Notes

NEW ANTIBIOTIC PRODUCED BY *MICROMONOSPORA GLOBOSA*

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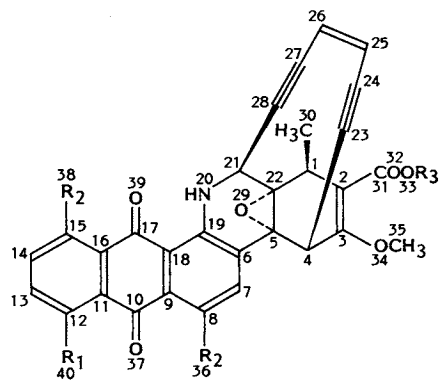
A new antibiotic, which we named deoxydynemicin A (**1**, Fig. 1), was produced together with dynemicin A[1] (**2**, Fig. 1) in the culture broth of a strain of actinomycetes, MG331-hF6, which was isolated from a soil sample collected on Mt. Minobu, Yamanashi Prefecture, Japan. The strain was classified as *Micromonospora globosa* MG331-hF6 by taxonomic studies and deposited in the Fermentation Research Institute of the Agency of Industrial Science and Technology, Japan, with the accession No. FERM P-10651. Antibiotic **1** inhibited the growth of bacteria very strongly. Here, we report the production, isolation, properties, structure, and biological activities of **1** and NMR studies of **2**.

The stock culture of the strain MG331-hF6 was cultured first in two 500-ml Erlenmeyer flasks each containing 100 ml of a medium consisting of glucose 1.0%, soybean flour (Prorich, Ajinomoto Co.) 2.0%, and dry yeast (Oriental Yeast Co.) 1.0%, at pH 7.0 before sterilization. Incubation was on a rotary shaker at 30°C for 6 days. Four ml of this culture broth was inoculated into each of 48-Erlenmeyer flasks of 500-ml capacity containing 100 ml of the same medium; the flasks were then shaken at 30°C for 4 days. Then 800 ml of the culture was inoculated into each of six jar fermenters containing 20 liters of a mixture consisting of 1 mM potassium iodide, silanized silica gel (Merck, Art. No. 7719) 1.0%, and Silicone oil (Shin-Etsu Chemical Co., KM-70) 0.025%, and fermented at 37°C for 66 hours with agitation at 100 rpm and aeration at the rate of 4 liters/minute. Inorganic or organic iodine compounds increased the production of **1** and **2**. In the presence of an iodine compound, nutrients were not needed for antibiotic production. Silanized silica gel was used to absorb **1** and **2**.

The cultured mycelia and silica gel were collected by filtration and extracted with 7 liters of MeOH. The MeOH extract was concentrated under reduced pressure and 4 liters of H_2O were added to the concentrate. The antibiotics were extracted at pH 7.0 with 4 liters of $CHCl_3$. The $CHCl_3$ extract, which contained **1** and **2**, was transferred into 4 liters of 25 mM sodium borate, pH 10.0, and after being adjusted to pH 8.0, this solution was extracted with 4 liters of $CHCl_3$. The $CHCl_3$ extract was concentrated under reduced pressure to give a blue oil (125 mg). The oil was mixed with a small amount of MeOH, and centrifuged, and the precipitate was dried to give a purple powder (34.0 mg). The powder was dissolved in a small amount of DMF, cooled to 5°C, and centrifuged, and the precipitate was collected. The supernatant was cooled to 5°C to obtain two further precipitates. The precipitates from three centrifugations were combined and dried to give a purple powder (6.5 mg). The powder was dissolved in a small volume of DMF, put on a Sephadex LH-20 column (70 ml), and developed

Fig. 1. Structures of **1**, **2**, **3**, and **4**.



