Notes

A51568A: *N*-DEMETHYLVANCOMYCIN

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(Received for publication March 22, 1984)

The first member of the glycopeptide class of antibiotics to be reported was vancomycin, in 1956[1]. A number of other glycopeptides have been isolated and characterized in recent years, and they share several similar or identical structural features. One feature which is not conserved is the nature of the *N*-terminal group of the peptide core; $-NHCH_3$ is present in vancomycin[2,3] and the avoparcins[4], while $-NH_2$ is present in many other cases, including the ristocetins[5], the actaplanins[6] and the A41030 antibiotics[7]. This paper presents the isolation and structure elucidation for a new glycopeptide antibiotic, A51568A (**1**); A51568A differs from vancomycin only in that $-NHCH_3$ at the peptide amino terminal of vancomycin has been replaced by $-NH_2$. The antimicrobial spectrum of A51568A is the same as that for vancomycin (Tables 1 and 2).

Isolation and Purification of A51568A

Isolation of Crude A51568A and B Mixture

A51568A and B are produced by a strain of *Nocardia orientalis*, designated NRRL 15232; the culture was grown under conditions reported by BOECK *et al.*[8]. Hyflo Supercel filter aid was added to three liters of fermentation broth con-

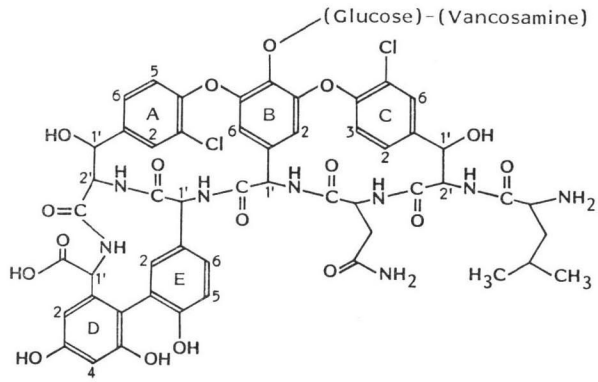

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Table 1. *In vitro* activities (MIC values) for A51568A and vancomycin against various organisms.

	Staphylococcus aureus				*S. epidermidis*		A	*Streptococcus* PW	D	
	X1.1	V41	X400	S13E	EP11	222	C203	PARK	X66	2041
A51568A	0.5	1.0	1.0	1.0	2.0	1.0	1.0	0.5	1.0	4.0
Vancomycin	1.0	1.0	1.0	1.0	2.0	1.0	1.0	0.5	1.0	4.0

	Haemophilus influenzae Sensitive	Resistant	*Escherichia coli*			*Klebsiella*		
	C.L.	76	N10	EC14	TEM	X26	KAE	X68
A51568A	64	128	>128	>128	64	>128	>128	>128
Vancomycin	128	128	>128	>128	>128	>128	>128	>128

Table 2. *In vivo* activity: A51568A and vancomycin.

	ED_{50} (mg/kg×2, sc)		
	Staphylococcus aureus	*Streptococcus pyogenes*	*Streptococcus pneumoniae*
A51568A	1.8	3.0	2.7
Vancomycin	1.3	1.2	1.8

Table 3. Retention times of A51568 factors (in minutes).

Column: Merck Hibar-II Lichrosorb C_{18}; 4.6 mm×250 mm.

Flow rate: 2.0 ml/minute.

	Solvent I	Solvent II
A51568A	7.5~7.7	9.6~9.8
A51568B	4.8~4.9	5.0~5.2

Solvent 1: H_2O-CH_3CN-THF, (93: 5: 2), plus 2.5 g of ammonium formate per liter; pH of the solution adjusted to 5.0 with formic acid.

Solvent II: Same as above except that the solution pH is adjusted to 3.0 with formic acid.

taining A51568 factors A and B. The mixture was filtered and the filtrate (2.7 liters, pH 7.8) was applied to a column (3×25 cm) of Diaion HP-20 resin (Mitsubishi Industries). The effluent was discarded and the column was washed with 1 liter of H_2O and 500 ml of 25% MeOH in H_2O. The antimicrobial activity (determined by assay on *Bacillus subtilis*) was eluted with two 500-ml portions of 50% MeOH in H_2O. The relative amounts of factors A and B were determined by HPLC (Table 3). The active fraction was concentrated to a small volume and lyophilized; 905 mg of crude A51568 containing two active factors (A and B) was obtained.

