Industria y Comercio
SUPERINTENDENCIA

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

No. 07-089192- -00002-0000

Fecha: 2008-03-28 09:19:31 Dep: 2020 NUECREACIONE
Tra: 11 PATENTEIYII Eve: 378 FASENACIONALI
Act: 400 OPOSIOBSERVIER Folios: 35

93 92

FORMULARIO ÚNICO DE OPOSICIÓN

① OPOSICION A :

- [X] Patente de Invención.
- [ ] Patente de Modelo de Utilidad.
- [ ] Diseño Industrial.
- [ ] Esquema de Trazado para Circuitos Integrados.
- [ ] Marcas de Productos o Servicios.
- [ ] Marca Colectiva.
- [ ] Marca de Certificación.
- [ ] Lema Comercial.

② SOLICITUD PUBLICADA

Número del expediente: **07-89192**
Solicitante: **WYETH**
Apoderado: **ALVARO CORREA ORDOÑEZ**
Título de la nueva creación o denominación del signo: **"PURIFICACIÓN DE RAPAMICINA.".**
Gaceta: **584**

③ OPOSICIÓN PRESENTADA POR:

SOLICITANTE

Nombre: **GYNOPHARM S.A**
Dirección: **CALLE 80 No. 78B -201**
Nacionalidad o Domicilio: **COLOMBIA**
Lugar de Constitución: **BARRANQUILLA**
Teléfono: **(571)3719900** Fax: **(571)3730818**
E-mail:

IDENTIFICACIÓN
C.C. [ ] NIT [X]
C.E. [ ] Otro [ ]
Cual
Número **802.006.279-4**

REPRESENTANTE O APODERADO

Nombre: **ELIANA ISAURA MORENO BOHORQUEZ**
Dirección: **CARRERA 13 No. 119-95 OF 104**
Teléfono: **(571)2131213** Fax: **(571)6121979**
E-mail: **negocios@optimum-co.com**

IDENTIFICACIÓN
C.C. [ ] NIT [X]
C.E. [ ] Otro [ ]
Cual
Número **51.975.664** TP **83823**

④ FUNDAMENTOS DE LA OPOSICIÓN:

Art.14, 16, 18, 20 y 30 de la Decisión 486 de la CAN

Causal: **No cumple con los requisitos de patentabilidad.**

Signo opositor:

Justificación:

La presente solicitud no cumple con los requisitos de patentabilidad dado que lo revelado, ya se encontraba en el estado de la técnica o es posible derivarlo de la misma tal y como se demostrará en el capítulo "Fundamento de Hechos".

93

⑤ **Comprobante de pago No.** _____ **Fecha** _____

⑥ **ANEXOS**

- [X] Comprobante de pago de la tasa.
- [X] Poderes, si fuere el caso.
- [X] Pruebas de los fundamentos de la oposición.
- [ ] Documentos que acrediten el legítimo interés, si fuere el caso.
- [X] Documento que acredita la existencia y representación legal de las partes, cuando sean personas jurídicas.

⑦

NOMBRE: ELIANA ISAURA MORENO BOHORQUEZ

FIRMA:

C.C. 51.975.664 **T.P.** 83823

Instrucciones para el diligenciamiento del presente formulario, ver reverso de esta página.

95 2
94

Señora Jefe,

DIVISIÓN DE NUEVAS CREACIONES

Superintendencia de Industria y Comercio

E. S. D.

Referencia	: Expediente N° 07.089.192
Asunto	: Oposición a la solicitud de patente de Invención titulada "PURIFICACIÓN DE RAPAMICINA"
Solicitante	: WYETH.
Opositora	: GYNOPHARM S.A.

Publicada en la Gaceta de la Propiedad Industrial

N° 584 del 31 de diciembre de 2007

Yo, *ELIANA ISAURA MORENO BOHORQUEZ,* abogada en ejercicio, identificada como aparece al pie de su firma, con tarjeta profesional No. 83823 del Consejo Superior de la Judicatura, en mi condición de apoderada de la sociedad ***Gynopharm S.A.*** conformada bajo las leyes de la República de Colombia y domiciliada en la ciudad de Barranquilla, Colombia, atentamente manifiesto que estando dentro del término de ley y por orden expresa de mi representada, presento oposición a la concesión del registro de la Patente de Invención titulada "PURIFICACIÓN DE

Carrera 13 N° 119-95, Oficina 104. Tel. (571) 2 13 12 13 – Fax. (571) 6 12 19 79
E-mail: negocios@optimum-co.com - www.optimum-co.com
Bogotá Colombia

RAPAMICINA", cuyo titular es **WYETH,** y pido a Usted declarar fundada esta oposición y en consecuencia negar el registro de la patente solicitada.

1. INTERÉS PARA ACTUAR

Conforme a lo dispuesto en el artículo 42 de la Decisión 486 de 2000 de la CAN, estando dentro del término estipulado por la ley, manifiesto el legitimo interés de nuestra representada para oponerse al registro de la patente de la referencia, atendiendo a que la solicitud pretendida no cumple con los requisitos dispuestos en la ley como se analizará más adelante y de ser concedida afectaría directamente la comercialización de algunos productos de mi representada, que contienen, en particular Rapamicina. Por lo tanto, solicito respetuosamente a ese Despacho se sirva tener en cuenta la presente oposición dentro del trámite administrativo que se surte en esta Superintendencia.

2. FUNDAMENTOS DE HECHOS

2.1. La solicitud objeto de la presente fue presentada el 30 de agosto de 2007, reivindicando prioridad bajo la solicitud US20050657910P

2005-03-02; y fue publicada en Colombia, en la Gaceta de la Propiedad Industrial N° 584 del 31 de diciembre de 2007, cuya publicación internacional corresponde a la WO2006093745 del 8 de septiembre de 2006.

2.2. La solicitud colombiana **07089192** objeto de la presente oposición, se refiere a Rapamicina que tiene una pureza mayor del 98% y un índice de color amarillo menor de 1; donde dicha Rapamicina tiene una proporción isomérica B:C mayor de 30:1; tiene una proporción isomérica B:C mayor de 35:1. Adicionalmente se refiere a una composición que comprende rapamicina y un portador fisiológicamente compatible; donde dicha composición que comprende rapamicina, tiene una proporción isomérica B:C mayor de 30:1; en donde la proporción isomérica es mayor de 35:1.

2.3. Ahora bien, con base en el artículo 14 de la Decisión 486 de la CAN consagra lo siguiente: "*Los países miembros otorgarán patentes para las invenciones, sean de producto o procedimiento, en todos los campos de la tecnología, siempre que sean nuevas, tengan nivel inventivo y sean susceptibles de aplicación industrial*". Así las cosas, las invenciones objeto de patente deben ser nuevas y tener nivel inventivo. Bien, pues antes de la fecha de prioridad de esta solicitud se encuentran los siguientes documentos que afecta la patentabilidad de la presente solicitud:

- D1: US2001039338 publicada el 8 de noviembre de 2001.
- D2: US3993749 publicada el 23 de noviembre de 1976.

2.4. Ahora bien, el documento D1 se refiere a un proceso regioselectivo para preparar 42-éster o éter de rapamicina y los intermedios de 31-silil éter. Particularmente en la página 1 párrafo 0003 se revela la existencia previa de la rapamicina demostrando que es conocida con anterioridad. Según esta anterioridad D1 se demuestra la pre-existencia de dicha sustancia, por lo que no podría ser novedoso que la misma ahora se tilde de novedosa por el simple hecho de tener un particular grado de pureza. Ahora bien, a partir de este documento D1 también es posible deducir una reacción de rapamicina con clorometilsilano en etil acetato en presencia de imidazol para sintetizar 31, 42 O-trimetilsilano rapamicina conforme se indica en el ejemplo 1, página 4. En este ejemplo se divide el grupo trimetilsilano con ácido sulfúrico para obtener rapimicina. Así la cosas como lo evidencia dicho documento D1 la presente solicitud no es novedosa pues lo revelado ya se encontraba en el estado de la técnica, particularmente en el documento D1.

2.5. El documento D2, por su parte revela un antibiótico de rapamicina que es obtenible por cultivo de Streptomyces hygroscopicus NRRL 5491 en un medio acuoso nutritivo. Como se evidencia, este

documento ya revelava con casi 30 años de anterioridad la existencia de la rapamicina. Particularmente, en la columna 5 líneas 29 a 41 este documento anterior revela la purificación de rapimicina por medio de carbón vegetal. Por lo tanto, cualquier persona versada en la materia, a partir de la materia revelada en dicho documento D2, en combinación con la materia definida en el documento D1, puede llegar de manera evidente a un proceso para purificar rapamicina usando productos naturales tal como carbón vegetal. Así las cosas, visto dicho documento D2, en combinación con el documento D1, la presente solicitud carece evidentemente de altura inventiva.

2.6. Por lo tanto, este documento objeto de oposición a la luz del documento D1 carece evidentemente de NOVEDAD y con base en el documento D2, carece de ALTURA INVENTIVA.

2.7. En consecuencia y de acuerdo a lo descrito anteriormente, se deriva que la solicitud colombiana **07089192** no puede pretender un monopolio por patente toda vez que lo que pretende no cumple con dos de los tres requisitos necesarios para su protección.

2.8. Que por consiguiente, respecto a la solicitud pretendida se tiene que:

2.8.1. <u>CARECE DE NOVEDAD</u>: Según la decisión 486 de la Comunidad Andina de Naciones, Artículo 16.- "*Una invención se*

Carrera 13 Nº 119-95, Oficina 104. Tel. (571) 2 13 12 13 - Fax. (571) 6 12 19 79
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Bogotá Colombia

considerará nueva cuando no está comprendida en el estado de la técnica. El estado de la técnica comprenderá todo lo que haya sido accesible al público por una descripción escrita u oral, utilización, comercialización o cualquier otro medio antes de la fecha de presentación de la solicitud de patente o, en su caso, de la prioridad reconocida.". A la fecha de prioridad, 2 de marzo de 2005 ya era conocido, pues como se demostró, era parte del arte anterior con base en el documento D1 y D2, la rapamicina sobre la cual busca protección en el capítulo reivindicatorio.

2.8.2. <u>CARENCIA DE ALTURA INVENTIVA</u>. Según la Decisión 486 de la Comunidad Andina de Naciones, Artículo 18.- *"Se considerará que una invención tiene nivel inventivo, si para una persona del oficio normalmente versada en la materia técnica correspondiente, esa invención no hubiese resultado obvia ni se hubiese derivado de manera evidente del estado de la técnica.".* A la fecha de prioridad, 2 de marzo de 2005 a partir del documento D2 en combinación con D1, puede llegar de manera evidente a un proceso para purificar rapamicina por medios naturales sobre el cual busca protección en el capítulo reivindicatorio.

2.9. Dado lo anterior, es posible deducir que la solicitud y lo reivindicado no es objeto de patentabilidad pues es contrario a lo que dicta la Decisión 486 de la Comunidad Andina de Naciones, Artículo

14.- *"Los Países Miembros otorgarán patentes para las invenciones, sean de producto o de procedimiento, en todos los campos de la tecnología, siempre que sean nuevas, tengan nivel inventivo y sean susceptibles de aplicación industrial."*. La solicitud pretendida no cumple con dos de los tres requisitos de patentabilidad.

2.10. Por todo lo anterior, atentamente solicito a usted que se sirva declarar fundada la presente oposición y negar el registro Patente de invención denominada **"PURIFICACIÓN DE RAPAMICINA"**, cuyo titular es **WYETH,** solicitud Colombiana que apareció publicada en la Gaceta de la Propiedad Industrial N° 584 del 31 de diciembre de 2007.

3. PRUEBAS

De conformidad con lo dispuesto por las normas vigentes, solicito a usted tener como prueba en este asunto los siguientes:

- D1: US2001039338 publicada el 8 de noviembre de 2001.
- D2: US3993749 publicada el 23 de noviembre de 1976.

