

**SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIRECCIÓN DE NUEVAS CREACIONES**

2020
Bogotá, D.C.,

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Apoderado

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



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	Folios	08

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Patente de Invención ☐ Patente de Modelo de Utilidad ☐

Vía Nacional ☐

Vía PCT (fase nacional) ☐ Cap.I ☐ Cap. II ☐

No. Sol. Internacional PCT/EP2005/011363
Fecha de Presentación Internal. 22 de octubre de 2005

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 le comunica:

1. Documentos estudiados

<u>Descripción:</u>	Folios: <u>61</u> a <u>125</u> y <u>130</u> como se radicó inicialmente
<u>Reivindicaciones:</u>	Folios <u>126</u> a <u>129</u> como se radicó inicialmente
<u>Prioridad:</u>	Folios: <u>01</u> 05/11/2004, DE, 102004053407.1

2. Análisis de la Prioridad

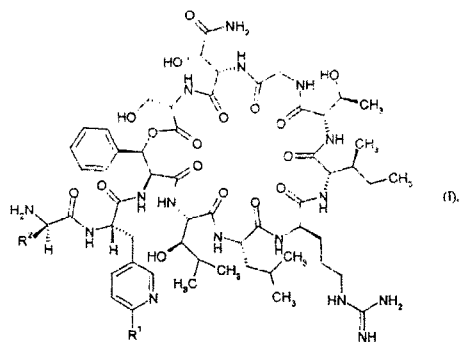
La fecha de prioridad del 05 de noviembre de 2004 y su correspondiente documento, corresponden con la materia solicitada de protección.

3. Objeto de la invención

Título de la solicitud: Nonadepsipéptidos acilados como derivados de lisobactina

El problema planteado en esta solicitud es la necesidad de obtener antimicrobianos derivados de lisobactina con menores efectos secundarios y mayor actividad biológica

Para solucionar este problema técnico la solicitud en estudio, el solicitante proporciona un compuesto de fórmula I:



IPC: C07K 11/02, 5/06

4. De la solicitud de Patente

Descripción (artículo 30 D 486)

La reivindicación 6 y 10 se encuentran caracterizadas por sus uso en un método de tratamiento.

5. Excepción a la patentabilidad (artículo 20 D486 literal a)-d))

La reivindicación 11 se refiere a métodos de tratamiento, los cuales no son patentables de acuerdo a los artículos mencionados de la normatividad vigente.

6. Reivindicaciones relativas a un uso o a un segundo uso (artículos 14 y 21 D 486)

Las reivindicaciones 7 y 8, se refieren al uso del compuesto, las cuales no son patentables de acuerdo a los artículos mencionados de la normatividad vigente.

7. Determinación del Estado de la Técnica

La fecha que se ha tenido en cuenta para determinar el Estado de la Técnica es:

- Fecha de la publicación en fase nacional: 05 de noviembre de 2004

Considerando que la fecha de presentación de la primera solicitud de patente de la cual se reivindica prioridad es: 05/11/2004 se encontraron los siguientes documentos del estado de la técnica relacionados, que pueden afectar la patentabilidad del objeto de la presente solicitud:

Nº	Documento	Título	Fecha de publicación
D1	Tetrahedron, Vol 53, No 41 pp 14021-14030	Monitoring the solid phase synthesis of analogues of lysobactin and the katanosins using in situ MALDI-TOF MS	1997
D2	J.Am. Chem Soc, 1997, 119, 6680-6681	WAP-8294 A ₂ , Anovel anti-MRSA antibiotic produced by <i>Lysobacter</i> sp	1997

D1 enseña un método de monitoreo de la síntesis en fase sólida de compuestos análogos de lisobactina

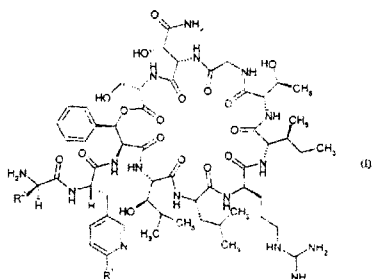
D2 enseña un antibiótico anti-MRSA, aislado de *Lysobacter* sp,

8. Análisis de patentabilidad (artículo 14 D486)

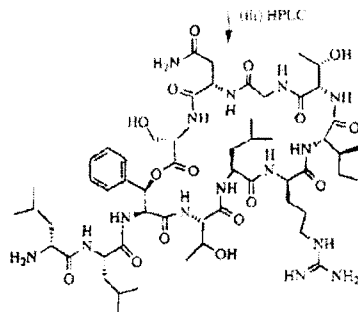
Nivel inventivo (artículo18 D486)

