A40926 AGLYCONE AND PSEUDOAGLYCONES: PREPARATION AND BIOLOGICAL ACTIVITY

ENRICO SELVA, BETH P. GOLDSTEIN*, PIETRO FERRARI, ROSA PALLANZA, ERNESTO RIVA, MARISA BERTI, ANGELO BORGHI, GRAZIA BERETTA, ROBERTO SCOTTI, GABRIELLA ROMANÒ, GIOVANNI CASSANI, VITTORIO ARIOLI and MAURIZIO DENARO

Merrell Dow Research Institute, Lepetit Research Center, Via R. Lepetit 34, 21040 Gerenzano (Varese), Italy

(Received for publication January 25, 1988)

A40926 antibiotics are new glycopeptides which are much more active than other members of this class against *Neisseria gonorrhoeae*. Their activity against Gram-positive bacteria, including coagulase-negative Staphylococci, is similar to that of other glycopeptides. An A40926 preparation containing factors A and B ("A40926 A+B complex") was hydrolyzed to the aglycone and to the mannosyl and *N*-acylaminoglucuronyl aglycones. The mannosyl aglycone and the aglycone were less active than A40926 A+B complex against Streptococci and Gram-positive anaerobes and lost the anti-gonorrheal activity. In contrast, the *N*-acylaminoglucuronyl aglycones were as active as the parent complex against these Gram-positive bacteria and were moderately active against *N. gonorrhoeae*. The aglycone and, even more so, the *N*-acylaminoglucuronyl aglycones, had better activity than the parent complex against coagulase-negative Staphylococci. In experimental septicemia in the mouse, A40926 A+B complex and its derivatives had activity proportional to their MIC for the test organism.

A40926 antibiotics are glycopeptides produced by *Actinomadura* strain ATCC 39727[1]. The same antibiotics were independently discovered by CHRISTENSEN *et al.*[2]. While their activity against Gram-positive bacteria is similar to that of other glycopeptides, they are much more active than these against *Neisseria gonorrhoeae*[1]. Some authors have found coagulase-negative Staphylococci (CNS) to be less sensitive to glycopeptide antibiotics, such as teicoplanin and vancomycin, than are *Staphylococcus aureus* strains[3]. In particular, many *Staphylococcus haemolyticus* isolates are relatively insensitive to teicoplanin[3~5]. In some cases, glycopeptide aglycones or pseudoaglycones have shown better activity against CNS than the parent compounds[6~8], while in others these derivatives had reduced activity (RIPAMONTI F., and B. GOLDSTEIN; unpublished data). We have now prepared the aglycone and pseudoaglycones of A40926 and determined their antimicrobial activities.

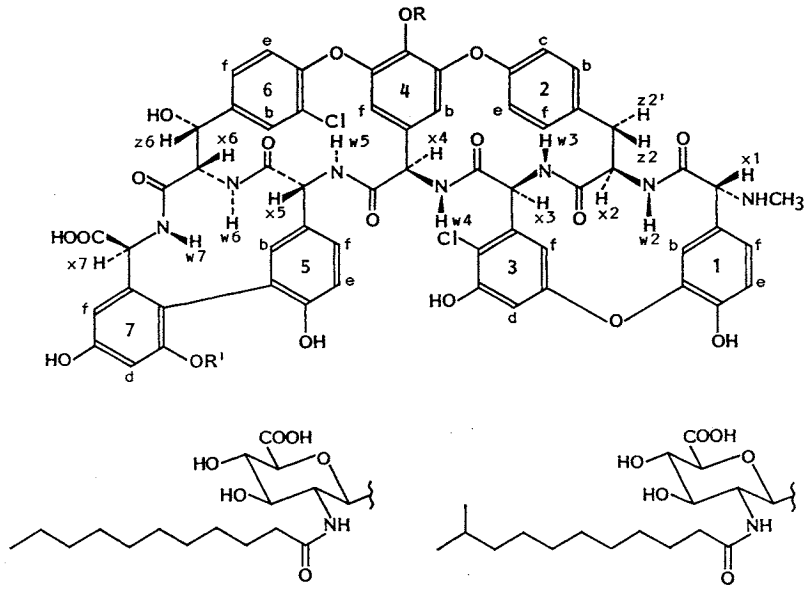
For this study we used as starting material A40926 A+B complex, which contains two stable antibiotics (factors A and B). These are the major components recovered during the normal purification procedure[1]. Each of these factors contains a neutral sugar (D-mannose) and a glycolipid derived from 2-aminoglucuronic acid. The fatty acids differentiate factors A (*n*-undecanoic acid) and B (10-methylundecanoic acid)[9].

