

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIRECCIÓN DE NUEVAS CREACIONES

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Patente de Invención ☐ Patente de Modelo de Utilidad ☐
Vía Nacional ☐
Vía PCT (Fase Nacional) ☐ Cap. I ☐ Cap. II ☐

No. Solicitud Internacional PCT/EP2007/008294
Fecha de Presentación Internacional 25 Septiembre 2007

Como resultado del Examen de Fondo realizado, con fundamento en el Artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se comunica:

1. Documentos estudiados

Descripción:	Folios 46 a 75	03/Abril/2009
Reivindicaciones:	Folios 76 a 80	03/Abril/2009
Prioridad:	EP 06020980,6	06/Octubre/2006

2. Análisis de la Prioridad

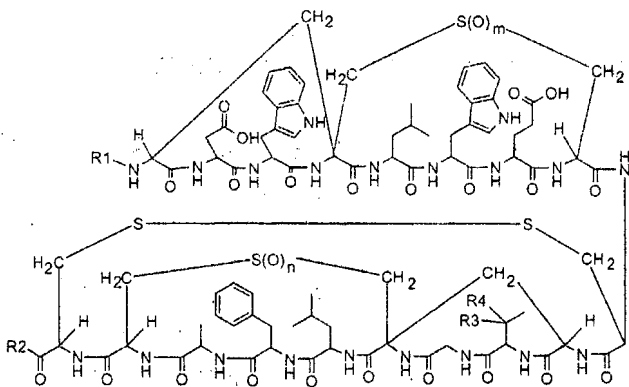
Se acepta la reivindicación de prioridad porque esta solicitud fue presentada dentro del plazo establecido (Art. 9 D 486) y su contenido técnico corresponde con el de la prioridad reivindicada.

3. Objeto de la invención

Título de la solicitud: "Péptidos antibacterianos y antivirales de *Actinomadura namibiensis*"

El problema planteado en esta solicitud consiste en la necesidad de suministrar compuestos útiles para tratar infecciones bacterianas, infecciones virales y/o dolor.

Para resolver este problema técnico la solicitud en estudio proporciona péptidos aislados de la cepa de microorganismos *Actinomadura namibiensis* (DSM 6313) de



El objeto de la presente Solicitud se relaciona con la Clasificación Internacional de Patentes (CIP): C 07 K 14/36, 7/08; A 61 K 38/10; A 61 P 31/04, 31/12; C 12 P 21/02.

4. No es una invención (Art.15 D 486 literal b)

El objeto de las reivindicaciones 1 a 13 que hace referencia a un compuesto aislado de *Actinomadura namibiensis* no se considera una invención, de acuerdo con el Art citado.

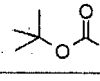
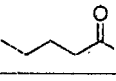
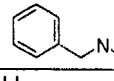
5. Reivindicaciones relativas a un uso o a un segundo uso (Art 14 y 21 D 486)

El uso de la reivindicación 14 no es patentable, de acuerdo con el Art 14.

6. De la Solicitud de Patente

Reivindicaciones (Art 30 D 486, Art 22 PCT)

En la descripción no se encuentra sustento para R2 = NH₂, NH-alquilo(C₁-C₆), NH-alquileno(C₁-C₄)-piridilo, m y n = 1 ó 2. Los radicales sustentados son los siguientes:

Radical	Ejemplos Sustentados en la Descripción
R1	H,  , 
R2	OH, 
R3	H, OH
R4	H, OH
R3 y R4	=O
n, m	0

Estas sustituciones pueden restringirse aún más teniendo en cuenta el compuesto preferido que se presenta en la reivindicación 12. Adicionalmente, sólo existe sustento de la evaluación de la actividad del péptido aislado de *Actinomadura namibiensis* de fórmula (III), donde R1= H, R2= OH, R3= H, R4= OH, n y m= 0. Aunque los demás sustituyentes están sustentados en la descripción como productos de la oxidación y de modificaciones en los extremos C y N terminal del compuesto de fórmula (III), no existe información suficiente para comprender la relevancia de dichas modificaciones ya que el único compuesto evaluado es el aislado de *Actinomadura namibiensis*. Se sugiere restringir la solicitud a una razonable generalización del tipo de compuestos que ha sido probado.

El capítulo reivindicatorio no es conciso porque contiene un excesivo número de reivindicaciones que son redundantes entre sí, lo cual hace difícil definir cuál es el

El capítulo reivindicatorio no es conciso porque contiene un excesivo número de reivindicaciones que son redundantes entre sí, lo cual hace difícil definir cuál es el objeto que se busca proteger. Por ejemplo la reivindicación 10 contiene la misma materia de la reivindicación 9 y esta a su vez es redundante con la reivindicación 7. Se sugiere eliminar las reivindicaciones que restan concisión al capítulo reivindicatorio.

La reivindicación 13 no es clara porque el compuesto se define por medio de parámetros cuando puede definirse de otra manera. Se sugiere definir el compuesto por sus características esenciales, estructurales.

La reivindicación 15 no es clara porque la expresión "ingrediente farmacéuticamente aceptable" no permite comprender el alcance de la invención. Se sugiere definir los "ingredientes farmacéuticamente aceptables" por medio de su nombre IUPAC e incluir las proporciones en las que cada una de ellas se encuentra dentro de la composición. Adicionalmente, no se encuentra soporte en la descripción para la preparación de una composición farmacéutica. Se sugiere eliminar la reivindicación 15 o modificar la descripción de tal forma que la composición farmacéutica se encuentre soportada.

