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Tubelactomicin A, a Novel 16-Membered Lactone Antibiotic, from *Nocardia* sp.

II. Structure Elucidation†

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A novel 16-membered lactone antibiotic named tubelactomicin A was isolated from the culture broth of *Nocardia* sp. MK703-102F1.

The structure of tubelactomicin A was assigned by spectroscopic analysis and the absolute configuration was determined by X-ray crystallographic analysis.

Tubelactomicin A (**1**, Fig. 1) is a novel 16-membered macrolide antibiotic produced by *Nocardia* sp. MK703-102F1. It showed strong and specific antibacterial activity against acid-fast bacteria. The taxonomy of the producing strain and fermentation, isolation and biological activities of **1** are reported in the preceding paper[1].

In this paper, we report the physico-chemical properties and structural elucidation of **1**.

Results

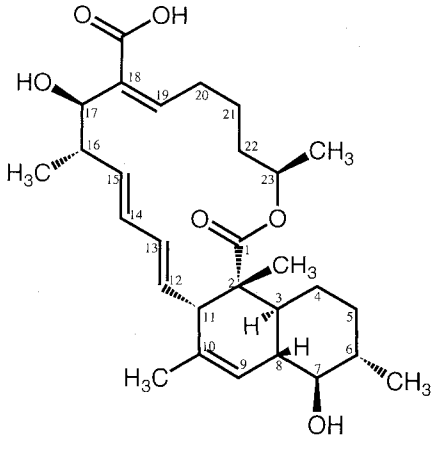
Structure Determination

The molecular formula of **1** was established as $C_{29}H_{42}O_6$ on the basis of HRFAB-MS and NMR analyses. The UV spectrum of **1** showed strong absorptions at 233 (ε 28,500) and 238 nm (ε 28,600) in MeOH due to a conjugated olefine system. IR absorption bands at 1718 and 1700 cm^{-1} implied the presence of a lactone and α,β-unsaturated carbonyl groups in the molecule. The physico-chemical properties of **1** are summarized in Table 1.

The ^{13}C NMR, DEPT and HMQC spectra of **1** showed the presence of 29 carbon signals; five methyls, five methylenes, five methines, three oxygen bearing methines, six olefinic methines, one sp^3 quaternary carbon, two olefinic quaternary carbons and two carbonyl carbons. The ^1H and ^{13}C NMR spectral data of **1** are shown in Table 2.

The ^1H-^1H COSY spectrum demonstrated that **1** had four partial structures represented by thick lines in Fig. 2. The connectivities of these fragments were determined by HMBC experiment. Both a methyl protons at δ_H 1.13 (2-CH_3) and a methine proton at δ_H 2.38 (11-H) coupled to a quaternary carbon at δ_C 49.1 (C-2). Both an oxygen bearing methine proton at δ_H 3.92 (17-H) and an olefinic proton at δ_H 5.80 (19-H) showed cross peaks with an

Fig. 1. Absolute structure of tubelactomicin A (**1**).



† This article is dedicated to Sir EDWARD P. ABRAHAM, a pioneer in the field of antibiotics.

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

Appearance	White powder
Molecular formula	$C_{29}H_{42}O_6$
FAB-MS (*m/z*)	509 $(M+Na)^+$ 485 $(M-H)^-$
HRFAB (*m/z*) Calcd	485.2903 (for $C_{29}H_{41}O_6$)
Found	485.2913 $(M-H)^-$
$[\alpha]_D^{25}$	+103°(*c* 0.64 , MeOH)
UV λ_{max}^{MeOH} nm(ε)	233 (28,500) 238 (28,600)
IR ν_{max}(KBr)cm^{-1}	3430, 2971, 2931, 2877, 1718(sh), 1700, 1648(sh), 1455, 1380, 1257, 1213, 1162, 1122, 1064, 1014, 993
TLC (Rf)	0.52* 0.23**
Color reaction positive	I_2, molybdophosphoric acid-sulfuric acid

* The Rf values of 1 on silica gel TLC (Kieselgel 60 F_{254}, art 5715, Merck) developed with $CHCl_3$: MeOH : AcOH=10 : 1 : 0.99).

** The Rf values of 1 on silica gel TLC (Kieselgel 60 F_{254}, art 5715, Merck) developed with Hexane : EtOAc=2 : 1).

olefinic quaternary carbon at δ_C 134.7 (C-18) and a carbonyl carbon at δ_C 168.6 (18-COOH), respectively. A long-range coupling from a methine proton at δ_H 4.71 (23-H) to a carbonyl carbon at δ_C 174.3 (C-1) clearly indicated the presence of 16-membered lactone ring.