- D1: US2001039338 publicada el 8 de noviembre de 2001.
- D2: US3993749 publicada el 23 de noviembre de 1976.

6. NOTIFICACIONES

Para efectos de notificaciones que por disposición del artículo 50 del Decreto 01 de 1984 debe hacer su Despacho, informo que mi representada y la suscrita recibiremos notificaciones en la Secretaría de su Despacho o en mis oficinas situadas en la Carrera 13 Nº 119-95, Of. 104 de esta ciudad de Bogotá.

Señor Superintendente,

ELIANA ISAURA MORENO BOHORQUEZ
C.C. 51'975.664 de Bogotá
T.P.A. 83823 del C.S. de la J.

104 TC
103

PODER ESPECIAL

Yo, **LUIS PALACIOS ARAGÓN,** mayor de edad y vecino de la ciudad de Barranquilla (Colombia), identificado como aparece al pie de mi firma, quien en el presente documento actúa en calidad de Representante Legal de la sociedad **GYNOPHARM S.A.** sociedad legalmente constituida y domiciliada en la ciudad de Barranquilla (Colombia), por el presente documento otorgo a la doctora **ELIANA ISAURA MORENO BOHORQUEZ,** abogada en ejercicio, identificada como aparece al pie de su firma, con tarjeta profesional No. 83823 **del C.S. de la J.**, poder especial, amplio y suficiente, para formular oposiciones u observaciones a la solicitud de patente No. **07.089.192,** titulada **"PURIFICACIÓN DE RAPAMICINA"**, cuyo titular es **WYETH.,** publicada en la gaceta No. 584 del 31 de Diciembre de 2007.

Nuestro apoderado queda facultado, de manera expresa, para realizar todo tipo de actos necesarios para llevar a cabo el trámite de la oposición, incluyendo, sin limitación, preparar y presentar solicitudes, hacer declaraciones, pagar tasas, contribuciones e impuestos, recabar los títulos o certificados. Asimismo quedan autorizados para impugnar administrativa y judicialmente todo lo que fuere resuelto en el expediente a que se hace expresa referencia en el presente poder, con todas las facultades generales y específicas que fuera requerido para ella.

De la misma manera, además de las facultades que le confiere la naturaleza del presente mandato, se encuentran facultados para recibir, desistir, transigir, sustituir, y reasumir el presente poder, incoar acciones, interponer recursos y demás facultades conferidas por la ley.

Dado y Firmado en Barranquilla (Colombia) a los 12 días del mes de Marzo de 2008.

GYNOPHARM S.A.

Cédula de Extranjería: No. 319788.
NIT: 802.006.279-4
Representante Legal

Acepto:

ELIANA ISAURA MORENO BOHORQUEZ
C.C. 51'975.664 de Bogotá
T.P.A. 83823 del C.S. de la J.

RECONOCIMIENTO DE DOCUMENTO PRIVADO

Ante mí, NIBARDO AGUSTIN FUERTES MORALES, Notario Veinticinco del C[illegible]

comparece Luis Palacios Arango

identificado con cédula de ciudadanía CE 319788 Bta

y declara que la firma puesta en el presente instrumento privado es suya y que el contenido del mismo es cierto.

Bogotá D.C. 12 MAR 2008

FIRMA

NIBARDO AGUSTIN FUERTES MORALES
NOTARIO VEINTICINCO(E)

PRESENTACION PERSONAL

El anterior escrito fue presentado ante la

NOTARIA DIECIOCHO DEL CIRCULO DE BOGOTA, D.C.

personalmente por Diana Isabel Bohorquez

quien exhibió la c.c. 51977669 y T.P.

y Tarjeta Profesional No. 82803

Fecha 13 MAR 2008

HECTOR FABIO CORTES DIAZ
NOTARIO ENCARGADO

01

6819232 7

105 104

CERTIFICADO NACIONAL

CAMARA DE COMERCIO DE BARRANQUILLA

Fecha : 20080312 Hora Certificado 18:25:01

Operacion : 02CP20312099 PAGINA No. 1

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CERTIFICADO DE EXISTENCIA Y REPRESENTACION LEGAL O DE INSCRIPCION DE DOCUMENTOS.

EN JUNIO DE ESTE A?O SE ELEGIR? JUNTA DIRECTIVA DE LA C?MARA DE COMERCIO. LAS INSCRIPCIONES DE CANDIDATOS DEBEN HACERSE EN LA PRIMERA QUINCENA DE MAYO. PARA INFORMACI?N DETALLADA DIRIGIRSE A LA SEDE PRINCIPAL O COMUNICARSE AL SIGUIENTE TELEFONO: 3303911.

EL SUSCRITO SECRETARIO DE LA CAMARA DE COMERCIO DE BARRANQUILLA, CON FUNDAMENTO EN LAS INSCRIPCIONES DEL REGISTRO MERCANTIL:

C E R T I F I C A

Que por Escritura P?blica No. 3,551 del 17 de Dic/bre de 1997, otorgada en la Notaria 3a. de Barranquilla, inscrito(as) en esta C?mara de Comercio, el 18 de Dic/bre de 1997 bajo el No. 72,965 del libro respectivo, fue constituida la sociedad----------------------- anonima denominada GYNOPHARM S.A. ----------------------------

C E R T I F I C A

C E R T I F I C A

.463.982, actuando como Presidente y represen-tante legal de la soci edad GYNOPHARM S.A., otorga Poder Especial, Amplio y Suficienta a KA REN JENNIFER FIGUEROA GARCIA C.C. No. 32.-732.070, para que en nombr e y representacion de la sociedad suscriba todas las declaraciones c ambiarias a que esta obligada GYNOPHARS.A., en el giro normal de sus negocios y operaciones sociales.---

C E R T I F I C A

Que dicha sociedad ha sido reformada por las siguientes escrituras y/o documentos privados:

Numero	aaaa/mm/dd	Notaria	No. Insc o Reg	aaaa/mm/dd
532	1999/03/25	Notaria 3a. de Barranquilla	80,373	1999/04/13

C E R T I F I C A

Que de acuerdo con la(s) escritura(s) arriba citada(s), la sociedad se rige por las siguientes disposiciones:

DENOMINACION O RAZON SOCIAL:

GYNOPHARM S.A.--

DOMICILIO PRINCIPAL: Barranquilla.

NIT No: 802.006.279-4.

MATRICULA MERCANTIL: 245,470.

C E R T I F I C A

*** CONTINUA ***

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Direccion para notificaciones judiciales:
CL 80 No 78 B - 201.
De la ciudad de Barranquilla.

C E R T I F I C A

DURACION: El t?rmino de duraci?n de la sociedad se fij? hasta el 16 de Dic/bre de 2047.

C E R T I F I C A

OBJETO SOCIAL: a) La fabricacion de capsulas y otro productos similares a partir de materiales tales como gelatinas u otros de distinta naturaleza, que puedan ser utilizados como envases o recipientes de productos y sustancias quimicas, farmaceuticas y cosmeticas, para uso y consumo humano y veterinario. b) La investigacion, desarrollo y fabricacion de toda clase de productos y sustancias quimicas, farmaceuticas y cosmeticas para uso y consumo humano, animal y vegetal. c) La compra y venta de meterias primas , insumos y productos intermedios necesarios para los procesos de fabricacion de sustancias quimicas, farmaceuticas y cosmeticas, asi como la comercializacion interna y externa de los productos terminados. d) La compra, venta, importacion, exportacion y distribucion de toda clase de elementos instrumentales y equipos, medicos, quirurgicos, odontologicos y hospitalarios para la salud humana y animal. e) Actuar como representante o agente de personas naturales y juridicas nacionales y extranjeras en la realizacion de operaciones o transacciones relacionadas con la industria farmaceutica y en especial con cualesquiera de las actividades descritas en los literales a), b), c) y d) antes transcritos. f) Prestar los servicios de asesoria y consultoria en las areas administrativa, de mercadeo, analisis clinico, asistencia tecnica, investigacion, costos y produccion exportaciones, importaciones, ventas, relacionadas con iguales o similares actividades a las que constituyen el objeto de GYNOPHARM S.A., asi como los de publicidad y promocion de productos quimicos, cosmeticos y farmaceuticos para uso humano, animal o industrial. g) Adquirir, operar, administrar y explotar, a cualquier titulo,empresas o establecimientos dedicados a la investigacion, desarrollo, fabricacion o comercializacion de productos quimicos, farmaceuticos, hospitalarios o cosmeticos. En desarrollo de su objeto principal, la sociedad podra tomar interes social como accionista, socio o fundador de empresas que tengan igual o similar objeto; comprar, vender, importar y exportar maquinarias, insumos y repuestos requeridos para el desarrollo de sus actividades; tomar dinero en prestamo,financiar y garantizar las operaciones comerciales que celebre con terceros relativas a su objeto; celebrar y ejecutar todo tipo de actos y contratos necesarios para desarrollar sus operacioes; girar, aceptar, otorgar y protestar todo tipo de titulos valores e instrumentos negociables; comprar,

*** CONTINUA ***

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vender y constituir gravamenes sobre bienes muebles e inmuebles; designar representantes o apoderados en el pais y en el exterior y, en general, realizar cualquier acto o negocio que sea necesario o conveniente para el desarrollo de su objeto social y para la mejor proteccion de sus intereses corporativos o lo de sus socios.----------

C E R T I F I C A

CAPITAL	Nro Acciones	Valor Acci?n
Autorizado		
$**********4,000,000,000	**********4,000,000	**********1,000
Suscrito		
$**********2,672,646,000	**********2,672,646	**********1,000
Pagado		
$**********2,672,646,000	**********2,672,646	**********1,000

C E R T I F I C A

ADMINISTRACION: Seran funciones de la junta directiva, entre otras: Autorizar cualquier acto o contrato cuya cuantia sea superior al equivalente en pesos Colombianos a cincuenta mil dolares de los Estados Unidos de America (US$50.000.oo), liquidados a la tasa de cambio representativa del mercado vigente el dia en que se surta la aprobacion y para enajenar o gravar bienes inmuebles y demas activos fijos de la sociedad, cualquiera que sea la cuantia de la transaccion. Las demas que le sean propias y no esten atribuidas a otro organo social y las que le correspondan en virtud de la ley. la sociedad tendra un Gerente General, quien ejercera la administracion y representacion legal de la sociedad y tendra las siguientes atribuciones, entre otras: Ejercer la representacion legal de la sociedad en todos los actos y contratos que se requieran para el desarrollo del objeto social, de conformidad con lo previsto en las leyes y en los presentes estatutos. Aprobar y celebrar en nombre de la sociedad todo acto o contrato cuya cuantia sea igual o inferior al equivalente en pesos Colombianos a cincuenta mil dolares de los Estados Unidos de America (US$50.000.oo), liquidados a la tasa de cambio representativa del mercado vigente el dia en que se surta la aprobacion o celebracion del acto. Para enajenar, a cualquier titulo, bienes inmuebles de la sociedad o constituir gravamenes sobre los mismos, debera obtener la aprobacion de la junta directiva, cualquiera que sea la cuantia de la transaccion; o las demas funciones que le correspondan como representante legal por disposicion de la ley, de estos estatutos o de la junta directiva. El Gerente General tendra un suplente, designado por la junta directiva, quien lo reemplazara con identicas facultades en sus faltas temporales, absolutas o meramente acciedentales, sin que sea necesario para ello acreditar la ausencia del principal.----------

*** CONTINUA ***

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C E R T I F I C A

Que seg?n Acta No. 15 del 18 de Febrero de 2008 correspondiente a la Asamblea de Accionistas en Bogota, de la sociedad: GYNOPHARM S.A.

cuya parte pertinente se inscribi? en esta C?mara de Comercio, el 25 de Febrero de 2008 bajo el No. 137,919 del libro respectivo, fueron hechos los siguientes nombramientos:

CLASE: JUNTA DIRECTIVA

Principales

1. Llobet Poal Manuel CC.******14,670,196
2. Zanatta Reherman Leonardo Andres CC.*******2,760,271
3. Munoz Marroquin Diego CC.*******7,688,292
4. Montealegre Gonzalez Andres Leonardo CC.******79,950,262

Suplentes

1. Agudelo Pelaez Luis Hernando CC.******14,238,997
2. Aroca Almanza Arleth Ayelina CC.******36,718,415
3. Penaranda Urbina Adriana Paola CC.******53,120,683
4. tovar Vasquez Ruth CC.******52,175,756

C E R T I F I C A

Que seg?n Acta No. 18 del 30 de Octubre de 2003 correspondiente a la Junta Directiva en Barranquilla, de la sociedad: GYNOPHARM S.A.

cuya parte pertinente se inscribi? en esta C?mara de Comercio, el 06 de Nov/bre de 2003 bajo el No. 107,783 del libro respectivo, fueron hechos los siguientes nombramientos:

Cargo/Nombre	Identificaci?n
Gerente General.	
Palacios Aragon Luis Alberto	CE.*********319,788

C E R T I F I C A

Que seg?n Acta No. 49 del 03 de Marzo de 2008 correspondiente a la Junta Directiva en Barranquilla, de la sociedad: GYNOPHARM S.A.

cuya parte pertinente se inscribi? en esta C?mara de Comercio, el 11 de Marzo de 2008 bajo el No. 138,342 del libro respectivo, fueron hechos los siguientes nombramientos:

Cargo/Nombre	Identificaci?n
Suplente del Gerente.	
Mollajoli Centurion Andres Fernando	CE.*********351,283

C E R T I F I C A

Que seg?n Acta No. 2 del 13 de Enero de 1998 correspondiente a la

*** CONTINUA ***

CAMARA DE COMERCIO DE BOGOTA

CERTIFICADO NACIONAL

CAMARA DE COMERCIO DE BARRANQUILLA

Fecha : 20080312 Hora Certificado 18:25:01

Operacion : 02CP20312099 PAGINA No. 5

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Asamblea de Accionistas en Barranquilla, de la sociedad: GYNOPHARM S.A. cuya parte pertinente se inscribi? en esta C?mara de Comercio, el 20 de Enero de 1998 bajo el No. 73,393 del libro respectivo, fueron hechos los siguientes nombramientos:

Cargo/Nombre	Identificaci?n
Revisor Fiscal Ppal.	
Barros Cera William Alberto	CC.*******8,715,941
Suplente del Revisor Fiscal.	
Duarte Diaz David	CC.******73,115,965

C E R T I F I C A

Que segun Documento Privado de fecha 10 de Octubre del 2.000, inscrito en esta Camara de Comercio el 23 de Noviembre del 2.000 bajo el No. 1.872 del libro repectivo, consta que el senor RUBEN MINSKI GONTOVNIK C.C. No. 7.463.982, actuando como Presidente y representante legal de la sociedad GYNOPHARM S.A., otorga Poder Especial, Amplio y Suficienta a KAREN JENNIFER FIGUEROA GARCIA C.C. No. 32.-732.070, para que en nombre y representacion de la sociedad suscriba todas las declaraciones cambiarias a que esta obligada GYNOPHARM S.A., en el giro normal de sus negocios y operaciones sociales.-----

C E R T I F I C A

Que segun Documento Privado de fecha 10 de Octubre del 2.000, inscrito en esta Camara de Comercio el 12 de junio del 2.002, bajo el 2.164 del libro respectivo,consta que el senor JORGE CASTELLANOS MARTINEZ,identificado con C.C.No.19.460.089,actuando como representante legal de la sociedad GYNOPHARM S.A, otorga Poder Especial Amplio y Suficiente a KAREN FIGUEROA GARCIA, identificada con la cedula de ciudadania No.32.732.070 de Barranquilla, mediante documento privado de octubre 10/2000, inscrito en la Camara de Comercio de Barranquilla bajo el No.1.872 del libro respectivo, para que en representacion de GYNOFARM S.A, suscriba las declaraciones cambiarias a los que esta obligada GYNOFARM S.A. en el giro ordinario de sus negocios y operaciones sociales.----------

C E R T I F I C A

.463.982, actuando como Presidente y represen-tante legal de la soci edad GYNOPHARM S.A., otorga Poder Especial, Amplio y Suficienta a KA REN JENNIFER FIGUEROA GARCIA C.C. No. 32.-732.070, para que en nombr e y representacion de la sociedad suscriba todas las declaraciones c ambiarias a que esta obligada GYNOPHARS.A., en el giro normal de sus negocios y operaciones sociales.----.----------

C E R T I F I C A

*** CONTINUA ***

CERTIFICADO NACIONAL

CAMARA DE COMERCIO DE BARRANQUILLA

Fecha : 20080312 Hora Certificado 18:25:01

Operacion : 02CP20312029 PAGINA No. 6

****

Que en esta C?mara de Comercio no aparecen inscripciones posteriores de documentos referentes a reforma, disoluci?n, liquidaci?n o nombramientos de representantes legales de la expresada sociedad.

C E R T I F I C A

Que su ?ltima Renovaci?n fue el: 23 de Marzo de 2007.

La informaci?n sobre embargos de establecimiento se suministra en Certificados de Matr?cula, la de contratos sujetos a registro, en Certificados Especiales.

C E R T I F I C A

Que los actos de registro aqu? certificados quedan en firme cinco d?as h?biles despues de la correspondiente inscripci?n, siempre que dentro de dicho t?rmino, no sean objeto de los recursos de reposici?n ante esta entidad, y/o de apelaci?n para ante la Superintendencia de Industria y Comercio.

108 16 109

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

NIT : 800176089-2

RECIBO OFICIAL DE CAJA : 08 - 25,223
FECHA : MARZO 28 DE 2008

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No. 07-039192- -00002-0000
Fecha: 2008-03-28 09:19:51 Dep: 2020 NULIDAD ACIONE
Trd: 51 PATENTES Liq: 378 PASENACIONALI
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GYNOPHARM S.A.	CONSIGNACION	BANCO DE BOGOTA	062754387	648410	27/03/2008	28,000.00
LAB. PROCAPS	CONSIGNACION	BANCO DE BOGOTA	062754387	4767284	06/03/2008	482,000.00
LAB. PROCAPS	CONSIGNACION	BANCO DE BOGOTA	062754387	4767287	14/03/2008	1,330,000.00

***** C O N C E P T O *****

CANT.	RENTISTICO		CONCEPTO		TOTAL CONCEPTO
1	50005-01-04	PRESENTACION DE OBSERVACIONES	12	MARCAS Y LEMAS COMERCIALES	266,000.00
				TOTAL : $	266,000.00

SON: DOSCIENTOS SESENTA Y SEIS MIL PESOS

RESPONSABLE : _____

RECIBO DE CAJA APLICADO AL EXPEDIENTE No. _____

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Shaw et al. (43) **Pub. Date: Nov. 8, 2001**

(54) **REGIOSELECTIVE SYNTHESIS OF RAPAMYCIN DERIVATIVES**

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Related U.S. Application Data

(63) Continuation of application No. 09/670,358, filed on Sep. 27, 2000, now Pat. No. 6,277,983, which is a non-provisional of provisional application No. 60/240,945, filed on Sep. 29, 1999, now abandoned.

Publication Classification

(51) **Int. Cl.**[7] **C07D 487/16**

(52) **U.S. Cl.** **540/456**

(57) **ABSTRACT**

This invention provides a regioselective process for preparing a 42-ester or ether of rapamycin, and 31-silyl ether intermediates.

REGIOSELECTIVE SYNTHESIS OF RAPAMYCIN DERIVATIVES

[0001] This application claims the benefit of U.S. Provisional Application No. Not Yet Known, which was converted from U.S. patent application No. 09/408,830, filed Sep. 29, 1999, pursuant to a petition filed under 37 C.F.R. 1.53(c)(2)(i).

BACKGROUND OF THE INVENTION

[0002] This invention relates to the regioselective synthesis of derivatives of rapamycin at the 42-position, which are useful for inducing immunosuppression, and in the treatment of transplantation rejection, graft vs. host disease, autoimmune diseases, diseases of inflammation, adult T-cell leukemia/lymphoma, solid tumors, fungal infections, and hyperproliferative vascular disorders.

[0003] Rapamycin is a macrocyclic triene antibiotic produced by *Streptomyces hygroscopicus*, which was found to have antifungal activity, particularly against *Candida albicans*, both in vitro and in vivo [C. Vezina et al., J. Antibiot. 28, 721 (1975); S. N. Sehgal et al., J. Antibiot. 28, 727 (1975); H. A. Baker et al., J. Antibiot. 31, 539 (1978); U.S. Pat. No. 3,929,992; and U.S. Pat. No. 3,993,749].

[0004] Rapamycin alone (U.S. Pat. No. 4,885,171) or in combination with picibanil (U.S. Pat. No. 4,401,653) has been shown to have antitumor activity. R. Martel et al. [Can. J. Physiol. Pharmacol. 55, 48 (1977)] disclosed that rapamycin is effective in the experimental allergic encephalomyelitis model, a model for multiple sclerosis; in the adjuvant arthritis model, a model for rheumatoid arthritis; and effectively inhibited the formation of IgE-like antibodies.

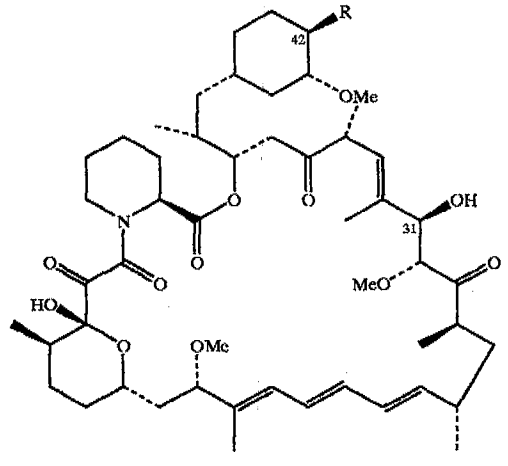
[0005] The immunosuppressive effects of rapamycin have been disclosed in FASEB 3, 3411 (1989). Cyclosporin A and FK-506, other macrocyclic molecules, also have been shown to be effective as immunosuppressive agents, therefore useful in preventing transplant rejection [FASEB 3, 3411 (1989); FASEB 3, 5256 (1989); R. Y. Calne et al., Lancet 1183 (1978); and U.S. Pat. No. 5,100,899].

[0006] Rapamycin has also been shown to be useful in preventing or treating systemic lupus erythematosus [U.S. Pat. No. 5,078,999], pulmonary inflammation [U.S. Pat. No. 5,080,899], insulin dependent diabetes mellitus [U.S. Pat. No. 5,321,009], smooth muscle cell proliferation and intimal thickening following vascular injury [U.S. Pat. No. 5,516, 781], adult T-cell leukemia/lymphoma [European Patent Application 525,960 A1], and ocular inflammation [U.S. Pat. No. 5,387,589].

[0007] Numerous rapamycin 42-derivatives are known, typically being esters (carbon and sulfur based) or ethers of the 42-hydroxyl group of rapamycin, that are produced by esterification or etherification of the 42-position. Esterification of rapamycin at the 42-position was commonly prepared by directly reacting rapamycin with acylating agents in order to afford the desired product. The chemistry appeared to be rather simple. However, as rapamycin contains two secondary hydroxyl groups at positions 31 and 42, attempts to discriminate between these two functional centers in order to achieve a selective synthesis of 42-monoacylated product, posed a difficult challenge. This type of non-regioselective reaction also produced a 31,42-bis-acylated by-product and as well, some unreacted rapamycin remained in the reaction mixture. The final result was a lower yield that required extensive purification to obtain pure 42-monoacylated product.