La reivindicación 16 no es clara porque los términos "aislamiento", "derivatización", "conversión en una sal", son etapas generales que no permiten comprender el alcance de la invención. De igual forma la expresión "condiciones adecuadas" no permite comprender cuales son las condiciones necesarias para llevar a cabo el proceso. Se sugiere caracterizar adecuadamente cada una de las etapas del proceso que se consideran novedosas y/o inventivas y las condiciones de reacción específicas. Adicionalmente, la expresión "o una de sus variantes y/o mutaciones" no es concisa. Se sugiere al solicitante especificar las cepas que hacen parte de la invención e incluir el certificado de depósito para cada una de éstas.

La reivindicación 19 no se encuentra sustentada en la descripción ya que no se divulga un proceso donde m y n son los dos 2, o m es 0 y n es 2, o m es 2 y n es 0. Se sugiere eliminar la reivindicación 19.

7. Determinación del Estado de la Técnica

El estado de la técnica se determinó con base en la fecha de presentación de la solicitud de prioridad (Octubre 06 de 2006) y se encontraron los siguientes documentos que anticipan el objeto de esta solicitud:

Ítem	Documento	Título	Fecha de Publicación
D1	Goldstein BP. Antimicrobial agents and chemotherapy. 1987;31(12):1961-6	A40926, a new glycopeptide antibiotic with anti-Neisseria activity	Diciembre/1987

D1 <http://www.ncbi.nlm.nih.gov/pubmed?term=Antimicrob%20Agents%20Chemother.%201987%20Dec%3B31%2812%29%3A1961-6> divulga (Abstract; Columna 1, párrafo 2; Folio 120, anverso) un procedimiento para aislar un compuesto con actividad antibacteriana de un actinomiceto del género *Actinomadura* que incluye las etapas de fermentación y purificación.

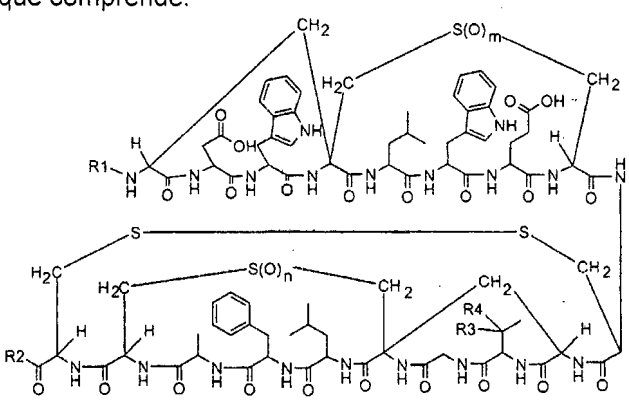
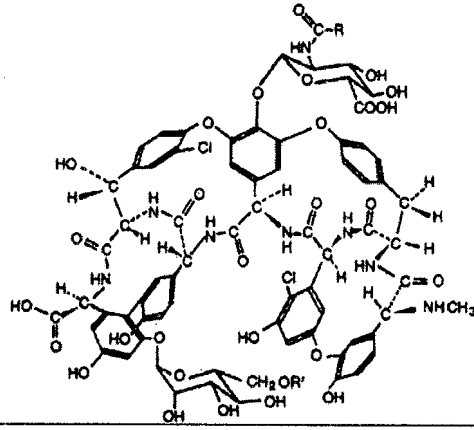
8. Examen de Patentabilidad (Art 14 D486)

Nivel Inventivo (Art18 D486)

La **reivindicación 16** consiste en un proceso para preparar un compuesto de la fórmula (I) de acuerdo con una cualquiera de las reivindicaciones 1 a 13, que comprende:

- a) Fermentación de la cepa *Actinomadura namibiensis* (DSM 6313), o una de sus variantes y/o mutaciones, en condiciones adecuadas en un medio de cultivo hasta que uno o más de los compuesto de la fórmula (I) se acumulen en el medio de cultivo.
- b) Aislamiento de un compuesto de la fórmula (I) a partir del medio de cultivo, y
- c) Derivatización del compuesto de la fórmula (I), cuando sea apropiado, y/o, cuando sea apropiado, conversión en una sal fisiológicamente tolerada

Comparación de las características técnicas **esenciales**, con las del Estado de la Técnica:

Características esenciales	D1
Un proceso para preparar un compuesto de fórmula (I) que comprende: 	Un proceso para preparar un compuesto que comprende: 
Fermentación de la cepa <i>Actinomadura namibiensis</i> (DSM 6313), o una de sus variantes y/o mutaciones, en condiciones adecuadas en un medio de cultivo hasta que uno o más de los compuesto de la fórmula (I) se acumulen en el medio de cultivo.	Fermentación de un actinomiceto del género <i>Actinomadura</i>
Aislamiento de un compuesto de la fórmula (I) a partir del medio de cultivo, y	Aislamiento del compuesto A40926 del medio de cultivo
Opcionalmente derivatización del compuesto de la fórmula (I) y/o conversión en una sal fisiológicamente tolerada	No se menciona

El documento **D1** se considera el estado de la técnica cercano a la invención definida en la reivindicación 1 porque divulga un procedimiento para aislar un compuesto con actividad antibacteriana de un actinomiceto del género *Actinomadura* (Abstract; columna 1, párrafo 2; ver folio 120, anverso). El compuesto A40926 se obtiene por fermentación y luego se purifica (Columna 1, párrafo 2; ver folio 120, anverso). El caldo de fermentación se pasa sobre una resina de afinidad Sefarosa-D-alanil-D-alanina y se eluye el compuesto adsorbido con amonio acuoso al 1% para obtener el compuesto puro en un paso (Columna 1, párrafo 3; ver folio 120, anverso).

