Mer-A2026A and B, Novel Piericidins with Vasodilating Effect

II. Physico-Chemical Properties and Chemical Structures

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The structure of vasodilating acitive substances, Mer-A2026A and B, produced by *Streptomyces pactum* Me2108 were determined on the basis of their spectral and chemical properties.

Mer-A2026A (**1**) and B (**2**) , new vasodilating acitive substances, were isolated from the culture broth of *Streptomyces pactum* Me2108 as described in the preceding paper.[1] This paper is concerned with the physico-chemical properties and structure elucidation of Mer-A2026A (**1**) and B (**2**).

Physico-chemical properties of **1** and **2** are summarized in Table 1. UV absorption spectrum of **1**, shown in Fig.1 is similar to those of piericidins.[2]

The partial structures of **1**, C-11 to C-13, C-7 to C-10, C-4 to C-6 and C-1 to C-3, including attached methyl groups were revealed by ^1H and H-H COSY spectrum (Table 2). These partial structures were combined to give a complete side chain of Mer-A2026A from its H-C COSY and COLOC spectral analysis. Long range couplings were observed between C-11$\sim$Me and C-12 or C-10, C-7$\sim$Me and C-8 or C-6 and C-3$\sim$Me and C-4 or C-2. The side chain of Mer-A2026B was deduced as illustrated in Fig. 2, using the same methods. The mass spectral diference between **1** (SI-MS *m/z* 414 $(M+H)^+$) and **2** (FAB-MS *m/z* 386 $(M+H)^+$), 28 mass unit, could have originated in the side chains.

The residual part of the structure, pyridine ring system, were determined as follows. ^{13}C NMR spectrum of **1** showed weak peaks for the carbons around the pyridine ring and no peak for pyridine carbons as listed in Table 2. Two derivatives of **1**, di-acetate (**3**, Ac_2O in pyridine) and methyl ether (**4**, CH_2N_2 in methanol) were prepared for further NMR spectral studies. The ^{13}C NMR spectra of **3** showed 30 carbons including two acetyl groups and all protons and carbons could be assigned (Table 2). Considering that **1** is an analogue of the piericidin group antibiotics, each one proton, methyl, methoxy, acetoxy and side chain exist on a pyridine ring. The position of above substituents is deduced from the chemical shifts (116.2; C-Me, 162.4; C-OMe, 158.3; C-OAc, 158.6; C-Side Chain, 100.7; C-H) of the pyridine carbons as follows. Methoxy, acetoxy and the side chain shoud be on 2- or 4-, besides, the proton and methyl on 3-position of pyridine. Three nuclear Overhauser effects (NOEs) were observed between C-1H_2 of side chain (δ 3.46 ppm) and the methyl (δ 2.01 ppm), acetyl (δ 2.32 ppm) and the methyl, and finally, methoxy (δ 3.89 ppm) and the proton (δ 6.31 ppm). Two possible structures, **3** and **5**, were proposed for the di-acetate of Mer-A2026A, as illustrated in Fig. 3. NOE experiment on methyl ether **4** indicated structure **3** as being correct. That is, NOEs were observed

Fig. 1. UV spectrum of Mer-A2026A (**1**).
—— MeOH, ------ acidic MeOH, – – – basic MeOH.

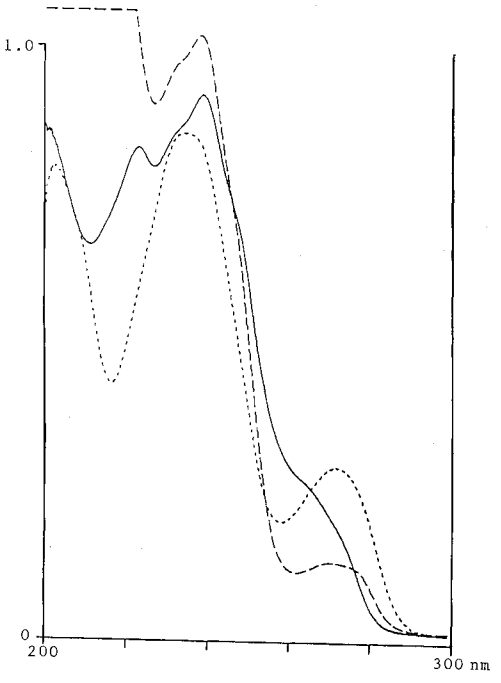


Table 1. Physico-chemical properties of Mer-A2026A (**1**) and B (**2**).

