Isolation and Structural Elucidation of AC326-α, A New Member of the Moenomycin Group

HAIYIN HE*, BO SHEN, JOSEPH KORSHALLA, MARSHALL M. SIEGEL and GUY T. CARTER

Wyeth-Ayerst Research,
401 N. Middletown Rd., Pearl River, NY10965, U.S.A.

(Received for publication September 13, 1999)

In the course of screening for new antibiotics to overcome resistance encountered in antibacterial chemotherapy, a new phosphoglycolipid antibiotic, designated as AC326-α (**1**), was isolated from an unidentified Actinomyces. This antibiotic showed potent antibiotic activity against Gram-positive bacteria and was inhibitory to cell wall biosynthesis. Structurally, AC326-α (**1**) is closely related to moenomycin A (**2**),[1,2] the major constituent of the flavomycin® complex which is utilized in animal nutrition.[3] In this paper, the production, isolation, biological activity, and structural characterization of the new antibiotic are reported.

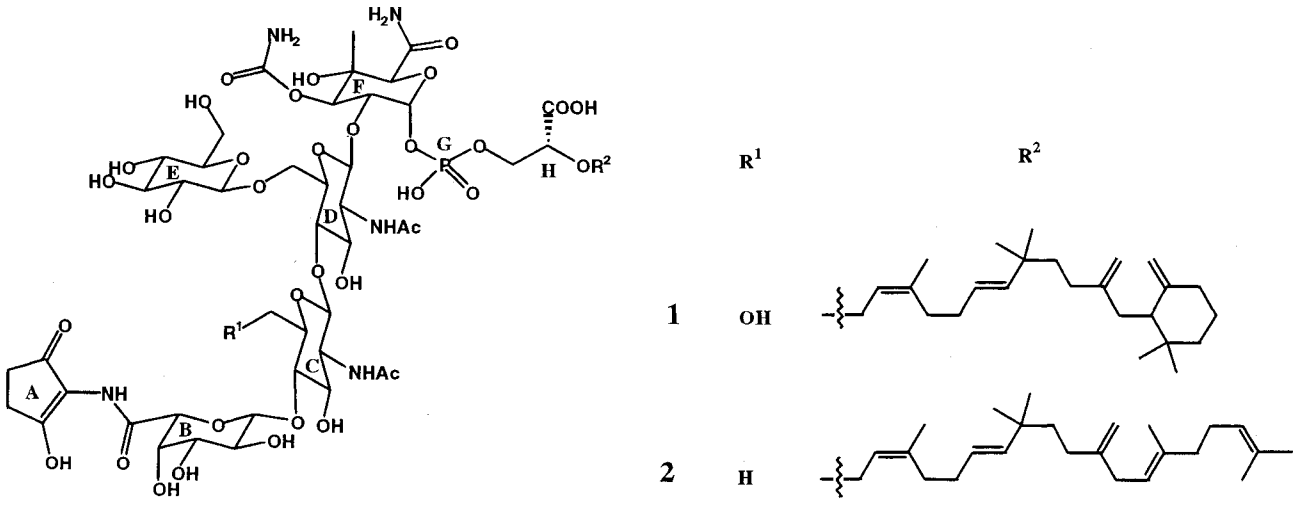
AC326-α (**1**) was produced by fermentation of culture AC326. Strain AC326 grown on an agar plate was transferred into a tube that contained 10 ml of a seed medium consisting of dextrose (10 g/liter), soluble starch (20 g/liter), yeast extract (5 g/liter), *N-Z* amine A (5 g/liter), $CaCO_3$ (1 g/liter), and Bacto agar (0.4 g/liter). The tube was shaken at 200 rpm at 28°C for 3 days. The first stage seed was inoculated into four 250-ml Erlenmeyer flasks, each containing 50 ml of the same medium and incubated for an additional 3 days. The second stage seed (200 ml) was added to a 10-liter fermentor that contained 10 liters of a production medium consisting of dextrin (50 g/liter), dextrose (5 g/liter), soy flour (35 g/liter), $CoCl_2 \cdot H_2O$ (0.25 mg/liter), and $CaCO_3$ (7 g/liter). Air was sparged at 10 liter/minute; agitation was set at 500 rpm; temperature was maintained at 28°C; dissolved oxygen was set to 99.9% at 0 time. The whole mash was harvested after it was incubated for 4 days.

