ACTINOPLANONES A AND B, NEW CYTOTOXIC POLYCYCLIC XANTHONES FROM *ACTINOPLANES* SP.

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(Received for publication September 22, 1987)

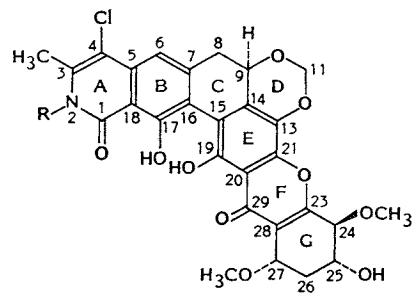
Two new cytotoxic polycyclic xanthones, actinoplanones A (**1**) and B (**2**) were isolated from the culture broth of *Actinoplanes* sp. R-304 by monitoring their bioactivity against HeLa cells. Compound **1** was extremely cytotoxic (IC_{50} 0.00004 μg/ml) against HeLa cells. The structures of **1** and **2** were established mainly by analyses of 2D heteronuclear correlation NMR experiments. The absolute configurations of the asymmetric carbons of the compounds have been assigned to be 9*R*, 24*S*, 25*R* and 27*S* by circular dichroism spectra and NMR analysis using chiral derivatives (esters of α-methoxy-α-(trifluoromethyl)acetic acid).

Our efforts to identify antitumor antibiotics from microorganisms[1] have resulted in the isolation of two potent cytotoxic compounds against HeLa cells from the culture broth of *Actinoplanes* sp. R-304 (Actinoplanaceae). Since the structure determination study of the compounds revealed that they were new polycyclic xanthones, we named them actinoplanones A (**1**) and B (**2**). Actinoplanone A (**1**) was found to be a potent cytotoxin from *in vitro* assay with HeLa cells. This paper deals with the fermentation, isolation and structure determination, including the absolute configurations, of **1** and **2**.

Actinoplanones A (**1**) and B (**2**) were produced in the culture broth of *Actinoplanes* sp. R-304 which was isolated from a forest soil sample collected in Okinawa Prefecture, Japan. The actinoplanones were isolated from the ethyl acetate extract of the broth through a combination of silica gel column chromatography and reversed-phase HPLC.

Actinoplanone A (**1**), mp 276~278°C, $[\alpha]_D^{25}$ −619.8° ($CHCl_3$), showed a 3:1 chlorine-containing ion pair at *m/z* 584 and 586 in the electron impact mass spectrum (EI-MS). A molecular formula of $C_{28}H_{25}N_2O_{10}Cl$ was given to **1** on the basis of the high-resolution fast atom bombardment mass spectral (HRFAB-MS) data.

Fig. 1. Structure of actinoplanones A (**1**) and B (**2**).



1 R = NH_2
2 R = H

Table 1. ^{13}C NMR spectral data[a] of actinoplanones A (**1**) and B (**2**).

Position	1	2	Position	1	2
C-1	163.30	165.86	C-17	157.38	157.95
C-3	138.57	135.10	C-18	108.96	109.51
3-CH_3	16.51	17.43	C-19	150.62	150.76
C-4	110.57	111.06	C-20	110.80[b]	110.59[c]
C-5	134.16	136.07	C-21	143.85	143.69
C-6	112.13	111.50	C-23	162.82	162.25
C-7	141.53	141.99	C-24	78.31	78.32
C-8	36.92	37.06	24-OCH_3	59.87	59.71
C-9	72.60	72.05	C-25	68.08	68.00
C-11	91.01	90.63	C-26	26.86	26.81
C-13	130.70	130.23	C-27	69.62	69.67
C-14	130.04	129.95	27-OCH_3	58.32	58.55
C-15	111.85[b]	111.39[c]	C-28	117.23	117.43
C-16	115.23	115.03	C-29	181.92	181.69

[a] Chemical shifts (ppm) are given relative to $CDCl_3$ signal as internal reference (77.00 ppm) at 100 MHz.
[b,c] Assignments bearing the same superscript could be reversed.

The IR spectrum indicated the existence of hydroxyl (3430 cm^{-1}), conjugated ketones (1642 and 1630 cm^{-1}) and γ-pyrone (1610 and 1572 cm^{-1}) functionalities. The UV spectrum showed absorption maxima at 229, 253, 306, 366 and 382 nm, the characteristic absorption maxima of the polycyclic xanthone skeleton, which had been observed previously in antibiotics such as albofungin (240, 255, 305 and 375 nm)[2] and chloroalbofungin (233, 254, 305, 371 and 384 nm)[2]. However, no identity was observed in the physico-chemical properties between **1** and the known polycyclic xanthone antibiotics.