	1	**2**
Apperance	Yellow oil	Yellow oil
Rf value (TLC Merck Art. 5715)		
Toluene - acetone (1 : 1)	0.54	0.49
$[\alpha]_D$	−12.3° (*c* 1.85, Methanol, 22°C)	−1.07° (*c* 0.36, Methanol, 24°C)
Molecular formula	$C_{26}H_{39}NO_3$	$C_{24}H_{35}NO_3$
MW	413	385
UV λ_{max}^{MeOH} nm (ε)	238.5 (37,400), 223.2 (33,700)	238.4 (37,600), 223.2 (33,400)
IR $\nu_{max}^{CHCl_3}$ cm^{-1}	3350, 2950, 2860, 1635, 1590, 1520, 1460, 1390, 970	3340, 2920, 2860, 1635, 1590, 1520, 1460, 1385, 970
Color reactions		
Positive	Iodine, 10% $H_2SO_4+\Delta$, phosphomolybdic acid, DRAGENDORFF's reagent, 2,4-dinitrophenylhydrazin reagent	Iodine, 10% $H_2SO_4+\Delta$, phosphomolybdic acid, DRAGENDORFF's reagent, 2,4-dinitrophenylhydrazin reagent
Negative	Ninhydrin reagent, $FeCl_3$	Ninhydrin reagent, $FeCl_3$

Table 2. ^1H and ^{13}C NMR spectra of Mer-A2026A (**1**), B (**2**) and di-acetate (**3**) in $CDCl_3$: δ (*J*/Hz).

Position	**1**		**2**		**3**	
	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C
1	3.36 d (7.0)	32.1[b] t	3.36 d (7.0)	[c]	3.46 d (7.0)	35.0 t
2	5.30 t (7.0)	120.1[b] d	5.32 t (7.0)	[c]	5.40 t (7.0)	121.5 d
3		138.0[b] s		[c]		135.5 s
4	2.81 d (7.0)	43.0 t	2.83 d (7.0)	43.0 t	2.77 d (7.0)	43.1 t
5	5.57 dt (15.4, 7.0)	125.8 d	5.58 dt (15.4, 7.0)	125.8 d	5.52 dt (15.4, 7.0)	125.7 d
6	6.09 d (15.4)	136.2 d	6.10 d (15.4)	136.3 d	6.04 d (15.4)	136.2 d
7		135.6 s		135.7 s		134.1 s
8	5.24 d (10.6)	133.5 d	5.24 d (9.9)	133.6 d	5.15 d (9.5)	132.9 d
9	2.67 m	36.9 d	2.69 m	36.9 d	2.81 m	35.1 d
10	3.61 d (8.8)	82.7 d	3.64 d (9.2)	82.8 d	4.93 d (8.8)	83.2 d
11		132.4 s		135.6 s		129.3 s
12	5.21 d (9.5)	136.9 d	5.49 m	123.5 d	5.26 d (9.2)	137.8 d
13	2.55 m	26.8 d	1.63 d (7.0)	13.1 q	2.51 m	26.9 d
14	1.71 s	16.5 q	—	—	1.73 s	16.5 q
15	1.79 s	13.1 q	—	—	1.74 s	12.9 q
16	0.82 d (6.6)	17.4 q	1.72 s	16.6 q	0.86 d (7.0)	17.2 q
17	1.64 s	10.8 q	1.81 s	13.1 q	1.62 s	11.8 q
18	0.94[a] d (6.6)	22.8 q	0.81 d (6.6)	17.4 q	0.94[a] d (6.6)	22.0 q
19	0.96[a] d (6.6)	22.7 q	1.64 s	10.6 q	0.92[a] d (6.6)	22.0 q
1′		[c]		[c]		158.6 s
2′		115.8[b] s		[c]		116.2 s
3′		[c]		[c]		158.3 s
4′	5.96 s	92.4[b] d	5.93 s	92.4[b] d	6.31 s	100.7 d
5′		160.4[b] s		[c]		162.4 s
6′	2.03 s	10.1[b] q	2.03 s	10.1[b] q	2.01 s	10.8 q
7′	3.78 s	54.6[b] q	3.79 s	54.6[b] q	3.89 s	53.4 q
3′-OAc	—	—	—	—	2.32 s	20.8 q
	—	—	—	—		168.1 s
10-OAc	—	—	—	—	1.94 s	21.1 q
	—	—	—	—		170.1 s

[a] Assignment may be interchanged.
[b] Weak intensity.
[c] Could not be observed.

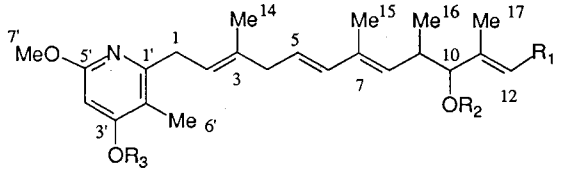
between the proton (δ 6.05 ppm) and both of methoxy groups (δ 3.89 and 3.80 ppm). In addition, as a result of NOE experiment on compound **3** the olefinic bonds of the side chain have *E*-configuration. The molecular formula of **1**, $C_{26}H_{39}NO_3$, was also confirmed from the mass spectrum (FAB-MS *m/z* 498 $(M+H)^+$) and the NMR spectra (^1H, ^{13}C) of **3**. The structure of Mer-A2026A was finally deduced as **1** from above findings. The absolute stereochemistry at C-9 and C-10 remains to be defined.