La reivindicación 1 consiste en:

Compuesto de fórmula



El Estado de la Técnica más cercano es **D1**, que da a conocer la síntesis de compuestos análogos de lisobactina, tales como el compuesto (10) (Ver página 14022, esquema 1)

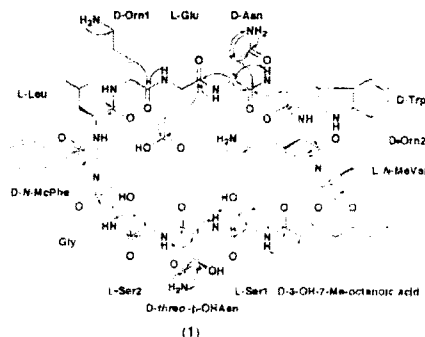


Los compuestos derivados de lisobactina, objeto de esta solicitud se diferencian de **D1**, en que presentan un residuo piridil-alanina.

El hecho de incluir un residuo piridil-alanina en los compuestos **D1**, no evidencia, comparativamente con el estado de la técnica, el aporte tecnológico, ni superior en el campo de los antimicrobianos.

El Problema Técnico Objetivo era obtener antimicrobianos derivados de lisobactina con menores efectos secundarios y mayor actividad biológica

D2 sugiere cómo resolver el problema técnico con el fin de obtener compuestos derivados de lisobactina con mayor actividad y menores efectos secundarios, ya que enseña compuestos análogos con un residuo de triptófano (indol), con una actividad antimicrobiana 14 veces más que la vancomicina. (Ver página 6681, columna 2)



La reivindicación es obvia, porque el Experto en la Materia enfrentado al problema de obtener antimicrobianos derivados de lisobactina con menores efectos secundarios y mayor actividad biológica, utilizaría análogos heterocíclicos del indol como es la piridina, como se sugiere para el compuesto de **D2**, en los compuestos que se divulgan en **D1**, logrando de esta forma los compuestos objeto de esta solicitud. Por tanto, la reivindicación 1 no tiene Nivel Inventivo. Las reivindicaciones dependientes 1-6 y 9-10, no mencionan alguna característica que haga inventivo al procedimiento de la reivindicación 1, por lo cual se considera que no tienen nivel inventivo.

9. Conclusión:

Los requisitos de patentabilidad de la presente solicitud se encuentran afectados así:

N°	Documento N°	Reivindicaciones afectadas	Requisito que afecta
D1	Tetrahedron, Vol 53, No 41 pp 14021-14030	1-6, 9-10	Nivel Inventivo
D2	J.Am. Chem Soc, 1997, 119, 6680-6681	1-6, 9-10	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 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 N° 10 de 2001.



JOSÉ LUIS SALAZAR LÓPEZ
Director de Nuevas Creaciones (E)

El anterior requerimiento fue notificado por fijación en lista, de fecha

10 DE ABRIL DE 2001

CONCEPTO TÉCNICO No. 04354	EXAMINADOR TÉCNICO Código No. 202070
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DL 242

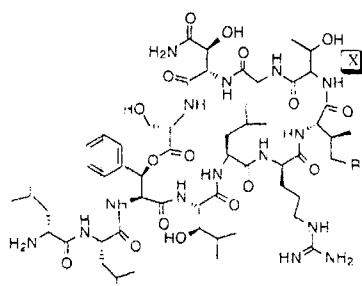
Monitoring the Solid Phase Synthesis of Analogues of Lysobactin and the Katanosins using *in situ* MALDI-TOF MS

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Abstract: A method of solid phase reaction analysis is described using an *in situ* cleavage process (TFA vapour) followed by MALDI-TOF MS analysis. The process is demonstrated by the solid phase synthesis of a depsipeptide based on the antibiotic Lysobactin. © 1997 Elsevier Science Ltd.

Lysobactin (1) is a depsipeptide antibiotic (Figure 1) isolated in 1988 from a species of *Lysobacter* (ATCC 53042) by a group from the Squibb Institute.¹⁻³ It was identified using a screen designed to detect antibiotics that bound to components found in a complex bacterial cell wall preparation, suggesting that it targets bacterial cell wall biosynthesis. It is suspiciously similar to katanosin A (2) and B (3) isolated from a culture broth of a strain related to the genus *Cytophaga* by a group from the Shionogi research laboratories also in 1988.⁴ Interestingly, the katanosins have been reported to have the opposite stereochemistry at the *L*-*allo*-threonine position (marked X in Figure 1) found in lysobactin yet they still retain potent antibacterial activity.