DESCRIPTION OF THE INVENTION

[0008] This invention provides a regioselective method for the preparation of a 42-ester or ether rapamycin having the structure



[0009] wherein R is an ester or ether, which comprises:

[0010] (a) treating rapamycin with a silylating agent to form rapamycin 31,42-bis-silyl ether;

[0011] (b) selectively hydrolyzing the 42-silyl ether in mild acid to provide rapamycin 31-silyl ether;

[0012] (c) treating the rapamycin 31-silyl ether with a suitable esterifying or etherifying reagent to form rapamycin 31-silyl ether 42-ester or ether; and

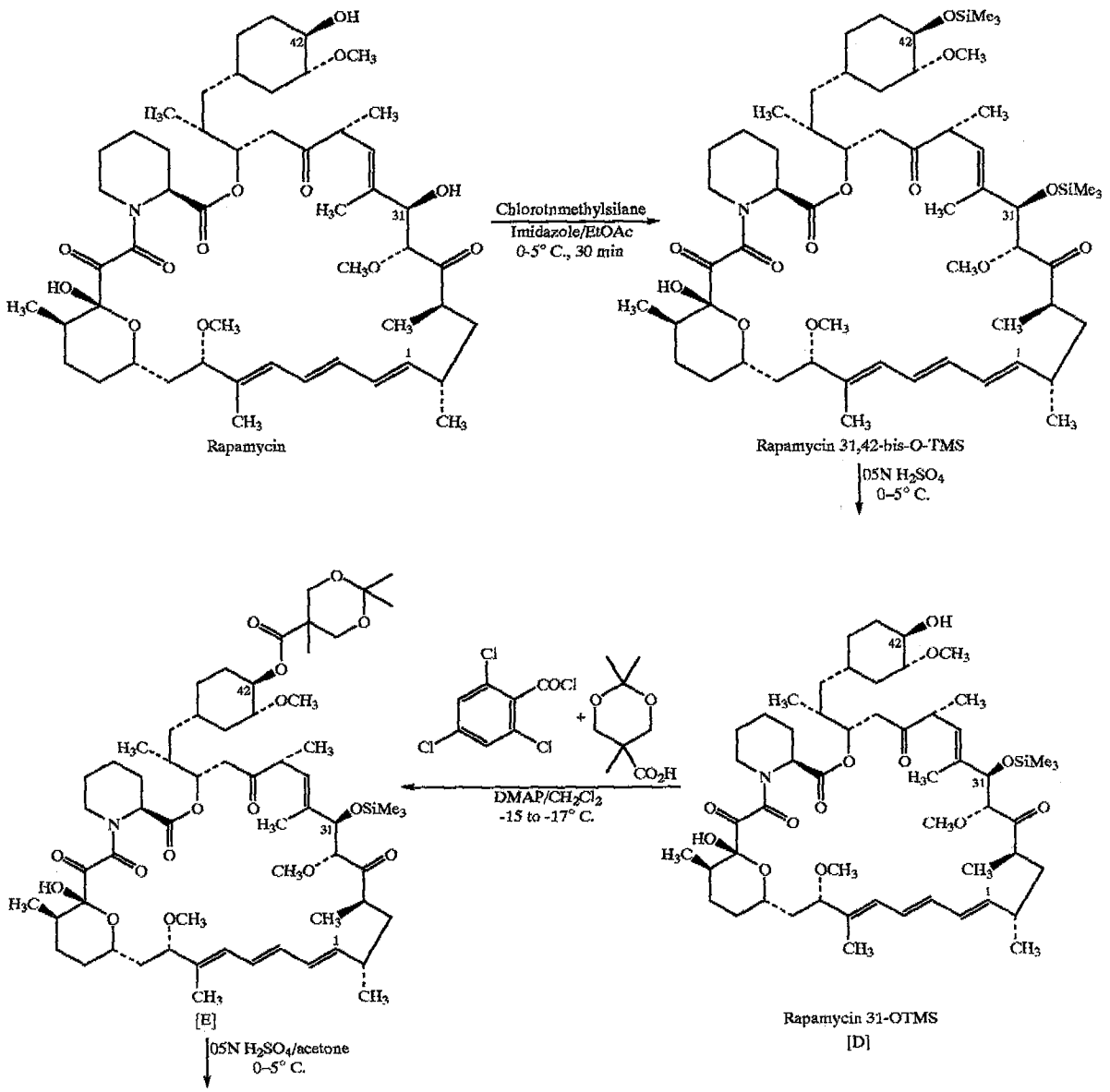
[0013] (d) selectively hydrolyzing the 31-silyl ether in mild acid to provide the desired rapamycin 42-ester or ether.

[0014] Preferred 42-esters and ethers of rapamycin which can be prepared by the method provided by this invention are disclosed in the following patents, which are all hereby incorporated by reference: alkyl esters (U.S. Pat. No. 4,316, 885); aminoalkyl esters (U.S. Pat. No. 4,650,803); fluorinated esters (U.S. Pat. No. 5,100,883); amide esters (U.S. Pat. No. 5,118,677); carbamate esters (U.S. Pat. No. 5,118, 678); silyl ethers (U.S. Pat. No. 5,120,842); aminoesters (U.S. Pat. No. 5,130,307); acetals (U.S. Pat. No. 5,51,413); aminodiesters (U.S. Pat. No. 5,162,333); sulfonate and sulfate esters (U.S. Pat. No. 5,177,203); esters (U.S. Pat. No. 5,221,670); alkoxyesters (U.S. Pat. No. 5,233,036); O-aryl, -alkyl, -alkenyl, and -alkynyl ethers (U.S. Pat. No. 5,258, 389); carbonate esters (U.S. Pat. No. 5,260,300); arylcarbonyl and alkoxycarbonyl carbamates (U.S. Pat. No. 5,262,

423); carbamates (U.S. Pat. No. 5,302,584); hydroxyesters (U.S. Pat. No. 5,362,718); hindered esters (U.S. Pat. No. 5,385,908); heterocyclic esters (U.S. Pat. No. 5,385,909); gem-disubstituted esters (U.S. Pat. No. 5,385,910); aminoalkanoic esters (U.S. Pat. No. 5,389,639); phosphorylcarbamate esters (U.S. Pat. No. 5,391,730); carbamate esters (U.S. Pat. No. 5,411,967); carbamate esters (U.S. Pat. No. 5,434,260); amidino carbamate esters (U.S. Pat. No. 5,463,048); carbamate esters (U.S. Pat. No. 5,480,988); carbamate esters (U.S. Pat. No. 5,480,989); carbamate esters (U.S. Pat. No. 5,489,680); hindered N-oxide esters (U.S. Pat. No. 5,491,231); biotin esters (U.S. Pat. No. 5,504,091); and O-alkyl ethers (U.S. Pat. No. 5,665,772). These patents also disclose methods for esterification or etherification utilized in step (c), above.

[0015] The following scheme illustrates the regioselective preparation of rapamycin 42-ester with 2,2-bis-(hydroxymethyl)propionic acid, as a representative 42-ester of rapamycin, which can be prepared according to the method provided in this invention. The original synthesis of rapamycin 42-ester with 2,2-bis-(hydroxymethyl)propionic acid is disclosed in U.S. Pat. No. 5,362,718.

Scheme I



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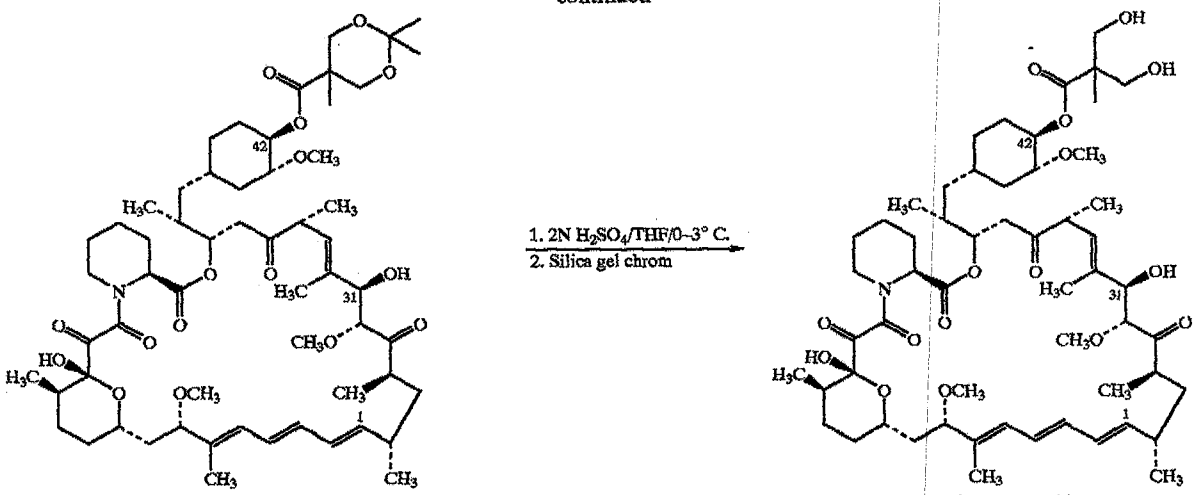


[B]

Rapamycin 42-ester with 2,2-bis-(hydroxymethyl)propionic acid

[C]

[0016] While the chemical preparation of rapamycin esters and ethers appears to be simple, and the desired products are obtainable, the synthetic yield of the esters and ethers is often poor. As rapamycin contains two secondary hydroxyl groups at positions 31 and 42, attempts to discriminate between these two functional centers in order to achieve a selective synthesis of 42-monoester such as compound [B] poses a difficult challenge. For example, the synthesis of rapamycin 42-ester with 2,2-bis-(hydroxymethyl)propionic acid described in U.S. Pat. No. 5,362,718, example 10, was non-regioselective, the 31,42-bisester by-product was also generated. As a result, the crude product [B] after work-up contains the desired product [B], 31,42-bisester by-product and unreacted rapamycin. In an effort to consume the remaining starting rapamycin, the reaction was allowed to proceed for a longer period with negative consequences, the quantity of the 31,42-bisester increased significantly. The resulting crude product [B] is contaminated with unreacted rapamycin and 31,42-bisester, and subsequent column chromatography purification effort has proved to be difficult as the 42,31-bisester has a very close retention time with product [B]. Overall, the major obstacle in large-scale production of compound [B] appears to be the non-regiospecificity that is further complicated by purification difficulties. This invention overcomes these difficulties by providing a regioselective synthesis of 42-esters or ethers of rapamycin by selectively protecting the 31-hydroxyl group as a silyl ether (i.e., compound [D]), leaving the 42-position hydroxyl accessible for regioselective esterification or etherification to produce 31-O-silyl, 42-esters or ethers (i.e., compound [E]). The 31-hydroxyl group can then be deprotected under mild acidic conditions (i.e., compound [B]).

[0017] In accordance with this invention, it is preferred that the 31, and 42-hydroxyl groups are protected as trialkyl silyl ethers. The 42-silyl protected hydroxyl group of the 31,42-bis-silylated rapamycin can be selectively cleaved under mildly acidic conditions to provide 31-silyl rapamycin. The silylating agents used for this transformation are common, commercially available chloroalkylsilanes, such as chlorotrimethylsilane, chlorotriethylsilane or chlorotripropylsilane. However, the bulkier the trialkylsilane, the more time is needed to deprotect in acid media during the penultimate chemical step to regenerate the 31-hydroxyl group. Also, a longer reaction time in the acid media generates more degradation by-products. Although, chlorotrimethylsilane, chlorotriethylsilane or chlorotripropylsilane can be used for the preparation of rapamycin 31-O-trialkylsilyl ethers, chlorotrimethylsilane is the preferred silylating agent. The trimethylsilyl group is more acid labile and therefore easier to de-protect during the transformation and in effect, this minimizes the formation of degradation products. The preparation of 31-O-trimethylsilyl rapamycin [D] is described in Example 1. Rapamycin is treated with excess chlorotrimethylsilane in ethyl acetate at 0-5° C. in the presence of an organic base and the 42- and 31- hydroxyl groups of rapamycin are silylated to form rapamycin 31,42-bis-O-trimethylsilyl ether in quantitative yield. The common organic bases such as imidazole, 1-methylimidazole, triethylamine and N,N-diisopropylethylamine can be used for the general silylation reaction. However, imidazole is found to be the preferred base for the silylation of rapamycin as the reaction is completed within 30 minutes.

