

Clarke, Modet & C^o
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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIVISION DE NUEVAS CREACIONES

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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



No. 06-023325-00006-0000

Fecha: 2008-08-01 14:50:14 Dep. 2020 NUECREACION
Tra. 11 PATENTEYII Eve: 378 FASENACIONALI
Act. 444 RESPUESTAREQUE Folios: 5

Re: Expediente No. 06.023.325

Solicitud de patente a nombre de ALFA WASSERMAN S.P.A.

Respuesta a la oposición presentada por PROCAPS S.A.

Yo, DILIA RODRIGUEZ D'ALEMAN, identificada como aparece al pie de mi firma, domiciliada en la carrera 11 No. 86-53 piso 6, obrando como apoderada de la sociedad ALFA WASSERMAN S.P.A. doy respuesta al oficio No. 3487 notificado por fijación en lista el 7 de marzo de 2008, por medio del cual se corrió traslado de la oposición presentada por la sociedad PROCAPS S.A., contra la solicitud de patente de la referencia, correspondiente a la invención titulada "Formas polimórficas de Rifaximina como antibióticos".

2. PRETENSIONES DE LAS OPOSICIONES:

Me opongo a la pretensión del escrito de oposiciones por cuanto, como se demuestra más adelante, la invención presentada por mi mandante cumple con los requisitos de novedad, nivel inventivo y aplicación industrial. En consecuencia, se debe otorgar el privilegio de patente de invención y expedir el título correspondiente.

2.1 Argumentos presentados por el opositor en el acápite fundamentos de hechos.

El opositor manifiesta que: "Los países miembros otorgarán patentes para las invenciones sean de productos o de procedimientos en todos los campos de la tecnología, siempre que sean nuevas, tengan nivel inventivo y sean susceptibles de aplicación industrial".

"Bien, pues antes de la fecha de prioridad de esta solicitud se encuentran (sic) el siguiente documento que afecta la patentabilidad de la presente solicitud:

- D1: EP0161534, publicada el 21 de noviembre de 1985.

De otra parte, el opositor manifiesta que "[...] el documento D1, particularmente en la descripción, revela métodos de obtención de la forma cristalina de rifaximina. Los ejemplos

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-Que la invención en estudio puede partir de conocimientos conocidos, caso en el cual habrá que hacer la comparación no de lo conocido sino de lo inédito y novedoso, teniendo que analizarse el valor agregado que aporta la invención con sus nuevas características al campo técnico al cual pertenece la invención en estudio.

Aplicando lo anterior al caso concreto, para que las razones que aduce el opositor para desvirtuar la novedad fuesen ciertas, sería forzoso que se demostrara que cada uno de los elementos que definen la invención en estudio esté comprendido en el estado de la técnica de forma TOTAL, lo cual no sucede como ahora se demostrará.

En primer lugar nada en D1 enseña o sugiere que se trate de las mismas formas cristalinas de la presente solicitud, particularmente porque los compuestos de D1 no comparten los mismos patrones de difracción de rayos X que los compuestos de la invención en cuestión. Al respecto resulta importante citar el "Manual para el examen de Solicitudes de Patentes de Invención en las Oficinas de Propiedad Industrial de los Países de la Comunidad Andina" del 2004, especialmente al numeral 9.1 de novedad, donde se manifiesta que: "Una descripción específica afecta la novedad de una general, pero no a la inversa.". Por lo tanto los compuestos enseñados por D1 no afectan de ninguna manera la novedad de la solicitud, ya que dichas enseñanzas son claramente generales frente a las características específicas divulgadas por la solicitud en cuestión. *debe probarse?*

Por otro lado el mismo manual en el numeral 9.4 también manifiesta que: "Para determinar la novedad de la invención, se debe comprobar si existen anticipaciones del estado de la técnica que contengan explícitamente todas las características técnicas esenciales de la invención. El examen de novedad se efectúa comparando elemento por elemento de la invención tal como está definido por las reivindicaciones con los del estado de la técnica." (El subrayado es mío). *2*
- literal?

La anterioridad D1 enseña compuestos de rifaximina, pero no enseña explícitamente los compuestos de rifaximina de la presente solicitud, particularmente en lo relacionado con los patrones de difracción de rayos X.

En D1 se manifiesta la intención de llevar a cabo la cristalización de rifaximina pero no hay indicio alguno de que el proceso aplicado haya sido exitoso. De hecho, no hay evidencia de que el material sólido que resulta del proceso sea cristalino. La única evidencia en D1 es que los compuestos obtenidos se encuentran en forma sólida, pero no necesariamente dicho material sólido se encuentra en estado cristalino.

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La cristalización es el proceso de formar cristales sólidos a partir de una solución uniforme. El cristal se define como un sólido en el cual los átomos, moléculas o iones que lo constituyen están empacados en un patrón regularmente ordenado y repetitivo que se extiende en las tres dimensiones. El material que no tiene un orden definido a largo plazo no es cristalino. Los materiales pueden clasificarse como amorfos, vítreos, o material vidrioso. La cristalización no es un proceso inevitable. Es sabido en el estado del arte que los intentos para obtener formas cristalinas pueden no ser exitosos.

En caso de un material en polvo, en particular cuando el polvo esta constituido por partículas finas, la cristalinidad del material puede determinarse solo por medio de técnicas analíticas apropiadas, tal como, por ejemplo, la medición de la difracción de rayos X. En caso de un polvo de partículas finas la inspección visual simple del material no proporciona información confiable sobre su cristalinidad.

En la literatura científica la cristalización también se reporta como una técnica de separación con el fin de obtener material de una solución o de obtener material purificado. En este respecto el término precipitación puede emplearse como sinónimo. En estos dos propósitos la naturaleza del material que viene del denominado proceso de cristalización, en otras palabras el material cristalino o no cristalino, no es relevante.

En D1 la cristalización es explícitamente descrita con el fin de lograr los dos propósitos antes mencionados: i) como alternativa a la extracción con solventes orgánicos para recuperar la rifaximina después de la reacción, ii) y para obtener material de rifaximina purificado, de ser necesario. La obtención de estos dos logros se llega en el primer caso obteniendo rifaximina sólida separable de la solución, en el segundo caso obteniendo rifaximina con suficiente pureza. En ninguno de los casos tiene que ver la cristalinidad.

De hecho, en ese momento no había evidencia de la cristalinidad de la rifaximina como lo sustenta el escrito de Brufani, M. y colaboradores "X-ray crystal strycture of 4-deoxy-3'bromopyridol[1',2'-1,2]imidazo[5,4-c]rifamycin S., J. Antibiot. 1984, 37(12), 1623-1627 en la cual se ha elucidado la estructura de un análogo de bromo para plantear una hipótesis sobre la estructura molecular de la rifaximina.

En vista del documento citado, EP 0161532, también quisiéramos señalar que en términos del potencial terapéutico del antibiótico rifaximina, la identificación de las formas polimórficas es una novedad, ya que EP 0161534 solo introduce una ruta novedosa de síntesis de rifaximina, pero se refiere a US 4.341.785 en lo relacionado con lo concerniente a las actividades antibacteriales *in vitro* e *in vivo*.

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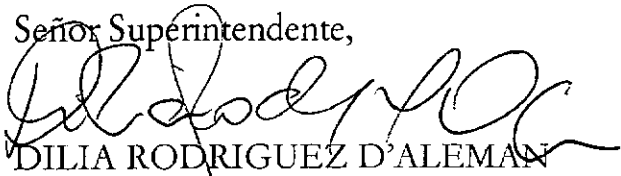
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De hecho, las formas polimórficas de rifaximina descritas en la presente solicitud que muestran rangos de valores de biodisponibilidad significativamente diferentes respecto a los valores reportados en US 4.341.785, deben ser consideradas desde el punto de vista terapéutico como novedosas y más útiles. El logro obtenido por la presente invención, que es la capacidad de modificar la biodisponibilidad de la rifaximina, debe ser considerado como una mejora técnica significativa comparado con US 4.341.785 y EP 0161534, ya que ahora es posible proporcionar un beneficio mejorado a los pacientes al tratarlos oportunamente con una forma polimórfica de rifaximina, seleccionada de entre aquellas desarrolladas, con el fin de optimizar el equilibrio de la eficacia de la terapia con los posibles efectos adversos.

En conclusión, la anterioridad D1 no contiene una divulgación explícita de todas y cada una de las características enseñadas por la presente solicitud por lo que dicha anterioridad no cumple los requerimientos establecidos por la Decisión 486 así la misma no puede afectar la novedad de la materia objeto de la invención en cuestión.

En consecuencia, solicito a ese despacho no tener presente la argumentación del opositor y continuar con el trámite administrativo en el presente caso.

Señor Superintendente,


DILIA RODRIGUEZ D'ALEMAN

C.C. 41.654.882 de Bogotá

T.P. 20824

Cód. 008

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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIVISIÓN DE NUEVAS CREACIONES

202012

Bogotá, D.C., 05 de mayo de 2009

Apoderado: Dilia Rodríguez D'Aleman

ASUNTO

Radicación 06-23325
Trámite 002
Evento 001
Actuación 430
Oficio
Folios 6129

Patente de Invención



Vía PCT (fase nacional)



Cap. I



Cap. II



No. Sol. Internacional

PCT/EP2004/012490

Fecha de Presentación Internal.

WO2005/044823

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:

Documentos estudiados

Descripción:

Folios: 04 a 35

Como se radicó inicialmente.

Reivindicaciones:

Folios 36 a 41

como se radicó inicialmente.

Oposiciones:

Folios 99 a 148: Opositor PROCAPS S.A.

Prioridad:

Folio1: IT MI2003A002144, 07-nov.-2003

Respuesta del solicitante Folios: 152 a 156.

Objeto de la invención

Título de la solicitud: FORMAS POLIMORFICAS DE RIFAXIMINA COMO ANTIBIOTICOS.

El objeto de la presente solicitud de patente de invención consiste en tres formas polimórficas de rifaximina denominadas α , β , γ .

-Reivindicación 1: Polimorfo α de roximina.

-Reivindicación 2: Polimorfo β de roximina.

-Reivindicación 3: Polimorfo γ de roximina.

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- Reivindicaciones 7-4: Procedimiento para la producción de los polimorfos α , β , γ .
 - Reivindicación 8: Procedimiento para la preparación del polimorfo α .
 - Reivindicación 9: Procedimiento para la producción del polimorfo β .
 - Reivindicación 10: Procedimiento para la preparación del polimorfo γ .
 - Reivindicación 11: Procedimiento para la preparación del polimorfo β .
 - Reivindicación 8: Procedimiento para la preparación del polimorfo α .
 - Las reivindicaciones 13 a 20: Se refieren al uso

La invención se catalogó, según la Clasificación Internacional de Patentes (IPC):C07D498/22, A61K31/437, A61P31/04

De la solicitud de Patente (según el caso)

Reivindicaciones (artículo 30 D 486 de la C.A.N.)

-En las reivindicaciones no es claro lo que se quiere significar con el termino "rifamixina cruda", debe quedar claro que es la rifamicina obtenida cristalizada del procedimiento original de síntesis sin que se le haya medido los patrones de difracción de rayos X, ó en su defecto aclarar a que se refiere esos términos.

Reivindicaciones relativas a un uso o a un segundo uso (artículos 14 y 21 D 486 de la C.A.N.)

-Las reivindicaciones 13-20 La reivindicación reclaman el uso, y de acuerdo con la legislación colombiana el uso reclamado no se considera para protección por patente, como se lee en el artículo 14 de la Decisión 486 de la Comisión de la Comunidad Andina"... Los Países Miembros otorgarán patentes para las invenciones sean de productos o procedimientos en todos los campos de la tecnología..."; aquí no se hace alusión al uso.

Los usos o segundos usos no son patentables, de conformidad con la sentencia del TJAC 89-AI-2000, los usos en general no son patentables.