La diferencia entre la invención, definida en la reivindicación 1 y el procedimiento de D1 consiste en que la cepa de *Actinomadura* usada para obtener el compuesto de fórmula (I) es específicamente *Actinomadura namibiensis*.

El efecto que logra el incluir esta cepa es que se obtiene un compuesto de fórmula (I).

Por lo tanto, el problema técnico objetivo que pretende resolver esta invención se puede formular así: "cómo modificar el procedimiento conocido en D1 para aislar péptidos alternativos de un actinomiceto del género *Actinomadura* con actividad antibacteriana".

Teniendo en cuenta que el documento D1 ya divulgaba un compuesto con actividad antibacteriana aislado de un actinomiceto del género *Actinomadura*, la persona del oficio normalmente versada en la materia, habría inferido la inclusión en el procedimiento de otra cepa de *Actinomadura* como *Actinomadura namibiensis* para obtener péptidos alternativos con la misma actividad; por lo tanto, la reivindicación 16 se considera obvia.

Puesto que las reivindicaciones dependientes 17 a 19 no mencionan características inventivas adicionales tampoco se pueden considerar inventivas.

Conclusión:

Las reivindicaciones 16 a 19 cumplen el requisito de novedad (Art. 16), pero no cumplen con el requisito de nivel inventivo (Art. 18).

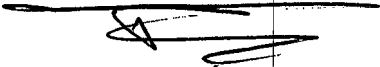
9. Conclusión

Los requisitos de patentabilidad de la presente solicitud están afectados así:

Ítem	Documento	Reivindicaciones afectadas	Requisito que afecta
D1	Goldstein BP. Antimicrobial agents and chemotherapy. 1987;31(12):1961-6	16 – 19	Nivel inventivo

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta (60) días contados a partir de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única No. 10 de 2001.


JOSÉ LUIS SALAZAR LÓPEZ

Director de Nuevas Creaciones (C)

El anterior requerimiento fue notificado por fijación en lista, de fecha 28 AGO. 2012

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Aprobado por: José Luis Salazar López



A40926, a New Glycopeptide Antibiotic with Anti-*Neisseria* Activity

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In the course of a search for glycopeptide antibiotics having novel biological properties, we isolated A40926. Produced by an actinomycete of the genus *Actinomadura*, A40926 is a complex of four main factors which contain a fatty acid as part of a glycolipid attached to the peptide backbone. Its activity was, in most respects, similar to that of other glycopeptides, such as vancomycin and teicoplanin. However, in addition to inhibiting gram-positive bacteria, A40926 was very active against *Neisseria gonorrhoeae*. A40926 was rapidly bactericidal for *N. gonorrhoeae* clinical isolates at concentrations equal to or slightly higher than the MIC. In mice, levels in serum were higher and more prolonged than those of an equivalent subcutaneous dose of teicoplanin. These properties suggest that A40926 may have potential in the therapy of gonorrhea.

The increased frequency of isolation of multiply antibiotic-resistant gram-positive bacteria has led to wider use of antibacterial agents which were originally rarely considered for first-line therapy, such as the glycopeptide vancomycin (18). One of the advantages of this antibiotic is that, thus far, resistance is rare and restricted to certain coagulase-negative staphylococci (25). The success of vancomycin has stimulated the development of new glycopeptides, such as teicoplanin (5, 13, 32, 33). These antibiotics inhibit cell wall biosynthesis by binding to the D-alanyl-D-alanine portion of nascent peptidoglycan (22). Specific binding to an affinity resin containing acyl-D-alanyl-D-alanine groups (9) formed the basis of our search for new glycopeptides.

Among the new glycopeptides identified, we focused our attention on those having novel biological properties. A40926 is the most interesting antibiotic which has emerged thus far from our screening campaign. In fermentation broths, we found it as a complex of four factors (PA, PB, A, and B). PA and PB, described here for the first time, predominated in the broth; during purification, these two compounds were largely converted to A and B, whose structures have been described recently (31a). One feature of all four factors, which relates them to teicoplanin and some other glycopeptides (aridicins and kibdellins) (3, 27, 12) while distinguishing them from vancomycin, ristocetin, and others, is the presence of a fatty acid moiety attached to one of the sugars. In terms of biological activity, the most striking difference between the components of the A40926 complex and other glycopeptide antibiotics is their activity against *Neisseria gonorrhoeae*.

MATERIALS AND METHODS

Isolation of A40926. We screened specifically for glycopeptide antibiotics by passing fermentation broths over the affinity resin Sepharose-D-alanyl-D-alanine (9) and eluting adsorbed glycopeptides with 1% aqueous ammonia. The strain producing A40926 was found in a soil sample collected in India. Batch adsorption to the same affinity resin described above yielded nearly pure A40926 complex in one step. Quick neutralization of the ammonia eluate yielded primarily the two factors of the complex (PA and PB) which

predominated in the fermentation broth. After prolonged incubation at high pH, these components were gradually converted into factors A and B. The A40926 complex used in the microbiological studies consisted of 65% B and 35% A.