[0018] Selective de-protection of the 42-O-trimethylsilyl group of rapamycin 31,42-bis-O-trimethylsilyl ether to form rapamycin 31-O-trimethylsilyl ether, is effected in situ at 0-5° C. with ethanol, ethanol-water mixtures, water, diluted inorganic or organic acids. Sulfuric acid (0.5 N) is preferred since the reaction is clean and can be completed in 4-5 h. A number of organic solvents can be used for silylation and in particular, DMF is often mentioned in the literature. However, in this invention, ethyl acetate is the preferred solvent.

[0019] The esterification or etherification of the 31-protected rapamycin can be carried out under conditions described in the patents listed above. For example, in Scheme I, the acylation of rapamycin 31-trimethylsilyl ether was accomplished using 2,4,6-trichlorobenzoyl mixed anhy-

dride of 2,2,5-trimethyl[1.3-dioxane]-5-carboxylic acid in the presence of 4-dimethylaminopyridine or a similar reagent. In addition, 2,2,5-trimethyl[1.3-dioxane]-5-carboxylic acid chloride was also found to be an effective acylation agent in this invention in the presence of 4-dimethylaminopyridine or a similar reagent. For the acylation conditions, methylene chloride is the preferred solvent rather than tetrahydrofuran which was described in the prior art. In addition, lower reaction temperature of less than 0° C., with −20 to −15° C. or lower being more preferred, provides better results than the room temperature acylation described in U.S. Pat. No. 5,362,718. In Scheme I, the acylation products, 31-O-TMS, 42-(protected-hydroxy) esters (compound [E]) can be further treated with diluted acid to convert them to 42-(protected-hydroxy) esters (compound [B]) or used directly to make final product 42-hydroxyesters (final product [C]). This methodology can be used to prepare other esters or ethers of rapamycin, by simply varying the esterfiying or etherifying agent used.

[0020] In Scheme I, conversion of compound [B] to rapamycin 42-ester with 2,2-bis-(hydroxymethyl)propionic acid [C], can be accomplished under mildly acidic conditions. It is preferred that aqueous sulfuric acid is used, as it minimizes the formation of impurities generated when aqueous hydrochloric acid is used, as described in the U.S. Pat. No. 5,362,718. The tetraene impurity formed when using hydrochloric acid has been reported to be difficult to separate from the desired product by column chromatography (Caufield et al, Tetrahedron Lett., 1994, 37,6835). It is also preferrable to carry out the hydrolysis at 0-5° C. rather than room temperature as described in U.S. Pat. No. 5,362,718.

[0021] The synthetic route disclosed in this invention provides several distinct advantages over the synthetic methodology which has been published for the preparation of rapamycin esters or ethers; mainly in the yield and ease of purification of the desired 42-esters or ethers. As this is a regioselective synthesis, the overall yields of the desired 42-esters or ethers is dramatically improved. For example, the synthetic methodology taught in U.S. Pat. No. 5,362,718 provides compound [B] in a 35% yield, whereas, the synthesis of [B] is accomplished in 85% yield using the methodology disclosed herein. Additionally, the conversion to rapamycin 42-ester with 2,2-bis-(hydroxymethyl)propionic acid from [B] is accomplished in approximately 75% yield using the process described herein, whereas only a 20% conversion is provided using the methodology of U.S. Pat. No. 5,362,718.

[0022] Using the same methodology, 42-ethers of rapamycin can be prepared in a regioselective manner. As an example, U.S. Pat. No. 5,665,772 discloses the preparation of 40-O-alkyl ethers of rapamycin in a non-regioselective manner. Owing to nomenclature differences, the 42-position of rapamycin (as named in this invention) is referred to as the 40-position in U.S. Pat. No. 5,665,772. These positons are identical. Using the methodology disclosed herein, rapamycin 31-O-trimethylsilyl ether can be treated with, for example, 2-(t-butyldimethylsilyl)oxyethyl triflate) to provide 31-O-trimethylsilyl, 42-O-[2-(t-butyldimethylsilyl)oxy]ethyl-rapamycin. Removal of the silyl protecting groups from the 31-hydroxyl group of rapamycin and from the 42-hydroxyethyl moiety can be accomplished under mildly acidic conditions, such as dilute sulfuric acid to provide 42-O-(2-hydroxy)ethyl rapamycin. The non-regioselective formation of other 42-ethers of rapamaycin is disclosed in U.S. Pat. No. 5,665,772. These also can be prepared regioselectively via rapamycin 31-O trimethylsilyl ether.

[0023] This invention also covers 31-silyl ethers of rapamycin, which are useful in the preparation of the 42-esters and ethers of rapamycin, as disclosed herein. The silicon moiety as represented by —SiR'R"R'", contains 3 groups which can be the same or different. Typical silyl ethers of this invention contain R', R", or R'" moieties which are alkyl of 1-6 carbon atoms, phenyl, or benzyl groups. The alkyl groups can be branched or straight chain. It is preferred that R', R", and R'" be alkyl groups, and more preferred that R', R", and R'" are methyl or ethyl. It is still more preferred that the 31-silyl ether is rapamycin 31-O-trimethylsilyl ether.

[0024] The following examples illustrate the preparation of rapamycin 31-silyl ethers and a rapamycin 42-ester, which is representative of the compound which can be prepared by the process of this invention.

EXAMPLE 1

Rapamycin 31-O-trimethylsilyl ether

[0025] A solution of rapamycin (25.0 g, 92.4% strength; 25.28 mmol) in 750 mL ethyl acetate was cooled to 0-5° C.; 7.5 g (110.20 mmol) of imidazole was added and stirred to form a solution. To this cold solution 11.0 g (101.25 mmol) of chlorotrimethylsilane was added dropwise over 30 min and stirred for a further 30 min at 0-5° C. in order to complete the formation of rapamycin 31,42-bis-O-trimethylsilyl ether. A 50 mL quantity of 0.5 N sulfuric acid was added dropwise over a 10 min period and the mixture was stirred for 2.5 h at 0-5° C. The reaction mixture was transferred into a separatory funnel and the aqueous layer was separated and extracted with 125 mL ethyl acetate. The organic layers were combined and successively washed with brine (125 mL), saturated sodium bicarbonate solution (100 mL), water (125 mL×2) then brine to pH 6-7. The organic layer was dried over anhydrous sodium sulfate and evaporated under reduced pressure to give a beige color foam product, 28.5 g (theory 24.94 g). HPLC analysis showed it contained 86% (by area %) of rapamycin 31-O-trimethylsilyl ether and 7% of rapamycin. The product was used directly for the subsequent reaction.

[0026] LC/MS electrospray (+) mode (M−H)=985. ^{1}H NMR (400 MHz, d-6 DMSO) δ 4.60 (m, 1H, (42C)OH), 4.10 (m, 1H, C(31) H), 3.09 (m, 1H, C(42) H), −0.027 (s, 9H, 31-O-TMS).

EXAMPLE 2

2,2,5-Trimethyl[1.3-dioxane]-5-carboxylic acid chloride

[0027] A solution of 2,2,5-trimethyl[1.3-dioxane]-5-carboxylic acid (17.42 g, 0.1 mol) in 200 mL of dry toluene was warmed to 40° C., and 26.0 mL of oxalyl chloride (37.83 g, 0.3 mol) added dropwise over a period of 30 min and then stirred at 40° C. for 2.5 h. The reaction mixture was evaporated under reduced pressure to remove solvent and excess oxalyl chloride. The residual product was evaporated twice with dry toluene (200 mL), then dried under high vacuum at 40° C. for 2 h to obtain 19.75 g of product as an

orange colored liquid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.28 (2H, d, J=10.5 Hz), 3.76 (2H, d, J=10.5 Hz), 1.46 (3H, s), 1.29 (3H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.43, 98.76, 66.06, 52.07, 25.82, 21.20, 18.10.

EXAMPLE 3

Rapamycin 31-O-trimethylsilyl ether, 42-ester with 2,2,5-trimethyl[1.3-dioxane]-5-carboxylic acid

[0028] Method A:

[0029] To a solution of the 2,2,5-trimethyl[1.3-dioxane]-5-carboxylic acid (9.77 g, 56.08 mmol) and N,N-diisopropylethylamine (12.00 g, 92.80 mmol) in 200 mL methylene chloride at room temperature under nitrogen, 2,4,6-trichlorobenzoyl chloride (13.35 g, 54.73 mmol) was added and the resulting mixture was stirred for 5 h at room temperature. The reaction mixture was cooled to −20 to −15° C. and a solution of rapamycin 31-O-trimethylsilyl ether (28.50 g, crude, made from 25.28 mmol of rapamycin) in 120 mL methylene chloride was added. A solution of 4-dimethylaminopyridine (11.68 g, 95.60 mmol) in 110 mL methylene chloride was added dropwise over a 2 h period. The reaction mixture was further stirred for 16 h at −15 to −16° C. The reaction mixture was quenched with 100 mL water and the organic layer was separated and washed with 0.5 N sulfuric acid (180 mL), followed by brine (100 mL), saturated sodium bicarbonate solution (100 mL), water (100 mL×2), brine (100 mL) to pH 6-7. The organic layer was dried over anhydrous sodium sulfate and evaporated under reduced pressure to afford the title compound (33.18 g) as a beige color foam.

[0030] LC/MS electrospray (+) mode ($M+NH_4$)=1160. ^{1}H NMR (400 MHz, d-6 DMSO) δ 4.57 (m, 1H, C(42)H), 4.10 (m, 1H, C(31) H), 4.03 (d, 2H), 3.57 (d, 2H), 1.34 (s, 3H), 1.24 (s, 3H), 1.13 (s, 3H), −0.023 (s, 9H, 31-O-TMS)

[0031] Method B:

[0032] A solution of rapamycin 31-O-trimethylsilyl ether (11.00 g; from 10.0 g of rapamycin; 11.15 mmol) in 120 mL methylene chloride, containing 2 mL of N,N,-dimethylformamide, was stirred under nitrogen and cooled to −15 ° C., 4-dimethylaminopyridine (4.80 g, 39.29 mmol) was added and the mixture was stirred to form a solution. To this cold solution a 7.5% solution of 2,2,5-trimethyl[1.3-dioxane]-5-carboxylic acid chloride (42.18 g; 16.42 mmol) in methylene chloride was added dropwise over a 2 h period. The solution was further stirred for 1 h at −15° C., and an additional 7.5% solution of acid chloride (14.06 g, 5.47 mmol) in methylene chloride was added over a 30 min period. The reaction mixture was further stirred for 16 h at −15 C. to −16° C. The reaction mixture was quenched with 100 mL brine and the organic layer was separated and washed with cold 0.5 N sulfuric acid (100 mL), brine (100 mL), saturated sodium bicarbonate solution (100 mL) water (100 mL), brine (100 mL) to pH 6-7. The organic layer was dried over anhydrous sodium sulfate and evaporated under reduced pressure to afford product (12.15 g) as a yellow foam.