The pellet obtained by centrifugation was extracted with methanol (6 liters) and the extract, upon concentration to a volume of 1 liter, was partitioned between dichloromethane and water (3 liters for both layers). The aqueous layer was then chromatographed over a Bondesil C18 open column (400 g, 40 micron particle size). The column was successively washed with water, 1 : 1 methanol in water, and methanol (3 liters each). The crude antibiotic contained in the methanol fraction was further purified by HPLC on a C18 column (YMC ODS-A, 10 micron, 30×250 mm). The column was eluted with a gradient of acetonitrile (27% to 100%) in water containing trifluoroacetic acid (0.01%) over 22 minutes at a flow rate of 20 ml/minute. The peak, centered at 20 minutes as detected by UV absorbance at 250 nm, was concentrated in vacuo to afford AC326-α (**1**) as trifluoroacetate (amorphous powder, 120 mg).[4]

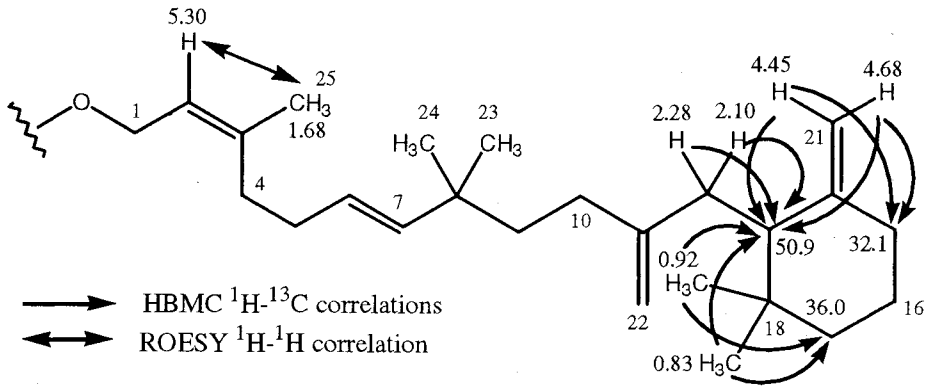
The molecular formula of AC326-α (**1**) was determined by high resolution electrospray Fourier transform mass spectrometry (ES-FT/MS) to be $C_{69}H_{108}N_5O_{35}P$. The analysis of ^1H and ^{13}C and 2-D NMR spectral data revealed the presence of a terpenoid substructure with a formula of $C_{25}H_{39}$ and five substituted sugar moieties, which in conjunction with the molecular formula directed our attention to moenomycin A (**2**). The ^1H and ^{13}C NMR spectral data for **1**, as determined by COSY, TOCSY, HMQC, and HMBC, are listed in Table 1. The NMR resonances assigned to sugar residues, B, D, E, F, and 2,3-dihydroxypropionic acid are consistent with the literature data[5] for **2** and the terpenoid or lipid portion was identified as diumycinol,[6,7] an unusual sesterterpenoid terminating in a methylene-cyclohexane. The 2- or 3-bond ^1H-^{13}C correlations in the HMBC spectrum, in particular, the correlations from C-13 at δ 50.9 to H_2-21 at 4.68 and 4.45, to H_3-19 at 0.83 and H_3-20 at 0.92, and to H-12 at 2.28, established the terminal ring structure. Selected 2-D NMR correlations are shown in Scheme 1.

The UV absorption maximum of **1** at 245 nm is also typical for moenomycin A (**2**), and is attributed to the chromophoric cyclopentenone moiety A. **1** lacks NMR signals for a 6-methyl group as in the 6-deoxy-*N*-acetylglucosamine (sugar C) of **2**, but possesses ^1H NMR signals at δ 3.83 (br d, 10.5 Hz), 3.65 (dd, 10.5, 5 Hz) and ^{13}C signal at 59.5 (t). Considering the additional oxygen required to fit the molecular formula, the sugar C in **1** is assigned to *N*-acetylglucosamine.

The high resolution ES-FT/MS data are consistent with the linkages between sugars, phosphate, 2,3-dihydroxypropionic acid, and the terpenoid moieties. In the positive ES-FT/MS/MS spectrum, the fragmentation between units H and R^2 gave rise to the strong product ion at *m/z* 1258.3526 ($MH^+ - R^2$), while the loss of sugar E resulted in the peak at 1436.6126 ($MH^+ - E$). The product ion, *m/z*



Scheme 1. Selected 2-D NMR data that determine diumycinol moiety (R^2) in **2**.



