.m. and $\delta_{C\text{-}20Me}$ 13.4 p.p.m.) and NOESY cross-peaks observed between H-15 and H-17, H-17 and H-19 and H-19 and H-21.

The sugar moiety was determined to be α-mannoside by proton–proton ($^{3}J_{H1'H2'}$ = 1.5 Hz, $^{3}J_{H2'H3'}$ = 3.3 Hz, $^{3}J_{H3'H4'}$ = 9.2 Hz and $^{3}J_{H4'H5'}$ = 9.2 Hz) and direct carbon–proton coupling constants ($^{1}J_{C1'H1'}$ = 170.0 Hz) and NOESY observed between H-1' and H-2', H-2' and H-3' and H-3' and H-5'. The stereochemistry of the sugar moiety was determined by the optical rotations of methanolysis product for quadoctomycin. Quadoctomycin (40 mg) was dissolved in hydrogen chloride-methanol at 65 °C for 3 h, which gave 1.6 mg of methyl-α-D-mannoside, for which NMR and optical rotation data were in good agreement with those of an authentic sample (see Supplementary figure S13 to S16). Figure 1 shows the structure of quadoctomycin elucidated from all the results. The absolute stereochemistry of the aglycon of quadoctomycin is currently being studied.

Quadoctomycin is structurally related to monazomycin A,[7] discovered by Professor Hamao Umezawa *et al.* in 1963.[8] NMR data for quadoctomycin were closely related to that for monazomycin A in methanol-d_4, but the NMR assignment for monazomycin A has not been published. We compared quadoctomycin and monazomycin A in pyridine-d_5 at 35 °C. The assignment of monazomycin A was established by analyzing 1D and 2D NMR spectra (not described in detail, see Supplementary Figure S17-22, Supplementary Table S1). The differences in chemical shifts were around C-10, and from C-50 to C-54 of the side chain, however the remaining ^{1}H and ^{13}C chemical shifts of monazomycin A were almost the same as those for quadoctomycin. Thus, quadoctomycin was determined to be a novel 48-membered ring macrolide having a methyl group at C-10 and a different side chain length from C-47 of monazomycin A.

Actinomycetes are known to produce many macrolides. Among them, the one with the largest ring structure is a 60-membered ring, quinolidomicin, reported by Hayakawa *et al.*[9,10] As far as the authors know, quadoctomycin has the second largest ring structure (48-membered) equal to that of monazomycin A,[7,8] B.[11]

Biological activities

Quadoctomycin showed potent antibacterial activity against *S. aureus* (including methicillin-resistant *S. aureus*) with minimum inhibitory concentrations (MICs) of 1–2 μg ml^{-1} (Table 2). Quadoctomycin also had antibacterial activity against other Gram-positive pathogenic bacteria such as *Enterococcus faecalis* and *E. faecium* (including drug-resistant strains), *Micrococcus luteus*, *Bacillus subtilis*, *B. cereus*, *Mycobacterium smegmatis* and *Corynebacterium bovis*, with minimum inhibitory concentrations of 0.5–2 μg ml^{-1}. Quadoctomycin did not show antimicrobial activity toward Gram-negative bacteria or *Candida albicans* (Table 2). Antimicrobial activities of quadoctomycin showed equal or two to fourfold more potent than those of monazomycin A as shown in Table 2.

MATERIALS AND METHODS

General

The optical rotations of compounds were obtained using a P-1030 polarimeter (JASCO, Tokyo, Japan). UV spectra were recorded with a U-2800 UV-Vis spectrophotometer (Hitachi High-Technologies, Tokyo, Japan). IR spectra were