Entonces las reivindicaciones analizadas no se pueden considerar invenciones y que por lo tanto no pueden obtener patente.

Análisis de la(s) Oposición(es): (artículo 43 D. 486):

El documento que se presento dentro del memorial de oposición EP0161534 es donde aparece la síntesis y de derivados de rifamicina y por primera vez la cristalización en etanol/agua en proporciones 7:3 referido en el ejemplo 1, que corresponde a cristales de 4-deoxi-4'-metil-prido[1',2':1,2]imidazo-[5,4-c]rifamicina SV, materia que corresponde a lo que en la presente solicitud se le denomina rifamicina, y particularmente es la forma como en la presente solicitud se denomina rifamixina γ . Por lo tanto se tomará en cuenta como uno de los documentos del estado de la técnica más próximo y se tendrá en cuenta la totalidad del documento patente presentado en el memorial de oposición, junto con la respuesta técnica del interesado.

Determinación del Estado de la Técnica

La fecha que se ha tenido en cuenta para determinar el Estado de la Técnica es la fecha de presentación de la solicitud prioritaria 07-nov-2003.

Nº	Documento	Título	Fecha de publicación*
D1	EP0161534	New process for the synthesis of pyrido-imidazo-rifamycins	21-vov-1985
D2	US4.341.785	Imidazo-rifamycin derivatives with antibacterial utility	27-Jul-1982
D3	GB1590732	Method for preparation of rifamycins	10-jun-1981

Análisis de patentabilidad (según requisito) (artículo 14 D486)

Novedad (artículo 16 D486 de la C.A.N.)

La solicitud en estudio reclama en las reivindicaciones 1,2,3 :

1-Se reivindica un polimorfo α cristalino de rifamixina:

Presenta un contenido de agua menos de un 4.5% y un diagrama de difracción de Rayos X en polvo, que presenta picos en valores de ángulos de difracción 2θ : 6.6°, 7.4°, 7.9°, 8.8°, 10.5°, 11.1°, 11.8°, 12.9°, 17.6°, 18.5°, 19.7°, 21.0°, 21.4°, 22.1°.

2-Un polimorfo β cristalino de rifamixina:

Presenta un contenido de agua mucho mayor de un 4.5% y un diagrama de difracción de Rayos X en polvo, que presenta picos en valores de ángulos de difracción 2θ : 5.4°, 6.4°, 7.0°, 7.8°, 9.0°, 10.4°, 13.1°, 14.4°, 17.1°, 17.9°, 18.3°, 20.9°.

3- Un polimorfo γ cristalino de rifamixina:

Presenta un contenido de agua entre 1.0% y 2.0% y un diagrama de difracción de Rayos X en polvo, que presenta picos en valores de ángulos de difracción 2θ :

-La anterioridad D1 (EP 0161534) especialmente en el ejemplo 1, describe la obtención cristalización y recristalización de 4-deoxi-4'-metil-pirido[1',2':1,2]imidazo-[5,4-c]rifamicina SV, que es lo que la solicitud en estudio denomina "rifamixina", igualmente enseña que los derivados de rifamicina se cristalizan y recristalizan en solvente etanol/agua. El ejemplo 1 (D1) el solvente es etanol:agua 7:3(v/v) a temperatura ambiente. Pero la anterioridad no reporta los valores de diagrama de difracción de Rayos X para los cristales obtenidos y tampoco para las recristalizaciones, en eso se diferencia con la presente solicitud.

-La anterioridad D2 (US4.341.785) en el ejemplo 6, describe la forma de obtención de de 4-deoxi-4'-metil-pirido[1',2':1,2]imidazo-[5,4-c]rifamicina SV, que es lo que la solicitud en estudio denomina "rifamixina", los solventes apropiados para las cristalizaciones están descritos en la columna 4, renglones 10-26, y también están los estudio de actividad sobre diferentes microorganismos. La diferencia con la presente solicitud es que la anterioridad no reporta datos de difracción de Rayos X para los cristales obtenidos y tampoco para las recristalizaciones.

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En Cuanto a los procesos de obtención a partir de rifamicina O la anterioridad describe ese procedimiento en las pags 10-11, y otros procesos de cristalización y recrystalización están descritos en toda la anterioridad. Sin embargo se diferencian el proceso de cristalización de la solicitud porque se introducen datos de tiempos y temperaturas, pero se llevan a cabo con los mismos solventes para la misma sustancia a recrystalizar.

Nivel inventivo (artículo 18 D486 de la C.A.N.)

-La solicitud reclama tres formas polimórficas de una sustancia del estado de la técnica rifamixina (4-deoxi-4'-metil-pirido[1',2':1,2]imidazo-[5,4-c]rifamicina SV), se sabe por D1 y D2 que la sustancia ha sido cristalizada y recrystalizada en etanol/agua, pero no se reportaron los patrones de difracción de Rayos X, en términos de los ángulos interplanares, determinados mediante la técnica de Difracción de Rayos X, técnica que permite poner de manifiesto sólo una propiedad física del estado sólido y no una definición de la molécula, porque debe tenerse en claro que la molécula es del estado de la técnica, al igual que el conocimiento de su actividad farmacológica como antibiótico.

-D3 describe derivados de rifamicina y los métodos de cristalizar y recrystalizar esta clase de moléculas, que se obtiene en formas cristalinas y que se recrystalizan en etanol/agua, lo que nos enseña esta solicitud es que los derivados de rifamicina son cristalizables y recrystalizables en etanol/agua. Pero sin el reporte de patrones de difracción de Rayos X para elucidar cual fue la forma de cristalina espontanea que se obtuvo de acuerdo con las condiciones termodinámicas en se desarrollo la cristalización.

Dentro de estas formas, la ordenación espacial de las moléculas puede ser diferente y cualquier persona normalmente versada en la materia sabe que para obtención de polimorfos se deben manipular los siguientes parámetros:

- i) Manipulando adecuadamente los índices de cristalización
- ii) Naturaleza del solvente
- iii) Temperatura
- iv) Velocidad de enfriamiento
- v) Agitación
- vi) Cantidad de solvente usado
- vii) Pureza del solvente

Así, si el formulador encuentra que la rifamixina o cualquier fármaco, se requiere mejorar la velocidad de disolución o cualquier propiedad mecánica obviamente la formulará como cristal; si es velocidad de disolución se requiere que guarde humedad, porque así lo enseña el acervo tecnológico, no es la primera vez que se obtiene un sólido cristalino de rifamixina donde se recrystalice en agua/ alcohol. Los otros factores que afectan la cristalización son elección del solvente, temperatura y control de la presión para hacer más lenta o más rápida la cristalización, para no esperar a que cristalice en condiciones ambientales, la elección de la óptima temperatura y del tiempo de crecimiento de los cristales, son ensayos de prueba y error que siempre deben

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hacerse, pero cuyo resultado no es patentable, porque por recristalizaciones jamás se obtiene un compuesto diferente, siempre es la misma estructura química y la misma actividad, para nuestro caso antibiótico y no parece obtenerse una característica nueva ni tampoco obtenida por el ingenio del "inventor."

Entonces así las cosas en la presente solicitud, no se observa el carácter inesperado de la invención, tampoco se observa la superación de las dificultades técnicas reales, o la solución original al problema de la biodisponibilidad.

Para una persona versada en la materia, las enseñanzas de D1, D2 y D3 individualmente tomadas, muestran que la rifamixina cristaliza y que se recristaliza en mezcla de solventes etanol/agua. Los controles de las condiciones termodinámicas de cristalización de rifamixina lo llevarán a cristalizar de la manera más estable a esas condiciones termodinámicas controladas, o sea, se obtendrá un empaquetamiento molecular espontaneo para esas condiciones, y para saber que clase de empaquetamiento fue se le realizan patrones de difracción de Rayos X, a la monocelda.

El procedimiento de cristalización de la presente solicitud no aporta nada al acervo tecnológico. Es decir una formas de empaquetamiento molecular de rifamixina con contenidos de humedad, que se diferencia de otro cristal por el patrón de difracción de rayos X, que se logra de acuerdo al solvente recristalizar controlando las condiciones termodinámicas de temperatura, velocidad de enfriamiento, agitación y en cuanto al solvente, cantidad de solvente usado y pureza del solvente; es evidente que el proceso de cristalización no constituye un aporte a los procesos de cristalización.

En cuanto a los patrones de difracción de rayos X el método detecta un fenómeno físico, a través del cual se manifiesta la interacción fundamental de los rayos X con los cristales, que consiste en medir las intensidades de la mayor cantidad posible de haces difractados del espectro tridimensional de difracción, de tal manera que se puedan obtener de ellas los módulos de los factores de estructura, y de sus valores mediante algún procedimiento de asignación de fases a cada uno de estos factores, también permite reconstruir la distribución electrónica en la celdilla elemental, cuyos máximos corresponderán a las posiciones atómicas y de esta manera obtener la información de la estructura atómica dentro de la celdilla unidad, si importar la sustancia. Es decir el método permite una descripción del patrón estructural de la red cristalina de manera detallada mediante la cuantificación de una propiedad inherente al estado sólido de los cristales, descripción que incluye desde las dimensiones de la unidad de celda, el tipo de retículo y el sistema cristalino hasta la determinación de los posibles grupos espaciales.

En este orden de ideas, si se reclama protección para una misma estructura química que cuenta con una capacidad particular de cristalización, es decir una propiedad inherente a la naturaleza de las moléculas de rifamixina, resulta ambiguo que al momento de reivindicar el hábito cristalino de la estructura,

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éste se defina en términos de una propiedad física, cuando en síntesis se pretende protección para el mismo núcleo químico pero caracterizado por un hábito particular de su estado sólido, en atención a su capacidad intrínseca de cristalizar como es conocido en D1, D2 y D3. Es decir, químicamente se trata del mismo núcleo estructural, solo que debido a su capacidad de estado sólido, es capaz de cristalizar en formas particulares, determinadas única y exclusivamente por la naturaleza del compuesto y no es producto de un prediseño ingenioso de agrupación de moléculas.

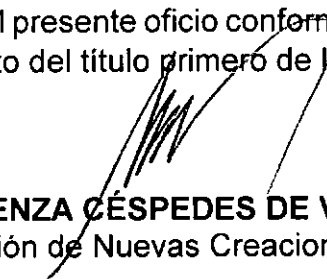
N°	Documento N°	Reivindicaciones afectadas	Requisito que afecta
D1	EP 0161534	1-12	Nivel Inventivo
D2	US 4,341,785	1-12	Nivel Inventivo
D3	GB 1590732	1-12	Nivel inventivo

Respuesta a los argumentos del solicitante:

-En cuanto a la materia contenida por la anterioridad ajunta al memorial de oposición, si bien es cierto que se trata de la rifamixina cristalizada e igualmente en etanol/agua, allí no se midió los patrones de difracción de rayos X, entonces nos faltó ese dato para decir que son consientes completamente, pero igualmente no esta mostrado el efecto técnico inesperado en el que se basa el nivel inventivo, toda vez que se sabe que la rifamixina y sus derivados son sustancias cristalinas y que para su recristalización se utilizo el mismo solvente hasta en las mismas proporciones, lo único que falto fue establecer las condiciones termodinámicas de cristalización, pero eso son del trabajo rutinario en recristalizaciones, por parte del formulador.

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 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 N° 10 de 2001.


ALIX CARMENZA CÉSPEDES DE VERGEL
Jefe de División de Nuevas Creaciones

El anterior requerimiento fue notificado por fijación en lista, de fecha
12 9 MAYO 2009

CONCEPTO TÉCNICO No.