Materials and Methods

Preparation of a Mixture of the Aglycone and the Two *N*-Acylaminoglucuronyl Aglycones

A40926 A+B complex (750 mg) was dissolved in 150 ml of DMSO - 37% HCl (9:1). After

Fig. 1. Structures of A40926 factors A and B and of the aglycone and pseudoaglycones.



Glycolipid A

Glycolipid B

Compound	R′	R	MW[a]	Formula
A40926 factor A	Mannose	Glycolipid A	1,716	$C_{82}H_{86}N_8O_{29}Cl_2$
A40926 factor B	Mannose	Glycolipid B	1,730	$C_{83}H_{88}N_8O_{29}Cl_2$
Aglycone (**1**)	H	H	1,211	$C_{59}H_{47}N_7O_{18}Cl_2$
N-Acylaminoglucuronyl aglycone of factor A (**2**)	H	Glycolipid A	1,554	$C_{76}H_{76}N_8O_{24}Cl_2$
N-Acylaminoglucuronyl aglycone of factor B (**3**)	H	Glycolipid B	1,568	$C_{77}H_{78}N_8O_{24}Cl_2$
Mannosyl aglycone (**4**)	Mannose	H	1,373	$C_{65}H_{57}N_7O_{23}Cl_2$

[a] Lowest isotope composition.

The nomenclature and numbering of the protons, proposed for glycopeptide antibiotics by BARNA *et al.*[13], were previously used in the structure elucidation of A40926[9].

5 hours at 65°C, factors A and B were completely hydrolyzed. The reaction mixture was then diluted with 600 ml of cold water and brought to pH 7 with NaOH. The solution contained a mixture of A40926 aglycone (**1** in Fig. 1) and the two *N*-acylaminoglucuronyl aglycones (**2** and **3**) derived from factors A and B.

Separation of the Aglycone from the *N*-Acylaminoglucuronyl Aglycone Complex by Affinity Chromatography

The reaction mixture was loaded onto a 100-ml column of Sepharose-D-alanyl-D-alanine[10] equilibrated with 10 mM Tris-HCl buffer, pH 7.5. The column was eluted first at pH 7.5 with 200 ml of solution A (2 M NaCl - 0.05 M NH_4OH, pH adjusted with HCl) and then 1,500 ml of solution B (same as A, but adjusted to pH 9.5). Solution B selectively eluted the aglycone. The pseudoaglycones were then eluted with 0.1 M aqueous ammonia. The solution B and ammonia eluates were each neutralized to pH 7.5 with HCl and desalted by readsorption to affinity columns. The columns were washed with distilled water to eliminate all inorganic salts and then eluted with 0.1 M aqueous ammonia. Pooled antibiotic-containing fractions were concentrated under vacuum by azeotropic distillation with BuOH and lyophilized. We recovered 236 mg of the aglycone (**1**) and 201 mg of the *N*-acylaminoglucuronyl aglycone complex (**2**+**3**).

Separation of the Two *N*-Acylaminoglucuronyl Aglycones

20 mg of the mixture were dissolved in 1 ml of 18 mM Na-phosphate buffer, pH 6 - acetonitrile (9 : 1). The solution was injected into an HPLC preparative column (7 mm i.d. × 250 mm) of Lichrosorb RP18 silanized silica gel having 7 μm particle size. The column was eluted in 55 minutes with a 10 to 55% linear gradient of phase A at a flow rate of 5 ml/minute. Phase A consisted of 18 mM Na-phosphate buffer, pH 6 - acetonitrile (3 : 7) and phase B was a 9 : 1 mixture. The eluates were analyzed by HPLC and the fractions containing the pseudoaglycones derived from factors A and B were collected. The appropriate fractions from 11 chromatographic runs were pooled and desalted by loading onto a 5-ml column of Sepharose-D-alanyl-D-alanine at pH 7, which was washed with 10 ml of 1 mM HCl followed by 50 ml of distilled water. Antibiotic was then eluted with 50 ml of aqueous ammonia and the eluates lyophilized. We recovered 15 mg of A40926 factor A *N*-acylaminoglucuronyl aglycone (**2**) and 51 mg of the factor B pseudoaglycone (**3**).