Experimental

General

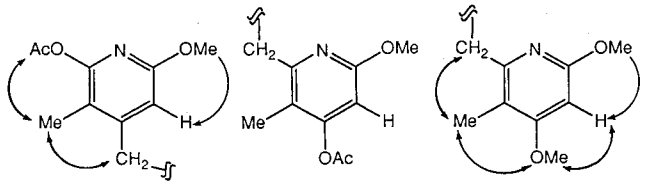
^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a JEOL JNM-GSX400 spectrometer using tetrametylsilane (TMS) as an internal standard and

Fig. 2. Structures of Mer-A2026A (**1**), B (**2**) and derivatives.



	R_1	R_2	R_3
1 (Mer-A2026A):	Me 18 / Me 19	H	H
2 (Mer-A2026B):	Me 13	H	H
3	Me / Me	Ac	Ac
4	Me / Me	H	Me

Fig. 3. Observed NOEs of di-acetate **3** and methyl ether **4**.



5 **3** **4**

are expressed as ppm (δ). J-Values are given in Hz. Splitting patterns are abbreviated as: s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, b=broad. Molecular weight was determined by MS spectometry (FAB technique) on a JEOL JMS-SX102A instrument. UV and IR spectra were taken on a Hitachi U-3210 and a JASCO FT/IR-5300 spectrophotometers, respectively. Optical rotations were measured with a JASCO DIP-370 polarimeter. Thin-layer chromatography (TLC) was performed using E. Merck silica gel 60 F-254 glass-backed plates (0.25 mm thick: analytical and 0.5 mm thick: preparative).

Acetylation of **1**

A solution of **1** (11 mg) in 0.2 ml of acetic anhydride and 1 ml of pyridine was stirred at room temperature for 20 hours. Water was added, and the resulting mixture was extracted with chloroform. The organic layers were washed with saturated aqueous $NaHCO_3$ and brine, dried (Na_2SO_4), and evaporated to give a residue. The residue was purified by preparative TLC (toluene-ethyl acetate, 7:1) to give 8.6 mg of di-acetate (**3**): Rf 0.76 (Toluene-ethyl acetate, 5:1); FAB-MS (NBA): positive m/z 498 $(M+H)^+$.

Methylation of **1**

To a solution of **1** (9.4 mg) in 1 ml of methanol, etheral solution of diazomethane (excess) was added at 0°C, and the mixture was stirred at ambient temperature for 5 hours and evaporated to give a residue. The residue was purified by preparative TLC (toluene-ethyl acetate, 4:1) to give 5.8 mg of methyl ether (**4**): Rf 0.65 (Toluene-ethyl acetate, 5:1); ^1H NMR (400 MHz, $CDCl_3$) δ 0.81 (3H, d, J=6.6 Hz, 16-H), 0.94* (3H, d, J=6.6 Hz, 19-H), 0.97* (3H, d, J=6.6 Hz, 18-H), 1.64** (3H, s, 17-H), 1.67 (1H, bs, 10-OH), 1.75 (3H, s, 14-H), 1.79** (3H, s, 15-H), 2.04 (3H, s, 6'-H), 2.56 (1H, m, 13-H), 2.66 (1H, m, 9-H), 2.78 (2H, d, J=7.0 Hz, 4-H), 3.42 (2H, d, J=7.0 Hz, 1-H), 3.58 (1H, d, J=8.8 Hz, 10-H), 3.80 (3H, s, 3'-OCH_3), 3.89 (3H, s, 7'-H), 5.21 (2H, bd, 8 and 12-H), 5.41 (1H, dt, J=1.1, 7.0 Hz, 2-H), 5.60 (1H, dt, J=15.8, 7.0 Hz, 5-H), 6.05 (1H, s, 4'-H), 6.09 (1H, d, J=15.8 Hz, 6-H); FAB-MS (glycerin): positive m/z 428 $(M+H)^+$. (*, **Assignment may be interchanged)

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References

1) KOMINATO, K.; Y. WATANABE, S. HIRANO, T. KIOKA, T. TERASAWA, T. YOSHIOKA, K. OKAMURA & H. TONE: Mer-A2026A and B, novel piericidins with vasodilating effect. I. Producing organism, fermentation, isolation and biological properties. J. Antibiotics 48: 99~102, 1995

2) YOSHIDA, S.; K. YONEYAMA, S. SHIRAISHI, A. WATANABE & N. TAKAHASHI: Isolation and physical properties of new piericidins produced by *Streptomyces pactum*. Agric. Biol. Chem. 41: 849~853, 1977