Separation of A51568 Factors A and B

To further purify the sample and separate factors A and B, the crude A51568 was dissolved in 50 ml of H_2O and applied to a 1.7×44 cm column of Sephadex CM-25 (NH_4^+ cycle). The effluent was discarded and the column was washed with 250 ml of H_2O. The column was eluted with a convex gradient of ammonium bicarbonate (H_2O to 1 M NH_4HCO_3; 100 ml mixing chamber). Fifty 25-ml fractions were collected and monitored by HPLC to determine the relative concentrations of factors A and B; fractions enriched in factor A were pooled. Further purification and characterization of factor B will be presented in a separate report.

Table 4. Comparison of ^1H NMR chemical shifts (δ, ppm) for A51568A and vancomycin (solvent DMSO; temperature 60°C; assignments refer to the numbering scheme shown in **1**).

Proton	A51568A	Vancomycin
A-NH	6.54	6.54
-2′	4.19	4.19
-1′	5.14	5.13
-OH	5.86	5.85
-2	7.86	7.85
-5	7.30	7.29
-6	7.47	7.47
B-NH	8.24	8.19
-1′	5.72	5.70
-2	5.63	5.61
-6	5.22	5.22
C-NH	?	?
-2′	4.78	4.84
-1′	5.18	5.17
-OH	5.74	5.70
-2	7.66	7.53
-3	7.18	7.21
-6	7.42	7.41
D-NH	8.45	8.43
-1′	4.47	4.47
-2	6.26	6.28
-4	6.42	6.41
E-NH	8.54	8.50
-1′	4.47	4.46
-2	7.17	7.17
-5	6.72	6.71
-6	6.79	6.78
Asn-NH	6.61	6.56
-α	4.32	4.33
-β	2.56,	2.51,
-β	2.14	2.14
-NH_2	7.29,	7.26,
〃	6.83	6.78
Leu(NCH_3)	—	2.34
-α	3.70	3.27
-β	1.65,	1.56,
-β	1.49	1.46
-γ	1.76	1.74
-δ	0.93,	0.91,
-δ	0.90	0.87
Glu-1	5.33	5.33
-2	3.58	3.59
-3	3.49	3.48
-4	3.29	3.29
-5	—	—
-6	3.70,	3.70,
-6	3.54	3.56
Van-1	5.30	5.29
-2	1.93,	1.92,
-2	1.77	1.76
(3-CH_3)	1.37	1.35
-4	—	—
-5	4.67	4.66
(5-CH_3)	1.09	1.08

Final Purification of A51568A

The pool containing factor A was applied to a 1.7×10 cm column of Diaion HP-20 resin equilibrated in H_2O. The column was washed with 200 ml of H_2O and eluted with 100 ml of 50% MeOH in H_2O. The MeOH eluate was evaporated to dryness and redissolved in a small amount of H_2O. The resulting solution was acidified with 0.1 N HCl and lyophilized. Approximately 25 mg of pure A51568A was obtained.

NMR Comparison of A51568A with Vancomycin

^1H NMR spectra of A51568A were recorded at 60°C in DMSO solution (roughly 2 mg in 0.4 ml), using a Bruker WH360 spectrometer; spectra of vancomycin were measured under similar conditions for comparison. Spectra of the two compounds are virtually superimposable in many regions; the chief difference noticeable by inspection is the absence of the NCH_3 resonance for (*N*-methyl)leucine in the spectrum of A51568A. (This difference is confirmed by amino acid analysis: A51568A gives aspartic acid and leucine, while vancomycin gives only aspartic acid among the common amino acids.) The A51568A NMR spectrum has been assigned, using homonuclear decoupling experiments to check the assignments of most of the non-aromatic resonances. Aromatic resonances were assigned by inspection, based on earlier decoupling studies on vancomycin. Assignments for A51568A and vancomycin are compared in Table 4.

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