1	R_1=H	R_2=OH	R_3=H
2	R_1=OH	R_2=OH	R_3=H
3	R_1=OCOCH$_3$	R_2=OCOCH$_3$	R_3=H
4	R_1=OCOCH$_3$	R_2=OCOCH$_3$	R_3=CH$_3$

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with DMF. The fractions that gave a blue band were collected. Antibiotics **1** and **2** were detected on silica gel TLC (Merck, Art. No. 5715) developed with $CHCl_3$-MeOH (10:1) and each fraction that showed a single spot on TLC was collected and dried under reduced pressure to give a total of 5.1 mg of purple powder. Then 3.2 mg of the powder was dissolved in 1.6 ml of DMF and successive one-tenth portions of the solution were put on a column for HPLC (Nucleosil $5C_{18}$, 4.6×250 mm, Macherey-Nagel Co.) and eluted with DMF-0.1% $(NH_4)_2CO_3$ aq soln (1:1) at a flow rate of 0.5 ml/minute. Antibiotics **1** and **2** eluted at 25 and 20 minutes, respectively. The antibiotics were concentrated separately under reduced pressure to yield reddish-purple powders (0.5 mg of **1** and 2.4 mg of **2**). The HPLC eluate of **1** was dissolved in a small volume of a DMF-$CHCl_3$ solution and cooled at 5°C. Reddish-brown crystals of **1** were obtained (0.2 mg).

Regarding solubility, antibiotic **1** was soluble in $CHCl_3$, DMF, and DMSO, and **2** was soluble in DMF and DMSO. The physico-chemical properties of the antibiotics are shown in Table 1. Treatment

Table 1. Physico-chemical properties of **1** and **2**.

	1	**2**
Molecular formula	$C_{30}H_{19}NO_8$	$C_{30}H_{19}NO_9$
FD-MS (M^+)	521	537
FAB-MS (*m/z*)		538 (MH^+), 537 (M^-)
UV λ_{max}^{MeOH} nm (log ε)	234 (4.10), 257 (sh, 4.01), 291 (sh, 3.73), 393 (sh, 3.27), 507 (sh, 3.54), 544 (3.65), 587 (sh, 3.55)	241 (4.29), 285 (sh, 3.73), 530 (sh, 3.73), 569 (3.92), 590~600 (3.91)
Solubility		
Soluble in:	DMSO, DMF, $CHCl_3$	DMSO, DMF
Insoluble in:	H_2O, hexane	H_2O, MeOH, $CHCl_3$, hexane

Table 2. ^1H NMR chemical shifts of **1**, **2**, **3**, and **4** (400 MHz).

Proton	Chemical shifts (δ) in ppm			
	1	**2**	**3**	**4**
1	3.81, q, 1H, J=7.4 Hz	3.56, q, 1H, J=7.0 Hz	3.69, q, 1H, J=7.2 Hz	3.68, q, 1H, J=7.5 Hz
4	4.41, s, 1H	4.85, s, 1H	4.91, s, 1H	4.20, s, 1H
7	7.52, s, 1H	8.01, s, 1H	7.90, s, 1H	7.43, s, 1H
12	7.85, dd, 1H, J=1.6, 8.0 Hz	—	—	—
13	7.65, d, 1H, J=8.0, 8.0 Hz	7.38, d, 1H, J=11.4 Hz	7.54, s, 2H	7.34, s, 2H
14	7.30, d, 1H, J=1.6, 8.0 Hz	7.33, d, 1H, J=11.4 Hz		
20	9.85, d, 1H, J=4.0 Hz	9.85, d, 1H, J=4.8 Hz	9.69, d, 1H, J=4.2 Hz	9.61, d, 1H, J=4.4 Hz
21	4.65, d, 1H, J=4.0 Hz	5.06, d, 1H, J=4.8 Hz	4.98, d, 1H, J=4.2 Hz	4.60, d, 1H, J=4.4 Hz
25	5.84, d, 1H, J=10.0 Hz	6.05, d, 1H, J=9.0 Hz	5.99, d, 1H, J=10.0 Hz	5.80, d, 1H, J=11.0 Hz
26	5.88, d, 1H, J=10.0 Hz	6.09, d, 1H, J=9.0 Hz	6.02, d, 1H, J=10.0 Hz	5.83, d, 1H, J=11.0 Hz
30	1.45, d, 3H, J=7.4 Hz	1.25, d, 3H, J=7.0 Hz	1.37, d, 3H, J=7.2 Hz	1.39, d, 3H, J=7.5 Hz
33				—
35	4.12, s, 3H	3.80, s, 3H	4.05, s, 3H	3.79, s, 3H
36	13.50, s, 1H	9.32, br s, 3H	—	—
38	12.88, s, 1H		—	—
40	—		—	—
33-CH_3	—	—	—	3.88, s, 3H
36-Acetyl	—	—	2.38, s, 3H	2.46, s, 3H
38-Acetyl	—	—	2.35, s, 3H	2.41, s, 3H
40-Acetyl	—	—	2.32, s, 3H	2.40, s, 3H
Solvent	$CDCl_3$	DMSO-d_6	Acetone-d_6	$CDCl_3$