1258.3526, underwent further fragmentation to give 1096.2980 (MH^+-R^2-E) by loss of sugar E, 1072.3589 (MH^+-R^2-H-G) by loss of H and G, and 910.3063 ($MH^+-R^2-H-G-E$) by loss H, G, and E. The fragmentation between D and F resulted in the ion at *m/z* 840.2884 ($MH^+-R^2-H-G-F$), which gave 678.2356 ($MH^+-R^2-H-G-F-E$) by loss of sugar E. The most abundant peak at *m/z* 475.1561 ($MH^+-R^2-H-G-F-E-D$) was derived from the fragmentation between C and D, and it gave the ion at 290.0878 ($MH^+-R^2-H-G-F-E-D-C$) and 272.0772 ($MH^+-R^2-H-G-F-E-D-C-H_2O$) by further loss of sugar C and additional H_2O. The molecular and fragmentation ions observed in ES-FT/MS/MS are listed in Table 2 and the fragmentation pattern is illustrated in Scheme 2.

AC326-α (**1**) exhibited potent antibiotic activity against Gram-positive bacteria, such as *Staphylococcus aureus*, but poor activity against Gram-negative bacteria and *Candida albicans*. **1** also showed some cross-resistance to piperacillin resistant *Enterococci*. The MIC data obtained from agar dilution method are listed in Table 3.

Diumycinol was originally obtained by acid hydrolysis of diumycin,[8,9] a phosphorus-containing glycolipid antibiotic isolated from the fermentation broth of *Streptomyces umbrinus*. Although a number of studies were conducted to demonstrate its inhibitory activity to cell wall biosynthesis,[10,11] the structure of diumycin has not yet been described. Obviously, AC326-α and diumycin are closely related compounds, because of the similar physical data and antibiotic profiles.

Table 1. ^{1}H and ^{13}C NMR data of AC326-α (**1**) in DMSO-d_6.

	^{13}C (75 MHz)	^{1}H (500 MHz, mult, J in Hz)		^{13}C	^{1}H
Diumycinol			E		
1	65.4	4.05 (dd, 6, 6)	1	102.2	4.39 (d, 7.5)
		3.90 (m)	2	54.5	3.48 (m)
2	122.0	5.30 (m)	2-NH		7.57 (d, 8)[a]
3	138.9		3	73.9	3.45 (m)
4	31.9	2.06 (m)	4	81.9	3.35 (m)
5	30.8	2.05 (m)	5	72.6	3.41 (m)
6	125.4	5.22 (ddd, 15.5, 3, 3)	6	67.6	3.96 (m)
7	139.9	5.34 (d, 15.5)			3.57 (m)
8	35.2		$COCH_3$	169.4	
9	40.7	1.32 (ddd, 13, 13, 5)		23.0	1.85 (3H, s)
		1.23 (m)			
10	29.8	1.83 (m)	D		
		1.78 (m)	1	103.0	4.32 (d, 8)
11	148.6		2	73.3	3.00 (dd, 8.5, 8)
12	33.4	2.28 (br dd, 14.5, 4)	3	76.7	3.25 (dd, 8.5, 8.5)
		2.10 (m)	4	70.2	3.05 (dd, 8.5, 8.5)
13	50.9	1.91 (m)	5	76.8	3.20 (m)
14	148.8		6	61.1	3.70 (br d, 11)
					3.45 (m)
15	32.1	2.07 (m)			
		1.90 (m)	C		
16	23.2	1.48 (2H, m)	1	101.5	4.49 (d, 7.5)
17	36.0	1.48 (m)	2	55.5	3.53 (m)
		1.20 (m)	2-NH		7.84 (d, 8)[a]
18	34.6		3	72.2	3.55 (m)
19	25.6	0.83 (3H, s)	4	75.1	3.40 (m)
20	28.3	0.92 (3H, s)	5	78.8	3.46 (m)
21	109.1	4.68 (br s)	6	59.8	3.83 (br d, 10.5)
		4.45 (br s)			3.65 (dd, 10.5, 5)
22	109.8	4.61 (br s)	$COCH_3$	169.4	
		4.59 (br s)		23.1	1.87 (3H, s)
23	27.0	0.95 (3H, s)			
24	27.2	0.95 (3H, s)	B		
25	23.2	1.68 (3H, s)	1	102.8	4.36 (d, 7.5)
			2	70.1	3.38 (m)
2, 3-Dihydroxylpropionic acid			3	73.4	3.40 (m)
1	171.3		4	69.2	3.90 (m)
2	77.3	3.95 (m)	5	74.2	4.21 (br s)
3	65.7	3.95 (m)	6	169.6	
		3.83 (m)	6-NH		8.75 (br s)[a]
F					
1	93.9	5.68 (br s)	A[b]		
2	76.8	3.47 (m)	4, 5		2.48 (4H, br s)
3	73.7	4.90 (d, 10.5)			
4	72.5				
4-CH_3	16.2	1.08 (3H, s)			
5	72.2	4.19 (m)			
6	171.6				
6-NH_2		7.54 (br s)[a]			
		7.15 (br s)[a]			
$OCONH_2$	156.4	6.35 (2H, br s)[a]			