- (1) Lysobactin R = Me, X = *L*-*allo*-Threonine
- (2) Katanosin A, R = H, X = *D*-*allo*-Threonine
- (3) Katanosin B, R = Me, X = *D*-*allo*-Threonine

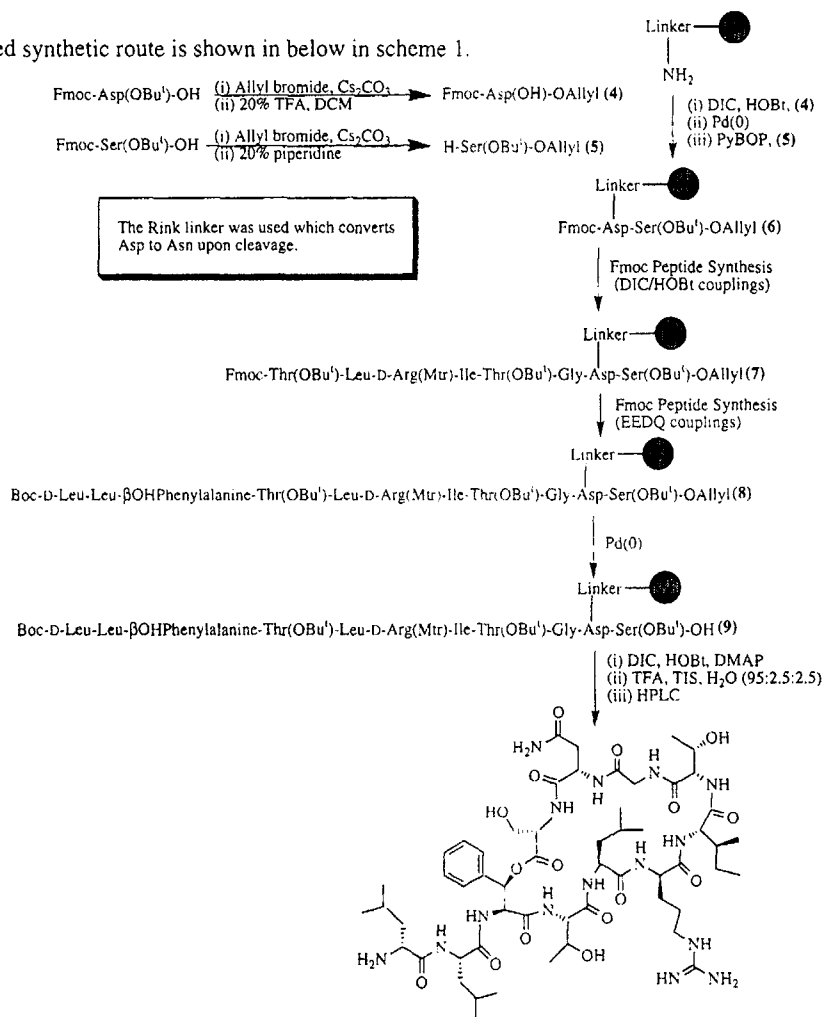
Figure 1

The spectrum of biological activity for lysobactin parallels that of vancomycin¹⁻³ although lysobactin is four times more potent (MIC mg/ml). The ED₅₀ values for lysobactin compare favourably with vancomycin while it has been shown to be several hundred times more efficacious in the treatment of surface wounds. It is however slightly more toxic. Unfortunately, due to the different bacterial strains used in the reports,¹⁻⁴ direct comparison of the biological action of katanosin A and lysobactin is impossible. Experiments have also shown that the activity of lysobactin is not due to membrane disruption and that unlike vancomycin its activity is undiminished in the presence of *D*-Ala-*D*-Ala containing peptides, with evidence suggesting that lysobactin probably affects a step prior to UDP-*N*-acetylglucosamine formation. Clearly lysobactin and the katanosins are targeting a distinct cell wall precursor and offer an exciting new antibacterial approach. A solid phase synthesis of lysobactin and the katanosins would allow simple access to peptides of the required size (11 residues), numerous analogues at every point of the synthesis and would be a rare example of a resin based macrolactonisation. Determination of the stereochemistry of the threonine residue would