of **2** in pyridine with acetic anhydride gave an *O*-triacetyl derivative (**3**). The molecular formula of **2** was deduced as $C_{30}H_{19}NO_9$ from the result of HRFAB-MS of **3**. Positive-ion HRFAB-MS of **3** showed a molecular ion at *m/z* 664.1448 ($(M+H)^+$, Calcd for $C_{36}H_{26}NO_{12}$, 664.1455) and negative-ion HRFAB-MS showed a molecular ion at *m/z* 663.1415 (M^-, Calcd for $C_{36}H_{25}NO_{12}$, 663.1376). The molecular formula of **1** was found from X-ray crystallography.

The methylation product of **3** by treatment with diazomethane gave a methyl ester of triacetyl derivative, **4**. Chemical shifts in the ^1H NMR spectra of **1**~**4** are shown in Table 2. Chemical shifts in the ^{13}C NMR spectra are shown in Table 3. Because of the lower solubility of **2**, further NMR studies of **3** were done. ^1H-^1H COSY, ^1H-^{13}C COSY, heteronuclear multiple-bond ^1H-^{13}C correlation spectroscopy (HMBC), NOESY, and differential NOE spectra of **3** were measured. The hydroxy proton signals at 9.32 ppm (36-OH, 38-OH, and 40-OH) in the ^1H NMR spectrum of **2** were absent in **3**, and these protons in **2** resonated at low field, so these signals were assignable to phenolic protons with hydrogen bonding. These results and the carbon chemical shifts of **2** and **3** from C-6 to C-19 suggested that part of **3** was an 8,9-substituted 1,4,6-hydroxy-5,10-anthraquinone. ^1H-^1H COSY and HMBC studies of **3** showed the partial structure labeled **3a** in Fig. 2. The methylation of **3** indicates that **3a** has a carboxylic group. The total number of oxygen atoms in **3** was 12, so an oxirane ring probably forms between C-5 and C-22 as shown in Fig. 2 (**3b**). Cross peaks between 4-H (4.91 ppm) and C-5 (64.2 ppm), 4-H and C-22 (72.7 ppm), 21-H (4.98 ppm) and C-5, 21-H and C-22, and 21-H and C-19 (145.5 ppm) were observed in the HMBC spectrum of **3**. These results suggest two possible structures, **3c** and **3d** (Fig. 2). In **3c**, there are bonds between C-4 and C-5 and between C-21 and C-22. In **3d**, there are bonds between C-4 and C-22 and between C-5 and C-21. In both cases, C-19 and N-20 are connected. In the ^1H-^1H COSY spectra, long-range coupling between 4-H (4.91 ppm) and 7-H (7.90 ppm) was observed, which suggested that the structure was likely to be **3c**. If the structure of **3d** were correct, cross peaks between 1-H and C-4, 4-H and C-1, and 21-H and C-6 would be observed in the HMBC spectrum, but such cross peaks were not found. The result of NOE between 4-H and 7-H and between 21-H (4.98 ppm) and 30-H (1.37 ppm) indicates that the structure, **3c** is reasonable. Therefore, the structures of **2** and **3** were as shown in Fig. 1, and **2** was identified as dynemicin A[1]. The stereochemistry of **2** was indicated by the relative configuration of **1**, because of similarities in the chemical shifts and coupling constants of the isoquinolyl units.