Preparation of the Mannosyl Aglycone (**4**)

A40926 A+B complex (400 mg) was dissolved in 50 ml of TFA - distilled water (9 : 1). After 64 hours at room temperature, the solution was concentrated under vacuum to about 20 ml. The residue was diluted with 80 ml of distilled water and extracted with 100 ml of EtOAc. The aqueous phase was neutralized to pH 7 with NaOH and concentrated under vacuum to 25 ml by azeotropic distillation with BuOH. 8 ml of this solution were injected into a column of Lichrosorb RP18 (10 μm, Merck) reverse phase silica gel equilibrated with acetonitrile - 18 mM Na-phosphate buffer, pH 6 (17 : 83). The resin was packed under a pressure of 14 atm into a 2-cm diameter stainless steel column that was part of a Jobin Yvon Modulprep preparative chromatograph. The column was eluted with the equilibration solution and the eluates analyzed by HPLC. Pooled fractions from three chromatographic runs were diluted 2-fold with 1 M NaCl. The solution was desalted by loading onto a 50-ml column of silanized silica gel, equilibrated with distilled water, which was then washed with distilled water until the inorganic salts were eluted. The mannosyl aglycone was eluted with acetonitrile - water (1 : 1). Upon lyophilization of the concentrated eluates, 66 mg of material were recovered.

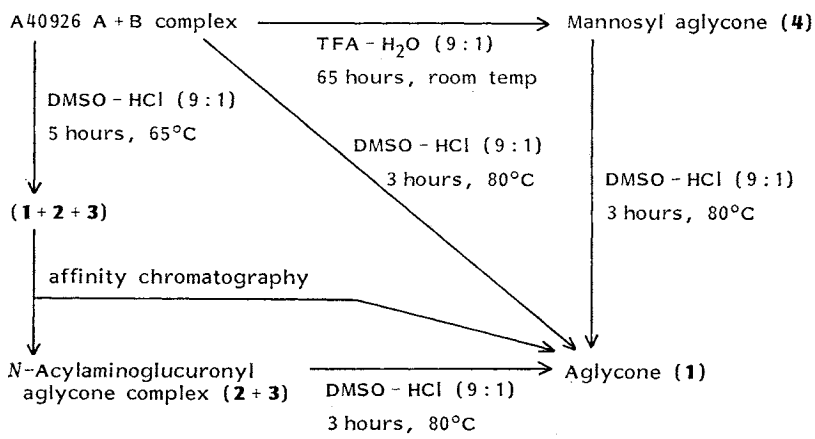
Selective Preparation of the Aglycone

Hydrolysis of A40926 A+B complex in DMSO - concentrated HCl (9 : 1) at 80°C for 3 hours yielded the aglycone (**1**) as the main reaction product. Under these conditions, the various pseudoaglycones (**2**, **3**, and **4**) were also converted to the aglycone. The complete scheme for the hydrolysis of A40926 is in Fig. 2.

Analytical Methods

The molecular weights of the hydrolysis products were determined by positive ion fast atom bom-

Fig. 2. Scheme of hydrolysis of A40926 A+B complex.



bardment mass spectroscopy (FAB-MS) using a VG model 70-70 EQ instrument. The experimental conditions were: 7 keV argon beam energy and 6 kV accelerating voltage. The samples were suspended in a thioglycerol matrix.

The ^1H NMR spectra and two-dimensional (2D) double quantum filtered phase sensitive homonuclear correlation spectroscopy (COSYPHDQ) were performed with a 250-MHz Bruker instrument equipped with an Aspect 3000 console. Samples were solubilized in DMSO-d_6 and spectra were recorded at 40°C. Stereochemical details were obtained from dipolar coupling through space by nuclear Overhauser effect phase sensitive spectroscopy (NOESYPH).

HPLC was performed using a Hewlett-Packard model 1090 liquid chromatograph connected to an HP3357 computing system. The chromatography conditions were:

Column: Altex Ultrasphere ODS 5 μm (250×4.6 mm); precolumn: RP18 5 μm, Brownlee Laboratories; mobile phase A: acetonitrile - 0.018 M NaH_2PO_4, pH 6 (7:3); mobile phase B: acetonitrile - 0.018 M NaH_2PO_4, pH 6 (1:9); gradient elution: linear 5 to 60% phase A gradient in 40 minutes; flow: 1.8 ml/minute; UV detection: 254 nm.

In Vitro Antimicrobial Activity

MIC for most organisms were determined by microbroth dilution methodology. MIC for *Clostridium difficile*, *Propionibacterium acnes* and *Bacteroides fragilis* and the data of Fig. 4 were obtained by the agar dilution method. Unless otherwise indicated, inocula were approximately 10^4 cfu/ml or per spot. Incubation times were 18~24 hours, except for: *N. gonorrhoeae*, *Haemophilus influenzae*, *C. difficile*, *P. acnes*, and *B. fragilis* (48 hours). All organisms were incubated at 37°C. *N. gonorrhoeae* and *H. influenzae* were incubated in a 5% CO_2 atmosphere; anaerobes were incubated in an anaerobic gas mixture. Media used were: Oxoid Iso-Sensitest broth (Staphylococci, *Streptococcus faecalis*, *Escherichia coli*); Difco Todd-Hewitt broth (other Streptococci); Difco GC base broth with 1% BBL IsoVitaleX for *N. gonorrhoeae*; Difco Brain-Heart Infusion Broth with 1% Difco Supplement C for *H. influenzae*; Difco AC medium without agar for *Clostridium perfringens*; Oxoid Wilkins - Chalgren agar for the other anaerobes.