allow the lysobactin/katanosin structural question to be addressed. The solid phase synthesis is complicated by three factors: (a) the cyclic nature of the peptide; (b) the lactone functionality and (c) the presence of several "unusual" amino acid residues. For our initial studies we decided to simplify the structure by incorporation of L-asparagine, rather than L-hydroxyasparagine (although the synthesis of protected L-threo-aspartic acid from maleic acid was readily achieved using literature procedures)⁵ and L-threonine or L-leucine rather than L-hydroxyleucine. The crucial L-threo- β -hydroxyphenylalanine residue was incorporated, to form part of the lactone, and was obtained in pure form by enzymatic resolution of commercially available DL-threo- β -hydroxyphenylalanine in excellent yield.⁶ D and L-allo-threonines could likewise be prepared in enantiomerically pure form by enzymic (acylase) resolution of commercially available DL-allo-threonine but were also commercially available. However due to the uncertainty about this residue it was anticipated all four isomers would eventually be incorporated.

Synthesis:

The proposed synthetic route is shown in below in scheme 1.



Scheme 1. The Solid Phase Synthesis of Analogues of Lysobactin

WAP-8294A₂, A Novel Anti-MRSA Antibiotic Produced by *Lysobacter* sp.

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Received March 20, 1997

Infectious diseases due to antibiotic-resistant bacteria pose a threat to public health around the world.¹ Especially, nosocomial infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) in hospitals have become a serious clinical problem.² Glycopeptide antibiotics, vancomycin and teicoplanin, are the drugs of choice for the treatment of MRSA infections. However, isolation of the glycopeptide-resistant *S. aureus* and *in vitro* transfer of the vancomycin-resistant genes from *Enterococcus faecalis* to *S. aureus* have been recently reported.³ Therefore, an antibacterial agent which differs from vancomycin in mode of action is clinically desirable. In the course of our screening for antibiotics, we found that a novel antibiotic, WAP-8294A₂ produced by a Gram-negative bacterium *Lysobacter* sp. showed higher *in vivo* activity against MRSA than vancomycin. In this paper, we report the structure and activity of this antibiotic.

The *Lysobacter* sp. strain WAP-8294, isolated from a soil sample collected at Shimoda, Shizuoka, Japan, was cultivated in a production medium consisting of glucose, soybean flour, NaCl, and CaCO₃ at 30 °C for 3 days. WAP-8294A₂ (**1**) was isolated from the culture broth as a white amorphous powder:⁴ ninhydrin-positive; mp 215–225 °C (dec.); [α]_D²⁰ +42° (c 0.5 H₂O); IR (KBr) 3300, 1720–1715, 1636, 1541, 1404, 1207, 1137 cm⁻¹; UV $\lambda_{\max}$ (nm) (ε) (H₂O) 275 (5000), 280 (5200), 287 (4600); HRFABMS *m/z* 1562.8224 (M + H)⁺, calcd for C₇₃H₁₁₂N₁₇O₂₁ Δ0.3 ppm.⁵ The UV spectrum indicated the presence of an indole moiety, and the dominant absorptions at 1636 and 1541 cm⁻¹ in the IR spectrum indicated the peptide nature.

Amino acid analysis of the acid hydrolysate (4 M methanesulfonic acid, 110 °C, 24 h) revealed the presence of nine amino acids, Asp, Glu, Gly, Leu, Trp, 2 × Orn, and 2 × Ser.

(1) (a) Tomasz, A. *N. Eng. J. Med.* **1994**, *330*, 1247–1251. (b) Winker, M. A.; Flanagan, A. *JAMA* **1996**, *275*, 245–246.

(2) (a) Mulligan, M. E.; Murray-Leisure, K. A.; Ribner, B. S.; Standiford, H. C.; John, J. F.; Korvick, J. A.; Kauffman, C. A.; Yu, V. L. *Am. J. Med.* **1993**, *94*, 313–328. (b) Voss, A.; Kresken, M. *Chemotherapy* **1996**, *42* (supplement 2), 13–18.

(3) (a) Watanakunakorn, C. *J. Antimicrob. Chemother.* **1990**, *25*, 69–72. (b) Sieradzki, K.; Tomasz, A. *FEMS Microbiol. Lett.* **1996**, *142*, 161–166. (c) Noble, W. C.; Virani, Z.; Cree, R. G. A. *FEMS Microbiol. Lett.* **1992**, *93*, 195–198.