The presence of octahydronaphthalene (octalin) moiety was revealed by the following observations in the HMBC spectrum. An olefinic proton at δ_H 5.85 (9-H), the methine proton of 11-H and the methyl protons of 2-CH_3 were coupled to a methine carbon at δ_C 46.6 (C-8), a methine carbon at δ_C 49.1 (C-2) and a methine carbon at δ_C 39.2 (C-3), respectively. Thus, the planar structure of tubelactomicin A was determined as shown in Fig. 2.

Relative Configuration of Tubelactomicin A

There were overlapping of signals for the octalin moiety; especially 3-H and 8-H resonated at the same field. Therefore, spin coupling constants for the octalin ring protons were not obtained from analyses of the ^1H NMR spectra of **1**. To reduce the number of overlapped signals, treatment of **1** with acetic anhydride and pyridine was carried out to give an acetyl derivative, 7-acetyl-16-dehydro-17-dehydroxytubelactomicin A (**2**) and its structure was confirmed by spectroscopic analyses. The ^1H NMR spectrum of **2** in acetone-d_6 showed desirable signal pattern for the octalin part than that of **1**. The large spin coupling constants of 7-H (δ_H 4.43, $J_{6,7}$=10.3, $J_{7,8}$=10.5) indicated the *trans*-diaxial relationship between 7-H and both 6-H (δ_H 1.6) and 8-H (δ_H 1.91). The axial 3-H (δ_H 1.78) was determined by NOESY correlation peak between 3-H and axial 7-H together with large J values ($J_{3,8}$=10.5, $J_{3,4ax}$=10.5, $J_{3,4eq}$=2.2). Thus, the junction of octalin ring is *trans* from the large coupling constant between 3-H and 8-H as shown in Fig. 3. In addition, NOESY correlation peaks between 2-methyl group (δ_H 1.16) and both 8-H and 11-H (δ_H 2.48) indicated that these protons were on the same side of the ring. Consequently, the cyclohexane ring in octalin moiety is a chair form having both equatorial methyl at C-6 and hydroxy group at C-7. The cyclohexene ring in octalin moiety takes a half-chair form with an axial methyl group at C-2 and *pseudo*-equatorial proton at C-11.

The configuration for the 16-membered ring part was studied by ^1H NMR and NOESY spectra of **1** and **2**. The methine proton, 11-H showed NOESY correlations with 2-CH_3 and 13-H. In addition, NOESY correlations between

Table 2. ^{13}C and ^{1}H NMR data of **1** and **2** in acetone-d_6.

	1				**2**			
No.	δ_C[a]	δ_H[b]		J (Hz)	δ_C[a]	δ_H[b]		J (Hz)
1	174.3 s				174.4 s			
2	49.1 s				49.7 s			
2-CH_3	17.2 q	1.13	s		17.4 q	1.16	s	
3	39.2 d	1.70~1.65	m		39.4 d	1.78	m	10.5, 10.5, 2.2
4	27.7 t	1.05~1.0	m		27.4 t	1.06	m	
		1.83	m	11.6, 6.0, 2.0		1.97~1.88	m	
5	34.4 t	1.18~1.08	m		33.9 t	1.22	m	
		1.75	ddd	12.0, 7.0, 2.6		1.82	m	
6	41.8 d	1.43~1.35	m		39.5 d	1.6	m	
6-CH_3	19.3 q	1.03	d	6.0	18.8 q	0.87	d	7.0
7	79.5 d	2.81	dd	9.0, 8.4	80.9 d	4.43	dd	10.3, 10.5
8	46.6 d	1.70~1.65	m		43.8 d	1.91	m	
9	122.7 d	5.85	s		120.7 d	5.28	s	
10	132.8 s				134.1 s			
10-CH_3	22.8 q	1.61	br s		22.9 q	1.59	br dd	1.5, 1.5
11	55.3 d	2.38	d	9.6	55.9 d	2.48	d	10.0
12	131.9 d	5.27	dd	14.0, 10.0	133.5 d	5.4	dd	15.0, 10.0
13	134.3 d	5.96	dd	14.0, 10.0	136.3 d	6.13	dd	15.0, 10.0
14	128.7 d	5.93	m		128.4 d	6.25	dd	15.5, 10.0
15	137.0 d	5.73	dd	15.0, 6.0	133.9 d	6.32	d	15.5
16	41.1 d	2.71	m		134.1 s			
16-CH_3	15.9 q	1.15	d	6.0	19.4 q	1.94	d	1.5
17	81.8 d	3.92	d	8.4	124.9 d	5.90	s	
18	134.7 s				130.9 s			
18-COOH	168.6 s				168.0 s			
19	144.6 d	5.80	dd	8.6, 4.4	147.5 d	6.87	dd	10.0, 7.0
20	29.0 t	2.47	ddd	16.0, 10.0, 4.4	31.2 t	2.13~2.08	m	
		2.58	m					
21	26.0 t	1.40~1.30	m		24.4 t	1.55	m	
		1.52	m					
22	35.8 t	1.18~1.08	m		37.8 t	1.43	m	
		1.68~1.60	m			1.52	m	
23	71.3 d	4.71	m		72.8 d	4.65	m	
23-CH_3	19.5 q	1.21	d	6.0	19.0 q	1.26	d	6.0
7-Ac(C=O)					170.9 s			
7-Ac(CH_3)					20.9 q	2.1	s	