Experimental Septicemia in the Mouse

Control and treatment groups contained five or ten CD-1 mice (Charles River) weighing 18~22 g. They were infected ip with 0.5 ml of bacterial suspension prepared by diluting an overnight culture of *Streptococcus pyogenes* C203 with sterile peptonized saline. Inocula were adjusted so that untreated animals died of septicemia within 48 hours. Antibiotics were administered sc immediately after infection. On the 7th day, the ED_{50} in mg/kg was calculated by the method of SPEARMAN and KÄRBER[11] from the percentages of surviving animals at each dose.

Results

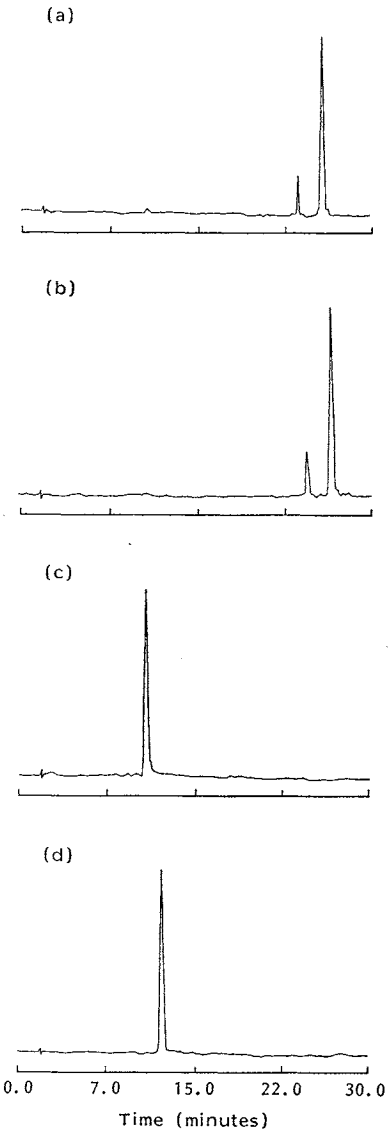
Chemical Characterization of Hydrolysis Products

The selective cleavage of mannose was obtained in a dimethyl sulfoxide - HCl mixture. As expected, loss of mannose from the A40926 A+B complex yielded a mixture of two *N*-acylaminoglucuronyl aglycones (**2** and **3** in Fig. 1). The fatty acid groups characteristic of factors A and B were retained in these pseudoaglycones. The time course of the reaction indicated that the antibiotics were first hydrolyzed to the *N*-acylaminoglucuronyl aglycones and then to the aglycone (**1** in Fig. 1). Therefore, the aglycone was easily obtained as the major hydrolysis product when the reaction time was prolonged. If instead the reaction was quenched as soon as the starting material disappeared, we obtained a mixture of the aglycone (about 50%) and two pseudoaglycones, which was fractionated into aglycone and pseudoaglycone preparations by differential elution from Sepharose-D-alanyl-D-alanine. Glycopeptides are less strongly bound to the peptide at basic than at acidic pH. At pH 9.5, in 2 M NaCl, the aglycone was eluted while the pseudoaglycones were retained.

The hydrolysis products of A40926 were analyzed by HPLC (Fig. 3) and structurally characterized by spectroscopic analysis. The molecular weights of these three products, determined by FAB-MS are consistent with the structures in Fig. 1.

Fig. 3. HPLC of A40926 complex and hydrolysis products.

(a) A40926 A+B complex, (b) *N*-acylaminoglucuronyl aglycone mixture, (c) mannosyl aglycone, (d) aglycone.



Interpretation of the NMR data was facilitated by comparison with our previous data for A40926 factors A and B[9] and for teicoplanin aglycone[12]. The complete assignment of A40926 aglycone, except for the phenolic and carboxylic OH's, is in Table 1 (here and in Fig. 1, the nomenclature and proton numbering are those proposed for glycopeptide antibiotics by BARNA *et al.*[13], and used previously by us for A40926[9]). As shown in the table, the NMR data for A40926 and teicoplanin aglycones are very similar. Two of the most important features which distinguish A40926 aglycone are the peak produced by the *N*-methyl group at the *N*-terminus of the peptide backbone (identified by a typical downfield shift in acidic solution) and the downfield shifts of the 4b and x3 protons due to the presence of a chlorine atom on ring 3 (instead of on ring 2).