(4) In a typical isolation, EtOH (5 L) was added to the culture broth (5 L), which was adjusted to pH 2.5 with 6 N HCl and then centrifuged. The supernatant was passed through a column of Diaion HP-20 (Mitsubishi Chemical, Tokyo, Japan). The column was washed with water, 80% acetone (pH 11.0), and 80% acetone and was eluted with 80% acetone [0.05% trifluoroacetic acid (TFA)]. The eluate was chromatographed on a Chromatorex ODS (Fuji Silysia Chemical, Kasugai, Japan) column [30% CH₃Cl₃ (0.05% TFA)]. The column was washed with 30% CH₃Cl₃ (0.05% TFA) and 40% CH₃Cl₃ (0.05% TFA) and then eluted with 45% CH₃Cl₃ (0.05% TFA). The fraction rich in **1** (monitored by HPLC) was applied to a column of SP-TOYOPEARL 650C (Tosoh, Tokyo). The column was washed with water and eluted in a stepwise manner with 0.1–0.8 M NaCl. The active eluate was further purified by Chromatorex ODS [45% CH₃Cl₃ (0.05% TFA)] furnishing 693 mg of pure bis(trifluoroacetate) salt **1**.

(5) HR-FAB mass spectra were recorded using a JEOL JMS-SX102A mass spectrometer [matrix, glycerol/*m*-nitrobenzyl alcohol (1:1)].

Additionally, three unusual amino acids were also detected by 2D-TLC from the hydrolysate and were isolated by ion-exchange chromatography. Their structures were determined as β -hydroxyaspartic acid (OHAsp), *N*-methylvaline (NMeVal), and *N*-methylphenylalanine (NMePhe) on the basis of NMR and FABMS experiments and were confirmed by direct comparison with authentic samples. The chiralities of the eight protein amino acids (except for Trp) and the two *N*-methylamino acids and Trp were determined by HPLC analysis of the (+)-1-(9-fluorenyl)ethyl chloroformate derivatives⁶ and chiral TLC analysis,⁷ respectively. The configuration of OHAsp was determined as *threo* by TLC analysis and *D* by chiral HPLC analysis.⁸ A fatty acid from the ether-extractable portion of the acid hydrolysate (6 N HCl, 110 °C, 2 h) was identified as 3-hydroxy-7-methyloctanoic acid (3-OH-7-Me-octanoic acid) on the basis of NMR spectral analysis and GC-MS analysis of its methyl ester, and its structure was finally confirmed by direct comparison with an authentic synthetic sample.⁹ The chirality of the fatty acid was determined as *D* (*R*) by HPLC of the 3,5-dinitroaniline (DA) derivatives on a chiral column.¹⁰

The determination of all constituent components from the hydrolysate and the molecular formula (C₇₃H₁₁₁N₁₇O₂₁) indicated that **1** has a cyclic peptide structure with two primary amides and one carboxylic acid in the side chains (Asx, Glx, and OHAsx). In fact, an increase of 14 mass units was observed in the FABMS of the methylation product of **1** with thionyl chloride and methanol.¹¹ Since treatment of **1** with Ac₂O/pyridine yielded a pentaacetate,¹² five out of the two amino and four hydroxyl groups (2 × Orn, and 2 × Ser, OHAsp and 3-OH-7-Me-octanoic acid) in **1** were estimated to be free. TLC analysis of the 2,4-dinitrofluorophenyl (DNP) derivative of the acid hydrolysate of **1** showed the presence of δ -DNP-Orn instead of Orn, indicating that the two Orn δ -amino groups are free. The amino acid and fatty acid analyses of the acid hydrolysate of **1** oxidized with chromic acid¹³ revealed the disappearance of three hydroxyl amino acids (2 × Ser and OHAsp) and the presence of intact 3-OH-7-Me-octanoic acid, indicating that the three hydroxyl groups of the amino acids are free. These results suggested that the hydroxyl group of 3-OH-7-Me-octanoic acid is involved in the cyclic structure formation.

Analysis of the ¹H, ¹³C, H–H COSY, and HSQC spectra of **1** (DMSO-¹²C₂, *d*₆) led to the assignment of proton signals except for the two Ser hydroxyl groups and the carbon signals

(6) Hayashi, T.; Sasagawa, T. *Anal. Biochem.* **1993**, *209*, 163–168.