Analyses of the ^{13}C NMR (Table 1) and ^{13}C distortionless enhancement by polarization transfer (DEPT) spectra of **1** revealed the presence of a methyl (δ 16.51), two methylene (δ 26.86 and 36.92), two methoxy (δ 58.32 and 59.87), four oxymethine (δ 68.08, 69.62, 72.60 and 78.31), a methylene (δ 91.01) adjacent to two oxygen atoms, and an olefinic methine (δ 112.13) carbons. In addition, **1** contains eleven quaternary sp^2 carbons (δ 108~142), four quaternary sp^2 oxycarbons and an amide carbon (δ 143~164), and a doubly α,β-unsaturated carbonyl carbon (δ 181.92).

The ^1H NMR spectrum of **1** (Table 2) indicated the existence of five D_2O exchangeable protons, which were identified as a hydroxyl (δ 4.25), a primary amino (δ 4.97) and two hydrogen-bonded OH protons (δ 12.92 and 13.48). The proton signals at δ 2.72 (3H), 3.59 (3H), 3.71 (3H) and 7.30 (1H) in the spectrum indicated the presence of a CH_3 and two OCH_3 groups and an aromatic proton, respectively. These spectral data suggested **1** to be a new polycyclic xanthone which was named actinoplanone A.

Since limited data were available for the ^1H-^1H connectivity for the skeletal protons of **1** by ^1H-^1H 2D homonuclear shift correlation spectrum (^1H-^1H COSY), long-range ^{13}C-^1H COSY (LR HETCOSY)[3] and long-range selective proton decoupling (LSPD)[4] experiments were carried out for confirming the carbon framework of **1**. Table 3 shows the observed ^{13}C-^1H long-range couplings in the LR HETCOSY experiments. Partial structures **I** and **II** (Fig. 2) were suggested by the data in Table 3.

Partial Structure I (A~D rings)

Analysis of the ^1H-^1H COSY spectrum of **1** revealed the connectivity from 6-H to 9-H *via* C-7, in which 6-H (δ 7.30), showing long-range coupling with 8-H_{ax}, was assignable to an aromatic proton.

Table 2. ^{1}H NMR spectral data[a] of actinoplanones A (1) and B (2).

Proton	1		2	
	δ[b]	Splitting	δ[b]	Splitting
2-H			11.87	s
2-NH_2	4.97	s		
3-CH_3	2.72	s	2.32	s
6-H	7.30	s	6.97	s
8-H_{ax}	2.97	dd (J=13.9, 12.8)[c]	2.87	dd (J=13.4, 12.7)
8-H_{eq}	3.32	dd (J=13.9, 4.7)	3.25	dd (J=13.4, 4.0)
9-H	4.91	dd (J=12.8, 4.7)	5.18	dd (J=12.7, 4.0)
11-H_{ax}	5.35	d (J=5.8)	5.34	d (J=5.7)
11-H_{eq}	5.59	d (J=5.8)	5.40	d (J=5.7)
17-OH	13.48	s	13.53	s
19-OH	12.92	s	12.85	s
24-H	4.19	d (J=2.1)	4.18	d (J=2.0)
24-OCH_3	3.71	s	3.62	s
25-H	4.31	dddd (J=8.6, 2.1, 2.0, 2.0)	4.33	br d (J=8.3)
25-OH	4.25	d (J=8.6)	4.39	d (J=8.3)
26-H_{ax}	2.00	ddd (J=15.0, 3.4, 2.0)	1.98	br d (J=13.7)
26-H_{eq}	2.43	ddd (J=15.0, 3.0, 2.0)	2.43	br d (J=13.7)
27-H	4.77	dd (J=3.4, 3.0)	4.68	br s
27-OCH_3	3.59	s	3.59	s