With the exception of the signals attributed to the glycolipid groups, there were no important differences between the NMR spectra of A40926 aglycone and the *N*-acylaminoglucuronyl aglycones (**2** and **3**). The spectra of compounds **2** and **3** showed a one-proton doublet at 5.4 ppm (J=8 Hz) assigned to the anomeric proton of the glycolipid. Other signals characteristic of these compounds were: A two-proton triplet at 2.0 ppm, a two-proton multiplet at 1.42 ppm and a broad multiplet from 1.13 to 1.26 ppm. These are assigned, respectively, to the α and β protons of the carbonyl group and to the remaining CH's of the aliphatic chain. As expected compound **2**, derived from factor A, showed a three-proton triplet at 0.85 ppm, while factor B-derived compound **3** showed a six-proton doublet at 0.85 ppm and a one-proton multiplet at 1.42 ppm. These signals are assigned to the methyl and isopropyl fatty acid termini which distinguish the two pseudoaglycones. Both the NMR and FAB-MS data indicated that the peptide core of A40926 was not affected by the acid treatment.

The single pseudoaglycone (**4** in Fig. 1) obtained from A40926 A+B complex by hydrolysis with

Table 1. NMR of A40926 aglycone.

Proton No.	A40926 aglycone		Teico aglycone		Proton No.	A40926 aglycone		Teico aglycone	
	δ	J (Hz)	δ	J (Hz)		δ	J (Hz)	δ	J (Hz)
x1	5.36	nd	5.47	nd	4b	5.76	s	5.50	2
x2	4.93	ddd	4.92	nd	4f	5.04	s	5.08	2
x3	6.05	10.5	5.35	10	5b	7.08	s	7.07	2
x4	5.54	8.2	5.60	8	5e	6.66	8.5	6.65	8
x5	4.34	5.6	4.33	5	5f	6.73	8.5, 3.1	6.68	8, 2
x6	4.09	12.2	4.10	12, 2					
x7	4.43	5.9	4.42	6	6b	7.75	2.0	7.77	2
z2	2.89	13.0	2.87	14, 3	6e	7.20	8.3	7.19	8
z2′	3.33	13.0, 6.0	3.35	14, 5	6f	7.43	8.3, 2.0	7.43	8, 2
z6	5.09	s	5.10	nd	7d	6.38	2.3	6.39	2
1b	6.76	2.1	6.77	2	7f	6.26	2.3	6.24	2
1e	7.01	8.3	7.02	8	NCH_3	2.50	s	—	—
1f	7.16	8.3, 2.5	7.20	8, 2	w1	9.21	nd	8.53	nd
					w2	8.02	8.3	8.10	8
2b	7.09	nd	7.20	2	w3	7.57	10.5	7.66	10
2c	7.12	8.04	—	—	w4	7.20	8.2	7.53	8
2e	6.98	8.4, 2.5	7.16	8	w5	8.38	5.6	8.38	5
					w6	6.61	12.2	6.62	12
2f	7.73	8.4, 1.9	7.86	8, 2	w7	8.37	5.9	8.40	6
					$NH_2^+(CH_3)$	9.20	nd	8.53	nd
3b	—	—	6.32	2					
3d	6.60	2.8	6.34	2					
3f	6.50	2.8	6.39	2					

s: Singlet, ddd: doublet of doublets of doublets, nd: not determined.
Teico: Teicoplanin.

trifluoroacetic acid has a MW of 1,373, as determined by FAB-MS. This is 343 atomic mass units less than the molecular weight of factor A and 357 less than that of B. These results were consistent with loss of the glycolipid groups. Further evidence for this conclusion came from the ^1H NMR spectrum of **4** and from comparison with the spectra of the starting materials. The spectrum of **4** showed a one-proton singlet at 5.31 ppm, assigned to the anomeric proton of mannose in A40926 factors A and B, but lacked the signals of the glycolipid groups. Further evidence for the presence of mannose at the original site came from the effect of the sugar on the resonances of nearby protons 7d and 7f, which are shifted downfield by 0.3 and 0.2 ppm, respectively, in comparison to the aglycone. In glycopeptide antibiotics, this behavior is typical of protons *ortho* or *para* to a phenol linked to a sugar or acyl-sugar[14]. Since no other significant differences were observed between the spectra of the mannosyl aglycone and the aglycone, the remainder of the molecule appeared to be unaffected by the hydrolysis procedure. Conversion of the *N*-acylaminoglucuronyl and mannosyl aglycones to the aglycone provided further evidence of the structural relationships of these hydrolysis products (see Fig. 2).

Biological Activity

We compared the activities (MIC) of the pseudoaglycones and the aglycone against various species of bacteria with that of the starting material (A40926 A+B complex) (Table 2). The *N*-acylamino-glucuronyl aglycone mixture had activity similar to that of the parent complex against all of these

Table 2. Spectrum of antibacterial activity of A40926 aglycone and pseudoaglycones.