EXAMPLE 4

Rapamycin 42-ester with 2,2,5-trimethyl[1.3-dioxane]-5-carboxylic acid

[0033] A solution of rapamycin 31-O-trimethylsilyl ether, 42-ester with 2,2,5-trimethyl[1.3-dioxane]-5-carboxylic acid (33.18 g; from example 3, method A) in 100 mL of acetone was stirred and cooled to 0-5° C. To this cold solution 17 mL of 0.5 N sulfuric acid was added dropwise over a 10 min period and the mixture was stirred for 2.5 h at 0-5° C. A solution of sodium bicarbonate (1.44 g) in 20 mL water was added over a period of 20 min followed by an additional 33 mL water over a period of 30 min; the product started to precipitated after about 1 h of stirring. The mixture was stirred overnight at 0-5° C. and after filtration the solid product was washed with 60 mL of acetone-water (1:1). The product was dried in a vacuum oven at 30° C. to obtain 28.85 g of product (83.9% strength, 89.3% overall yield from rapamycin). The ^{1}H NMR of the product was identical to the product described in U.S. Pat. No. 5,362,718 example 10.

EXAMPLE 5

Ragamycin 42-ester with 2,2-bis-(hydroxymethyl)propionic acid

[0034] Method A:

[0035] A solution of rapamycin 42-ester with 2,2,5-trimethyl[1.3-dioxane]-5-carboxylic acid (28.85 g; from example 4) in 276 mL of tetrahydrofuran was stirred and cooled to 0-5° C. To this cold solution 83 mL of cold 2 N sulfuric acid was added dropwise over a 30 min period and the mixture was stirred for 60 h at 0-5° C. The reaction mixture was diluted with 600 mL of ethyl acetate and washed with 120 mL brine. The aqueous layer was extracted once with 120 mL of ethyl acetate and the organic extracts were combined and washed with saturated sodium bicarbonate solution (120 mL), water (200 mL×2) and brine (120 mL) to pH 6-7. The organic phase was dried over anhydrous sodium sulfate and evaporated under reduced pressure at room temperature to obtain product (28.42 g), as a beige color foam. The crude product was chromatographed on a silica gel column and eluted with 30% acetone in heptane to give 18.06 g of pure product, a white solid (69.4% overall from rapamycin). The ^{1}H NMR of the product is identical to the product described in U.S. Pat. No. 5,362,718 example 11.

[0036] Method B:

[0037] A solution of rapamycin 31-O-trimethylsilyl ether, 42-ester with 2,2,5-trimethyl[1.3-dioxane]-5-carboxylic acid (23.25 g, prepared from 20.06 g of rapamycin, strength 92.7%, 20.34 mmol) in 230 mL of tetrahydrofuran was stirred and cooled to 0-5° C. To this cold solution 115 mL of cold 2 N sulfuric acid was added dropwise over a 45 min period and the mixture was stirred for 88 h at 0-5° C. The reaction mixture was diluted with 500 mL of ethyl acetate and washed with 100 mL brine. The aqueous layer was extracted once with 100 mL of ethyl acetate and the organic extracts were combined and washed with saturated sodium bicarbonate solution (80 mL), water (80 mL×2) and brine (100 mL) to pH 6-7. The organic phase was dried over anhydrous sodium sulfate and evaporated under reduced pressure at room temperature to afford product (22.48 g), a beige color foam. The crude product was chromatographed on a silica gel column and eluted with 30% acetone in heptane to give 16.50 g of pure product as a white solid (78.4% overall from rapamycin). The ^{1}H NMR of the product is identical to the product described in U.S. Pat. No. 5,362,718 example 11.

EXAMPLE 6

Rapamycin 31,42-bis-O-trimethylsilyl ether

[0038] A solution of rapamycin (10.0 g, 94.3% strength; 10.3 mmol) in 150 mL ethyl acetate was cooled to 0-5° C.; 3.0 g (44 mmol) of imidazole was added and stirred to form a solution. To this cold solution, 4.4 g (40.5 mmol) of chlorotrimethylsilane was added dropwise over a 20 min period and following this, the solution was stirred at 0-5° C. for a further 30 min. The reaction mixture was filtered to remove the imidazole HCl and the filtrate was evaporated under reduced pressure to obtain a yellow foam. Heptane (200 mL) was added and stirred at room temperature for 20 min and the mixture was filtered. The filtrate was washed with 40 mL of saturated sodium bicarbonate solution, then twice with water (80 mL), then brine (50 mL).

[0039] The organic layer was dried over anhydrous sodium sulfate and evaporated to obtain the product, a yellow foam of 11.42 g (98.6%).

[0040] LC/MS electrospray (−) mode (M−H)=1057. ^{1}H NMR (400 MHz, d-6 DMSO) δ 4.10 (m, 1H, C(31) H), 3.31 (m, 1H, C(42) H), 0.057 (s, 9H, 42-O-TMS), −0.027 (s, 9H, 31-O-TMS).

EXAMPLE 7

Rapamycin 31-O-triethylsilyl ether

[0041] A solution of rapamycin (5.00 g, 92.7% strength; 5.07 mmol) in 75 mL ethyl acetate was cooled to 0-5° C.; 1.50 g (22.03 mmol) of imidazole was added and stirred to form a solution. To this cold solution, 3.05 g (20.23 mmol) of chlorotriethylsilane was added dropwise over a 10 minutes period. The mixture was stirred for 30 min at 0-5° C., then stirred at room temperature overnight to complete the formation of rapamycin 31,42-bis-O-triethylsilyl ether. Following filtration of the reaction mixture, the filtrate was evaporated under reduced pressure at room temperature to remove most of the solvent. The residual solution (ca. 10 mL) was dissolved in 60 mL acetone and 15 mL of 0.15 N sulfuric acid was added and the mixture stirred for 25 h at 0-5° C. The rapamycin 31,42-bis-O-triethylsilyl ether disappeared at this stage. The reaction mixture was diluted with 80 mL of ethyl acetate and successively washed with brine (60 mL×2), saturated sodium bicarbonate solution (40 mL), water (60 mL×2), brine (60 mL) to pH 6-7. The organic layer was dried over anhydrous sodium sulfate and evaporated under reduced pressure to obtain a product of light yellow gum, 6.92 g (theory 5.21 g). HPLC analysis showed it contained 95.2% (by area %) rapamycin 31-O-triethylsilyl ether and 0.9% rapamycin.

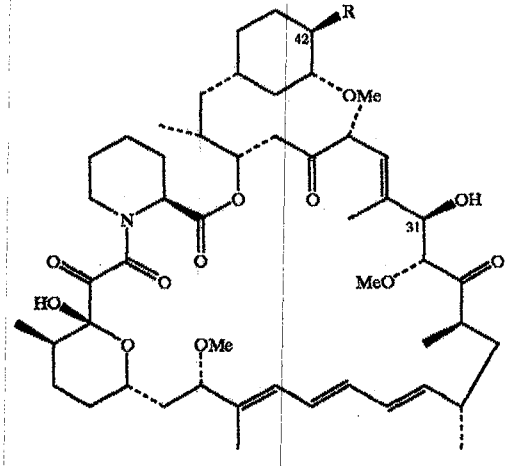
EXAMPLE 8

Rapamycin 31-O-tripropylsilyl ether

[0042] A solution of rapamycin (5.00 g, 92.7% strength; 5.07 mmol) in 75 mL ethyl acetate was cooled to 0-5° C.; 1.50 g (22.03 mmol) of imidazole was added and stirred to form a solution. To this cold solution, 3.91 g (20.3 mmol) of chlorotripropylsilane was added dropwise over 10 min period. The mixture was stirred for 30 min at 0-5° C., then at room temperature for 21 h to complete the formation of rapamycin 31,42-bis-O-tripropylsilyl ether. Following filtration of the reaction mixture, the filtrate was evaporated under reduced pressure at room temperature to remove most of the solvent and the residual solution was dissolved in 60 mL acetone. A 15 mL quantity of 0.25 N of sulfuric acid was added and the mixture was stirred for 45 h at 0-5° C.; the rapamycin 31,42-bis-O-tripropylsilyl ether disappeared at this stage. The reaction mixture was diluted with 100 mL of ethyl acetate, and successively washed with brine (40 mL×2), saturated sodium bicarbonate solution (40 mL), water (40 mL×2), and brine (50 mL) to pH 6-7. The organic layer was dried over anhydrous sodium sulfate and evaporated under reduced pressure to obtain a product of light yellow gum, 8.07 g (theory 5.43 g). HPLC analysis showed it contained 96.7% (by area %) of rapamycin 31-O-tripropylsilyl ether and 1% of rapamycin

What is claimed is:

1. A process for preparing a 42-ester or ether of rapamycin having the structure



wherein R is an ester or ether, which comprises:

(a) treating rapamycin with a silylating agent to form rapamycin 31,42-bis-silyl ether;

(b) selectively hydrolyzing the 42-silyl ether in mild acid to provide rapamycin 31-silyl ether;

(c) treating the rapamycin 31-silyl ether with a suitable esterifying or etherifying reagent to form rapamycin 31-silyl ether 42-ester or ether; and

(d) selectively hydrolyzing the 31-silyl ether in mild acid to provide the desired rapamycin 42-ester or ether.

2. The process according to claim 1, wherein the silylating agent in step (a) is a trialkylsilyl halide.

3. The process according to claim 2, wherein the silylating agent in step (a) is trimethylsilyl chloride.

4. The process according to claim 3, wherein the acid used to perform the hydrolysis in step (b) is dilute sulfuric acid.

5. A process for preparing rapamycin 31-trimethylsilyl ether, which comprises:

(a) treating rapamycin with chlorotrimethylsilane in an inert solvent in the presence of a suitable base to provide rapamycin 31,42-bis-trimethylsilyl ether; and

(b) treating the 31,42-bis-trimethylsilyl ether with dilute acid to provide rapamycin 31-trimethylsilyl ether.

6. The process according to claim 5, wherein the base in step (a) is imidazole, 1-methylimidazole, triethylamine, or N,N-diisopropylethylamine.

7. The process according to claim 6, wherein the acid in step (b) is sulfuric acid.

8. A process for preparing rapamycin 42-ester with 2,2-bis-(hydroxymethyl)propionic acid, which comprises:

(a) treating rapamycin with a silylating agent to form rapamycin 31,42-bis-silyl ether;

(b) selectively hydrolyzing the 42-silyl ether in mild acid to provide rapamycin 31-silyl ether;

(c) acylating the rapamycin 31-silyl ether with 2,2,5-trimethyl[1.3-dioxane]-5-carboxylic acid chloride or the 2,4,6-trichlorobenzoyl mixed anhydride of 2,2,5-trimethyl[1.3-dioxane]-5-carboxylic acid to give rapamycin-31-O-trimethylsilyl ether, 42-ester with 2,2,5-trimethyl[1.3-dioxane]-5-carboxylic acid;

(d) selectively hydrolyzing the 31-silyl ether in mild dilute acid to provide rapamycin 42-ester with 2,2,5-trimethyl[1.3-dioxane]-5-carboxylic acid; and

(e) treatment of rapamycin 42-ester with 2,2,5-trimethyl [1.3-dioxane]-5-carboxylic acid with mild acid to provide 42-ester with 2,2-bis-(hydroxymethyl)propionic acid.

9. The process according to claim 8, wherein the silylating agent is a trialkylsilyl halide.

10. The process according to claim 9, wherein the silylating agent is chlorotrimethylsilane.

11. The process according to claim 10, wherein the acid used in steps (b) and (d) is sulfuric acid.

12. The process according to claim 11, wherein the acylation in step (c) is carried out at less than 0° C.

13. A process for preparing 42-O-(2-hydroxy)ethyl-rapamycin which comprises:

(a) treating rapamycin with a silylating agent to form rapamycin 31,42-bis-silyl ether;

(b) selectively hydrolyzing the 42-silyl ether in mild acid to provide rapamycin 31-silyl ether;

(c) treating the rapamycin 31-silyl ether with a an ethylene glycol equivalent containing an acid labile hydroxyl protecting group protecting on one terminus of the ethylene glycol equivalent and a leaving group suitable of alkylating a hydrxyl group as the other terminus of the ethylene glycol equivalent.

(d) hydrolying the protecting groups on the 31-postion and on the 42-hydroxyethyl position under mildly acidic conditions.