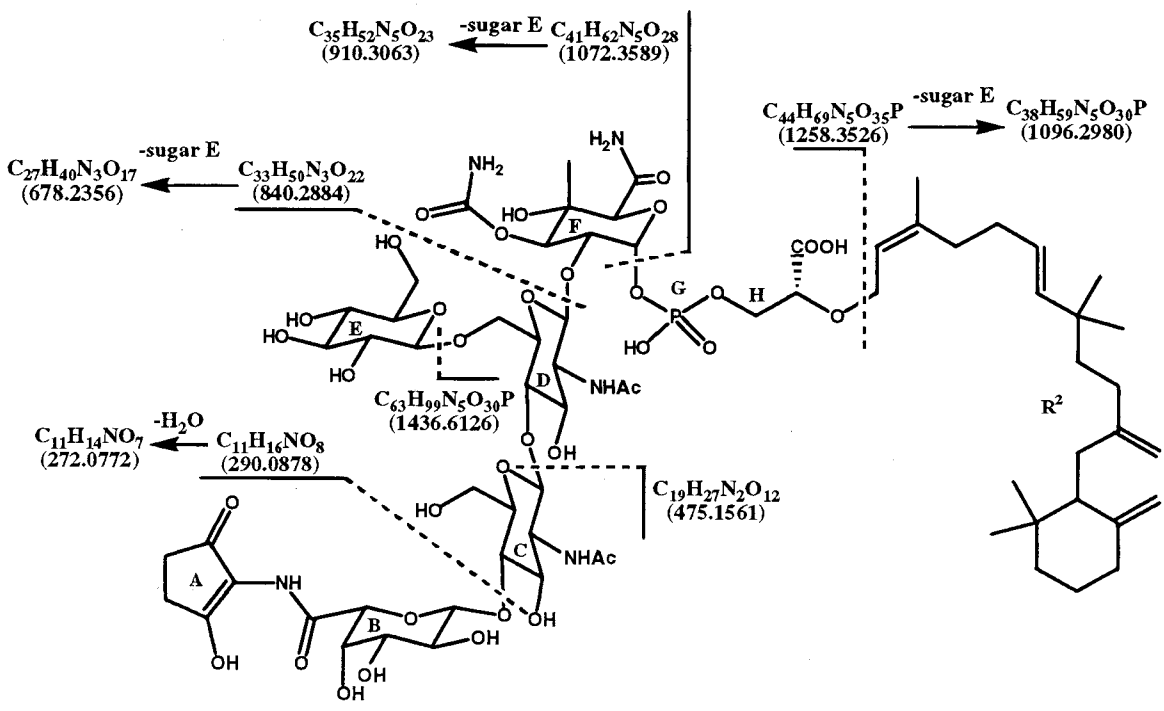
[a] D_2O exchangeable.

[b] Carbon signals of unit A were not observable, which is attributed to the processes of rapid proton exchange and slow rotation around the C–NH–CO bonds.

Table 2. Positive electrospray fourier transform mass spectral data for AC326-α (**1**).

Observed mass	Composition	Calculated value	Assignment
1620.6464	$C_{69}H_{108}N_5O_{35}PNa$	1620.6455	MNa^+
1598.6644	$C_{69}H_{109}N_5O_{35}P$	1598.6635	MH^+
1436.6126	$C_{63}H_{99}N_5O_{30}P$	1436.6107	MH^+-E
1258.3526	$C_{44}H_{69}N_5O_{35}P$	1258.3505	MH^+-R^2
1096.2980	$C_{38}H_{59}N_5O_{30}P$	1096.2977	MH^+-R^2-E
1072.3589	$C_{41}H_{62}N_5O_{28}$	1072.3576	MH^+-R^2-H-G
910.3063	$C_{35}H_{52}N_5O_{23}$	910.3047	$MH^+-R^2-H-G-E$
840.2884	$C_{33}H_{50}N_3O_{22}$	840.2897	$MH^+-R^2-H-G-F$
678.2356	$C_{27}H_{40}N_3O_{17}$	678.2352	$MH^+-R^2-H-G-F-E$
475.1561	$C_{19}H_{27}N_2O_{12}$	475.1558	$MH^+-R^2-H-G-F-E-D$
290.0878	$C_{11}H_{16}NO_8$	290.0870	$MH^+-R^2-H-G-F-E-D-C$
272.0772	$C_{11}H_{14}NO_7$	272.0765	$MH^+-R^2-H-G-F-E-D-C-H_2O$

Scheme 2. Fragmentation pattern of AC326-α from EM-FT/MS/MS.