Organism	MIC (μg/ml)			
	A40926 A+B			
	Complex	AcNGluc-aglycone[a]	Mannosyl aglycone	Aglycone
Staphylococcus aureus Tour	0.13	0.13	0.25	0.13
S. epidermidis ATCC 12228	0.25	0.13	0.5	0.13
Streptococcus pyogenes C203	0.06	0.06	1	0.5
S. pneumoniae UC41	0.13	0.13	1	1
S. faecalis ATCC 7080	0.13	0.13	1	0.5
S. mitis L796[b]	0.06	0.06	1	0.5
Clostridium perfringens ISS 30543	0.004	0.008	0.13	0.25
C. difficile ATCC 9689	0.25	0.25	2	1
Propionibacterium acnes ATCC 6919	0.06	0.06	1	1
Bacteroides fragilis ATCC 23745	32	16	64	32
Neisseria gonorrhoeae ISM 68/126	1	4	32	16
Haemophilus influenzae ATCC 10418	128	128	64	32
Escherichia coli SKF 12140	>128	>128	>128	128

[a] Mixture of demannosylated derivatives of A40926 factors A and B.
[b] Clinical isolate.
AcNGluc: *N*-Acylaminoglucuronyl.

Table 3. *In vitro* activity of A40926 aglycone and pseudoaglycones against coagulase-negative Staphylococci (clinical isolates).

Species	Strain	MIC (μg/ml)			
		A40926 A+B			
		Complex	AcNGluc-aglycone	Mannosyl aglycone	Aglycone
Staphylococcus epidermidis	L393[a]	0.5	0.06	2	0.13
S. epidermidis	L408	0.5	0.13	1	0.13
S. epidermidis	L410	1	0.06	2	0.13
S. haemolyticus	L381[a]	2	0.25	8	1
S. haemolyticus	L382[a]	1	0.13	8	0.5
S. haemolyticus	L383[a]	8	0.5	16	2
S. haemolyticus	L403	0.25	0.25	2	0.25

[a] Methicillin-resistant strains.
AcNGluc: *N*-Acylaminoglucuronyl.

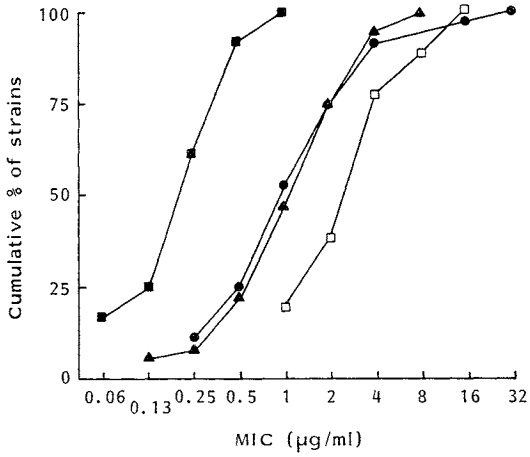
organisms, with the exception of a moderate reduction in anti-*Neisseria* activity. The mannosyl aglycone and the aglycone were less active against Streptococci and Gram-positive anaerobes and lost the anti-*Neisseria* activity of the parent complex.

Table 3 contains MIC data for a series of 7 CNS, all clinical isolates. Four of the strains were methicillin-resistant. The mannosyl aglycone was less active and the aglycone somewhat more active than the A40926 A+B complex. The mixture of *N*-acylaminoglucuronyl aglycones was significantly more active than the parent complex (4- to 16-fold for all but one strain) and than the other hydrolysis products against this group of bacteria.

The pseudoaglycones were further tested against a group of 36 CNS (Fig. 4). The strains, all clinical isolates, included: 12 *Staphylococcus epidermidis*, 8 *S. haemolyticus*, 4 *S. saprophyticus*, 3 *S. hominis*, 2 *S. simulans*, 2 *S. capitis*, 2 *S. warneri*, 2 *S. cohnii*, 1 *S. xylosus*. The *N*-acylaminoglucuronyl

Fig. 4. Activity of the pseudoaglycones against coagulase-negative Staphylococci.

▲ A40926 complex, ■ *N*-acylaminoglucuronyl aglycone, □ mannosyl aglycone, ● teicoplanin.



Bacteria were inoculated onto Oxoid Iso-Sensitest agar with a multipoint replicator. The data are expressed as the cumulative percentage of strains inhibited at each concentration.

Table 4. Activity of A40926 aglycone and pseudoaglycones in mouse septicemia (*Streptococcus pyogenes* C203).