14. The process according to claim 13, wherein the silylating agent is is a trialkylsilyl halide.

15. The process according to claim 14, wherein the silylating agent is chlorotrimethylsilane.

16. The process according to claim 15, wherein the ethylene glycol equivalent is 2-(t-butyldimethylsilyl)oxyethyl triflate).

17. The process according to claim 16, wherein the acid used in steps (b) and (d) is sulfuric acid.

18. A compound which is a rapamycin 31-O-silyl ether.

19. The compound of claim 18, in which the 31-O-silyl ether is a trialkylsilyl ether.

20. The compound of claim 19, which is rapamycin 31-O-trimethylsilyl ether.

* * * * *

United States Patent [19]

Sehgal et al.

[11] **3,993,749**

[45] **Nov. 23, 1976**

[54] **RAPAMYCIN AND PROCESS OF PREPARATION**

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[73] Assignee: **Ayerst McKenna and Harrison Ltd.**, Montreal, Canada

[22] Filed: **Sept. 25, 1975**

[21] Appl. No.: **616,695**

Related U.S. Application Data

[60] Division of Ser. No. 460,665, April 12, 1974, Pat. No. 3,929,992, which is a continuation-in-part of Ser. No. 293,699, Sept. 29, 1972, abandoned.

[52] **U.S. Cl.** **424/122**

[51] **Int. Cl.²** **A61K 35/74**

[58] **Field of Search** 424/122

[56] **References Cited**

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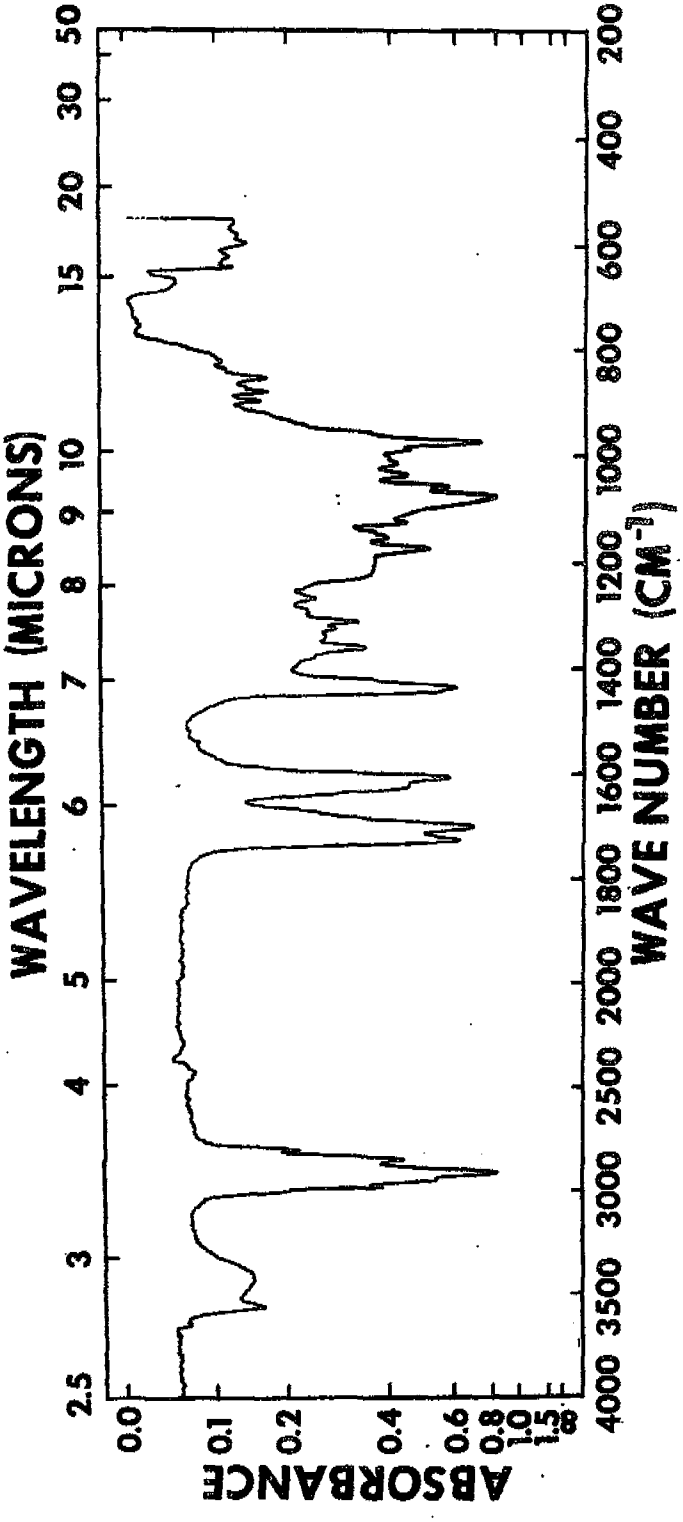
Millev, The Pfizer Handbook of Microbial Metabolites, McGraw-Hill Book Co., Inc., N.Y., N.Y., 1961, p. 580.

Primary Examiner—Jerome D. Goldberg

[57] **ABSTRACT**

Antibiotic rapamycin is producible by culturing *Streptomyces hygroscopicus* NRRL 5491 in an aqueous nutrient medium. Rapamycin has antifungal properties. Methods for its preparation and use are disclosed.

2 Claims, 2 Drawing Figures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3
4
5
6
7
8
9
10
15
20
30
50
WAVE NUMBER (CM⁻¹)
4000
3500
3000
2500
2000
1800
1600
1400
1200
1000
800
600
400
200
ABSORBANCE
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0.1
0.2
0.4
0.6
0.8
1.0
1.5
2.0

FIG. 1

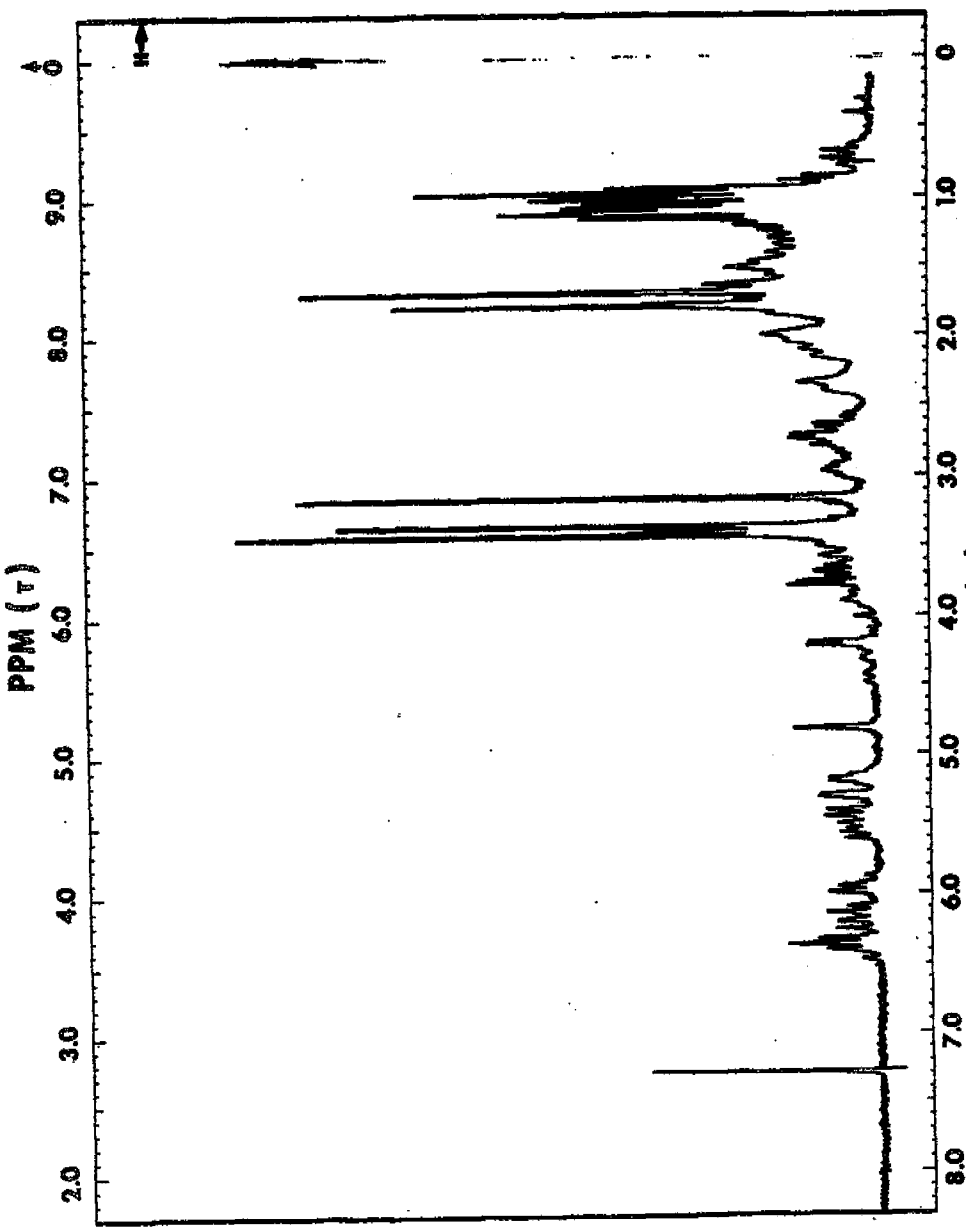

FIG.2

RAPAMYCIN AND PROCESS OF PREPARATION

This application is a division of application Ser. No. 460,665, filed Apr. 12, 1974, now U.S. Pat. No. 3,929,992, granted Dec. 30, 1975, which, in turn, is a continuation in part of Ser. No. 293,699, filed Sept. 29, 1972, now abandoned.

BACKGROUND OF THE INVENTION

A. Field of Invention

This invention relates to an antibiotic, a new composition of matter called rapamycin, and to a process for its preparation.

B. Description of Prior Art

The antibiotic of this invention is readily distinguished from prior art compounds of its class by its profound antifungal activity and its relatively low order of toxicity.

More explicitly, the ultra violet spectrum of rapamycin, noted herein, indicates that this compound belongs to the class of antibiotics known as triene antibiotics. In this particular class there are only five compounds reported previously, Trienine, A. Aszalos et al., J. Antibiotics, 21, 611 (1968) is a triene antibiotic with antitumor activity which also shows marked activity against gram positive organisms and only marginal activity against Candida strains. The antifungal triene reported by J. J. Armstrong, et al., Nature, 206, 399 (1965) and Mycotrienin reported by C. Coronelli et al., J. Antibiotics, 20, 329 (1967) are probably identical. Both have low antifungal activity (MIC against C. albicans: 5 μg/ml) and high toxicity (LD_{50} in mice: 15 mg/kg). The remaining two antibiotics — Resistaphylin, S. Aezaiva et al., J. Antibiotics, 14, 393 (1971) and Proticin, G. Nesemann et al., Naturwissenschaften, 59, 81 (1972)- are readily distinguished from the compound of the present invention in that they exhibit antibacterial without any antifungal activity.

BRIEF SUMMARY OF THE INVENTION

Rapamycin is a chemical compound producible by culturing a rapamycin-producing organism in an aqueous nutrient medium. The compound has the property of adversely affecting the growth of fungi, for example, *Candida albicans* and *Microsporum gypseum*. Accordingly, rapamycin may be used to prevent the growth of or reduce the number of certain fungi in various environments.

The rapamycin — producing organism used for this invention, *Streptomyces hygroscopicus* NRRL 5491, was obtained from Easter Island soils and samples thereof have been deposited without restrictions with the Northern Utilization and Research Division, Agricultural Research Service, U.S. Department of Agriculture, Peoria, Illinois, U.S.A.