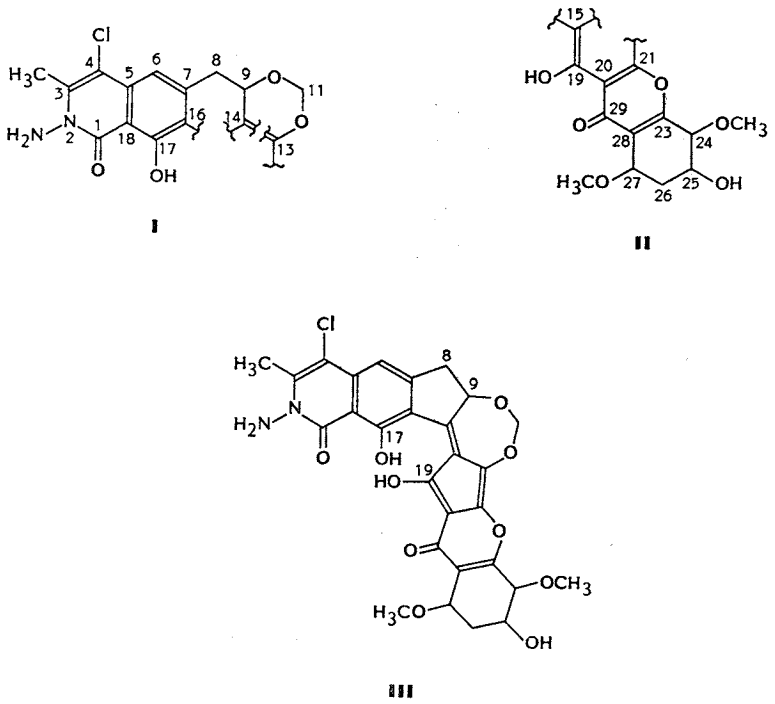
[a] Spectra were recorded at 400 MHz.
[b] Chemical shifts (ppm) are given relative to TMS signal.
[c] Coupling constants in parentheses are in Hz.

Table 3. Long-range couplings observed in long-range ^{13}C-^{1}H COSY (LR HETCOSY) spectrum of actinoplanone A (1) (400 MHz).

Proton	δ	Correlated carbon		
		Two-bond coupling	Three-bond coupling	Four-bond coupling
2-NH_2	4.97		C-1, C-3	
3-CH_3	2.72	C-3	C-4	C-5
6-H	7.30		C-4, C-8, C-16, C-18	C-1
8-H_{ax}	2.97	C-7, C-9	C-14	
8-H_{eq}	3.32	C-7	C-6, C-14, C-16	
11-H_{eq}	5.59		C-9, C-13	
17-OH	13.48	C-17	C-16, C-18	
19-OH	12.92	C-19	C-15, C-20	
24-H	4.19	C-23, C-25	24-OCH_3, C-26, C-28	
24-OCH_3	3.71		C-24	
25-H	4.31	C-24	C-23	
25-OH	4.25	C-25		
26-H_{eq}	2.43	C-27	C-28	
27-H	4.77	C-28	C-23, C-25, 27-OCH_3	
27-OCH_3	3.59		C-27	

In the LR HETCOSY (Table 3), 6-H showed three-bond couplings with C-8 (δ 36.92), C-4, C-16 and C-18, and the OH proton at δ 13.48 with C-16, C-17 and C-18. These proton-carbon connectivities indicated the location of the OH on C-17, a carbon in the aromatic B ring. Further connectivities of the methylene (8-H_{ax} and 8-H_{eq}) and the dioxymethylene (11-H_{eq}) protons with carbons (see Table 3) allowed the structure to be expanded from the B to the D ring. The correlation of 11-

Fig. 2. Partial structures I and II, and a plausible structure III of actinoplanone A (1).



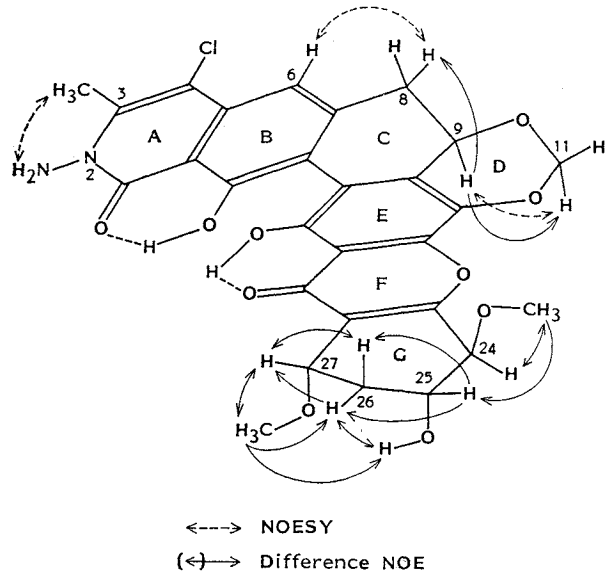
H_{eq} with C-9 was also shown by a long-range coupling ($^3J_{CH}$=3.4 Hz) observed in the LSPD experiment. Nuclear Overhauser effects (NOE's) observed in the 2D homonuclear NOE (NOESY) and the difference NOE experiments (Fig. 3) of **1** provided confirmatory evidence for the above atomic relation on the B~D rings.