EXAMINADOR TÉCNICO Código No.202012

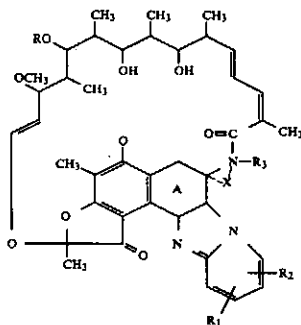
IMIDAZO-RIFAMYCIN DERIVATIVES WITH ANTIBACTERIAL UTILITY

BACKGROUND OF THE INVENTION

Rifamycin derivatives bearing a heterocyclic ring which is condensed at the 3,4-positions are known from the art literature, including the patent literature. As an example, in South African Pat. No. 68/0903 there are claimed pyrrolo [5,4-c] rifamycin SV derivatives, whereas German Patent Publications Nos. 2,739,671 and 2,739,623 describe some imidazo [5,4-c] rifamycin SV compounds which bear substituent at the positions 1 and 2. Thiazolo [5,4-c] rifamycin SV (rifamycin F) derivatives are reported in the German Patent Publication No. 2,741,066.

SUMMARY OF THE INVENTION

The present invention refers to new imidazo-rifamycin derivatives of the following general formula



wherein:
A may be the structure



—x— is a chemical bond or nil;
R is hydrogen or acetyl

R₁ and R₂ independently represent hydrogen, (C₁₋₄) alkyl, benzyloxy, mono- and di-(C₁₋₃)alkylamino-(C₁₋₄)alkyl, (C₁₋₃)alkoxy-, (C₁₋₄)alkyl, hydroxy-methyl, hydroxy-(C₂₋₄)-alkyl, nitro or R₁ and R₂ taken together with two consecutive carbon atoms of the pyridine nucleus form a benzene ring optionally substituted by one or two methyl or ethyl groups; R₃ is a hydrogen atom or nil;

with the proviso that, when A is A₁, —x— is nil and R₃ is a hydrogen atom; with the further proviso that, when A is A₂, —x— is a chemical bond and R₃ is nil.

The compounds possess antibacterial utility.

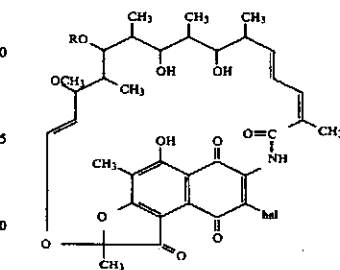
A preferred group of compounds comprises those compounds of formula I wherein A may be the structure A₁ or the structure A₂ as above indicated, —x— is

a chemical bond or nil, R is hydrogen or acetyl, R₁ and R₂ independently represent hydrogen, (C₁₋₄)alkyl, benzyloxy, hydroxy (C₂₋₄) alkyl, di-(C₁₋₃) alkylamino-(C₁₋₄) alkyl, nitro or R₁ and R₂ taken together with two consecutive carbon atoms of the pyridine nucleus form a benzene ring and R₃ is a hydrogen atom or nil; with the proviso that, when A is A₁, —x— is nil and R₃ is a hydrogen atom; with the further proviso that, when A is A₂, —x— is a chemical bond and R₃ is nil.

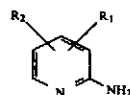
A second preferred group of compounds comprises those compounds of formula I wherein A may be the structure A₁ or the structure A₂ as above indicated, —x— is a chemical bond or nil, R is acetyl, R₁ and R₂ independently represent hydrogen, (C₁₋₄) alkyl or R₁ and R₂ taken together with two consecutive carbon atoms of the pyridine nucleus form a benzene ring and R₃ is a hydrogen atom or nil; with the proviso that, when A is A₁, —x— is nil and R₃ is a hydrogen atom; with the further proviso that, when A is A₂, —x— is a chemical bond and R₃ is nil.

As used herein, the terms (C₁₋₃) alkyl, (C₂₋₄)alkyl and (C₁₋₄) alkyl identifies linear or branched alkyl radicals containing from 1 to 3 or 2 to 4 or 1 to 4 carbon atoms, such as, for instance, methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, isobutyl or tert-butyl, whereas the term (C₁₋₃) alkoxy essentially refers to methoxy, ethoxy, propoxy or isopropoxy groups.

The compounds of the invention are prepared according to methods which essentially depends on the type of compound one wishes to obtain. Thus, for instance, the compounds of formula I wherein A represents structure A₂, R, R₁ and R₂ are as above defined, —x— is an additional bond and R₃ represents nil are conveniently prepared by reacting a 3-halorifamycin S of formula



wherein hal represents a halogen atom, preferably bromine or iodine, and R is defined as above, with a compound of formula



wherein R₁ and R₂ have the same meaning as before.

In the actual practice, the reaction is carried out by contacting a molar amount of the compound of formula II with a molar excess of the compound of formula III.

IMIDAZO-RIFAMYCIN DERIVATIVES WITH ANTIBACTERIAL UTILITY

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[52] U.S. Cl. 424/256; 260/239.3 P

[58] Field of Search 260/239.3 P; 424/256

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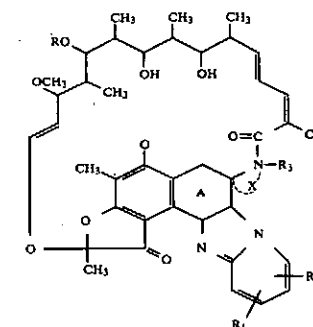
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Primary Examiner—Robert T. Bond
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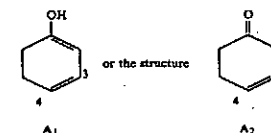
ABSTRACT

New rifamycin derivatives of the following general formula



wherein:

A may be the structure



—x— is a chemical bond or nil;
R is hydrogen or acetyl

R₁ and R₂ independently represent hydrogen, (C₁₋₄) alkyl, benzyloxy, mono- and di-(C₁₋₃)alkylamino-(C₁₋₄)alkyl, (C₁₋₃)alkoxy-, (C₁₋₄)alkyl, hydroxy-methyl, hydroxy-(C₂₋₄)-alkyl, nitro or R₁ and R₂ taken together with two consecutive carbon atoms of the pyridine nucleus form a benzene ring optionally substituted by one or two methyl or ethyl groups; R₃ is a hydrogen atom or nil;

with the proviso that, when A is A₁, —x— is nil and R₃ is a hydrogen atom; with the further proviso that, when A is A₂, —x— is a chemical bond and R₃ is nil.

The compounds possess antibacterial utility.

13 Claims, No Drawings

102/160/163

used in the therapy, are scarcely absorbed from animal organs and tissues when administered by oral route, and are found unaltered in the stool in a remarkable percentage with respect to the administered dosage. Thus, for instance, in a representative experiment carried out on groups of four rats fasted and normally fed, which were orally given 100 mg/kg of the compound of Example 6, it was found that, after four hours from the administration only 0.2 µg/ml and 6.5 µg/g were absorbed respectively by the serum and the liver in the fasted rats, and only 0.1 µg/ml and 0.7 µg/g were absorbed respectively by the serum and the liver in the fasted rats.

In a further representative experiments aiming at evaluating the extent of the urinary and faecal elimination of the compounds of the invention, groups of six rats were orally given 25 mg/kg of the compound of Example 6. Stools and urine were collected for 72 hours, then the content of compound was determined by microbiological route.

After this period of time, almost 60% of the compound was found unaltered in the stools, whereas the amount of said compound in the urine could not be determined because of its very low concentration. These data, coupled with the remarkable *in vitro* activity of the compounds of the invention against Gram negative bacteria present in the intestinal bacterial flora in pathological situations indicate that the compound of formula I are useful as intestinal antibacterial agents. These properties were confirmed by experiments carried out on rats by determining the total number (total bacterial charge) in stool samples collected both from animals which received no compounds and from animals which daily received by oral route predetermined amounts of the compounds of the invention.

The experiments lasted seven days and were conducted on groups of six animals. The choice of the animal species (rat) is absolutely justified, as rats have an intestinal bacterial flora similar to that of humans.

The obtained results are reported in the following table, wherein the total bacterial charge is expressed as the decimal logarithm of the number of bacteria in one gram of collected fresh stools. As the representative compounds, those of Examples 6 and 11 were taken into consideration.

TABLE 2

Compound of Ex-ample	Dose					
	1 mg/kg/die per os total bacterial charge		10 mg/kg/die per os total bacterial charge		30 mg/kg/die per os total bacterial charge	
	aerobic	anaerobic	aerobic	anaerobic	aerobic	anaerobic
6	4.50	6.00	4.50	3.60	4.50	3.30
11	3.50	4.83	5.00	2.83	8.20	3.75
Controls	6.00	9.83	5.40	7.33	9.67	6.50

The results reported in this table are self-explanatory. They clearly show that the compounds of the invention cause a marked reduction of the bacterial intestinal flora of the laboratory animals. It was also discovered that this action displayed by the compounds of the invention is even higher than that possessed by neomycin, an aminoglycoside antibiotic employed as intestinal antibacterial agent (see, for instance, Remington's Pharmaceutical Sciences, page 1126, 16th Edition, Mack Publishing Company, Pennsylvania, 1980) which, however,

displays a lot of dangerous side effects (see again Remington's Pharmaceutical Sciences, page 1274).

These favorable biological properties are coupled with a very low toxicity, being the LD₅₀ values per os both in rats and in mice always higher than 2000 mg/kg.

The use of the compounds of the invention as antibacterial agents refers to all industrially applicable acts and aspects of said use, including their incorporation into pharmaceutical compositions.

The pharmaceutical compositions containing the active ingredient are in fact a further specific object of the invention.

The compounds of the invention can therefore be administered by several routes, as an example by oral, topical or intramuscular route. For such administrations, the substances are embodied into conventional pharmaceutical dosage formulations. These formulations contain, in admixture with the active ingredient, the usual additives such as, for instance, sweetening, flavoring, coloring, coating and preservative agents, inert diluents such as, for instance, calcium or sodium carbonate, lactose and talc, binding agents, e.g. starch, gelatin and polyvinylpyrrolidone, suspending agents, e.g. methylcellulose or hydroxyethylcellulose, and wetting agents such as, for instance, lecithin, polyoxyethylene stearates and polyoxymethylene sorbitan monooleate. The preparation useful for the topical and intramuscular administration may contain the active ingredient dissolved or suspended in distilled and pyrogen-free water, in admixture with the commonly employed pharmaceutical carriers.

The invention can be better illustrated by means of the following examples which, however, must in no way be construed as a limitation of the scope of the invention itself.

The U.V. spectra have been recorded in absolute methanol with a Parkin-Elmer 552 spectrophotometer.

The I.R. spectra have been recorded in KBr with a Perkin-Elmer 281-B spectrophotometer.

The ¹H-NMR and ¹³C-NMR, where not expressly specified, have been recorded in CDCl₃ with a Varian XL 100 spectrophotometer, using tetramethylsilane as the reference substance.

The reported data are in agreement with the proposed structures.

EXAMPLE 1

N-Dehydro-4-deoxy-2-imino-4'-methyl-pyrido[1',2':1,2]imidazo[5,4-c]rifamycin S

A solution of 1.54 g (0.002 mole) of 3-bromo-rifamycin S dissolved in 10 ml of ethanol was added under stirring at room temperature with 0.430 g (0.004 mole) of 2-amino-4-methyl-pyridine. The reaction mixture was kept at this temperature for about two hours until complete disappearance of 3-bromo-rifamycin S [TLC-monitoring; eluting system CHCl₃/CH₃OH=40/1 (v/v)], then 250 ml of ethyl acetate were added. The organic phase was separated, washed first with 5% aqueous citric acid, then with water until pH 7 and subsequently dried over sodium sulfate. After evaporating to dryness, the obtained residue was taken up with ethanol from which the title compound separated. Yield: 1.35 g (86% of theoretical). M.p.: 228°-32° C. (with decomposition).