a: 125 MHz, chemical shifts in ppm, multiplicity.
b: 500 MHz, chemical shifts in ppm, multiplicity.

12-H and 14-H, between 14-H and 16-CH_3, and between 17-H and 19-H were observed, respectively. These observations indicated that 12-H, 14-H, 16-CH_3, 17-H and 19-H were on the same side of the 16-membered ring, and the geometry of the double bond at C-18 was assigned to be *Z*. The geometry of other double bonds were assigned as *E*

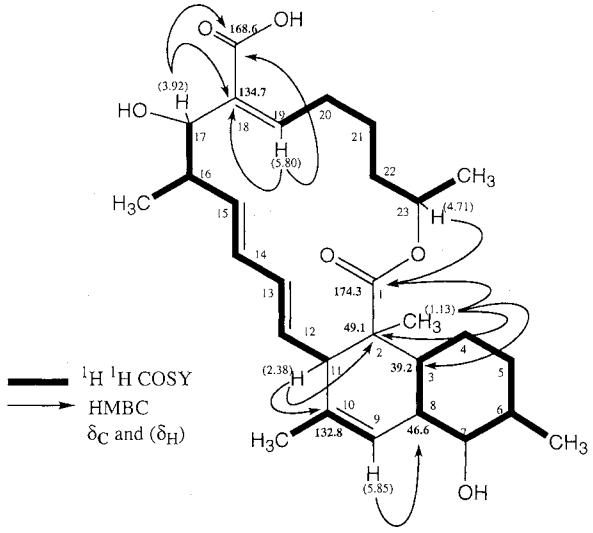
on the basis of large J values; $J_{12,13}$=15.0 Hz and $J_{14,15}$=15.5 Hz which were clearly observed in the ^{1}H NMR spectrum of **2**.

Absolute Configuration of Tubelactomicin A

Since tubelactomicin A (**1**) was amorphous powder, we tried preparing several derivatives suitable for X-ray crystallographic analysis. Treatment of **1** with L-phenylalanine methylester hydrochloride, triethylamine and dicyclohexylcarbodiimide in $CHCl_3$ at room temperature for 16 hours gave a carboxamide derivative (**3**). Compound **3** was crystallized from hexane-EtOAc solution as colorless prisms. The molecular formula and structure of **3** were confirmed by FAB-MS and NMR analyses.

The absolute configuration of **3** was totally elucidated by the X-ray analysis and stereoisomerism of the L-phenylalanine moiety. The ORTEP drawing of **3** is shown in Fig. 4.

Fig. 2. HMBC correlations of **1** in acetone-d_6.



Experimental

General

Optical rotation was measured with a Perkin-Elmer model 241 polarimeter. UV spectra were recorded with a Hitachi 557 spectrophotometer. IR spectra were recorded with a Horiba FT-210 fourier transform infrared spectrometer. The ^{1}H and ^{13}C NMR spectra were measured with a JEOL JNM-A500 spectrometer at 24°C, using TMS as an internal reference. The mass spectrum was recorded with a JEOL JMS-SX102 mass spectrometer.

Preparation of 7-Acetyl-16-dehydro-17-dehydroxy-tubelactomicin A (2)

To a solution of 8.2 mg of tubelactomicin A (0.017 mmol) in pyridine (0.5 ml), 14 mg of acetic anhydride was added and stirred for 23 hours at room temperature. The reaction mixture was extracted with 10 ml of ethyl acetate and the extract was washed with 2 ml of 0.2 M citric acid. The organic layer was collected and concentrated *in vacuo*, which was purified by preparative TLC (Kieselgel 60 F_{254}, art 5715, Merck) developing with $CHCl_3$-MeOH (19 : 1, Rf 0.51) to give 6.7 mg of 7-acetyl-16-dehydro-17-dehydroxytubelactomicin A (**2**, yield 78%). **2**; FABMS *m/z* 511 $(M+H)^+$; Molecular formula $C_{31}H_{42}O_6$; UV λ_{max}^{MeOH} nm (log ε): 225 (sh, 4.19), 230 (4.19), 237 (sh,

Fig. 3. Relative stereochemistry of **2**.