Long-range couplings were observed between the CH_3 protons and two quaternary sp^2 carbons (C-3 and C-4), and between the NH_2 protons and C-3, as well as the amide carbonyl carbon at δ 163.30. These couplings suggested the existence of a six-membered A ring in which the carbonyl carbon was C-1 and this carbon was judged to connect with C-18 from the observation of W-coupling between C-1 and 6-H *via* C-18 and C-5 in the LR HETCOSY. Therefore, the location of the NH_2 group was reasonable on the amide nitrogen (N-2), since the NH_2 protons showed long-range couplings with C-1 and C-3 as mentioned above.

On the other hand, an NOE observed between the NH_2 and the CH_3 protons (Fig. 3) indicated a vicinal substitution of the two groups, which led to locate the CH_3 group on C-3. Based on the substitution pattern noted above, a quaternary sp^2 carbon at δ 134.16 was assigned to C-5 because the cross peak due to W-coupling between this quaternary carbon and the CH_3 protons was observed in the LR HETCOSY. The chlorine atom was, hence, assigned on the remaining quaternary sp^2 carbon, C-4, of the A ring. This substitution pattern of the A ring is same as that of chloroalbofungin[5].

The splittings of 8-H_{ax}, 8-H_{eq} and 9-H (Table 2) indicated an axial configuration of 9-H, because 9-H exhibited axial-axial (J=12.8 Hz) and axial-equatorial (J=4.7 Hz) couplings with 8-H_2. The NOE experiment confirmed the stereochemistry of C-9 (Fig. 3). Thus, the partial structure I for the A~D rings of **1** was determined.

Fig. 3. Observed NOE's in actinoplanone A (1) in the NOESY and difference NOE experiments.



Partial Structure II (E~G rings)

The ^{1}H-^{1}H COSY spectrum of **1** indicated the connectivity from 24-H to 27-H. On the other hand, the ^{13}C-^{1}H COSY spectrum indicated that 24-H, 25-H and 27-H were located on oxymethine carbons. In the LR HETCOSY of **1** (Table 3), 24-H signal (δ 4.19) showed cross peaks with C-23 (quaternary carbon), C-25, C-26, C-28 and a OCH_3 carbon (δ 59.87). The OCH_3 proton signal at δ 3.71 showed a cross peak with C-24. Therefore, the OCH_3 group was located on C-24. The presence of a OH group on C-25 was shown by the long-range coupling of the proton with C-25 and also by the coupling of the proton with 25-H (δ 4.31) ($J_{25\text{-}OH,25\text{-}H}$=8.6 Hz). The location of a second OCH_3 group was determined to be at C-27 from the cross peaks observed for 27-H (δ 4.77) and the OCH_3 proton signals (see Table 3). Furthermore, 25-H showed long-range couplings with C-23 and C-24. These NMR evidence provided the structure of the G ring.

In the LSPD experiment, the doubly α,β-unsaturated carbonyl carbon (δ 181.92) displayed a long-range coupling ($^{3}J_{CH}$=2.4 Hz) with 27-H. However, the carbon signal showed no observable simplification, when 24-H was irradiated, indicating unreasonable linkage of the carbonyl carbon to C-23. Thus, only C-29 was assignable to the position for the carbonyl carbon.

The OH proton at δ 12.92 showed long-range couplings with C-15, C-19 and C-20. These proton-carbon correlations and the low ^{13}C chemical shift of C-19 (δ 150.62) among the quaternary sp^2 carbons implied that the OH group was on C-19. The very low ^{1}H chemical shift of the OH proton indicated the existence of a hydrogen bond between the proton and C-29 carbonyl oxygen. This hydrogen bonding indicated that the γ-pyrone ring should be situated between the E and G rings as the F ring, in this way the partial structure **II** was established.