U.V. Spectrum:	λ _{max} (mμ)	E _{1%} ^{1cm}
	242	544

This molar excess varies from about 2 to about 8 or more equivalents calculated on the compound of formula II. The reaction is performed in the presence of an inert organic solvent such as, for instance, an aliphatic alkanol containing from 1 to 4 carbon atoms, a lower halogenated hydrocarbon from 1 to 2 carbon atoms, dioxane, tetrahydrofuran or mixture thereof, at a temperature which may vary within wide limits, though the room temperature is the preferred one. The reaction is completed within a period of time comprised between about 1 and about 4 hours.

The so obtained compounds may undergo further chemical reactions having the purpose of preparing other substances falling within the scope of the above formula I. Thus, for instance, compounds of formula I can be obtained where A is structure A₁, R₁, R₂ and R₃ have the same meanings as before, —x— represents nil and R₃ stands for a hydrogen atom, by treating the corresponding substance in which A represents structure A₂ with a suitable reducing agent such as, for instance, L(-)-ascorbic acid.

This step, which can even be performed without isolating the compound deriving from the condensation of the starting substances of formula II and III, occurs essentially at room temperature, though a gentle heating can sometimes favor the reaction course, for a period of time varying from about 10 minutes to about 1 hour, in an inert organic solvent again selected, for instance, from an aliphatic alkanol from 1 to 4 carbon atoms, a lower halogenated hydrocarbon from 1 to 2 carbon atoms, dioxane, tetrahydrofuran or mixtures thereof. In turn, the so obtained compounds of formula I wherein A is structure A₁, R₁, R₂ and R₃ have the above meanings, —x— represents nil and R₃ stands for a hydrogen atom, can be transformed into the corresponding products in which A represents structure A₂ by treatment with an appropriate oxidizing agent such as, for instance, manganese dioxide, lead tetracetate, dichloro-dicyano-benzoquinone, 2,3,4,5-tetrachloro-benzoquinone, 2,3,5,6-tetrachloro-benzoquinone and 40 (y/ml).

in the presence of a solvent, generally an aliphatic alkanol from 1 to 4 carbon atoms, treating a molar amount of the selected compound with a molar excess of an alkali agent, e.g. sodium or potassium hydroxide or carbonate, at room temperature, for a period of time varying from about 1 to about 5 hours. The so obtained compounds of formula I in which R is hydrogen may in turn undergo the above illustrated reduction or oxidation reactions, depending on the structure A represents.

The substances according to the invention are recovered from the reaction medium by means of techniques entirely familiar to a skilled technician. These techniques comprise the extraction with a suitable organic solvent, e.g. ethyl acetate, chloroform, methylene chloride and analogs or mixtures thereof, the evaporation to dryness of the organic extract and taking up the residue with an appropriate solvent from which the final product separates. Alternatively, the reaction mixture can be directly evaporated to dryness and the obtained residue is in turn taken up with a suitable solvent from which the final product separates. Crystallization solvents which can advantageously be employed are selected from water, methanol, ethanol, n-propanol, isopropanol, n-hexane, ethylacetate, methylene chloride, chloroform, ethylene glycol monomethyl ether or mixtures thereof.

As stated above, the compounds of the invention are useful antibacterial agents. More exactly, they possess a remarkable *in vitro* activity both against Gram positive (as an example, various *Staph. aureus* strains) and Gram negative bacteria, some of them from clinical isolation, which can be found in the intestinal bacterial flora in pathological conditions.

The results obtained with representative compounds of the invention are summarized in the following table. They represent the minimum concentration of active substance capable of inhibiting the growth *in vitro* of the pathogenic bacterium (M.I.C.) and are expressed as micrograms of substance per ml of culture medium (y/ml).

TABLE 1 (M.C.I.)

STRAIN	COMPOUND OF EXAMPLE											
	1	2	3	5	6	7	8	10	11	12		
<i>Staph. aureus</i> 209 P FDA	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
<i>Staph. aureus</i> Colliva	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.001
<i>Pseud. aeruginosa</i> ATCC 10145	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.7+1.5	6+12	3+6	3+6
<i>Esch. coli</i> Beltrami	0.7	0.7	0.7-1.3	~12	0.7-1.5	<0.7	0.7-1.5	6+12	0.3	0.3+0.7		
<i>Esch. coli</i> 47/9	12+25	~12	12+25	~12	12+25	6+12	12+25	~12	6+12	6+12	6+12	6+12
<i>Esch. coli</i> ML/35	6+12	6+12	12+25	~25	~6	6+12	12+25	12+25	3+6	3+6	3+6	3+6
<i>Klebs. pneumoniae</i> G24viani	12+25	6+12	12+25	~12	12+25	6+12	12+25	6+12	3+6	6+12	6+12	6+12
<i>Proteus mirabilis</i> ATCC 29484	12+25	~6	12	n.d.	~12	~6	6+12	n.d.	6+12	6+12	6+12	6+12
<i>Sal. typhimurium</i> ATCC 13311	n.d.	12+25	12	3+6	~6	6+12	6+12	~3	3+6	3+6	3+6	3+6
<i>Sal. typhimurium</i> ATCC 13311	n.d.	12+25	~6	12+25	~6	6+12	6+12	~3	3+6	3+6	3+6	3+6
<i>Enter. cloacae</i> ATCC 13047	n.d.	n.d.	n.d.	6+12	n.d.	n.d.	12+25	6+12	6+12	6+12	6+12	6+12
<i>Shig. sonnei</i> ATCC 9290	6+12	12+25	6+12	~12	6+12	12+25	6+12	6+12	~1.5	~1.5	~1.5	~1.5
<i>Shig. flexneri</i>	n.d.	~12	3+6	n.d.	6+12	~12	3+6	n.d.	3+6	3+6	3+6	3+6

< = less than
~ = about
n.d. = not determined

analogs. This reaction is carried out in an inert organic solvent like those indicated above, at temperature comprised between room temperature and the boiling temperature of the reaction mixture. A period of time varying from about 10 minutes to about 1 hour is sufficient for having the reaction completed.

Finally, the compounds of formula I wherein R is hydrogen are conveniently prepared by hydrolyzing, under alkaline conditions, the corresponding compounds where R is acetyl. This hydrolysis is carried out

The compounds of the invention possess also a noteworthy *in vivo* activity against the experimental infection provoked by *Staphylococcus aureus* when administered by subcutaneous route. This *in vivo* activity, expressed as an ED₅₀ value, may vary between about 0.1 and about 0.5 mg/kg.

It has also been found that the compounds of the invention, unlike other rifamycin derivatives widely

mole) of 3-iodo-rifamycin S and 0.288 g (0.002 mole) of 1-amino-isoquinoline. Yield: 0.510 g (62% of theoretical). M.p.: 198°-203° C. (with decomposition).

U.V. Spectrum:	λ_{max} (m μ)	E _{1%} ^{1cm}
	256	765
	322	264
	338	280
	413	107

I.R. Spectrum: characteristic absorption bands were observed at the following frequencies (in cm⁻¹): 3460; 3120 (w); 3060 (w); 2980 (s); 2930 (s); 2880; 2820; 1735 (s); 1715 (s); 1660; 1625 (vw); 1600; 1525.

¹H-NMR Spectrum: characteristic resonance peaks were observed at the following δ (expressed as p.p.m.): 0.06 (d, 3H); 0.22 (d, 3H); 0.5 (d, 3H); 0.83 (d, 3H); 1.79 (s, 3H); 2.00 (s, 3H); 2.26 (s, 3H); 2.3 (s, 3H); 2.5-3.00 (m, 2H); 3.10 (s, 3H); 3.45 (s, 1H); 3.6 (d, 1H); 4.00 (s, 1H); 4.76 (d, 1H); 5.39 (q, 1H); 6.8 (m, 4H); 7.38 (d, 1H); 7.6-7.9 (m, 3H); 9.00 (m, 1H); 9.20 (d, 1H).

s=singlet; d=doublet; q=quartet; m=multiplet.

EXAMPLE 6

4-Deoxy-4'-methyl-pyrido[1',2':1,2]imidazo[5,4-c]rifamycin SV

1 Gram (0.00127 mole) of the compound of Example 1 was dissolved in 50 ml of absolute ethanol and the resulting solution was added with 20 ml of a 5% aqueous solution of L(-)-ascorbic acid. The mixture was kept at room temperature for about 1 hour until disappearance of the compound of Example 1 [TLC-monitoring; eluting system CHCl₃/CH₃OH=23/2 (v/v)], then it was extracted with 50 ml of ethyl acetate. After washing with water to pH 7 and drying over sodium sulfate, the solvent was evaporated off and the obtained residue was taken up with a 70/30 (v/v) mixture of glycol monomethyl ether/water. The title compound separated, which was recovered by filtration: Yield: 0.840 g (85% of theoretical). M.p.: 200°-5° C. (with decomposition).

U.V. Spectrum:	λ_{max} (m μ)	E _{1%} ^{1cm}
	232	489
	260	339
	292	295
	320	216
	370	119
	450	159

I.R. Spectrum: characteristic absorption bands were observed at the following frequencies (in cm⁻¹): 3440 (b); 2960 (s); 2920 (s); 2860 (w); 2820 (vw); 1705 (s); 1640 (s); 1580 (s); 1500 (s).

b=broad; s=strong; w=weak; vw=very weak

¹H-NMR Spectrum: characteristic resonance peaks were observed at the following δ (expressed as p.p.m.): -0.56 (d, 3H); 0.14 (d, 3H); 0.74 (d, 3H); 0.94 (d, 3H); 1.94 (s, 3H); 1.98 (s, 3H); 2.02 (s, 3H); 2.26 (s, 3H); 2.63 (s, 3H); 3.00 (s, 3H); 3.2-3.9 (m, 3H); 4.15-5.20 (m, 2H); 5.9-6.9 (m, 4H); 7.06 (dd, 1H); 7.38 (s, 1H); 8.39 (s, 1H); 8.43 (d, 1H); 11.0 (s, 1H); 13.12 (s, 1H).

s=singlet; d=doublet; m=multiplet; dd=doublet of doublet.

¹³C-NMR Spectrum: characteristic resonance peaks were observed at the following (expressed as p.p.m.): 6.98; 8.06; 8.21; 10.76; 17.56; 20.43; 20.78; 21.44; 22.35;

32.91; 36.93; 37.78; 38.59; 56.99; 72.65; 73.91; 76.75; 77.86; 97.83; 103.86; 104.09; 108.97; 109.99; 112.03; 114.96; 115.52; 117.61; 119.26; 122.99; 125.35; 128.44; 128.96; 136.21; 138.87; 141.75; 142.10; 147.74; 155.10; 170.63; 171.89; 182.19; 188.84.

EXAMPLES 7-10

The following compounds were prepared substantially as described in Example 6.

EXAMPLE 7

4-Deoxy-5'-methyl-pyrido [1',2':1,2] imidazo [5,4-c] rifamycin SV, from 1 g (0.00127 mole) of the compound of Example 2. Yield: 0.940 g (95% of theoretical). M.p.: 185°-90° C. (with decomposition).

U.V. Spectrum:	λ_{max} (m μ)	E _{1%} ^{1cm}
	217	470
	235	544
	262	333
	273	303
	292	295
	320	205
	356	99
	373	122
	440	164
	454	166

I.R. Spectrum: characteristic absorption bands were observed at the following frequencies (in cm⁻¹): 3440 (b); 3300 (b); 3200 (b); 2960 (s); 2920 (w); 2850 (vw); 1730 (s); 1710 (w); 1640 (s); 1595 (s); 1580 (b); 1555 (w).

b=broad; s=strong; w=weak; vw=very weak

¹H-NMR Spectrum: characteristic resonance peaks were observed at the following δ (expressed as p.p.m.): 0.64 (d, 3H); 0.02 (d, 3H); 0.45 (d, 3H); 0.90 (d, 3H); 1.75 (s, 3H); 1.94 (s, 3H); 1.97 (s, 3H); 2.23 (s, 3H); 2.45 (s, 3H); 2.95 (s, 3H); 2.6-5.8 (m, 5H); 4.5-5.25 (m, 2H); 5.5-7.0 (m, 4H); 7.25-7.75 (m, 2H); 8.27 (s, 1H); 8.47 (s, 1H); 14.86 (s, 1H); 16.77 (s, 1H).

s=singlet; d=doublet; m=multiplet

EXAMPLE 8

4-Deoxy-3'-methyl-pyrido [1',2':1,2] imidazo [5,4-c] rifamycin SV, from 1.5 g (0.00191 mole) of the compound of Example 3. Yield: 1.46 g (96.3% of theoretical). M.p. 193°-98° C. (with decomposition).