The structures of factors PA and PB were elucidated by fast atom bombardment mass spectrometry and ¹H nuclear magnetic resonance spectroscopy at 250 MHz in dimethyl sulfoxide d-6 as described for factors A and B (31a).

Other glycopeptide antibiotics. Actaplanin, A35512B, A41030A, and A47934 were obtained from Eli Lilly & Co.; avoparcin was from Lederle Laboratories. Ristocetin was purchased from Sigma Chemical Co., and aridicin (14) was prepared by fermentation of *Kibdellosporangium aridum* ATCC 39323.

Preliminary antibacterial activity of the factors of the complex. The MICs for *Clostridium difficile*, *Propionibacterium acnes*, *Bacteroides fragilis*, and *Haemophilus influenzae* were determined by agar dilution (inocula, 10⁴ CFU). MICs for other organisms were determined by broth microdilution (inocula, 10⁴ to 10⁵ CFU/ml) for *Clostridium perfringens* and by broth microdilution for other bacteria. Incubation times were 18 to 24 h, except for *N. gonorrhoeae*, *H. influenzae*, *C. difficile*, *Propionibacterium acnes*, and *B. fragilis* (48 h). All organisms were incubated at 37°C. *N. gonorrhoeae* and *H. influenzae* were incubated in a 5% CO₂ atmosphere; anaerobic bacteria were incubated in an anaerobic gas mixture. The media used were Iso-Sensitest broth (Oxoid Ltd.) (staphylococci, *Streptococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*); Todd-Hewitt broth (Difco Laboratories) (other streptococci); GC base broth (Difco) plus 1% IsoVitaleX (BBL Microbiology Systems) (*N. gonorrhoeae*); GC base agar plus 1% IsoVitaleX and hemin (10 µg/ml) (*H. influenzae*); AC medium (Difco) (*C. perfringens*); Wilkins-Chalgren agar (Oxoid) (other anaerobic bacteria).

MIC of A40926 complex for gram-positive bacteria and neisseriae. MIC studies were performed by agar dilution with inocula of 10⁴ CFU. The media used were Iso-Sensitest agar for staphylococci, *Streptococcus faecalis*, and *Streptococcus faecium*; Todd-Hewitt agar (Difco) for other streptococci; and GC base agar (Difco) plus 1% IsoVitaleX and hemin (10 µg/ml) for neisseriae. Neisseriae were incubated for 48 h in 5% CO₂; *Streptococcus sanguis*, *Streptococcus salivarius*, and *Streptococcus mutans* strains were incubated

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for 48 h in an anaerobic gas mixture; *Streptococcus pneumoniae* was incubated for 40 h and other species were incubated for 24 h in a normal atmosphere.

Bactericidal activity. Inocula were at least 10^6 CFU/ml. The time-kill experiment with *Streptococcus faecalis* was performed in Todd-Hewitt broth with log-phase inocula. At the indicated sampling times, duplicate 0.1-ml samples of suitable dilutions (at least 10-fold in saline with peptone) were plated, by inclusion in 2.5 ml of Todd-Hewitt soft agar, on Todd-Hewitt agar plates. For *N. gonorrhoeae* MBC and time-kill studies, contact with antibiotics was in GC base broth with 1% IsoVitaleX. Inocula were prepared from 24-h slants; cultures were grown to log phase before addition of antibiotics. At the indicated sampling times, triplicate 0.01-ml samples were dripped across the surface of plates containing GC base agar plus 1% IsoVitaleX and hemin (10 μ g/ml). The lowest concentration which killed at least 99.9% of the initial inoculum was considered the MBC. Preliminary studies in which antibiotic was added immediately before plating showed that under our experimental conditions, initial concentrations of up to 16 times the MIC had no effect on the plating efficiency of staphylococci, *Streptococcus faecalis*, or *N. gonorrhoeae*.

Experimental septicemia in mice. Each control and treatment group contained five male and five female CD-1 mice (Charles River Breeding Laboratories) weighing 18 to 22 g. They were infected intraperitoneally with 0.5 ml of bacterial suspension prepared by diluting an overnight culture with sterile saline plus peptone (*Streptococcus pyogenes* and *Streptococcus pneumoniae*) or with 5% bacteriological mucin (Difco) (*Staphylococcus aureus*). Inocula were adjusted so that untreated animals died of septicemia within 48 h (2). Antibiotics were administered subcutaneously immediately after infection. The 50% effective dose (ED_{50}) (dose at which 50% of animals survived) was calculated by the method of Spearman and Kärber (11) from the percentages of surviving animals at each dose on day 7.

Pharmacokinetics. For each sampling time, five male and five female mice were treated subcutaneously with 10 mg of A40926 factor B per kg. Blood (0.5 ml per animal) was collected by cardiac puncture; serum from pooled blood was assayed by the agar diffusion method on antibiotic medium 3 (Difco) (pH 6) supplemented with 1% Noble agar (Difco), 6.65% NaCl, 10% (vol/vol) 1 M pH 4.2 phosphate buffer, and 0.5% (vol/vol) bovine serum. The test organism was *S. aureus* ATCC 6538; the assay is sensitive to 1 μ g of factor B per ml.