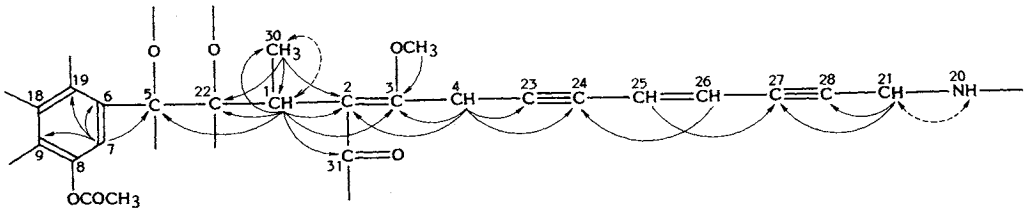
Table 3. ^{13}C NMR chemical shifts of **2** and **3** (100 MHz).

Carbon	Chemical shifts (δ) in ppm	
	2	**3**
1	35.8 d	36.7 d
2	(113.1) s	115.0 s
3	(156.2) s	156.3 s
4	30.8 d	32.4 d
5	63.3 s	64.2 s
6	(138.6) s	141.2 s
7	128.8 d	130.7 d
8	(157.2) s	(145.4) s
9	(114.6) s	126.0 s
10	(179.8) s	(181.8) s
11	(114.8) s	(127.3) s
12	(157.2) s	(148.6) s
13	(127.0) d	(131.5) d
14	(127.6) d	(131.8) d
15	(157.2) s	(148.1) s
16	(115.0) s	(127.5) s
17	(186.6) s	(184.4) s
18	(135.2) s	(131.3) s
19	(142.8) s	145.5 s
21	44.0 d	45.4 d
22	70.2 s	72.7 s
23	(99.3) s	99.0 s
24	(89.5) s	90.5 s
25	(124.4) d	125.4 d
26	(124.3) d	124.3 d
27	(88.8) s	90.7 s
28	(98.0) s	98.2 s
30	18.6 q	19.2 q
31	162.9 s	166.4 s
35	57.8 q	58.6 q
36-38-40 (acetyl-CH_3)		21.1, 20.9, 20.9 q
36-38-40 (acetyl-C=O)		169.8, 169.7, 169.6 q
Solvent	DMSO-d_6	Acetone-d_6

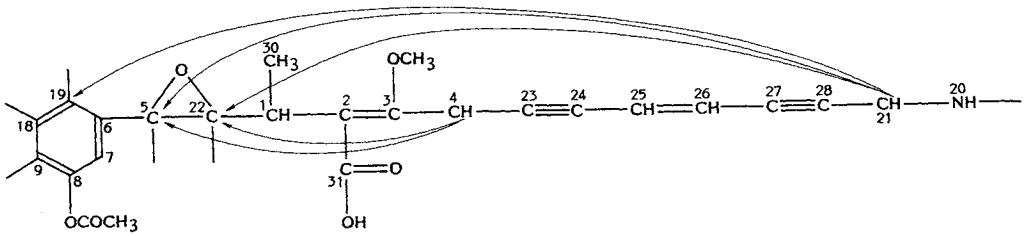
The chemical shifts in parentheses may be interchanged.

The structure of **1** was established by X-ray crystallography (Fig. 3). However due to the very small size of the crystals, the number of reflections measured was small and hydrogen atoms were not detected. Accordingly, the results did not show whether position 20 was N or O, whether position 29 was O or C, and whether position 32 was O or N. These atoms were identified by comparison of

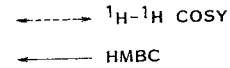
Fig. 2. Partial structures of **3**.

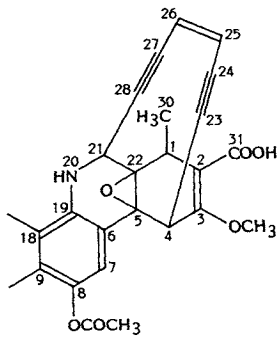


3a

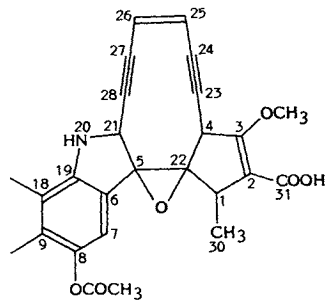


3b





3c



3d

Fig. 3. Molecular structure of **1**.