U.V. Spectrum:	λ_{max} (m μ)	E _{1%} ^{1cm}
	218	493
	244	433
	258	518
	274	301
	294	315
	304	207
	360	104
	373	123
	448	166

I.R. Spectrum: characteristic absorption bands were observed at the following frequencies (in cm⁻¹): 3340 (b); 3300 (b); 2960 (s); 2925 (s); 2870 (vw); 2850 (s); 1730 (s); 1710 (vw); 1650 (vw); 1640 (s); 1600 (vw); 1585 (s); 1565 (w); 1525 (vw); 1505 (s).

b=broad; s=strong; w=weak; vw=very weak

¹H-NMR Spectrum: characteristic resonance peaks were observed at the following δ (expressed as p.p.m.): -0.7 (d, 3H); 0.05 (d, 3H); 0.68 (d, 3H); 0.87 (d, 3H); 1.73

-Continued

U.V. Spectrum:	λ_{max} (m μ)	E _{1%} ^{1cm}
	272	391
	320	204
	342	190
	375	76
	415	116

I.R. Spectrum: characteristic absorption bands were observed at the following frequencies (in cm⁻¹): 3440 (b); 2960 (s); 2920 (w); 2860 (w); 2800 (w); 1728 (s); 1708 (s); 1635 (w); 1590 (s); 1500 (w).

b=broad; s=strong; w=weak.

¹H-NMR Spectrum: characteristic resonance peaks were observed at the following δ (expressed as p.p.m.): 0.02 (d, 3H); 0.04 (d, 3H); 0.53 (d, 3H); 0.91 (d, 3H); 1.77 (s, 3H); 2.06 (s, 3H); 2.25 (s, 3H); 2.28 (s, 3H); 2.54 (s, 3H); 2.75-3.05 (m, 2H); 3.10 (s, 3H); 3.45 (s, 1H); 3.58 (d, 1H); 4.02 (d, 1H); 4.82 (d, 1H); 5.36 (dd, 1H); 6.32 (dd, 1H); 6.5-6.8 (m, 3H); 7.00 (dd, 1H); 7.80 (s, 1H); 9.32 (d, 1H); 13.4 (s, 1H).

s=singlet; d=doublet; m=multiplet; dd=doublet of doublet

¹³C-NMR Spectrum: characteristic resonance peaks were observed at the following δ (expressed as p.p.m.): 7.35; 8.18; 9.55; 10.61; 14.18; 20.69; 21.44; 21.80; 22.26; 34.23; 36.45; 37.05; 39.80; 57.30; 73.98; 76.18; 77.06; 78.47; 108.20; 109.82; 110.45; 111.22; 117.63; 118.31; 118.89; 120.90; 125.13; 127.09; 128.44; 132.83; 138.61; 139.71; 142.03; 142.44; 146.45; 146.52; 150.01; 170.98; 172.57; 180.62; 181.92.

EXAMPLES 2-5

The following compounds were prepared substantially according to the same procedure of the above Examples, starting from an appropriate 3-halo-rifamycin S of formula II and a predetermined compound of formula III.

EXAMPLE 2

N-Dehydro-4-deoxy-2-imino-5'-methyl-pyrido[1',2':1,2]imidazo[5,4-c]rifamycin S, from 1.54 g (0.002 mol) of 3-bromo-rifamycin S and 0.432 g (0.004 mol) of 2-amino-3-methyl-pyridine. Yield: 1.2 g (78% of theoretical). M.p.: 208°-12° C. (with decomposition).

U.V. Spectrum:	λ_{max} (m μ)	E _{1%} ^{1cm}
	238	574
	270	370
	310	298
	370	103
	422	131
	440	122

I.R. Spectrum: characteristic absorption bands were observed at the following frequencies (in cm⁻¹): 3450 (b); 2980 (s); 2920 (s); 2870 (s); 2820 (s); 1735 (s); 1710 (s); 1660 (s); 1630 (s); 1600 (s); 1555 (s).

b=broad; s=strong.

¹H-NMR Spectrum: characteristic resonance peaks were observed at the following δ (expressed as p.p.m.): 0.0 (d, 3H); 0.54 (d, 3H); 0.88 (d, 3H); 1.3 (d, 3H); 1.73 (s, 3H); 2.02 (s, 3H); 2.23 (s, 3H); 2.27 (s, 3H); 2.4 (s, 3H); 2.5-3.0 (m, 2H); 3.05 (s, 3H); 3.42 (s, 1H); 3.52 (d, 1H); 3.9 (d, 1H); 4.74 (d, 1H); 5.3 (q, 1H); 6.3 (d, 1H); 6.4-7.0

(m, 3H); 7.35 (d, 1H); 7.58 (d, 1H); 9.22 (s, 1H); 13.25 (s, 1H).

s=singlet; d=doublet; q=quartet; m=multiplet

EXAMPLE 3

N-Dehydro-4-deoxy-2-imino-3'-methyl-pyrido[1',2':1,2]imidazo[5,4-c]rifamycin S, from 1.54 g (0.002 mole) of 3-bromo-rifamycin S and 0.432 g (0.004 mole) of 2-amino-3-methyl-pyridine. Yield: 1.05 g (67% of theoretical).

U.V. Spectrum:	λ_{max} (m μ)	E _{1%} ^{1cm}
	242	459
	274	352
	322	260
	345	153
	384	79
	418	99
	510	36

I.R. Spectrum: characteristic absorption bands were observed at the following frequencies (in cm⁻¹): 3410 (b); 3340 (b); 2960 (s); 2920 (s); 2880 (w); 2840 (w); 1735 (s); 1710 (w); 1655 (s); 1620 (vw); 1598 (s); 1505 (s).

s=strong b=broad w=weak vw=very weak

¹H-NMR Spectrum: characteristic resonance peaks were observed at the following δ (expressed as p.p.m.): 0.04 (d, 3H); 0.55 (d, 3H); 0.93 (d, 3H); 1.1-1.5 (m, 4H); 1.8 (s, 3H); 2.09 (s, 3H); 2.25 (s, 3H); 2.32 (s, 3H); 2.48 (s, 3H); 2.7-3.3 (m, 2H); 3.13 (s, 3H); 3.52 (s, 1H); 3.62 (d, 1H); 4.1 (d, 1H); 4.9 (d, 1H); 5.45 (q, 1H); 6.48 (d, 1H); 6.6-6.9 (m, 3H); 7.55 (d, 1H); 8.08 (d, 1H); 9.55 (s, 1H); 13.35 (s, 1H).

s=singlet; d=doublet; m=multiplet; q=quartet.

EXAMPLE 4

5'-Benzyloxy-N-dehydro-4-deoxy-2-imino-pyrido[1',2':1,2]imidazo[5,4-c]rifamycin S, from 1.54 g (0.002 mole) of 3-bromo-rifamycin S and 1.6 g (0.008 mole) of 2-amino-3-benzyloxy-pyridine. Yield: 0.840 g (46% of theoretical). M.p. 198°-203° C. (with decomposition).

U.V. Spectrum:	λ_{max} (m μ)	E _{1%} ^{1cm}
	245	536
	275	349
	320	238
	420	95

I.R. Spectrum: characteristic absorption bands were observed at the following frequencies (in cm⁻¹): 3450 (b); 2980 (w); 2970 (s); 2930 (s); 2920 (vw); 1730 (s); 1710 (s); 1655 (s); 1625 (vw); 1595 (s); 1545 (s); 1505 (s).

b=broad; s=strong; w=weak; vw=very weak

¹H-NMR Spectrum: characteristic resonance peaks were observed at the following δ (expressed as p.p.m.): 0.08 (d, 3H); 0.53 (d, 3H); 0.94 (d, 3H); 1.1-1.6 (m, 4H); 1.82 (s, 3H); 1.12 (s, 3H); 1.3 (s, 3H); 1.34 (s, 3H); 2.7-3.2 (m, 2H); 3.15 (s, 3H); 3.3-3.8 (m, 2H); 4.10 (d, 1H); 4.88 (d, 1H); 5.42 (q, 1H); 5.9 (s, 2H); 6.42 (d, 1H); 6.5-6.8 (m, 3H); 6.9-7.2 (m, 2H); 7.3-7.8 (m, 5H); 9.14 (q, 1H); 13.39 (s, 1H).

s=singlet; d=doublet; m=multiplet; q=quartet

EXAMPLE 5

N-Dehydro-4-deoxy-2-imino-isoquinolino[2',1':1,2]imidazo[5,4-c]rifamycin S, from 0.820 g (0.001

108
105

was obtained which was taken up with chloroform and n-hexane. The title compound separated, which was recovered by filtration. Yield: 1.45 g (97% of theoretical). M.p. 207°-12° C. (with decomposition).

U.V. Spectrum:	λ_{max} (m μ)	$E_{1\%}^{1cm}$
	233	429
	272	281
	315	255
	342	175
	410	116

I.R. Spectrum: characteristic absorption bands were observed at the following frequencies (in cm^{-1}): 3460 (b), 3340 (b), 2960 (s), 2930 (s), 2880 (w), 2850 (w), 1735 (s), 1710 (vw), 1655 (s), 1625 (s), 1600 (s), 1505 (s).

b=broad; s=strong; w=weak; vw=very weak

¹H-NMR Spectrum: characteristic resonance peaks were observed at the following δ (expressed as p.p.m.): 0.02 (d, 3H); 0.5 (d, 3H); 0.9 (d, 3H); 1.77 (s, 3H); 2.04 (s, 3H); 2.26 (s, 3H); 2.29 (s, 3H); 2.75-3.05 (m, 2H); 3.10 (s, 3H); 3.42 (s, 1H); 3.58 (d, 1H); 4.01 (d, 1H); 4.82 (d, 1H); 5.37 (q, 1H); 6.34 (dd, 1H); 6.45-6.8 (m, 3H); 7.18 (m, 1H); 7.58 (m, 1H); 8.04 (d, 1H); 9.50 (d, 1H); 13.28 (s, 1H).

s=singlet; d=doublet; dd=doublet of doublet; m=multiplet; q=quartet

¹³C-NMR Spectrum: characteristic resonance peaks were observed at the following δ (expressed as p.p.m.): 7.36; 8.22; 9.58; 10.71; 14.14; 20.70; 21.43; 22.25; 34.24; 36.45; 37.01; 39.75; 57.31; 73.94; 76.2; 76.91; 78.38; 108.19; 109.99; 110.36; 111.40; 116.52; 117.79; 119.55; 120.75; 125.01; 128.01; 128.32; 130.09; 132.67; 138.67; 139.99; 142.40; 146.31; 146.64; 149.48; 171.22; 172.46; 172.60; 180.26; 181.82; 192.98.

EXAMPLE 13

25-Desacetyl-4-deoxy-4'-methyl-pyrido [1',2':1,2]imidazo [5,4-c]rifamycin SV

A solution of 1.57 g (0.002 mole) of the compound of Example 6 and 0.240 g of NaOH in 60 ml of methanol was kept at room temperature for about three hours, until disappearance of the starting compound [TLC-monitoring; eluting system: $CHCl_3/CH_3OH=23/2$ (v/v)]. The reaction mixture was then extracted with 300 ml of $CHCl_3$ and the organic extract was first washed with a 10% aqueous solution of citric acid and then with water to neutrality. After drying over sodium sulfate and removing the solvent by evaporation, the obtained residue was taken up with ethyl acetate. The title compound separated, which was recovered by filtration. Yield: 1.07 g (72% of theoretical). M.p. 199°-200° C. (with decomposition).