The stereochemistry of the G ring was determined by analysis of the coupling constants (Table 2) of the ring protons. Both 25-H and 27-H were assigned to equatorial configurations, because of their equatorial-axial couplings with 26-H_{ax} (J=2.0 and 3.4 Hz, respectively) and equatorial-equatorial couplings with 26-H_{eq} (J=2.0 and 3.0 Hz, respectively). The equatorial configuration of 24-H was indicated

by a cross peak between this proton and 26-H_{eq} due to W-coupling in the ^1H-^1H COSY spectrum in which the cross peak caused by W-coupling between 25-H_{eq} and 27-H_{eq} was also observed. The stereochemistry of the G ring was supported by NOE experiments (Fig. 3). The relative configurations of the asymmetric carbons in the G ring were consequently assigned to be 24*S**, 25*R** and 27*S**.

Connection of the Partial Structures I and II

Possibilities for connection of the partial structures **I** and **II** were examined by considering the quaternary sp^2 character of the five carbons (C-13 to -16 and C-21). As a result, two structures **1** and **III** (Fig. 2) remained for consideration.

Inspection of the Dreiding model of structure **III** gave two possible conformers around 8-H-C-C-9-H. One conformer showed dihedral angles 8-H_{ax}-C-C-9-H=155° and 8-H_{eq}-C-C-9-H=30°, while the other one showed angles of approximately 140° and 10°. However, the observed *J* values between 8-H_2 and 9-H protons ($J_{8ax,9}$=12.8 and $J_{8eq,9}$=4.7 Hz) of **1** were unexplainable for either of the conformers. Furthermore, inspection of the Dreiding model of **III** demonstrated that the two oxygen atoms on C-17 and C-19 were spatially close (about 1.2 Å distance between the oxygen atoms), suggesting the probable existence of strong van der Waals repulsion between the atoms. Therefore, structure **III** is unacceptable. Only the structure **1** was consistent with all the spectral data and was assigned to actinoplanone A (**1**).

Structure of Actinoplanone B (**2**)

The second active component (**2**), mp 240~243°C, $[\alpha]_D^{25}$ −649.4° ($CHCl_3$), showed a striking resemblance to actinoplanone A (**1**) in its UV, IR ^1H and ^{13}C NMR spectra (see Tables 1 and 2, and Experimental section). The presence of a chlorine atom in **2** was shown by the EI-MS, and the molecular formula, $C_{28}H_{24}NO_{10}Cl$, was assigned to **2** by HRFAB-MS, which suggested the replacement of the NH_2 group of **1** by a hydrogen atom. In the ^1H NMR spectrum of **2** (Table 2), the NH_2 proton signal was not observed, while a D_2O exchangeable amide proton signal appeared at δ 11.87. Since **2** appeared to be a deamino derivative of **1**, it was named actinoplanone B.

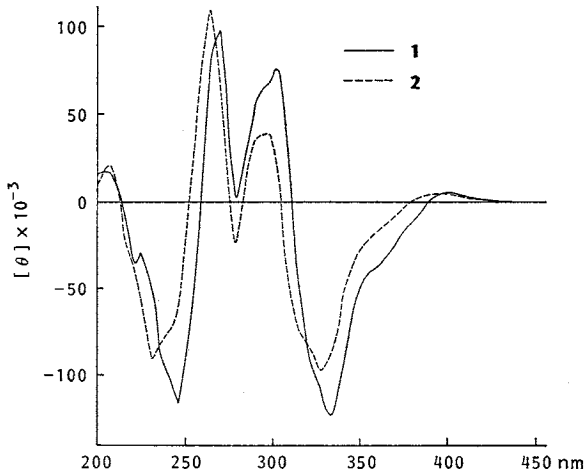
The structure of **2** was confirmed by cross peaks in its LR HETCOSY. Observation of the cross peak between the amide proton and C-4 permitted the location of the proton to be at N-2.

The ^1H NMR of **2** in $CDCl_3$ (Table 2) showed unclear coupling patterns for the protons on the G ring, which were clarified by D_2O treatment. The coupling constants for the protons on the C, D and G rings of **2** were essentially identical with those of **1** (Table 2), and W-couplings were observed between 24-H and 26-H_{eq}, and between 25-H and 27-H. Through the mutual irradiation of 24-H and 26-H_{eq}, the broad doublet of 26-H_{eq} was changed to a doublet of doublet of doublets (*J*=14.8, 3.0 and 2.3 Hz), while the doublet ($W_{1/2}$=5.05 Hz) of 24-H was sharpened to a doublet with $W_{1/2}$= 4.25 Hz. In addition, this decoupling experiment verified the equatorial configurations of 25-H (ddd, *J*=3.0, 2.5 and 2.4 Hz) and 27-H (dd, *J*=3.3 and 2.3 Hz). Thus, the asymmetric carbons (C-9, C-24, C-25 and C-27) of **2** possessed the same relative configurations as the carbons of **1**.