(7) CHIRALPLATE (Macherey-Nagel), MeOH/H₂O/CH₃CN (50:50:200). *R_f* values: NMeVal, *L*-isomer 0.49, *D*-isomer 0.41; NMeVal from **1**, 0.49; NMePhe, *L*-isomer 0.69, *D*-isomer 0.50; NMePhe from **1**, 0.50; Trp, *L*-isomer 0.69, *D*-isomer 0.54; Trp from **1**, 0.54.

(8) Silica gel TLC (Merck), 2-PrOH/NH₄OH/H₂O (4:2:1). *R_f* values: *threo*-OHAsp 0.52; *erythro*-OHAsp 0.39; OHAsp from **1**, 0.52. HPLC, CHIRALPAK WH (4.6 × 250 mm, Daicel Chemical, Tokyo) using CH₃CN/2 mM CuSO₄ (4:96) as eluent at 254 nm. Retention time (flow rate 1.0 mL/min, 50 °C): *L*-isomer 9.1 min, *D*-isomer 5.9 min; OHAsp from **1**, 6.1 min.

(9) (a) 3-OH-7-Me-octanoic acid was synthesized by the method of Fritzsche (Fritzsche, K.; Lenz, R. W.; Fuller, R. C. *Int. J. Biol. Macromol.* **1990**, *12*, 92–101). (b) The ether extract of the hydrolysate was methylated with (trimethylsilyl)diazomethane and was subjected GC-MS: GC, HP-5890II, column, J&W Scientific DB-5 (0.25 mm × 30 m); MS, JEOL JMS-AX505WA, ion mode, EI (+). For the methyl ester of authentic 3-OH-7-Me-octanoic acid: retention time, 9.68 min; *m/z* 103 (base peak), 170 (*M*⁺ – 18). For the methyl ester of 3-OH-7-Me-octanoic acid from **1**: retention time, 9.72 min; *m/z* 103 (base peak), 170 (*M*⁺ – 18).

(10) (a) Nakagawa, Y.; Matsuyama, T. *FEMS Microbiol. Lett.* **1993**, *108*, 99–102. (b) DA-Derivatives were analyzed on TSK gel Enantio P2 (4.6 × 250 mm, Tosoh) using hexane/1,2-dichloroethane/EtOH (35:15:0.5) as eluent at 254 nm. Retention time (flow rate 1.0 mL/min): *L*-isomer 54.2 min, *D*-isomer 56.9 min. DA-derivative of the fatty acid from **1**, 57.1 min.

(11) FABMS, JEOL JMS-AX505WA, methyl ester of **1**: *m/z* 1576.8 (*M* + H)⁺. HRFABMS: *m/z* 1576.8326 (*M* + H)⁺, calcd for C₇₄H₁₁₄N₁₇O₂₁, Δ0.8 ppm.

(12) Pentaacetate of **1**: *m/z* 1773.0 (*M* + H)⁺.

(13) Shoji, J.; Hinoo, H.; Katayama, T.; Nakagawa, Y.; Ikenishi, Y.; Iwatani, K.; Yoshida, T. *J. Antibiot.* **1992**, *45*, 824–831.

D2
244

285

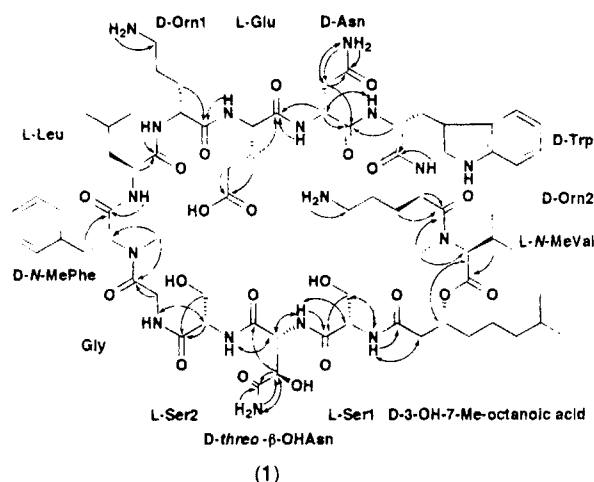


Figure 1. Structure of WAP-8294A₂ (1). Arrows show HMBC (→) and ROESY (↔) correlations.