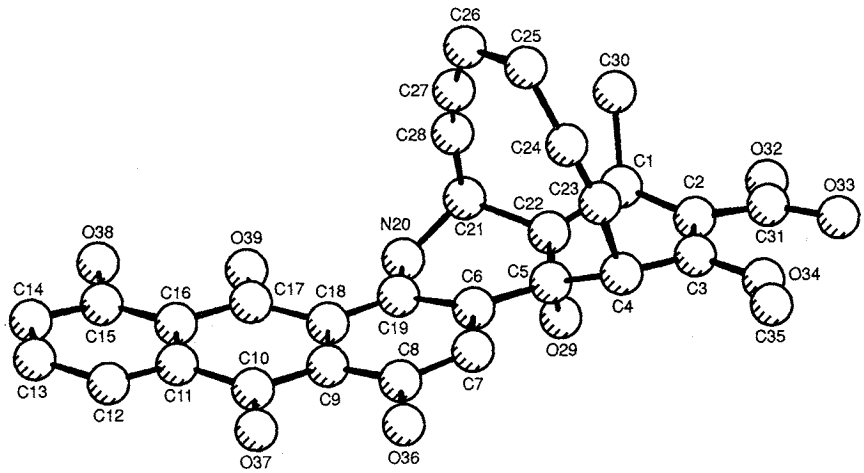


Table 4. Antimicrobial activities of **1** and **2**.

Test organisms	Medium*	MIC (μg/ml)	
		1	**2**
Staphylococcus aureus FDA 209P	a	<0.00006	<0.00006
S. aureus Smith	a	<0.00006	<0.00006
S. epidermidis 109	a	<0.00012	0.00006
Micrococcus luteus FDA 16	a	0.001	0.00012
M. luteus PCI 1001	a	0.002	0.00025
Bacillus anthracis	a	0.00025	0.00012
B. subtilis PCI 219	a	<0.00006	<0.00006
B. subtilis NRRL B-558	a	<0.00006	<0.00006
B. cereus ATCC 10702	a	0.002	0.0005
Corynebacterium bovis 1810	a	0.00025	<0.00006
Escherichia coli NIHJ	a	0.008	0.004
E. coli K-12	a	0.03	0.004
E. coli K-12 ML1629	a	0.03	0.015
Kleibsiella pneumoniae PCI 602	a	0.015	0.03
Shigella dysenteriae JS11910	a	0.008	0.008
Salmonella typhi T-63	a	0.03	0.03
Proteus rettgeri GN466	a	0.008	0.002
Serratia marcescens	a	0.03	0.015
Pseudomonas aeruginosa A3	a	0.015	0.004
Mycobacterium smegmatis ATCC 607	a	0.03	0.06
Aeromonas punctata IAM 1646	b	0.004	0.004
Vibrio anguillarum NCMB6	b	0.002	0.004
Pseudomonas fluorescens	b	0.008	0.008
Erwinia aroideae	b	0.015	0.015
Candida pseudotropicalis F-2	c	1.0	1.0
C. albicans 3147	c	>1.0	>1.0
Saccharomyces cerevisiae F-7	c	>1.0	>1.0
Cryptococcus neoformans F-10	c	>1.0	>1.0
Xanthomonas citri	c	0.001	0.004
X. oryzae	c	0.001	0.0005
Aspergillus niger F-16	c	>1.0	>1.0
Trichophyton mentagrophytes F-15 (833)	c	>1.0	>1.0

* a: Mueller-Hinton agar, 37°C; b: Mueller-Hinton agar, 27°C; c: nutrient agar + glucose 1%, 27°C.

^{1}H NMR spectra and structures with those of **2**. The structure of **1** was that of 12-deoxy **2**.

Recently, cyclic conjugated endiyne antibiotics, including esperamicin[2] and calicheamicins[3], have been reported. The structure of the neocarzinostatin chromophore[4] is similar to that of these compounds. Antibiotics **1** and **2** are similar in their endiyne unit, but other parts of the chromophore are quite different. The physico-chemical and biological properties of the antibiotics M-92 VA-2[5,6] and T-42318[7] resemble the properties of **2**. There are some differences in the UV spectra between these antibiotics and **1**. Their structures have not been determined, but they may be closely related to **2**.

The antimicrobial activities of **1** and **2** are shown in Table 4. Antibiotic **1** had potent antimicrobial activities like those of **2** and inhibited Gram-positive bacteria, in particular, at extremely low doses.

Acknowledgment

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