Absolute Configurations of **1** and **2**

The absolute configuration of the C-9 of the actinoplanones was determined from their CD curves. The CD spectra of **1** and **2** (Fig. 4) showed similar curves to that of albofungin[6], and exhibited a negative Cotton effect in the 235~245 nm region and a positive Cotton effect in the 205~210 nm region. These Cotton effects imply an *R* configuration for C-9, as reported in albofungin[6].

Fig. 4. CD curves of actinoplanones A (**1**) and B (**2**).



For reliable biological assay results, a large amount of **1** had been consumed. In order to determine the absolute stereochemistry of the G ring, the MTPA method[7] was, therefore, applied to **2**. The determination of the same relative configuration of the G ring for **1** and **2** was described above.

Table 4. ^{1}H NMR chemical shifts for the selected proton signals of (*R*)-(+)-MTPA diester (**3**) and (*S*)-(−)-MTPA diester (**4**) of actinoplanone B (**2**).

	3	**4**	$\Delta\delta=\delta_3-\delta_4$
24-H	4.25	4.13	+0.12
26-H_{ax}	2.10	2.20	−0.10
26-H_{eq}	2.50	2.63	−0.13

Spectra were recorded at 500 MHz.
Chemical shifts (ppm) are given relative to TMS signal.

Actinoplanone B (**2**) was converted to the corresponding (*R*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetic acid ((*R*)-(+)-MTPA) diester (**3**) and (*S*)-(−)-MTPA diester (**4**) according to MOSHER's method[8]. Chemical shifts of 24-H and 26-H_2 of **3** and **4** in the ^{1}H NMR spectra are given in Table 4. The differences of the chemical shift values between **3** and **4** ($\Delta\delta=\delta_3-\delta_4$) were calculated to be positive (+0.12 ppm) for 24-H, while $\Delta\delta$ values for 26-H_2 were negative (−0.10 and −0.13 ppm). Therefore, an *R*-configuration was assigned to C-25 by the general rule of the MTPA method[7]. The absolute configurations of both C-24 and C-27 were accordingly assigned to *S* configuration. From the NMR and CD evidence, the absolute configurations of 9*R*, 24*S*, 25*R* and 27*S* were determined for **2**, and accordingly for **1**.

The polycyclic xanthone antibiotics, albofungin[5], chloroalbofungin[5], lysolipin I[9], cervinomycins (A_1 and A_2)[10] and LL-D42067 (α and β)[11], have been found to possess antibacterial, antifungal, antimycoplasmal and anti-protozoal activities. Among them, albofungin (P-42-1) possessed cytotoxicity against HeLa cells at concentrations of 0.005 to 0.01 μg/ml, and also exhibited antitumor activity against Ehrlich ascites tumor in mice[12].

In vitro assay of actinoplanones A (**1**) and B (**2**) indicated much stronger cytotoxicities against HeLa cells (0.00004 and 0.005 μg/ml in IC_{50}, respectively) as compared with albofungin. *In vivo* antitumor assay results of **1** and **2** will be reported elsewhere.

Experimental

General Methods

MP was measured with a Yanagimoto micro melting apparatus, and were uncorrected. Optical rotations were measured in $CHCl_3$ at 25°C with a Jasco DIP-360 digital polarimeter. UV and CD spectra were recorded in EtOH at 25°C with a Cary 17 spectrometer and with a Jasco J-40A instrument, respectively. IR spectra were run in KBr on a Perkin-Elmer 1730 fourier transformation (FT)-IR spectrometer. ^1H and ^{13}C NMR spectra were taken in $CDCl_3$ on a Jeol JNM-GX400 or on a Bruker AM-500 spectrometer. Mass spectra were obtained with a Jeol JMS-HX100 spectrometer, and HRFAB-MS with a Shimadzu GC-MS 9020-DF(FAB) spectrometer. TLC and preparative TLC were carried out on Pre-coated TLC plates (Silica gel 60 F_{254}, Merck) eluting with a solvent mixture of 4% MeOH in $CHCl_3$.

Microorganism

Actinoplanes sp. R-304 was isolated from a forest soil sample collected in Okinawa Prefecture, Japan, and has been deposited in the Fermentation Research Institute, Agency of Industrial Science and Technology, Japan, under the name *Actinoplanes* sp. R-304 with the accession No. FERM P-9132. Morphological, cultural and physiological characteristics of this strain will be described in detail elsewhere.