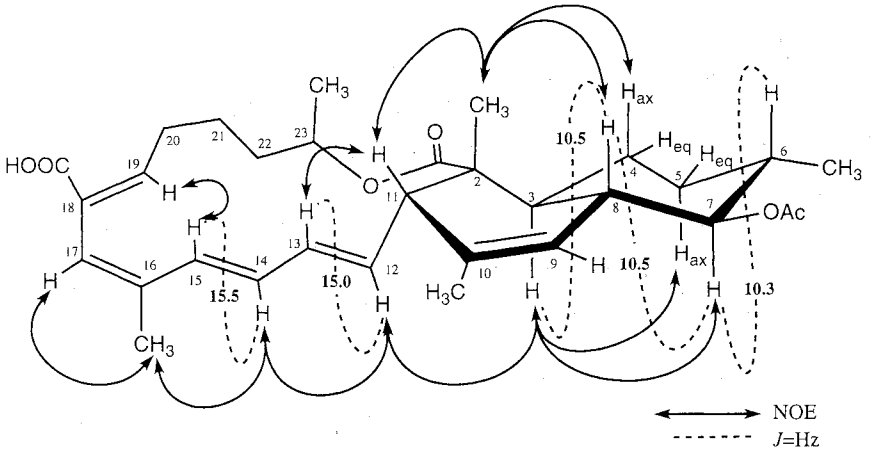
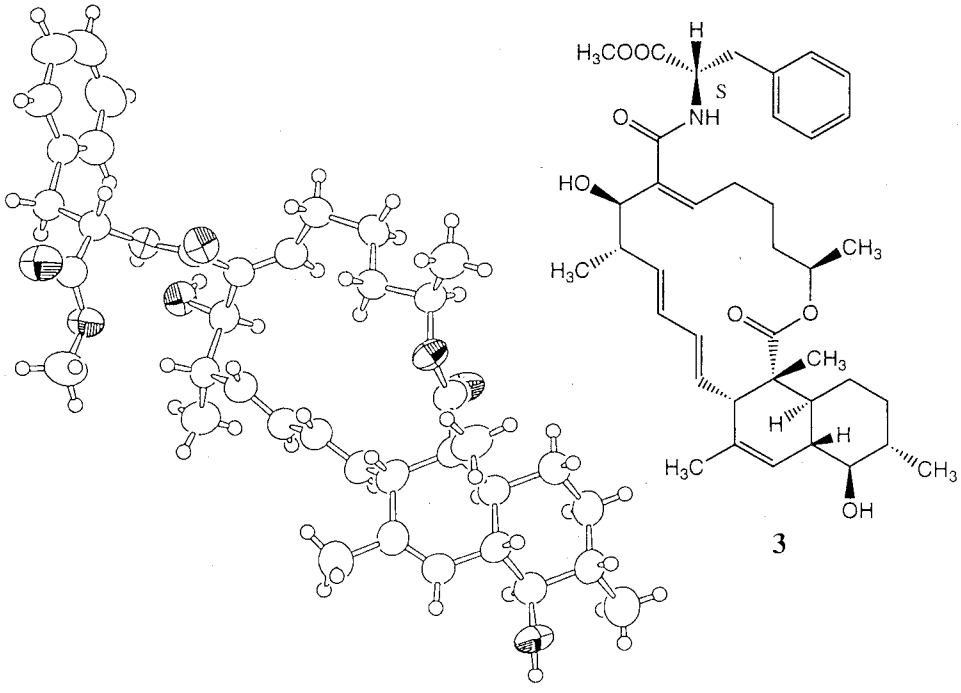


Fig. 4. ORTEP drawing of **3**.



3

4.17), 247 (sh, 4.10), 266 (sh, 4.20), 278 (4.28), 286 (sh, 4.23).