Acknowledgments

The authors wish to thank Dr. JOSEPH ASHCROFT for NMR spectral data, Drs. NELSON HUANG, KASIA JANOTA, and GARY KRUPPA for mass spectral measurements, and PETE PETERSEN for *in vitro* antimicrobial data.

References

1) WELZEL, P.; B. WIETFELD, F. KUNSCH, TH. SCHUBERT, K. HOBERT, H. DUDDECK, D. MULLER, G. HUBER, J. E. MAGGIO & D. H. WILLIAM: Moernomycin A: further structural studies and preparation of the simple derivatives. Tetrahedron 39: 1583~1591, 1983

Table 3. Antibacterial activity of AC326-α (**1**).

Test organism	MIC (μg/ml)[a)]
Staphylococcus aureus (9 strains)	<0.06~0.25
S. haemolyticus GC 4546	0.25
Enterococcus faecalis (8 strains, including vancomycin resistant strains)	0.12~0.5
E. faecalis GC 2243 (piperacillin resistant)	32
E. faecium (2 strains)	<0.06~0.25
E. faecium (2 piperacillin resistant strains)	32~64
Streptococcus pyogenes GC 4563	0.25
S. pneumoniae (3 strains)	4~16
Escherichia coli (2 strains)	>64
Micrococcus luteus GC 4562	>64
Candida albicans GC 3066	>64

[a] Agar dilution method in Mueller-Hinton AII, incubated at 35°C for 18 hours.

2) FERSE, K.-T.; K. KLOEDER, L. HENNIG, M. FINDEISEN, P. WELZEL, D. MULLER & J. V. HEIJENOORT: Acceptor site recognition of transglycosylase inhibitors, a β-D-glucopyranosyl-(1→2)-α-D-glucopyranuronamide-derived moenomycin anologue. Tetrahedron 55: 3749~3766, 1999

3) HUBER, G.; Antibiotics. *ed.*, HAHN F. E. Springer, Berlin, Vol. 1, p. 135, 1979

4) $[\alpha]_D$=+2.2 (*c* 0.7, MeOH); UV λ_{max} (0.01% trifluoroacetic acid in 1 : 5 acetonitrile/water) 245 nm; IR ν_{max} KBr cm^{-1} 3196, 3064, 1667, 1557, 1434, 1203.

5) KURZ, M.; W. GUBA & L. VERTESY: Three-dimensional structure of moenomycin A, a potent inhibitor of penicillin-binding protein 1b. Eur. J. Biochem. 252: 500~507, 1998

6) SLUSARCHYK, W. A.; J. A. OSBAND & F. L. WEISENBORN: The structure of a novel lipid from the antibiotic diumycin. J. Am. Chem. Soc. 92: 4486~4488, 1970

7) SLUSARCHYK, W. A.; J. A. OSBAND & F. L. WEISENBORN: The structure of a novel lipid from the antibiotic diumycin. Tetrahedron 29: 1465~1472, 1973

8) MEYERS, E.; D. S. SLUSARCHYK, J. L. GOUCHARD & F. L. WEISENBORN: The diumycins, new members of an antibiotic family having prolonged *in vivo* activity. J. Antibiotics 22: 490~493, 1969

9) MEYERS, E.; D. S. SLUSARCHYK, P. E. PANSY, H. I. BASCH, W. H. TREJO & P. J. DEPAECKE: Biological characterization of diumycin, a phosphorus-containing glycolipid antibiotic. J. Antibiotics 25: 163~170, 1972

10) VILLEMEZ, C. L. & P. L. CARLO: Properties of a soluble polyprenyl phosphate. UDP-D-*N*-acetylglucosamine *N*-acetylglucosamine-1-phosphate transferase. J. Biol. Chem. 255: 8174~8178, 1980

11) KEAN, E. D. & Z. WEI: Stimulation as well as inhibition by antibiotics of the formation of GlcNAc-lipids of the dolichol pathway. Glycoconjugate J. 15: 405~414, 1998