except for those of the quaternary carbons.¹⁴ The assignment of the quaternary carbons was based on the HMBC correlation. The locations of the two primary amides were confirmed as Asn and OHAsn from the HMBC and ROESY data. The HMBC correlations from the α -amide protons or *N*-methyl protons to the neighboring carbonyl carbons were observed between OHAsn/Ser1, NMePhe/Gly, Leu/NMePhe, Orn1/Leu, Glu/Orn1, Asn/Glu, Orn2/Trp, and NMeVal/Orn2. The HMBC correlation from the α -methine proton of Asn to the carbonyl carbon of the neighboring Trp was also observed. In addition, the HMBC correlation of the α -amide proton of Ser1 was found to the carbonyl carbon of 3-OH-7-Me-octanoic acid, supporting the fact that the *N*-terminus of 1 is Ser1 and the amino group is acylated with 3-OH-7-Me-octanoic acid. Diagnostically important ROESY correlations from the α -methine protons to the neighboring α -amide protons were seen between Ser1/

OHAsn, OHAsn/Ser2, Ser2/Gly, and Asn/Trp. Furthermore, the ROESY correlation from the 3-methine proton of 3-OH-7-Me-octanoic acid to an α -methine proton of NMeVal indicated that the lactone linkage is formed between the 3-hydroxyl group of 3-OH-7-Me-octanoic acid and the α -carboxyl group of NMeVal. The presence of α -linked Glu, Asn, and OHAsn was shown by the HMBC and ROESY spectral analysis. The HMBC correlation from the α -amide proton of the neighboring residue to the α -carboxyl group of Glu was observed. The primary amide protons of Asn and OHAsn showed HMBC correlations with their own β -carbons. Furthermore, the α -methine protons of Asn and OHAsn showed ROESY cross peaks with the neighboring α -amide protons. Thus, the structure 1 was elucidated as shown in Figure 1.

Among the known antibiotics of bacterial origin, plusbasins (*Pseudomonas* sp.) and empedopeptin (*Empedobacter halodii* nov. sp.) are somewhat similar to 1, because they are cyclic depsipeptides containing one 3-hydroxy fatty acid.^{13,15} However, 1 differs from them in constituent amino acids and a fatty acid. Especially, 3-OH-7-Me-octanoic acid is the first to be isolated from antibiotics. Lysobactin, an antibiotic produced by *Lysobacter* sp. also differs from 1 in constituent amino acids and the absence of a fatty acid.¹⁶ While 1 was as active as vancomycin against MRSA clinical isolates (MIC: 0.78 μ g/mL), interestingly, the activity of 1 was highly enhanced by the addition of 10% human serum (MIC: 0.1 μ g/mL). No activity of 1 was observed against Gram-negative bacteria, yeasts, and fungi. *In vivo* efficacies of 1 and vancomycin were assessed in the experimental systemic MRSA infection of mice. The mean ED₅₀ values of 1 and vancomycin against nine MRSA strains were 0.38 and 5.3 mg/kg, respectively, indicating that 1 is 14 times more active than vancomycin. Very recently, vancomycin dimers with activity against vancomycin-resistant enterococci (VRE) have been reported (MIC: 19–55 μ g/mL).¹⁷ Against VRE, 1 was more active than these vancomycin analogs (MIC: 6.3 μ g/mL), suggesting that the mode of action of 1 is different from that of vancomycin. Moreover, 1 was active against *S. aureus* protoplasts as well as the intact cells, whereas vancomycin was not active against the protoplasts. In the disc diffusion test, the anti-MRSA activity of 1 was inhibited by the addition of phosphatidylglycerol or cardiolipin to the disc. These results suggest that 1 interacts selectively with phospholipids in the target cell membrane resulting in membrane damage.

Acknowledgment. We thank Mrs. N. Suzuki and Y. Aiba and Ms. N. Kokubo for their assistance during the course of this work. We also thank Mrs. A. Kusai and K. Nojima, JEOL and Co., Ltd., for the measurement of HR-FABMS.

Supporting Information Available: ¹H, ¹³C, H–H COSY, HSQC, HMBC, and ROESY spectra for 1 (8 pages). See any current masthead page for ordering and Internet access instructions.

JA970895O

(15) (a) Konishi, M.; Sugawara, K.; Hanada, M.; Tomita, K.; Tomatsu, K.; Miyaki, T.; Kawaguchi, H.; Buck, R. E.; More, C.; Rossomano, V. Z. *J. Antibiot.* **1984**, *37*, 949–957. (b) Sugawara, K.; Numata, K.; Konishi, M.; Kawaguchi, H. *J. Antibiot.* **1984**, *37*, 958–964.

(16) Iymak, A. A.; McCormic, T. J.; Unger, S. E. *J. Org. Chem.* **1989**, *54*, 1149–1157.