Preparation of Carboxamide Derivative (**3**)

To a solution of 10.0 mg of **1** free acid (0.02 mmol) in $CHCl_3$ (3 ml), 43 mg of (*S*) phenylalanine methyl ester HCl salt (0.2 mmol), 22 mg of trimethylamine (0.22 mmol) and a solution of 6.5 mg of dicyclohexylcarbodiimide (0.032 mmol) in $CHCl_3$ was added at room temperature, and the reaction mixture was stirred for 16 hours. The reaction mixture was detected by molybdophosphoric acid-sulfuric acid positive spot arising from **3** on a silica gel TLC (Kieselgel 60 F_{254}, art 5715, Merck) developing with toluene - acetone (1 : 1) as a solvent system. **2** showed the Rf value of 0.70. The reaction mixture was suspended in EtOAc, filtered and then concentrated. The concentrated material was chromatographed by using silica gel column (toluene, toluene : acetone=9 : 1, and 4 : 1). Compound **3** was collected and concentrated *in vacuo* to give a 9.7 mg (0.015 mmol) of **3** as colorless prism crystal. Compound **3** was crystallized from EtOAc-Hexane solution. **3**: FABMS *m/z* 648 $(M+H)^+$; Molecular formula $C_{39}H_{53}NO_7$; $[\alpha]_D^{23}$ +83.1° (*c* 0.78, MeOH); m.p. (°C) 201~202; IR ν_{max} (KBr) 3430, 2973, 2929, 2873, 1743, 1722, 1704, 1662 (sh), 1635, 1521, 1455, 1380, 1255, 1213, 1162, 1120, 1065, 993 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ (*J*=Hz) 1.03~0.97 m (2H), 1.04 d (3H, *J*=6.0), 1.12 s (3H), 1.35~1.20 m (2H), 1.16 d (3H, *J*=6.0), 1.19 d (3H, *J*=6.0), 1.20 m (2H), 1.40 m (1H), 1.53 m (2H), 1.65 s (3H), 1.67 m (1H), 1.77 ddd (1H, *J*=13.0, 7.0, 3.0), 1.82 m (1H), 2.05 ddd (1H, *J*=16.0, 8.0, 4.0), 2.33 d (1H, *J*=10.0, 2.5), 2.58~2.48 m (1H), 2.9 m (1H), 3.09 dd (1H, *J*=14.0, 7.0), 3.23 dd (1H, *J*=14.0, 6.0), 3.58 dd (1H, *J*=9.0, 5.0), 3.76 s (3H), 4.62 ddd (1H, *J*=9.0, 6.0, 2.6), 4.92 ddd (1H, *J*=7.4, 7.0, 6.0), 5.28 m (1H, *J*=14.0, 10.0), 5.28 dd (1H, *J*=14.0, 10.0), 5.43 dd (1H, *J*=10.0, 3.0), 5.58 dd (1H, *J*=15.0, 6.0), 5.72 br s (1H), 5.85 m (2H), 6.82 d (1H, NH, *J*=7.4), 7.32~7.23 m (5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 15.8 q, 16.7 q, 18.7 q, 19.2 q, 22.6 q, 25.2 t, 26.9 t, 27.0 t, 33.4 t, 34.7 t, 37.6 t, 38.2 d, 39.2 d, 40.9 d, 45.6 d, 48.5 s, 52.4 q, 53.0 d, 54.3 d, 71.4 d, 79.7 d, 84.2 d, 120.5 d, 127.2 d, 127.8 d, 128.67×2 d, 129.17×2 d, 131.0 d, 131.0 d, 133.3 d, 135.8 s, 136.0 d, 136.0 s, 139.8 d, 167.8 s, 172.2 s, 174.5 s.

X-Ray Crystallographic Analysis

A prism crystal having approximate dimensions of 0.10×0.15×0.30 mm was chosen for X-ray crystallography. All measurements were made on Rigaku AFC7R diffractometer with graphite monochromated Cu-Kα radiation and a rotation anode generator. The crystal data

are as follows: Empirical formula $C_{39}H_{53}NO_7$; F.W. 647.85; Crystal system Orthorhombic; Space group $P2_12_12_1$; Lattice parameters $a=17.033(1)$ Å, $b=34.489(3)$ Å, $c=6.306(1)$ Å, $V=3704.7(8)$ Å^3; $Z=4$. Dcalc 1.161 g/cm^3, μ(CuKα) 6.32 cm^{-1}. Of the 4191 reflections which were collected, 3922 were unique. No decay correction was applied. The structure was solved by direct method (SIR92)[2]. The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included but not refined. The final cycle of full-matrix least-squares refinement was based on 3139 observed reflections ($I>\sigma(I)$) and 424 variable parameters and converged with unweighted and weighted agreement factors of R=0.049 and Rw=0.057. The maximum and minimum peaks on the final difference Fourier map corresponded to 0.10 and −0.14 e$^-$/Å^3, respectively. All calculations were performed using the teXsan crystallographic software package of Molecular Structure Corporation.

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