Fermentation

Actinoplanes sp. R-304 on an agar slant was inoculated on a seed medium (40 ml) in a 200-ml Erlenmeyer flask. The seed medium (pH 7.0) was composed of maltose syrup 4%, soybean oil 0.3%, soybean meal 2%, Fermamedia 1%, Sungrowth L (Sungrowth Co., Osaka) 0.5%, $CaCO_3$ 0.3%, $FeSO_4 \cdot 7H_2O$ 0.001%, $CoCl_2 \cdot 6H_2O$ 0.0001% and $NiCl_2 \cdot 6H_2O$ 0.0001%. The flasks were incubated on a rotary shaker (210 rpm) for 4 days at 26°C. Two hundred ml of the seed culture was transferred into 15 liters of the medium described above in a 30-liter jar fermentor. The fermentation was carried out for 6 days at 26°C with aeration of 10 liters/minute and with agitation of 210 rpm.

Biological Assay

The method for cytotoxicity against HeLa cells was essentially according to that of MIRABELLI *et al.*[13].

Isolation of Actinoplanones

The isolation procedures were monitored by cytotoxicity against HeLa cells. The filtered culture broth (27 liters) was adjusted to pH 2.0 with dil HCl, and extracted three times with EtOAc (3 liters). The extracts were dried over $MgSO_4$, filtered and concentrated *in vacuo* to give a brown paste (10.31 g), which was dissolved in EtOAc (500 ml). After three partition between saturated $NaHCO_3$ (200 ml), the EtOAc extract was dried over $MgSO_4$, filtered and concentrated to yield a brown paste (1.44 g).

The brown paste was applied to a Silica gel column (Merck, Kieselgel 60, 70 g) and eluted with a mixture of $CHCl_3$ and MeOH containing 0.5% AcOH, in which the concentration of MeOH in $CHCl_3$ was successively increased in the following mixing ratios; 99.5:0.5, 99:1, 98:2, 96:4 and 90:10 (300 ml each). One hundred fractions of 15 ml each were collected. Cytotoxic activity was observed in fractions 27~45 which contained **1** (Rf 0.40) as the major component on TLC. Activity was also observed in fractions 46~62, in which **2** (Rf 0.35) was the major component. The former fractions were concentrated to afford an oily residue (382 mg) which was purified by reversed-phase HPLC (column: 10.7×250 mm packed with Unisil Q C8 (5 μm) (Gasukuro Kogyo Co., Tokyo); eluent: 55% MeCN; flow rate: 5 ml/minute; detection: RI detector). A solid component (100 mg) corresponding to a retention time of 9.8 minutes was found to be active. The solid was finally purified by preparative TLC (20×20 cm, 1 mm thickness, 3 plates) to yield a yellowish solid (95 mg) which was recrystallized from Me_2CO to give actinoplanone A (**1**) (81 mg) as yellow needles.

The brown oily residue (393 mg) obtained from active fractions 46~62 was purified by HPLC (the same conditions as performed for **1** except that 50% MeCN was used as eluent). The concentration of active component corresponding to a retention time of 12.8 minutes gave a yellowish solid (73 mg). Final purification of the solid by preparative TLC (the same conditions as for **1**) gave actino-

planone B (**2**) (62 mg) as a yellowish amorphous solid.

Actinoplanone A (**1**): MP 276~278°C; $[\alpha]_D^{25}$ −619.8° (*c* 0.29); CD, see Fig. 4; UV λ_{max} nm (log ε) 229 (4.49), 253 (4.52), 306 (4.13), 366 (4.36), 382 (4.40); IR ν_{max} cm^{-1} 3430, 1642, 1630, 1610, 1572, 1100; ^1H NMR, see Table 2; ^{13}C NMR, see Table 1; EI-MS *m/z* 584, 586 (relative intensity 3:1); HRFAB-MS *m/z* 585.1342 (calcd for $C_{28}H_{26}N_2O_{10}{}^{35}Cl$, mmu error +6.8), 587.1222 (calcd for $C_{28}H_{26}N_2O_{10}{}^{37}Cl$, mmu error −2.3).