Table 2 Antimicrobial activities of quadoctomycin and monazomycin A

			MIC ($\mu g\,ml^{-1}$)					MIC ($\mu g\,ml^{-1}$)	
Test organisms			*Quadoctomycin*	*Monazomycin A*	*Test organisms*			*Quadoctomycin*	*Monazomycin A*
Staphylococcus	*aureus*	FDA 209P	1	2	*Enterococcus*	*faecium*	JCM 5804	2	4
Staphylococcus	*aureus*	Smith	1	2	*Enterococcus*	*faecium*	NCTC12202	2	8
Staphylococcus	*aureus*	MS9610	2	4	*Enterococcus*	*faecium*	NCTC12204	2	4
Staphylococcus	*aureus*	MRSA No.5	2	4	*Escherichia*	*coli*	NIHJ	>64	>64
Staphylococcus	*aureus*	MRSA No.17	2	4	*Escherichia*	*coli*	K-12	128	>128
Staphylococcus	*aureus*	MS16526(MRSA)	1	4	*Escherichia*	*coli*	K-12 ML1629	>64	>64
Staphylococcus	*aureus*	TY-04282(MRSA)	2	4	*Escherichia*	*coli*	BEM11	>64	32
Staphylococcus	*aureus*	Mu50	2	8	*Escherichia*	*coli*	BE1121	64	32
Micrococcus	*luteus*	FDA 16	0.5	1	*Escherichia*	*coli*	BE1186	64	32
Micrococcus	*luteus*	IFO 3333	0.5	1	*Shigella*	*dysenteriae*	JS11910	>64	>64
Micrococcus	*luteus*	PCI 1001	0.5	1	*Salmonella*	*enteritidis*	1891	>64	>64
Bacillus	*subtilis*	NRRL B-558	2	4	*Proteus*	*vulgaris*	OX19	>64	>64
Bacillus	*subtilis*	PCI 219	2	4	*Proteus*	*mirabilis*	IFM OM-9	>64	>64
Bacillus	*subtilis*	ATCC23857 (168)	2	4	*Serratia*	*marcescens*	B-0524	>64	>64
Bacillus	*cereus*	ATCC 10702	1	2	*Pseudomonas*	*aeruginosa*	A3	>64	>64
Corynebacterium	*bovis*	1810	2	8	*Klebsiella*	*pneumoniae*	PCI 602	>64	>64
Enterococcus	*faecalis*	JCM 5803	1	4	*Candida*	*albicans*	3147	>64	>64
Enterococcus	*faecalis*	NCTC12201	2	8	*Mycobacterium*	*smegmatis*	ATCC607*	2	2
Enterococcus	*faecalis*	NCTC12203	2	4					

Agar dilution streak method (twofold dilution).
Medium & Culture condition: Mueller-Hinton agar (Difco) 37 °C 18 h., *37 °C 42 h. "DYNATEC".

recorded with a FT/IR-4100 Fourier-transform infrared spectrometer (JASCO). ^{1}H and ^{13}C NMR spectra, including 2D NMR, were measured with an ECZ600R spectrometer (JEOL Resonance, Tokyo, Japan) and an AVANCE III 500 spectrometer (Bruker, Billerica, MA, USA) using solvent signals as an internal reference. Mass spectra were recorded using a LTQ Orbitrap XL mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA). The novelty of quadoctomycin was searched using the Dictionary of Natural Products on DVD databases (Chapman & Hall/CRC Press, Boca Raton, FL, USA) and SciFinder (Chemical Abstracts Service, Columbus, OH, USA). Standard methyl-α-D-mannoside was purchased from Sigma Aldrich, St Louis, MO, USA. Monazomycin A was obtained from our inhouse library.

Taxonomic studies of the producing strain MM168-141F8

Morphological properties were observed following incubation at 30°C for 21 days on yeast extract-malt extract agar (ISP medium No. 2), oatmeal agar (ISP medium No. 3) and inorganic salt starch agar (ISP medium No. 4). Detailed observation of mycelial morphology was performed using a scanning electron microscope (SU-1510, Hitachi High-Technologies) after strain MM168-141F8 was incubated on ISP medium No. 3 at 30 °C for 8 days. The type of diaminopimelic acid isomers in whole-cell hydrolysates was determined by the method of Staneck and Roberts.[12] The total DNA of MM168-141F8 was prepared using a Genomic DNA Extraction Kit Mini (RBC Bioscience Co., New Taipei, Taiwan) according to the manufacturer's instructions. 16S rRNA (positions 56–1495, *Escherichia coli* numbering system[13]) was amplified by PCR and sequenced. A search for the most closely related sequences was performed using the EZ Taxon database (http://www.ezbiocloud.net/).[14]