U.V. Spectrum:	λ_{max} (m μ)	$E_{1\%}^{1cm}$
	234	465
	260	305
	232	274
	310	232
	372	93
	436	138

I.R. Spectrum: characteristic absorption bands were observed at the following frequencies (in cm^{-1}): 3420 (b), 3330 (b), 2960 (s), 2920 (s), 2850 (s), 1735 (vw), 1725 (s), 1655 (w), 1650 (w), 1630 (vw), 1595 (s), 1585 (vw), 1505 (w).

b=broad; s=strong; w=weak; vw=very weak

¹H-NMR Spectrum: characteristic resonance peaks, recorded in $CDCl_3+CD_3OD$, were observed at the following δ (expressed as p.p.m.): -0.68 (d, 3H); -0.20 (d, 3H); 0.74 (d, 3H); 0.92 (d, 3H); 1.88 (s, 3H); 2.03 (s, 3H); 2.20 (s, 3H); 2.58 (s, 3H); 3.05 (s, 3H); 2.75-3.95 (m, 4H); 4.75-5.14 (m, 1H); 6.00-6.5 (m, 4H); 7.05 (d, 1H); 7.48 (d, 1H); 8.40 (d, 1H).

s=singlet; d=doublet; m=multiplet

EXAMPLE 14

25-Desacetyl-N-dehydro-4-deoxy-2-imino-4'-methyl-pyrido [1',2':1,2]imidazo [5,4-c]rifamycin S

This compound was prepared substantially as described in Example 12, starting from 0.740 g (0.001 mole) of the compound of Example 13. Yield: 0.720 g (97% of theoretical). M.p.: 200°-5° C. (with decomposition).

U.V. Spectrum:	λ_{max} (m μ)	$E_{1\%}^{1cm}$
	234	465
	260	305
	292	274
	310	232
	372	93
	436	138

I.R. Spectrum: characteristic absorption bands were observed at the following frequencies (in cm^{-1}): 3400 (b), 2960 (s), 2920 (s), 2870 (s), 1725 (s), 1655 (w), 1645 (w), 1630 (vw), 1595 (s), 1585 (vw), 1505 (s).

b=broad; s=strong; w=weak; vw=very weak

¹H-NMR Spectrum: characteristic resonance peaks were observed at the following δ (expressed as p.p.m.): 0.25 (d, 3H); 0.42 (d, 3H); 0.65 (d, 3H); 0.90 (d, 3H); 1.76 (s, 3H); 2.20 (s, 6H); 2.48 (s, 3H); 3.15 (s, 3H); 2.8-4.1 (m, 7H); 5.24 (q, 1H); 5.8-6.75 (m, 4H); 6.94 (d, 1H); 7.12 (d, 1H); 9.25 (d, 1H); 13.25 (s, 1H).

s=singlet; d=doublet; m=multiplet; q=quartet

By operating substantially according to the procedure illustrated in the above examples, the following compounds falling within the scope of the general formula I can be prepared.

A	x	R	R ₁	R ₂	R ₃
A ₁	nil	CH ₂ CO	H	4'-CH ₂ -CH ₂ -OH	H
A ₁	nil	CH ₂ CO	H	4'-CH ₂ -CH ₂ -CH ₂ OH	H
A ₁	nil	CH ₂ CO	H	4'-CH ₂ -CH ₂ -N(CH ₃) ₂	H
A ₁	nil	CH ₂ CO	H	4'-CH ₂ -N(CH ₃) ₂	H
A ₁	nil	CH ₂ CO	H	4'-CH ₂ -CH ₂ -CH ₂ -N(CH ₃) ₂	H
A ₁	nil	CH ₂ CO	3'-CH ₃	4'-CH ₂ -CH ₂ -CH ₂ -N(CH ₃) ₂	H
A ₁	nil	CH ₂ CO	4'-C ₂ H ₅	3'-CH ₂ -CH ₂ -CH ₂ -OH	H
A ₁	nil	CH ₂ CO	5'-CH ₃	3'-CH ₂ -CH ₂ -N(CH ₃) ₂	H
A ₁	nil	H	H	3'-CH ₂ CH ₂ -N(CH ₃) ₂	H
A ₁	nil	H	4'-CH ₃	3'-CH ₂ CH ₂ -N(CH ₃) ₂	H
A ₂	bond	CH ₃ CO	H	4'-CH ₂ -CH ₂ -N(CH ₃) ₂	nil
A ₂	bond	CH ₃ CO	H	4'-CH ₂ -CH ₂ -CH ₂ -N(CH ₃) ₂	nil
A ₁	nil	CH ₂ CO	H	4'-NO ₂	H
A ₂	bond	CH ₃ CO	H	4'-NO ₂	nil

The compounds of formula II were prepared according to the method described in U.S. Pat. No. 4,179,438.

The substances of formula III are commercial products.

We claim:

1. A compound of formula

(s, 3H); 1.92 (s, 3H); 1.97 (s, 3H); 2.23 (s, 3H); 2.63 (s, 3H); 2.92 (s, 3H); 3.25-4.00 (m, 5H); 4.6-5.10 (m, 2H); 5.9-6.8 (m, 4H); 7.13 (q, 1H); 7.6 (q, 1H); 8.48 (q, 1H); 14.14 (s, 1H); 16.65 (s, 1H)

s=singlet; d=doublet; m=multiplet; q=quartet

EXAMPLE 9

5'-Benzyloxy-4-deoxy-pyrido [1',2':1,2]imidazo [5,4-c]rifamycin SV, from 1.5 g (0.00167 mole) of the compound of Example 4. Yield: 1.6 g (96.4% of theoretical). M.p. 175°-80° C. (with decomposition).

U.V. Spectrum:	λ_{max} (m μ)	$E_{1\%}^{1cm}$
	238	561
	260	339
	282	311
	324	194
	353	100
	370	129
	432	153
	452	167

I.R. Spectrum: characteristic absorption bands were observed at the following frequencies (in cm^{-1}): 3520 (w), 3410 (w), 3310 (w), 3210 (w), 3140 (w), 3060 (vw), 2960 (s), 2920 (s), 1750 (vw), 1730 (vw), 1710 (w), 1650 (s), 1600 (w), 1575 (s), 1505 (s).

s=strong; w=weak; vw=very weak

¹H-NMR Spectrum: characteristic resonance peaks were observed at the following δ (expressed as p.p.m.): the spectrum was recorded in $CDCl_3+CD_3OD$: -0.65 (d, 3H); -0.25 (d, 3H); 0.73 (d, 3H); 0.90 (d, 3H); 1.92 (s, 3H); 1.97 (s, 3H); 2.02 (s, 3H); 2.28 (s, 3H); 2.83 (d, 1H); 2.97 (s, 3H); 3.1-4.0 (m, 1H); 4.5-5.2 (m, 2H); 5.4 (s, 2H); 5.8-6.95 (m, 4H); 7.0-7.75 (m, 7H); 8.21 (d, 1H)

s=singlet; d=doublet; m=multiplet

EXAMPLE 10

4-Deoxy-isoquinolino [2',1':1,2]imidazo [5,4-c]rifamycin SV, from 0.410 g (0.0005 mole) of the compound of Example 5. Yield: 0.400 g (97.5% of theoretical). M.P.: 181°-86° C. (with decomposition)

U.V. Spectrum:	λ_{max} (m μ)	$E_{1\%}^{1cm}$
	253	332
	286	363
	300	346
	320	290
	382	120
	430	120

I.R. Spectrum: characteristic absorption bands were observed at the following frequencies (in cm^{-1}): 3440 (b), 3140 (b), 2910 (s), 2850 (w), 1700 (s), 1630 (b), 1610 (b), 1580 (w), 1555 (vw), 1535 (vw).

b=broad; s=strong; w=weak; vw=very weak

¹H-NMR Spectrum: characteristic resonance peaks were observed at the following δ (expressed as p.p.m.): -0.65 (d, 3H); 0.04 (d, 3H); 0.7 (d, 3H); 0.88 (d, 3H); 1.55 (s, 3H); 1.92 (s, 3H); 2.02 (s, 3H); 2.27 (s, 3H); 2.77 (d, 1H); 2.94 (s, 3H); 3.00-3.90 (m, 4H); 4.78 (d, 1H); 4.93 (q, 1H); 5.75-7.00 (m, 4H); 7.34 (d, 1H); 7.6-8.0 (m, 6H); 16.6 (m, 1H).

s=singlet; d=doublet; m=multiplet; q=quartet

EXAMPLE 11

4-Deoxy-pyrido [1',2':1,2]imidazo [5,4-c]rifamycin SV

A solution of 2.32 g (0.003 mole) of 3-bromo-rifamycin S in 50 ml of ethanol was added at room temperature under stirring with 1.41 g (0.015 mole) of 2-amino-pyridine. The resulting mixture was kept at the same temperature for 4 hours until complete disappearance of 3-bromo-rifamycin S (TLC-monitoring, eluting system: ethyl acetate), then it was added with 300 ml of ethyl acetate. After washing the organic phase with a 5% aqueous solution of citric acid and, subsequently, with water to pH 7, and drying over sodium sulfate, the solvent was evaporated off, the obtained residue was dissolved in some ethanol and the resulting solution was added with 20 ml of a 5% aqueous solution of L(-) ascorbic acid. The reaction mixture was kept at room temperature for 15 minutes, added with 50 ml of $CHCl_3$, and subsequently washed with water to pH 7. After drying over sodium sulfate and evaporating off the solvent, the obtained residue was taken up with ethyl acetate. The title product separated, which was recovered by filtration.

Yield: 1.8 g (77.7% of theoretical). M.p.: 170°-75° C. (with decomposition).

U.V. Spectrum:	λ_{max} (m μ)	$E_{1\%}^{1cm}$
	234	521
	258	329
	293	301
	373	122
	450	160

I.R. Spectrum: characteristic absorption bands were observed at the following frequencies (in cm^{-1}): 3440 (b), 3300 (b), 3200 (b), 2970 (s), 2930 (s), 2880 (w), 2820 (w), 1635 (s), 1605 (w), 1585 (w), 1575 (w), 1605 (s).

b=broad; s=strong; w=weak

¹H-NMR Spectrum: characteristic resonance peaks were observed at the following δ (expressed as p.p.m.): -0.54 (d, 3H); 0.18 (d, 3H); 0.76 (d, 3H); 0.96 (d, 3H); 1.92 (s, 3H); 1.96 (s, 3H); 1.98 (s, 3H); 2.27 (s, 3H); 2.88 (d, 1H); 3.00 (s, 3H); 3.34 (d, 1H); 3.66 (d, 1H); 4.92 (d, 1H); 5.06 (m, 1H); 5.5-5.9 (m, 3H); 6.6-7.0 (m, 1H); 7.1-7.4 (m, 1H); 7.6-8.0 (m, 2H); 8.39 (s, 1H); 8.66 (d, 1H); 13.8 (s, 1H); 15.4 (s, 1H).

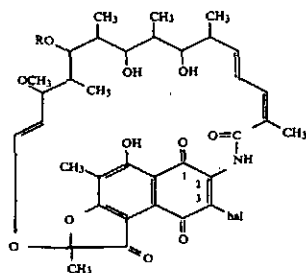
s=singlet; d=doublet; m=multiplet

¹³C-NMR Spectrum: characteristic resonance peaks were observed at the following δ (expressed as p.p.m.): 6.98; 8.37; 10.84; 17.53; 20.41; 20.79; 21.29; 32.96; 36.93; 37.86; 38.56; 57.08; 72.82; 74.00; 76.88; 77.87; 97.93; 104.23; 104.35; 108.94; 111.49; 112.28; 114.96; 115.02; 115.31; 119.69; 123.33; 125.42; 128.31; 129.77; 134.31; 137.06; 138.54; 141.97; 142.27; 155.14; 170.61; 171.86; 171.98; 182.32; 188.79.