	ED_{50} (mg/kg)	MIC (μg/ml)
A40926 A+B complex	0.35[a]	0.06
N-Acylaminoglucuronyl aglycone mixture	0.54[b]	0.06
Mannosyl aglycone	3.8[a]	1
Aglycone	6.2[b]	0.5
Teicoplanin	0.15[b]	0.06

[a] 5 animals/treatment group.
[b] 10 animals/treatment group.

aglycone mixture was significantly more active than either teicoplanin or the parent A40926 A+B complex against these strains.

The pseudoaglycones and aglycone were also tested for *in vivo* activity in mouse septicemia (Table 4). In this *S. pyogenes* infection, the *N*-acylaminoglucuronyl aglycone mixture had activity similar to that of A40926 A+B complex while the mannosyl aglycone and the aglycone were much less effective. The relative *in vivo* activities of these preparations appeared to reflect their MIC for the infecting strain.

Discussion

Acid hydrolysis of glycopeptide antibiotics under suitable conditions yields pseudoaglycones, which retain at least one of the original sugars, and aglycones[8,15~18]. These derivatives are often biologically active and in some cases (*e.g.* ristocetin and aridicin) they have improved antimicrobial activity against certain microorganisms[6~8]. In contrast, we have found vancomycin aglycone to be significantly less active than the parent compound against a variety of Gram-positive bacteria, including coagulase-negative Staphylococci (RIPAMONTI F., and B. GOLDSTEIN; unpublished data).

We have described procedures for preparing A40926 derivatives in which mannose and/or the glycolipid are removed. The selective removal of mannose by mild acid hydrolysis, to produce *N*-acylaminoglucuronyl aglycones has also been described for aridicins[8], which are structurally related to A40926 antibiotics in containing both mannose and a glycolipid[19].

We found that the demannosylated products could be easily separated into aglycone and *N*-acylaminoglucuronyl aglycone fractions by using the same affinity resin, Sepharose-D-alanyl-D-alanine[10], which we have used extensively in screening for glycopeptides and for purification of A40926 from fermentation broths[1]. This resin is thus a powerful tool for separating glycopeptide mixtures on the basis of their binding capacities. Interestingly, the *N*-acylaminoglucuronyl aglycones, which bound more strongly to D-alanyl-D-alanine, had better activity against several bacterial species (Streptococci, anaerobes, and *N. gonorrhoeae*) than did the more weakly bound aglycone.

The *N*-acylaminoglucuronyl aglycones of A40926 A+B complex (and to a lesser extent the aglycone) had better activity than the parent compound against CNS. The same observation has been made for a pseudoaglycone of ristocetin[6] and for the aglycone and pseudoaglycones of aridicin[8]. CNS isolates are often resistant to several classes of antibiotics[3,4,20] and some, mainly *S. haemolyticus* strains, have exhibited rather high MIC for glycopeptide antibiotics, particularly teicoplanin[3~5,21].

The latter organisms are increasingly being isolated from certain types of patients (*e.g.* those in peritoneal dialysis)[21]. New glycopeptide derivatives with improved activity against CNS may thus prove to be useful in therapy.

The *N*-acylaminoglucuronyl aglycones of A40926 A+B complex were effective *in vivo* against *S. pyogenes* septicemia in the mouse. While they were somewhat less active than teicoplanin and A40926 in this model, their activities seemed to correlate well with the *in vitro* sensitivity of the infecting bacterium to the various preparations.