RESULTS

Characterization and taxonomic identification of the producer strain. The substrate mycelium is composed of branched hyphae. The aerial hyphae form short and compact closed spirals, some of them with a hooklike structure, containing chains of 10 to 20 spores. The spores are irregularly shaped, roughly globular-cylindrical, with an average size of 0.8 by 1.2 μ m. The strain grows well on most media used to study its cultural characteristics (26, 31) and produces a red-violet pigment on oatmeal agar.

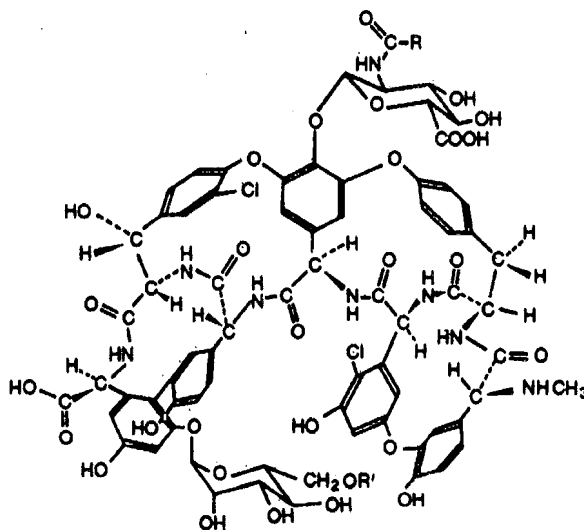
In the whole-cell hydrolysate (4, 21), madurose was the diagnostic sugar. Analysis of cell wall preparations (17, 19, 29) showed the presence of *meso*-diaminopimelic acid and the absence of glycine. These features identified the strain as an actinomycete of cell wall type IIIB (20). This information, together with the morphological characteristics of the strain and the absence of mycolic acids, led us to classify it in the

genus *Actinomadura*. The strain has been deposited with the American Type Culture Collection as ATCC 39727.

Structure of the components of the complex. Figure 1 shows the structures of A40926 factors PA, PB, A, and B. All four contained two chlorine atoms and two sugars, mannose, and *N*-acylaminoglucuronic acid. The acyl groups were *n*-undecanoic acid in PA and A and isododecanoic acid in PB and B. Among the glycopeptides, teicoplanin, aridicins, and kibdellins also contain fatty acid moieties (3, 12, 27). Another similarity between A40926 and the aridicins was the presence of two carboxylic acid functions which gave these molecules a net negative charge at neutral pH. From the molecular weight differences and the nuclear magnetic resonance data, we determined that A and B differed from PA and PB in the loss of an acetyl group at position C-6 of mannose.

Preliminary characterization of antibacterial activity. We compared the MICs of A40926 A+B complex and of pure factors A, B, PA, and PB with those of teicoplanin for single strains of various bacterial species (Table 1). Against the gram-positive bacteria, the MICs of the four A40926 factors were similar to those of teicoplanin. The activity of A40926 against *N. gonorrhoeae* stood out from a spectrum otherwise similar to that of teicoplanin. In common with other glycopeptides, A40926 was not active against the other gram-negative organisms tested (Table 1) or against mycoplasmas (data not shown).

As the anti-*Neisseria* activity of glycopeptides had not been previously described, we tested all the available compounds of this class under our own experimental conditions. Activity might have been missed if chocolate agar had been used; we have observed that the denatured hemoglobin



Factor	R	R'	MW
A	(CH ₂) ₁₀ CH ₃	H	1716
PA	(CH ₂) ₁₀ CH ₃	COCH ₃	1758
B	(CH ₂) ₁₀ CH(CH ₃) ₂	H	1730
PB	(CH ₂) ₁₀ CH(CH ₃) ₂	COCH ₃	1772

FIG. 1. Structures of the four principal factors of A40926 complex. MW, Molecular weight.

TABLE 1. Spectrum of antibacterial activity of A40926

Organism	MIC (µg/ml)					
	Teicoplanin	A40926 ^a				
		A+B complex	Factor A	Factor B	Factor PA	Factor PB
<i>Staphylococcus aureus</i> Tour	0.25	0.13	0.13	0.13	0.5	1
<i>Streptococcus pyogenes</i> C203	0.06	0.06	0.06	0.06	0.06	0.03
<i>Streptococcus pneumoniae</i> UC41	0.06	0.06	0.06	0.06	0.06	0.06
<i>Streptococcus faecalis</i> ATCC 7080	0.13	0.13	0.13	0.13	0.06	0.13
<i>Clostridium perfringens</i> ISS 30543	0.03	0.004	0.016	0.016	0.008	0.008
<i>Clostridium difficile</i> ATCC 9689	0.25	0.13	0.25	0.13	0.25	0.13
<i>Propionibacterium acnes</i> ATCC 6919	0.13	0.016	0.03	0.016	0.03	0.03
<i>Bacteroides fragilis</i> ATCC 23745	128	64	64	64	64	64
<i>Neisseria gonorrhoeae</i> ISM 68/126	32	1	1	0.5	4	2
<i>Haemophilus influenzae</i> ATCC 19418	128	64	64	64	64	64
<i>Escherichia coli</i> SKF 12140	>128	>128	>128	>128	>128	>128
<i>Proteus vulgaris</i> ATCC 881	>128	>128	>128	>128	>128	>128
<i>Pseudomonas aeruginosa</i> ATCC 10145	>128	>128	>128	>128	>128	>128

^a The four factors of the complex were isolated by reverse-phase high-pressure liquid chromatography.

present in this medium reduces the activity of glycopeptides (R. Pallanza and R. Scotti, unpublished data). The MICs of these antibiotics for test strains of various species are shown in Table 2. The activities of most of these compounds against several gram-positive species have been compared previously (14). Among the glycopeptides we tested, A40926 was the most active against our standard test strain of *N. gonorrhoeae*. A47934, which is an aglycone (7) (i.e., it contains no sugars), was moderately active against *N. gonorrhoeae*, but less so than A40926. The other glycopeptide-aglycone, A41030A (10), was not active.