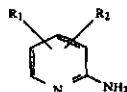
EXAMPLE 12

N-Dehydro-4-deoxy-pyrido [1',2':1,2]imidazo [5,4-c]rifamycin S

1 Gram of manganese dioxide was added to a solution of 1.5 g (0.00194 mole) of the compound of Example 11 in 30 ml of $CHCl_3$. The reaction mixture was kept at room temperature until disappearance of the starting compound (TLC-monitoring; eluting system: ethyl acetate). The oxidizer was then removed by filtration, the precipitate was washed with methanol, the methanolic and chloroform phases were joined together and washed with a 5% aqueous solution of citric acid and then with water to neutrality. After drying over sodium sulfate, the solvent was evaporated off and a residue



wherein hal stands for a halogen atom and R is hydrogen or acetyl, with a molar excess of a compound of formula



where R₁ and R₂ are as above defined, in the presence of an inert organic solvent, at room temperature, for a period of time comprised between about 1 and about 4 hours, whereby a compound of formula I is obtained in which A is A₂, R, R₁, and R₂ are as above defined, —x— is a covalent chemical bond and R₃ is nil, which can be transformed into the corresponding compounds of formula I wherein A is A₁, R, R₁ and R₂ are as above defined, —x— is nil and R₃ stands for a hydrogen atom, by treatment with a suitable reducing agent, in an inert organic solvent; said process being further characterized in that:

(a) the compound of formula I wherein A identifies the structure A₁, R, R₁ and R₂ are as above defined, —x— is nil and R₃ stands for a hydrogen atom, is transformed into the corresponding compounds of formula I wherein A is A₂, R, R₁ and R₂ are as above defined, —x— is a covalent chemical bond and R₃ represents nil, by treatment with a suitable oxidizing agent selected from manganese dioxide, lead tetracetate, dichloro-dicyano-benzoquinone, 2,3,4,5-tetrachloro-benzoquinone, 2,3,5,6-tetrachloro-benzoquinone and analogs;

(b) the compound of formula I wherein R is acetyl and is converted into the corresponding compound of formula I wherein R is hydrogen, by mild alkaline hydrolysis.

7. A process as defined in claim 6, wherein from about 1 to about 8 molar equivalents of the compound of formula III are used for each molar equivalent of the rifamycin derivative of formula II.

8. A process as defined in claim 6, wherein hal represents bromine or iodine.

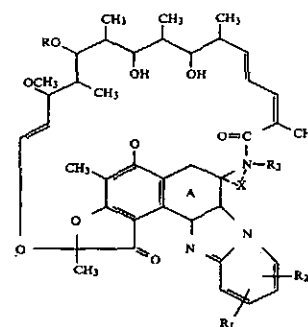
9. A process as defined in claim 6, wherein the organic solvent is selected from the group consisting of aliphatic alcohols from 1 to 4 carbon atoms and halogenated hydrocarbons from 1 to 2 carbon atoms.

10. A pharmaceutical antibacterial composition containing, as the active ingredient, an antibacterial effective amount of a compound as defined in claim 1 in admixture with one or more solid or liquid pharmaceutically acceptable carriers.

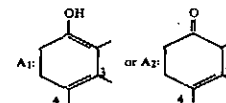
11. A pharmaceutical antibacterial composition as defined in claim 10, wherein the active ingredient is 4-deoxy-4'-methyl-pyrido[1',2':1,2]imidazo[4,5-c]rifamycin SV.

12. A pharmaceutical antibacterial composition as defined in claim 10, wherein the active ingredient is 4-deoxy-pyrido[1',2':1,2]imidazo[5,4-c]rifamycin SV.

13. The method of treating antibacterial diseases, which comprises administering to mammals suffering from said disease an antibacterial effective amount of a compound as defined in claim 1.



wherein:
A is



—x— is a covalent chemical bond or nil;
R is hydrogen or acetyl;

R₁ and R₂ independently represent hydrogen, (C₁₋₄) alkyl, benzyloxy, mono- and di-(C₁₋₃) alkylamino-(C₁₋₄) alkyl, (C₁₋₃)alkoxy-, (C₁₋₄)alkyl, hydroxymethyl, hydroxy-(C₂₋₄)-alkyl, nitro or R₁ and R₂ taken together with two consecutive carbon atoms of the pyridine nucleus form a benzene ring unsubstituted or substituted by one or two methyl or ethyl groups; R₃ is a hydrogen atom or nil; with the proviso that, when A is A₁, —x— is nil and R₃ is a hydrogen atom; with the further proviso that, when A is A₂, —x— is a covalent chemical bond and R₃ is nil.

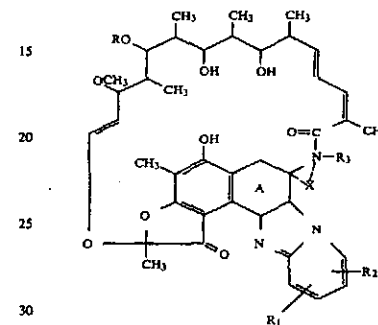
2. A compound as defined in claim 1, wherein A is A₁ or A₂ as above indicated, —x— is a covalent chemical bond or nil, R is hydrogen or acetyl, R₁ and R₂ independently represent hydrogen, (C₁₋₄)alkyl, benzyloxy, hydroxy-(C₂₋₄) alkyl, di-(C₁₋₃) alkylamino-(C₁₋₄) alkyl, nitro or R₁ and R₂ taken together with two consecutive carbon atoms of the pyridine nucleus form a benzene ring and R₃ is a hydrogen atom or nil; with the proviso that, when A is A₁, —x— is nil and R₃ is a hydrogen atom; with the further proviso that, when A is A₂, —x— is a covalent chemical bond and R₃ is nil.

3. A compound as defined in claim 1, wherein A is A₁ or A₂ as above indicated, —x— is a covalent chemical bond or nil, R is acetyl, R₁ and R₂ independently represent hydrogen, (C₁₋₄) alkyl or R₁ and R₂ taken together with two consecutive carbon atoms of the pyridine nucleus form a benzene ring and R₃ is a hydrogen atom or nil; with the proviso that, when A is A₁, —x— is nil and R₃ is a hydrogen atom; with the further proviso that, when A is A₂, —x— is a covalent chemical bond and R₃ is nil.

4. A compound as defined in claim 1, which is 4-deoxy-4'-methyl-pyrido[1',2':1,2]imidazo[5,4-c]rifamycin SV.

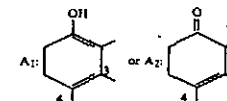
5. A compound as defined in claim 1, which is 4-deoxy-pyrido[1',2':1,2]imidazo[5,4-c] rifamycin SV.

6. A process for preparing a compound of formula



wherein:

A is



—x— is a covalent chemical bond or nil;

R is hydrogen or acetyl;

R₁ and R₂ independently represent hydrogen, (C₁₋₄) alkyl, benzyloxy, mono- and di-(C₁₋₃)alkylamino-(C₁₋₄)alkyl, (C₁₋₃)alkoxy-, (C₁₋₄)alkyl, hydroxymethyl, hydroxy-(C₂₋₄)-alkyl, nitro or R₁ and R₂ taken together with two consecutive carbon atoms of the pyridine nucleus form a benzene ring unsubstituted or substituted by one or two methyl or ethyl groups; R₃ is a hydrogen atom or nil; with the proviso that, when A is A₁, —x— is nil and R₃ is a hydrogen atom; with the further proviso that, when A is A₂, —x— is a covalent chemical bond and R₃ is nil, which consists of reacting a molar amount of a 3-halorifamycin S of formula:

Handwritten signature and date: 16/7

PATENT SPECIFICATION

(11) 1 590 732

1 590 732

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(62) Divided Out of No. 1590731

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C2C	1390	1450	1485	1530	1532	1590
	1626	200	213	214	215	220 226
	22Y	247	250	251	252	255 256 25Y
	281	28X	30Y	313	314	31Y 323 326
	327	328	32Y	332	338	339 342 346
	34Y	351	352	354	355	360 362 364
	365	366	367	368	36Y	373 376 37Y
	385	394	396	39Y	510	51X 536 601
	620	624	628	62X	62Y	643 650 656
	657	658	667	672	677	67X 689 699
	701	718	719	720	742	744 801 FJ
	KN	LY	MB	ME	MF	MG MV RR SF



(54) METHOD FOR PREPARATION OF RIFAMYCINS

(71) We, INTREPRINDEREA DE ANTIBIOTICE IASI, of Valea Lupului No. 1, Iasi, Romania, a Romanian body corporate do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:

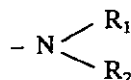
5 This application relates to derivatives of 3-formylrifamycin SV and to a method of preparing same. 5

Many semisynthetic rifamycins are known but only one: 3-(4'-methyl-1'-piperazinyliminomethyl)-rifamycin SV, known usually as rifampicin, has been applied in therapy, exclusively as an antimicrobial agent and especially in tuberculosis treatment.

10 As far as is known, the clinical use of rifamycin has revealed a number of side-effects. Research during prolonged treatment has revealed that in the liver, the selective solubility of rifamycin in bile alters the hepatic cell content and raises the level of bilirubin in the blood. At the same time resistance to rifampicin increases rapidly, thus restricting its use in long term therapy such as is required in antituberculous treatments. 10

15 Experimental investigations performed over the last few years have shown that rifampicin has a limited potency in its antiviral, immunosuppressive and antileukaemic properties, while other rifamycin derivatives exhibit higher potencies. 15

From the literature are known many derivatives of 3-formylrifamycin SV, which have in position 3 of the rifamycin SV ring a -CH = N-R group, where R may be the group 20



25 or the group -NH - CO - R₃ or the group - O - R₄ where R₁, R₂, R₃ and R₄ may be the same or different alkyl, acyl or aralkyl groups. 25

These rifamycins can be prepared by condensing 3-formyl-rifamycin SV with a nucleophilic reagent having an amino group, suitable for reaction with the carbonyl group, the general formula of the nucleophilic reagent being IA: 30



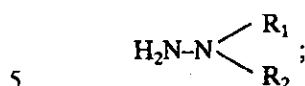
(IA)

35 where R is as defined above. U.S.A. Serial No. 3,342,810; and 3,865,812 and Canadian Serial No. 966,481, Journal of Medicinal Chemistry Vol. 16 Nr. 10, pp. 1071-1075 and vol. 17, nr. 9 pp. 948-953). These procedures have some disadvantages. 35

Thus, using tetrahydrofuran as the reaction medium is disadvantageous by reason of its high inflammability, and difficulty is encountered in the isolation of the final products.

40 Similarly, the use in some processes of a single solvent, such as tetrahydrofuran, benzene, ethyl acetate, chloroform or dioxan as a reaction medium in which one of the two reactants 40

famycin SV with a nucleophilic reactant of formula III or IV or V.



5

(III)



(IV)



(V)

5

in which R^1 , R^2 , R^3 and R^4 are as defined above, the method being carried out in an organic liquid reaction medium selected from aliphatic alcohols of 1 - 5 carbon atoms, halogenated aliphatic hydrocarbons of from 1 - 3 carbon atoms, water and mixtures thereof and in which a solution of the nucleophilic reactant is added to a suspension of the 3-formylrifamycin S.V. in the reaction medium.