References

1) GOLDSTEIN, B. P.; E. SELVA, L. GASTALDO, M. BERTI, R. PALLANZA, F. RIPAMONTI, P. FERRARI, M. DENARO, V. ARIOLI & G. CASSANI: A40926, a new glycopeptide antibiotic with anti-*Neisseria* activity. Antimicrob. Agents Chemother. 31: 1961~1966, 1987
2) CHRISTENSEN, S. B.; H. S. ALLAUDEEN, M. R. BURKE, S. A. CARR, S. K. CHUNG, P. DEPHILLIPS, J. J. DINGERDISSEN, M. DIPAOLO, A. J. GIOVENELLA, S. L. HEALD, L. B. KILLMER, B. A. MICO, L. MUELLER, C. H. PAN, B. L. POEHLAND, J. B. RAKE, G. D. ROBERTS, M. C. SHEARER, R. D. SITRIN, L. J. NISBET & P. W. JEFFS: Parvodicin, a novel glycopeptide from a new species, *Actinomadura parvosata*: Discovery, taxonomy, activity and structure elucidation. J. Antibiotics 40: 970~990, 1987
3) WILSON, A. P. R.; M. D. O'HARE, D. FELMINGHAM & R. N. GRÜNEBERG: Teicoplanin-resistant coagulase-negative staphylococci. Lancet 1986-II: 973, 1986
4) DELBENE, V. E.; J. F. JOHN, J. A. TWITTY & J. W. LEWIS: Antistaphylococcal activity of teicoplanin, vancomycin and other antimicrobial agents: The significance of methicillin resistance. J. Infect. Dis. 154: 349~352, 1986
5) ARIOLI, V. & R. PALLANZA: Teicoplanin-resistant coagulase-negative staphylococci. Lancet 1987-I: 39, 1987
6) NIELSEN, R. V.; F. HYLDIG-NIELSEN & K. JACOBSEN: Biological properties of ristocetin-ϕ-aglycone. J. Antibiotics 35: 1561~1564, 1982
7) PHILIP, J. E.; J. R. SCHENCK, M. P. HARGIE, J. C. HOLPER & W. E. GRUNDY: The increased activity of ristocetins A and B following acid hydrolysis. *In* Antimicrobial Agents Annual-1960. *Ed.*, P. GRAY *et al.*, pp. 10~16, Plenum Press, New York, 1961
8) SITRIN, R. D.; G. W. CHAN, F. CHAPIN, A. J. GIOVENELLA, S. F. GRAPPEL, P. W. JEFFS, L. PHILLIPS, K. M. SNADER & L. J. NISBET: Aridicins, novel glycopeptide antibiotics. III. Preparation, characterization, and biological activities of aglycone derivatives. J. Antibiotics 39: 68~75, 1986
9) WALTHO, J. P.; D. H. WILLIAMS, E. SELVA & P. FERRARI: Structure elucidation of the glycopeptide antibiotic complex A40926. J. Chem. Soc. Perkin Trans. I 1987: 2103~2107, 1987
10) CORTI, A. & G. CASSANI: Synthesis and characterization of D-alanyl-D-alanine-agarose: A new bioselective adsorbent for affinity chromatography of glycopeptide antibiotics. Appl. Biochem. Biotechnol. 11: 101~109, 1985
11) FINNEY, D. J.: The spearman-kärber method. *In* Statistical Method in Biological Assay. pp. 524~530, Charles Griffin & Co., Ltd., London, 1952
12) MALABARBA, A.; P. FERRARI, G. G. GALLO, J. KETTENRING & B. CAVALLERI: Teicoplanin, antibiotics from *Actinoplanes teichomyceticus* nov. sp. VII. Preparation and NMR characteristics of the aglycone of teicoplanin. J. Antibiotics 39: 1430~1442, 1986
13) BARNA, J. C. J.; D. H. WILLIAMS, D. J. M. STONE, T.-W. C. LEUNG & D. M. DODDRELL: Structure elucidation of the teicoplanin antibiotics. J. Am. Chem. Soc. 106: 4895~4902, 1984
14) HUNT, A. H.; R. M. MOLLOY, J. L. OCCOLOWITZ, G. G. MARCONI & M. DEBONO: Structure of the major glycopeptide of the teicoplanin complex. J. Am. Chem. Soc. 106: 4891~4895, 1984
15) MCGAHREN, W. J.; R. A. LEESE, F. BARBATSCHI, G. O. MORTON, N. A. KUCK & G. A. ELLESTAD: Components and degradation compounds of the avoparcin complex. J. Antibiotics 36: 1671~1682, 1983
16) MALABARBA, A.; P. STRAZZOLINI, A. DEPAOLI, M. LANDI, M. BERTI & B. CAVALLERI: Teicoplanin, antibiotics from *Actinoplanes teichomyceticus* nov. sp. VI. Chemical degradation: Physico-chemical and biological properties of acid hydrolysis products. J. Antibiotics 37: 988~999, 1984
17) DEBONO, M. (Eli Lilly): Pseudo-aglycone of actaplanin. U.S. 4,322,343, Mar. 30, 1982
18) NAGARAJAN, R. & A. SHABEL (Eli Lilly): Improvements in and relating to novel glycopeptide antibiotics. Eur. Pat. Appl. 0159863, Apr. 16, 1984
19) SITRIN, R. D.; G. W. CHAN, J. J. DINGERDISSEN, W. HOLL, J. R. E. HOOVER, J. R. VALENTA, L. WEEB &

K. M. SNADER: Aridicins, novel glycopeptide antibiotics. II. Isolation and characterization. J. Antibiotics 38: 561~571, 1985

20) BRUMFITT, W.; J. M. T. HAMILTON-MILLER & L. O. NEVILLE: Teicoplanin-resistant coagulase-negative staphylococci. Lancet 1987-I: 328, 1987

21) GRANT, A. C.; R. W. LACEY, A. M. BROWNJOHN & J. H. TURNEY: Teicoplanin-resistant coagulase-negative staphylococcus. Lancet 1986-II: 1166~1177, 1986