(17) Sundram, U. N.; Griffin, J. H.; Nicas, T. I. *J. Am. Chem. Soc.* **1996**, *118*, 13107–13108.

(14) NMR spectra were taken on a JEOL A600 (600 MHz) instrument. ¹H NMR of 1: 3-OH-7-Me-octanoic acid δ 2.31 (H₂), 2.55 (H_{2'}), 4.94 (H₃), 1.50 (H₄), 1.23 (H₅), 1.11 (H₆), 1.47 (H₇), 0.81 (H₈, H_{8'}); Ser1 δ 4.82 (H α), 3.45 (H β), 3.55 (H β'), 7.93 (NH); OHAsn δ 4.96 (H α), 4.25 (H β), 7.34 (NH γ'), 7.41 (NH γ), 8.08 (NH), 5.65 (OH β); Ser2 δ 4.82 (H α), 3.64 (H β), 8.29 (NH); Gly δ 3.88 (H α), 8.19 (NH); NMePhe δ 4.41 (H α), 2.93 (H β), 3.26 (H β'), 7.15 (H₂, 6), 7.26 (H₃, 5), 7.19 (H₄), 2.56 (NMe); Leu δ 4.27 (H α), 1.37 (H β), 1.51 (H β'), 1.38 (H γ), 0.67 (H δ), 0.70 (H δ'), 7.93 (NH); Orn1 δ 4.65 (H α), 1.64 (H β), 1.70 (H β'), 1.55 (H γ), 2.85 (H δ), 7.72 (NH₂), 7.48 (NH); Glu δ 4.63 (H α), 1.70 (H β), 1.82 (H β'), 2.17 (H γ), 8.51 (NH), 12.0 (OH δ); Asn δ 4.77 (H α), 2.42 (H β), 2.60 (H β'), 6.93 (NH γ'), 7.32 (NH γ), 8.19 (NH); Trp δ 4.73 (H α), 2.77 (H β), 3.11 (H β'), 7.13 (H₂), 7.44 (H₄), 6.87 (H₅), 6.95 (H₆), 7.30 (H₇), 10.5 (NH₁), 8.73 (NH); Orn2 δ 4.58 (H α), 1.55 (H β), 1.66 (H β'), 1.45 (H γ), 2.77 (H δ), 7.72 (NH₂), 7.98 (NH); NMeVal δ 4.62 (H α), 2.06 (H β), 0.71 (H γ), 0.86 (H γ'), 2.51 (NMe). ¹³C NMR: 3-OH-7-Me-octanoic acid δ 169.4 (CO), 38.3 (C₂), 71.0 (C₃), 33.1 (C₄), 22.0 (C₅), 38.2 (C₆), 27.3 (C₇), 22.3 (C₈, 8'); Ser1 δ 169.6 (CO), 53.9 (C α), 62.8 (C β); OHAsn δ 169.2 (CO), 54.7 (C α), 71.3 (C β), 173.5 (CO γ); Ser2 δ 169.8 (CO), 54.9 (C α), 62.1 (C β); Gly δ 169.0 (CO), 42.1 (C α), NMePhe δ 169.4 (CO), 63.3 (C α), 33.9 (C β), 138.4 (C₁), 129.1 (C₂, 6), 128.3 (C₃, 5), 126.3 (C₄), 35.1 (NMe); Leu δ 171.5 (CO), 51.2 (C α), 39.3 (C β), 24.1 (C γ), 20.8 (C δ), 22.9 (C δ'); Orn1 δ 170.9 (CO), 50.7 (C α), 30.3 (C β), 23.0 (C γ), 38.5 (C δ); Glu δ 169.8 (CO), 51.3 (C α), 28.5 (C β), 29.7 (C γ), 174.0 (CO δ); Asn δ 170.5 (CO), 49.2 (C α), 38.2 (C β), 171.8 (CO γ); Trp δ 170.7 (CO), 52.7 (C α), 27.4 (C β), 123.3 (C₂), 109.3 (C₃), 118.1 (C₄), 118.0 (C₅), 120.7 (C₆), 111.4 (C₇), 127.2 (C₈), 135.9 (C₉); Orn2 δ 170.3 (CO), 48.3 (C α), 28.1 (C β), 23.2 (C γ), 38.7 (C δ); NMeVal δ 169.1 (CO), 60.9 (C α), 25.6 (C β), 18.0 (C γ), 19.1 (C γ'), 29.2 (NMe).