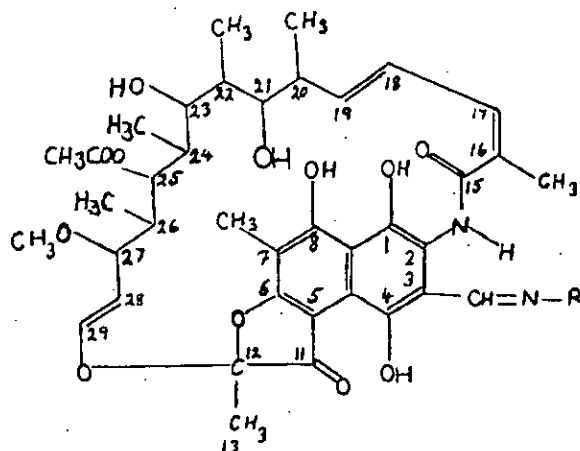
The 3-formylrifamycin S.V. is reacted in crystalline form with the reaction medium containing the nucleophilic reactant.

Advantageously the molar ratio of the nucleophilic reactant to the 3-formylrifamycin S.V. is from 1.05:1 - 1.25:1.

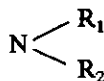
Preferably also the condensation reaction is conducted at a temperature of from $5^\circ\text{C}.$, to the boiling point of the reaction medium.

The nucleophilic reactant is suitably in the form of a salt in which case the condensation is preferably conducted in the presence of an organic base in equimolar amount to the said salt.

Attention is directed to our copending Application No, 27782/77 Serial No 1590731 which describes and claims novel semisynthetic rifamycins having a chemical structure according to general formula II



where R is



50

or $-\text{NH}-\text{CO}-\text{R}^3$ or $-\text{O}-\text{R}^4$ in which R^1 is a hydrogen atom;

R^2 is an arylpyridazinyl group of which the aryl group is unsubstituted or substituted by one or more alkyl groups or halogen atoms; a benzthiazolyl group; a 4-sulphophenyl group; a phenyl group, unsubstituted or substituted by one or more alkoxy or aryloxy groups, a substituted or unsubstituted arylthio group, or an arylsulphonyl group, or a piperidinyl group unsubstituted or in which the nitrogen atom is substituted by an alkyl or aralkyl group; or R^2 is a 2-hydroxypropyl, a 2-dimethylaminoethyl, a 3-dimethylaminopropyl, a 2,5-dihydroxybenzoyl, a 2,4-dihydroxybenzoyl or a 1-phenyl-2-benzyl-1,3,4-triazolyl-5-mercaptoacetyl group.

R^3 is a phenyl group, unsubstituted or substituted by one or more, acylamino or aminosulphonyl groups; a heteroaromatic group containing nitrogen; a phenoxyalkyl group, unsubstituted or in which the phenoxy group is substituted by one or more halogen atoms or alkyl, aminosulphonyl, alkylaminosulphonyl or dialkylaminosulphonyl groups; a benzhydryl group unsubstituted or substituted by one or more alkoxy groups or a

triazolomercaptomethyl group; or an ethoxy group; and R⁴ is a haloalkyl group.

These derivatives are solid crystalline substances, which are various shades of orange or red colours, and which generally decompose on melting. They are highly soluble in aliphatic hydrocarbons and haloalkyls, soluble or slightly soluble in lower alkanols, ketones, aliphatic esters, aliphatic ethers, cyclic ethers and dimethylformamide, and insoluble or very slightly soluble in aliphatic hydrocarbons and water.

The new method of preparing rifamycins avoids the disadvantages of previous known procedures by the following:

- crystalline 3-formylrifamycin SV is added directly to a reaction medium which contains the nucleophilic reagent suitable for the carbonyl group, or is suspended in a liquid and then is added to the nucleophilic reagent solution;
- in order to increase the reaction rate, to obtain purer products and to reduce the dangers related to inflammability, there are used as the reaction medium lower aliphatic alcohols with 1 to 5 carbon atoms, especially ethyl alcohol, alone or in admixture with halogenated aliphatic hydrocarbons with 1-3 carbon atoms (especially dichloromethane) and in some cases water (up to 10% by volume), thus permitting high concentrations of the reactants;
- in order to ensure the complete conversion of 3-formylrifamycin SV, the nucleophilic reagent is used in a ratio of 1.05 to 1.25 mole to 1 mole of 3-formylrifamycin SV.
- the condensing step is conducted at a temperature range between 5°C and the reflux temperature of the solvent until total condensation of 3-formylrifamycin SV is achieved;
- the nucleophilic reactant may be used also as the salt form, in which case an organic base is added in an approximately stoichiometric quantity.
- the isolation of high purity reaction products may be performed by precipitation from the solvent system used, possibly modified by partial removal of one component and/or by water addition.

The invention will now be described, by way of illustration, in the following Examples.

EXAMPLE 1

3-[3'-(3'',4''-dimethylphenyl)-pyridazinyl-6'-hydrazonomethyl]-rifamycin SV. 30

To a solution of 257 mg (1.2 mmol) 3-(3',4'-dimethylphenyl)-6-hydrazinopyridazine in 10 ml ethanol, heated at 45°C, are added 34 mg of 3-formylrifamycin SV, 99% purity, (1 mmol) with stirring; the solution is kept 5 minutes at 45°C at which time crystallization begins. Cool to 20°C and after 2 hours filter off the product.

The crystalline product is washed with ethanol and dried. Results 790 mg crystalline, dark red coloured product; the yield is 85%.

The residual filtrate is treated with 3 ml water and yields 90 mg crystalline product, similar in chromatogram to the main product; Overall yield is 95%.

A sample recrystallized from ethanol has melting point 200-203°C (with decomposition) and shows absorption maxima in U.V. and visible range at 356 nm ($E_{1cm}^{1\%} = 331$) and 487 nm ($E_{1cm}^{1\%} = 168$).

In a parallel experiment using 235.5 mg (1.1 mmol) of hydrazine compound, the final product contains 3-formylrifamycin SV.

EXAMPLE 2

3-[3'-(p-chlorophenyl)-pyridazinyl-6'-hydrazonomethyl]-rifamycin SV. 45

The working procedure is similar to that described in Example 1, using 265 mg (1.2 mmol) 3-p-chlorophenyl-6-hydrazino-pyridazine; and there is obtained 740 mg cherry-red or brown coloured product; the yield is 80%.

A sample recrystallised from ethanol has a melting point of 191 203°C (with decomposition) and shows absorption maxima in U.V. and visible range at 358 nm ($E_{1cm}^{1\%} = 337$) and 487 nm ($E_{1cm}^{1\%} = 168$).

EXAMPLE 3

3-[3'-(p-isopropylphenyl)-pyridazinyl-6'-hydrazonomethyl]-rifamycin SV. 55

The working procedure is similar with that described in Example 1, using 251 mg (1.1 mmol) of 3-p-isopropylphenyl-6-hydrazinopyridazine; and there is obtained 880 mg crystalline red brick coloured product. The yield is 93%.

A sample recrystallised from ethanol has a melting point of 202 205°C and shows absorption maxima in U.V. and visible range at 358 nm ($E_{1cm}^{1\%} = 291$) and 487 nm ($E_{1cm}^{1\%} = 164.5$).

EXAMPLE 4

3-(Benzthiazolyl-2'-hydrazono-methyl)-rifamicin SV. 65

In 7 ml of ethanol there are dissolved, in warm conditions, 173 mg (1.05 mmol) of

174
171

5 benzthiazolyl-2-hydrazine, 734 mg of 3-formylrifamycin SV of 99% purity (1 mmol) are added and after dissolution the reaction mass is kept for 5 minutes at 40°C. By subsequent addition of 1.5 ml water, which is added stepwise over a period of time, a crystalline product separates which, after cooling to room temperature and standing for 1 hour, is filtered off, washed with aqueous ethanol, and dried under vacuum. There is obtained 720 mg of crystalline dark red cherry product; the yield is 82%. 5

10 The product has the melting range of 193-197°C (with decomposition) and shows absorption maxima in U.V. and visible range at 360 nm ($E_{1\text{cm}}^{1\%} = 291.5$) and 494 nm ($E_{1\text{cm}}^{1\%} = 179.5$). 10

EXAMPLE 5

3-(phenoxyacetylhydrazonomethyl)-rifamycin SV
(reaction in ethanol)

15 To a solution of 174 mg (105 mmol) phenoxyacetohydrazide in 5 ml ethanol at approximately 40°C is added under stirring 726 mg (1 mmol) 3-formylrifamycin SV. After a few minutes crystallisation of the product begins and the solution is kept for 3 hours at room temperature before filtering. The product is then rinsed with alcohol and dried. There is obtained 810 mg of dark red coloured product. The yield is 92%. 15

20 A recrystallised sample from ethanol has a melting point at 160-164°C (with decomposition) and shows absorption maxima at 336 nm ($E_{1\text{cm}}^{1\%} = 290$) and 478 nm ($E_{1\text{cm}}^{1\%} = 164$). 20

EXAMPLE 6

3-(isonicotinoylhydrazonomethyl)-rifamycin SV
(reaction in methanol).

25 To a solution of 151 mg (1.1 mmol) isonicotinohydrazide in 6 ml methanol at 45°C is added 726 mg (1 mmol) 3-formylrifamycin SV, under stirring. 25

The mixture is kept for 15 minutes, at 45 - 50°C, when the absence of 3-formylrifamycin SV can be confirmed, and then cooled down slowly to room temperature.

30 The crystallisation is improved by gradually adding 1 ml water and stirring for 2 hours. The mixture is filtered, then, the crystalline red coloured product. The yield is 85%. A sample recrystallised from ethanol decomposes at 200 - 205°C and shows absorption maxima at 337 nm ($E_{1\text{cm}}^{1\%} = 287$) and 483 nm ($E_{1\text{cm}}^{1\%} = 166.5$). 30

35 After performing the condensation with an equimolar amount of hydrazide, one can observe chromatographically the presence in the reaction medium of the dimethylacetal of 3-formylrifamycin SV which disappears when extra amounts of hydrazide are added. 35

EXAMPLE 7

3-(nicotinoylhydrazonomethyl)-rifamycin SV.

40 The procedure is similar to that of Example 6, using 144 mg (1.05 mmol) nicotinohydrazide dissolved in 15 ml methanol, and for completion of crystallisation are added 3 ml water. There is obtained 680 mg crystalline, bright red coloured product. The yield is 80%. 40

45 A sample recrystallised from dimethylformamide-ethanol-water has melting point at 200-204°C (with decomposition) and shows absorption maxima at 338 nm ($E_{1\text{cm}}^{1\%} = 285$) and 483 nm ($E_{1\text{cm}}^{1\%} = 165$). 45

EXAMPLE 8

3-(p-chlorophenoxyacetylhydrazonomethyl)-rifamycin SV.

50 The working procedure is similar to that described in Example 6. 210 mg (1.05 mmol) of p-chlorophenoxyacetohydrazide is dissolved in 10 ml of methanol and the product is precipitated with water (2.5 ml). 50

There is obtained 770 mg of crystalline, dark red coloured product. The yield is 84%. Melting point is 156-160°C (with decomposition) and shows absorption maxima at 333 nm ($E_{1\text{cm}}^{1\%} = 270$) and 486 nm ($E_{1\text{cm}}^{1\%} = 152$).

55 EXAMPLE 9 55

3-(o-chlorophenoxyacetylhydrazonomethyl)-rifamycin SV.

60 The working procedure is as described in Example 5, using 210 mg (1.05 mmol) of o-chlorophenoxyacetohydrazide. After cooling, the product is precipitated in crystalline form by stepwise addition of 1.5 ml water. 60

A sample recrystallised from ethanol has a melting point at 191 - 193°C (with decomposition) and shows absorption maxima at 335 nm ($E_{1\text{cm}}^{1\%} = 277$) and 477 nm ($E_{1\text{cm}}^{1\%} = 159$).

EXAMPLE 10

65 3-(o-chloro- α -phenoxypropionylhydrazonomethyl)-rifamycin SV. 65