Fermentation and purification of quadoctomycin

A slant culture of *Streptomyces* sp. MM168-141F8 was inoculated into a 500-ml baffled Erlenmeyer flask containing 110 ml of a seed medium consisting of 2.0% galactose, 2.0% dextrin, 1.0% Bacto Soytone (Becton Dickinson and Company, Franklin Lakes, NJ, USA), 0.5% corn steep liquor (Kogo Starch, Chiba, Japan), 0.2% $(NH_4)_2SO_4$ and 0.2% $CaCO_3$, pH 7.4 before sterilization. The culture was incubated at 27 °C for 3 days on a rotary shaker at 180 rpm. Production was performed using a solid medium (15 g of pressed barley and 25 ml of deionized water). Portions of 7 ml of this seed culture were inoculated into 14 K-1 flasks containing solid medium. The culture was incubated statically at 30 °C for 14 days. The whole fermentation culture (560 g) was extracted with acetone (560 ml, twice) and the acetone extract obtained by filtration. The solvent was concentrated *in vacuo*; water (300 ml) was added and extracted with an equivalent volume of *n*-BuOH. The organic layer was evaporated and partitioned by *n*-hexane/MeOH (10 ml each). The MeOH layer was concentrated to dryness to obtain a brown solid (1 g). The residue was suspended in a small amount of chloroform/MeOH (1:1) and applied to a silica gel column (50 g, Silica gel 60 Art 1.07734; Merck KGaA, Darmstadt, Germany) and washed with chloroform/MeOH/H_2O (v/v 3:1:0.15) and eluted with chloroform/MeOH/H_2O (v/v 10:5:1) to give 339 mg of light-brown solid. The solid containing the quadoctomycin was further purified by HPLC (600E System, Waters, Milford, MA, USA) using an ODS column (Shiseido, Capcell Pak UG120, 30 mm id × 250 mm; Tokyo, Japan) developed with 35% acetonitrile aqueous solution containing 0.01% trifluoroacetic acid to give 55 mg of quadoctomycin: white powder; $[\alpha]_D^{28}$ +15° (*c* 0.5, MeOH); IR ν_{max} (KBr) cm^{-1} 3406, 2965, 2934, 1780, 1677, 1458, 1434, 1385, 1092, 1070, 1049, 974; UV λ_{max} (MeOH) end absorption; ^{1}H NMR (pyridine-d_5, 600 MHz), ^{13}C NMR (pyridine-d_5, 150 MHz) data are given in Table 1; HRESI/MS $[M+H]^+$ *m/z* 1436.9961 (calcd. for $C_{76}H_{142}NO_{23}$, 1436.9967).

Methanolysis of quadoctomycin

Quadoctomycin (40 mg) was dissolved in 3 M hydrogen chloride-MeOH and stirred at 65 °C for 3 h. The solution was concentrated *in vacuo*. The residue was dissolved in a small amount of MeOH and applied to Sephadex LH-20 (GE Healthcare Bio-Sciences, Uppsala, Sweden). The fractions containing methyl glycoside were collected and dried. The residue was subjected to hydrophilic interaction liquid chromatography (XBridge Amide, 3.5 μm, 10 mm id × 150 mm, Waters, 90–50% acetonitrile, three times). The pure fraction containing methyl glycoside was collected and concentrated to dryness to give 1.6 mg of methyl-α-mannoside: colorless solid; $[\alpha]_D^{28}$ +71° (*c* 0.1, H_2O); ^{1}H NMR (D_2O, 500 MHz) δ 4.74 (1H, d, 1.7, H-1), 3.91 (1H, dd, 3.4, 1.7, H-2), 3.88 (1H, dd 12.3, 2.0, H-6b), 3.73 (1H, dd, 12.3, 5.7, H-6a), 3.73 (1H, m, H-3), 3.62 (1H, t 9.7, H-4), 3.59 (1H, m, H-5), 3.39 (3H, s, H-1OMe); ^{13}C NMR (D_2O, 125 MHz) δ 103.5 (d, C-1), 75.2 (d, C-5), 73.2 (d, C-3), 72.6 (d, C-2), 69.4 (d, C-4), 63.6 (t, C-6), 57.4 (q, C-1OMe); HRESI/MS $[M+Na]^+$ *m/z* 217.0685 (calcd for $C_7H_{14}O_6Na$, 217.0683).

Antimicrobial activity

Minimum inhibitory concentrations were determined by the standard agar dilution method per Clinical Laboratory Standards Institute guidelines.[15] Bacteria were incubated on Mueller-Hinton agar (Becton Dickinson) at 37 °C for 18 h, and yeast were incubated for 42 h.

DEDICATION

This article is dedicated to Professor Dr. Hamao Umezawa in honor of his profound contributions to basic science and the improvement of human health.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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