In vitro activity against staphylococci and streptococci. We compared the MICs of A40926 A+B complex and teicoplanin for a total of 132 clinical isolates (Table 3). More than half of the *Staphylococcus* strains were methicillin resistant (MR). In our analysis, we combined MR and methicillin-susceptible (MS) strains because previous studies with our collection failed to reveal methicillin-related differences in susceptibilities to glycopeptides (1). Nine of 10 *Staphylococcus haemolyticus* (including two MS strains), three MR *Staphylococcus epidermidis*, five of the six MR *S. aureus*, and two other MS coagulase-negative staphylococci were resistant to gentamicin. With one exception, the species or groups of species showed the same median MIC for A40926 and teicoplanin; their median MICs differed (by a factor of 2) only for *Streptococcus faecalis*.

The bactericidal activities of these two antibiotics against staphylococci and *Streptococcus faecalis* were also similar.

A representative experiment, in which a *Streptococcus faecalis* strain was exposed to antibiotic concentrations 8 and 16 times the MIC, is shown in Fig. 2. Vancomycin was not bactericidal for this strain under these conditions.

Anti-*Neisseria* activity of A40926. Table 4 summarizes our MIC data for *N. gonorrhoeae* clinical isolates, including penicillinase-producing *N. gonorrhoeae*, chromosomally determined penicillin-resistant *N. gonorrhoeae*, and spectinomycin-resistant strains. The median MIC of A40926 for the 37 strains was 1 µg/ml, compared with 16 µg/ml for teicoplanin and 32 µg/ml for vancomycin. The activity of A40926 against *N. gonorrhoeae* was thus quite similar to its activity against coagulase-negative staphylococci (Table 3). The MBC, determined for seven strains of *N. gonorrhoeae*, was equal to or twice the MIC even after only 6 h of contact (data not presented).

The bactericidal activity of A40926 against *N. gonorrhoeae* was fairly rapid. From 99.9 to 99.99% killing of four clinical isolates (including a spectinomycin-resistant and a penicillinase-producing strain) occurred within 5 h of exposure to concentrations four to eight times the MIC (Fig. 3).

A40926 was somewhat less active against *Neisseria meningitidis*. The MIC range for five standard strains from serological groups A through D was 4 to 16 µg/ml.

Activity of A40926 in experimental septicemia in mice. We compared the ED₅₀s of teicoplanin and A40926 factor B in animals infected with various gram-positive species (Table 5). A40926 was slightly less active than teicoplanin in these

TABLE 2. Comparison of antibacterial activity of various glycopeptides

Compound	MIC (µg/ml) for ^a :				
	<i>Neisseria gonorrhoeae</i>	<i>Streptococcus pyogenes</i>	<i>Streptococcus faecalis</i>	<i>Staphylococcus aureus</i>	<i>Staphylococcus epidermidis</i>
A40926	2	0.06	0.06	0.06	0.06
A47934	8	0.13	0.13	0.06	0.03
Teicoplanin	32	0.06	0.13	0.13	0.13
Vancomycin	32	0.13	0.5	0.25	0.5
Ristocetin	64	0.25	1	4	2
A35512B	64	0.13	0.5	1	0.5
A41030A	64	0.13	0.13	0.03	0.008
Aridicin	64	0.13	2	1	4
Avoparcin	128	0.25	0.25	2	2
Actaplanin	>128	0.13	0.5	1	2

^a Same strains as in Table 1, plus *Staphylococcus epidermidis* (ATCC 12228).

TABLE 3. MICs of A40926 for staphylococci and streptococci

Organism (no. of isolates) ^a	MIC (μg/ml) ^b					
	Teicoplanin			A40926 A + B complex		
	Range	50%	90%	Range	50%	90%
<i>Staphylococcus aureus</i> (6/6)	0.5–1	0.5	1	0.25–0.5	0.25	0.5
<i>Staphylococcus epidermidis</i> (7/5)	0.25–4	1	2	0.13–4	1	4
<i>Staphylococcus haemolyticus</i> (9/3)	1–32	4	16	0.5–16	4	8
Other staphylococci ^c (9/7)	0.25–4	1	2	0.13–4	1	4
<i>Streptococcus pyogenes</i> (11)	0.25	0.25	0.25	0.25	0.25	0.25
<i>Streptococcus pneumoniae</i> (10)	0.25	0.25	0.25	0.25	0.25	0.25
<i>Streptococcus faecalis</i> (10)	0.25–0.5	0.25	0.25	0.06–0.25	0.13	0.25
<i>Streptococcus faecium</i> (10)	0.13–0.5	0.5	0.5	0.06–0.5	0.5	0.5
<i>Streptococcus agalactiae</i> (10)	0.25–0.5	0.25	0.5	0.25–0.5	0.25	0.5
<i>Streptococcus mutans</i> (13)	0.25–2	1	2	0.13–1	1	1
Other streptococci ^d (16)	0.25–1	0.5	0.5	0.25–1	0.5	1

^a For staphylococci, shown as number MR/number MS.
^b 50% and 90%, MIC for 50 and 90% of isolates, respectively.
^c *S. simulans*, *S. capitis*, *S. warneri*, *S. cohnii* (two strains each); *S. xylosus* (one strain) *S. hominis* (three strains), and *S. saprophyticus* (four strains).
^d *S. salivarius* (seven strains), *S. sanguis* (six strains), and *S. bovis* (three strains).