Actinoplanone B (**2**): MP 240~243°C; $[\alpha]_D^{25}$ −649.4° (*c* 1.54); CD, see Fig. 4; UV λ_{max} nm (log ε) 226 (4.40), 252 (4.46), 303 (4.12), 336 (sh), 363 (4.25), 377 (4.29); IR ν_{max} cm^{-1} 3450, 1654, 1633, 1610, 1580, 1089; ^1H NMR, see Table 2; ^{13}C NMR, see Table 1; EI-MS *m/z* 569, 571 (relative intensity 3:1); HRFAB-MS *m/z* 570.1091 (calcd for $C_{28}H_{25}NO_{10}{}^{35}Cl$, mmu error −7.4).

Esterification of Actinoplanone B (**2**) with MTPA-Cl

Compound **2** (2.3 mg) was added to a mixture of undistilled MTPA-Cl (prepared with 55 mg of (*R*)-(+)- or (*S*)-(−)-MTPA and excess thionyl chloride by refluxing for 5 hours and then removing the excess thionyl chloride *in vacuo*) dissolved in CCl_4 (80 μl) and pyridine (80 μl). The mixture was stirred for 12 hours at room temp and the solvent was evaporated. The residue was purified by reversed-phase HPLC (column: 4.6×150 mm packed with Unisil Q C8 (5 μm); eluent: 75% MeCN; flow rate: 1 ml/minute; detection: UV at 240 nm) to give the 17, 25-di-MTPA ester (1.2 mg) of **2** (retention time: 4.4 minutes).

17,25-Di-(*R*)-(+)-MTPA Ester (**3**) of Actinoplanone B (**2**): ^1H NMR δ 2.10 (1H, ddd, *J*=15.5, 4.7 and 3.2 Hz, 26-H_{ax}), 2.42 (3H, s, 3-CH_3), 2.50 (1H, ddd, *J*=15.5, 2.6 and 1.9 Hz, 26-H_{eq}), 2.83 (1H, dd, *J*=14.0 and 12.9 Hz, 8-H_{ax}), 3.29 (1H, dd, *J*=14.0 and 4.6 Hz, 8-H_{eq}), 3.35, 3.57, 3.70, 4.18 (each s, OCH_3×4), 4.25 (1H, d, *J*=2.6 Hz, 24-H), 4.41 (1H, dd, *J*=4.7 and 1.9 Hz, 27-H), 4.82 (1H, dd, *J*=12.9 and 4.6 Hz, 9-H), 5.29 (1H, d, *J*=5.8 Hz, 11-H_{ax}), 5.47 (1H, ddd, *J*=3.2, 2.6 and 2.6 Hz, 25-H), 5.55 (1H, d, *J*=5.8 Hz, 11-H_{eq}), 6.82, 7.16, 7.41, 7.59 (10H, aromatic protons of MTPA moiety), 7.77 (1H, s, 6-H), 8.43 (1H, br s, 2-H), 12.40 (1H, s, 17-OH); FAB-MS *m/z* 1,002 $(M+H)^+$.

17,25-Di-(*S*)-(−)-MTPA Ester (**4**) of Actinoplanone B (**2**): ^1H NMR δ 2.20 (1H, ddd, *J*=15.1, 4.8 and 3.1 Hz, 26-H_{ax}), 2.33 (3H, s, 3-CH_3), 2.63 (1H, ddd, *J*=15.1, 3.1 and 1.9 Hz, 26-H_{eq}), 2.90 (1H, dd, *J*=14.0 and 13.3 Hz, 8-H_{ax}), 3.33 (1H, dd, *J*=14.0 and 4.7 Hz, 8-H_{eq}), 3.47, 3.51, 3.56, 3.63 (each s, OCH_3×4), 4.13 (1H, d, *J*=3.1 Hz, 24-H), 4.60 (1H, dd, *J*=4.8 and 1.9 Hz, 27-H), 4.91 (1H, dd, *J*=13.3 and 4.7 Hz, 9-H), 5.32 (1H, d, *J*=5.9 Hz, 11-H_{ax}), 5.37 (1H, ddd, *J*=3.1, 3.1 and 3.1 Hz, 25-H), 5.54 (1H, d, *J*=5.9 Hz, 11-H_{eq}), 7.20, 7.36, 7.55, 7.58 (10H, aromatic protons of MTPA moiety), 7.79 (1H, s, 6-H), 8.95 (1H, br s, 2-H), 12.67 (1H, s, 17-OH); FAB-MS *m/z* 1,002 $(M+H)^+$.

Acknowledgments

We are indebted to Dr. SEIJI TAKAHASHI, Shimadzu Seisakusho Company, for measuring the HRFAB-MS.

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