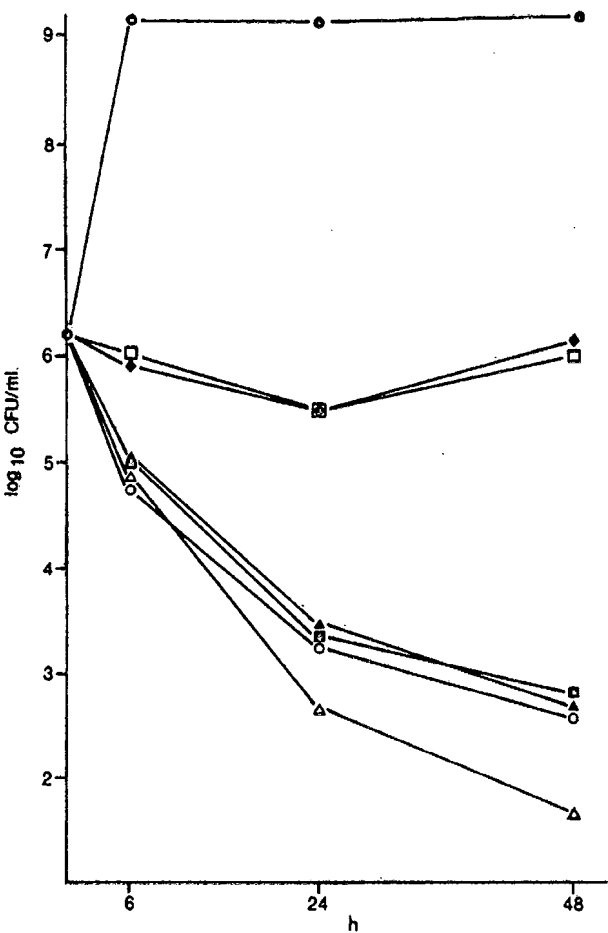


FIG. 2. Bactericidal effect of A40926 against *Streptococcus faecalis* ATCC 7080. Concentrations of antibiotics used were 8 and 16 times the MIC determined under the same culture conditions used in this experiment: A40926 A+B complex, 8 (○) and 16 (Δ) μg/ml; teicoplanin, 8 (◻) and 16 (▲) μg/ml; vancomycin, 16 (◻) and 32 (◆) μg/ml; ●, untreated control.

model systems. In *Streptococcus pneumoniae* infections, A40926 complex and factors A, PA, and PB all had activity similar to that of factor B (data not presented).

Pharmacokinetics of A40926 in mice. We compared the levels of A40926 factor B and teicoplanin in serum after a single subcutaneous administration of 10 mg/kg (Fig. 4). At this dose, the peak level in serum of A40926 was much higher than that of teicoplanin, and significant concentrations of A40926 were maintained for a longer period of time.

DISCUSSION

By using an affinity resin to screen fermentation broths, we were able to identify strains which produced glycopeptides even when other types of antibiotics were produced by the same strain. This approach was therefore more efficient than methods based on the reversal of antibacterial activity when antibiotic preparations are mixed with acyl-D-alanyl-D-alanine (23, 28). The latter method may miss glycopeptides when other nonsuppressed antibiotic activities are present.

A40926 complex emerged from this screening campaign. Although structurally very similar to some other previously described glycopeptide antibiotics, in particular the aridicins (27), A40926 was unique in its activity against *N. gonorrhoeae*. In all other respects, its in vitro antibacterial activity appears, thus far, to be similar to that of teicoplanin and vancomycin. Another novel feature of A40926 was its high levels in serum and long half-life in the mouse compared with teicoplanin and vancomycin (5, 30).

A40926 was somewhat less active than teicoplanin in the treatment of gram-positive septicemia in mice, even though the infecting organisms had the same in vitro susceptibilities

TABLE 4. MICs of A40926, teicoplanin, and vancomycin for 37 clinical isolates of *N. gonorrhoeae*^a

Antibiotic	MIC (μg/ml) ^b		
	Range	50%	90%
A40926	0.5–4	1	2
Teicoplanin	4–64	16	32
Vancomycin	8–>128	32	64

^a The isolates included penicillin-resistant and spectinomycin-resistant strains.
^b 50% and 90%, MIC for 50 and 90% of isolates, respectively.

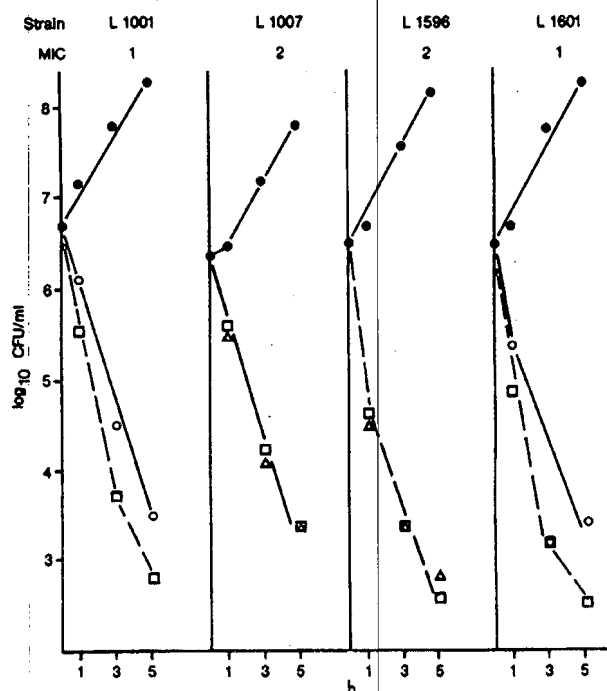


FIG. 3. Bactericidal effect of A40926 against four clinical isolates of *N. gonorrhoeae*. Concentrations of A40926 A+B complex were four and eight times the MIC for each strain, determined under the same culture conditions used in this experiment. Symbols: ●, untreated control; ○, 4 µg/ml; □, 8 µg/ml; △, 16 µg/ml. Strain L1596 is spectinomycin resistant, and L1601 is penicillin resistant.

to both antibiotics. One feature of our infection model is rapid dissemination of bacteria to various organs (2). In our experience, differences in ED₅₀ as low as two- to threefold are of little significance in this system. The pharmacokinetics of A40926 and teicoplanin in the mouse have thus far been compared at the single dose of 10 mg/kg. The peak levels of the two antibiotics might be more similar at the lower doses used to determine the ED₅₀. (It is not possible to assay blood levels at these doses.) Furthermore, the potential advantages of a long-lasting antibiotic may fail to emerge in such an acute infection. Another factor which may affect the ED₅₀ is serum binding. Preliminary data, based only on the MIC in the presence of various concentrations of bovine serum, indicate that A40926 may be bound somewhat more strongly than teicoplanin. Any or all of these factors might account for the modest differences in ED₅₀.

Although we cannot yet fully account for the unusual activity of A40926 against *N. gonorrhoeae*, the antibiotic has

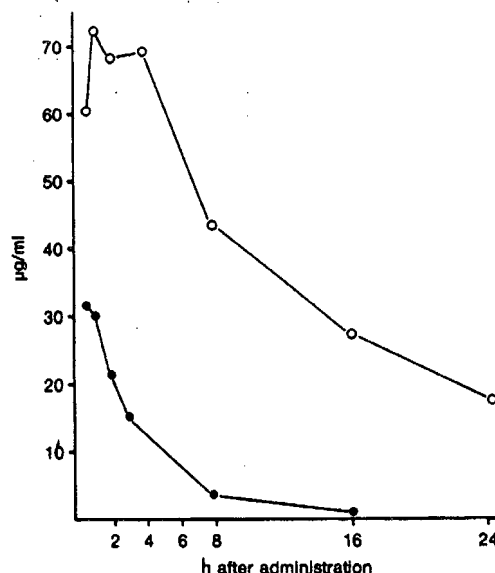


FIG. 4. Levels of A40926 factor B and teicoplanin in mouse serum. Groups of five male and five female mice were treated once with antibiotic at 10 mg/kg. Serum samples from pooled blood samples were assayed. Symbols: ○, A40926 factor B; ●, teicoplanin.

two structural features which correlate with its longer half-life of elimination (14). One is its net negative charge at neutral pH; in this, A40926 resembles the aridicins (27). The second feature is the lipophilicity conferred by the fatty acid moiety attached to the *N*-aminoglucuronic acid. Along with teicoplanin, aridicins, and kibdellins, A40926 might be better described as a lipoglycopeptide.

N. gonorrhoeae is an organism in which resistance to two of the most widely used classes of therapeutic drugs, penicillins and tetracyclines, has been spreading worldwide (6, 15). Some penicillin-resistant strains are also relatively resistant to cephalosporins and erythromycin (24). Spectinomycin is an effective antgonorrheal agent which, like penicillin, has the advantage of single-dose therapy. Sporadic occurrences of primary resistance, as well as of resistance secondary to therapy, are, however, cause for concern (8, 16). A totally new class of antgonorrheal drug could thus be of great interest. In the field of gram-positive infections, in which vancomycin has been used for a number of years, resistance to glycopeptide antibiotics is rare among normally sensitive bacterial species. The pharmacokinetics of A40926 and its activity against *N. gonorrhoeae* suggest that this new glycopeptide may have potential in single-shot therapy of gonorrhea.

TABLE 5. Activity of A40926 in experimental septicemia in mice^a

Organism	ED ₅₀ (mg/kg)	
	Teicoplanin	A40926 factor B
<i>Staphylococcus aureus</i> Tour	0.72	1.9
<i>Streptococcus pyogenes</i> C203	0.17	0.33
<i>Streptococcus pneumoniae</i> UC41	0.12	0.38

^a Single subcutaneous administration of antibiotic immediately after intraperitoneal infection. The ED₅₀ was calculated at day 7 from the percentages of animals surviving at each treatment level (11).

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