It is to be understood that the invention is not limited to the use of the particular organism herein described, but includes variations and mutants obtained by natural selection or by treatment of the microorganism with, for instance, ultraviolet rays, X-rays, N-methyl-N'-nitro-N-nitroso-guanidine, manganese chloride, camphor, nitrogen mustards, and the like, as well as polyploids of the varous mutants.

Streptomyces hygroscopicus NRRL 5491 develops abundantly in culture media usually employed for cultivation of other organisms of the same genus. It is capable of growing at temperatures ranging from 20° to 35° C., preferably at about 28° C, on Czapek's agar, glucose asparagine agar, glycerol asparagine agar, starch agar and peptone beef agar. Also, the organism grows very well on yeast extract agar, malt extract agar, starch-inorganic salts agar, oatmeal agar, oatmeal-tomato agar and Bennet's agar. On potato slices there is no aerial mycelium, but substrate growth is well developed and buff in color. On all media, the aerial growth is at first white then grayish with black spots. Sporophores are often compact, forming a spiral of more than ten spores. Substrate growth is light yellow to almost colorless and in some media pale brown. Ocasionally a yellowish pigment is produced. The organism is H_2S- and melanine-negative.

Carbohydrate utilization by *Streptomyces hygroscopicus* NRRL 5491 was studied in carbon utilization agar (ISP Medium 9) according to the procedure standardized by the International Streptomyces Project (ISP).

The best utilized carbohydrates were D-glucose, inositol, D-fructose and D-mannitol; less well utilized carbohydrates were rhamnose, raffinose, xylose, starch and arabinose. Carbohydrates not utilized were sucrose and cellulose.

The environment and nutritional requirements for the fermentation of *Streptomyces hygroscopicus* NRRL 5491 are similar to those necessary for the production of antibiotics by other aerobic microorganisms. Thus, aerobiosis can be sustained in a liquid nutrient medium inoculated with a sterile culture incubated in flasks placed on shaking machines. For industrial production, metal tanks with internal aeration and agitation by means of paddles can be substituted. Rapamycin is also produced by surface cultivation. The microorganism requires as nutrient elements assimilable carbon and organic nitrogenous substances. The presence of mineral salts is desirable. Cultivation is best effected when the initial pH of the culture medium is between 6.5 and 7.5, the optimum pH being aroudn 6.8–7.3.

The utilizable sources of assimilable carbon for the production of the antibiotic are very diverse, there being included sugars (for example, glucose, D-fructose, D-mannitol, maltose, arabinose, rhamnose, raffinose, xylose, and the like), dextrin, starches of different types of origina, glycerol (and other polyalcohols), inositol and animal and vegetable fats, as well as esters thereof. The sources of organic assimilable nitrogen which actively stimulate growth and favor production of rapamycin are substances such as soybean meal, cotton meal and other vegetable meals (whole or partially or totally defatted), meat flours or animal viscera, various peptones, casein hydrolysates, soybean hydrolysates, yeast hydrolysates, lactalbumin, wheat glutins, distillers solubles, corn steeps, molasses, urea and amino acids.

Mineral salts, such as the chlorides, nitrates, sulfates, carbonates and phosphates of sodium, potassium, ammonium and calcium, should be included in appropriate concentrations. The nutritive medium should contain a number of trace elements such as magnesium, iron, manganese, and zinc.

The inoculum of the above medium for the fermentation is provided with a fresh slant of *Streptomyces hygroscopicus* NRRL 5491.

Under the described conditions and with the temperature of cultivation at about 20°–35° C, preferably at about 25° C, maximum production of rapamycin in tanks is obtained in from about two to about eight days. Alternatively, the pH may be controlled during fermen-

tation in tanks and maintained at about pH 6.0, and glucose may be added continuously from about 2 days after beginning to the end of fermentation, thus obtaining maximum yields in about four to five days.

Thereafter a variety of procedures may be employed in the isolation and purification of rapamycin, for example, solvent extraction, partition chromatography, silica gel chromatography, liquid-liquid distribution in a Craig apparatus, and crystallization from solvents. Solvent extraction procedures are preferred for commercial recovery inasmuch as they are less time consuming and less expensive.

Generally speaking, rapamycin may be harvested by one of the following methods.

a. The fermentation mixture is extracted with a substantially water-immiscible solvent, preferably a lower alkanol, for example n-butanol, n-pentanol or the commercial mixture of pentanols known as "Pentasol" or n-hexanol, or a substantially water-immiscible lower alkyl lower alkanoate, for example, ethyl acetate, buryl acetate, amyl acetate or the commercially available mixture of amyl acetates, or a substantially water-immiscible halogenated aliphatic hydrocarbon, for example, chloroform, methylene dichloride or dichloroethane. The extracts are dried and concentrated under reduced pressure to yield an oily residue which is in turn extracted with a water-miscible solvent, preferably a lower alkanol, for example methanol or ethanol. Said last-named extracts are filtered through diatomaceous earth ("Celite"), and the filtrate concentrated under reduced pressure to yield an oily residue containing crude rapamycin.

b. The fermentation mixture is filtered through a pad of diatomaceous earth ("Celite") and the filter cake containing the mycelium is extracted as described below under (c). The filtrate, i.e. the mycelium-free fermentation mixture, is extracted several times with a substantially water-immiscible solvent, for example, a lower alkanol, lower alkyl lower alkanoate or halogenated aliphatic hydrocarbon as exemplified above in section (a). The extracts are dried and concentrated under reduced pressure to yield an oily residue which is extracted with a water-miscible solvent, preferably a lower alkanol, for example methanol or ethanol. Said last-named extracts are treated in the same manner as described above under (a) to yield an oily residue containing crude rapamycin.

c. The mycelium is separated from the fermentation mixture and extracted with a suitable water-miscible solvent, preferably a lower alkanol, for example methanol or ethanol. The extract is concentrated by evaporation to the aqueous phase, which in turn is extracted with a substantially water-immiscible solvent, such as a lower alkyl lower alkanoate, halogenated aliphatic hydrocarbon or a substantially water-immiscible lower alkanol as described above or an aromatic hydrocarbon, for example benzene or toluene. The latter extract is evaporated under reduced pressure to yield an oily residue containing crude rapamycin.

The crude rapamycin obtained by any of the processes described in sections (a), (b), or (c) is then purified by a variety of methods, for example, see above. Preferred methods include absorption of the crude rapamycin on an absorbent, for instance charcoal or silica gel from a solution in a substantially nonpolar, first solvent, followed by elution therefrom with a second solvent, more polar than said first solvent.

DETAILS OF THE INVENTION

Rapamycin is useful as an antifungal agent against a number of pathogenic fungi; for example, *Candida albicans*, and other candida species, *Microsporum gypseum*, *Trichophyton mentagrophytes*, *Asperqillus sp.*, and *Sporotrichum sp.*.

The inhibitory activity of rapamycin is especially pronounced against *Candida albicans* and said last organism may be used advantageously for assay purposes.

The antifungal activity of this compound is demonstrable in standard tests used for this purpose, for example, in the tests described in "Antiseptics, Disinfectants, Fungicides and Sterilization", G. F. Reddish, Ed., 2nd ed., Lea and Febiger, Philadelphia, 1957 or by D. C. Grove and W. A. Randall in "Assay Methods of Antibiotics", Med. Encycl. Inc., New York 1955.

When the antibiotic of this invention is employed as an antifungal agent in warm-blooded animals, e.g. rats, it may be used alone or in combination with pharmaceutically acceptable carriers, the proportion of which is determined by the solubility and chemical nature of the compound, chosen route of administration and standard biological practice. For example, an antifungally effective amount of the antibiotic may be administered orally in solid form containing such excipients as starch, sugar, certain types of clay and so forth. Similarly, such an amount may also be administered orally in the form of solutions or suspensions, or the antibiotic may be injected parenterally. For parenteral administration the antibiotic may be used in the form of a sterile solution or suspension containing other solutes or suspending agents, for example, enough saline or glucose to make the solution isotonic, bile salts, acacia, gelatin, sorbitan monoleate, polysorbate 80 (oleate esters of sorbitol and its anhydrides copolymerized with ethylene oxide) and the like.

The dosage of the present antibiotic will vary with the form of administration and the particular compound chosen. Furthermore, it will vary with the particular host under treatment. Generally, treatment is initiated with small dosages substantially less than the optimum dose of the compound. Thereafter, the dosage is increased by small increments until the optimum effect under the circumstances is reached. In general, the compound of this invention is most desirably administered at a concentration level that will generally afford antifungally effective results without causing any harmful or deleterious side effects and preferably at a level that is in a range of from about 1.0 mg to about 250 mg per kilo per day, although as aforementioned variations will occur. However, a dosage level that is in the range of from about 10 mg to about 100 mg per kilo per day is most desirably employed in order to achieve effective results.

In addition, the agent may be employed topically. For topical application it may be formulated in the form of solutions, creams, or lotions in pharmaceutically acceptable vehicles containing 0.1 – 5 percent, preferably 2 percent of the agent, and may be administered topically to the infected area of the skin.

Rapamycin may also be used for cleaning and disinfecting laboratory equipment, surgical instruments, locker rooms, or shower rooms of sensitive fungus organisms. For such purposes it is preferred to use 0.1 – 10% solutions of rapamycin in a lower alkanol, preferably methanol, diluted with 10 – 100 volumes of water containing 0.001 – 0.1% of a non-ionic surface-

active agent, for example, polysorbate 80 U. S. P., immediately before applying it to the objects to be cleaned and disinfected.

PREPARATION

In one embodiment of this invention rapamycin is prepared in the following manner:

A suitable fermenter is charged with production medium 8KM (see Example 1). After sterilization and cooling, the medium is inoculated with a first stage inoculum preparation of *Streptomyces hygroscopicus* NRRL 5491.

A maximum titre of 20 to 100 μg/ml of the antibiotic is reached in the fermentation mixture after 2–8 days, usually after about 5 days, as determined by the cup plate method and *Candida albicans* as the test organism. The mycelium is harvested by filtration with diatomaceous earth. Rapamycin is then extracted from the mycelium with a water-miscible solvent, for example a lower alkanol, preferably methanol or ethanol. The latter extract is then concentrated, preferably under reduced pressure, and the resulting aqueous phase is extracted with a water-immiscible solvent. A preferred water-immiscible solvent for this purpose is methylene dichloride although chloroform, carbon tetrachloride, benzene, n-butanol and the like may also be used. The latter extract is concentrated, preferably under reduced pressure, to afford the crude product as an oil.

The product may be purified further by a variety of methods. Among the preferred methods of purification is to dissolve the crude product in a substantially non-polar, first solvent, for example petroleum ether or hexane, and to treat the resulting solution with a suitable absorbent, for example charcoal or silica gel, so that the antibiotic becomes absorbed on the absorbant. The absorbant is then separated and washed or eluted with a second solvent more polar than the first solvent, for example ethyl acetate, methylene dichloride, or a mixture of methylene dichloride and ether (preferred). Thereafter, concentration of the wash solution or eluate affords substantially pure rapamycin. Further purification is obtained by partial precipitation with a non-polar solvent, for example, petroleum ether, hexane, pentane and the like, from a solution of the rapamycin in a more polar solvent, for example, ether, ethyl acetate, benzene and the like. Still further purification is obtained by column chromatography, preferably employing silica gel, and by crystallization of the rapamycin from ether.

In another preferred embodiment of this invention a first stage inoculum of *Streptomyces hygroscopicus* NRRL 5491 is prepared in small batches in a medium containing soybean flour, glucose, ammonium sulfate, and calcium carbonate incubated at about 25° C at pH 7.1 – 7.3 for 24 hrs. with agitation, preferably on a gyrotary shaker. The growth thus obtained is used to inoculate a number of somewhat larger batches of the same medium as described above which are incubated at about 25° C and pH 7.1 – 7.3 for 18 hrs. with agitation, preferably on a reciprocating shaker, to obtain a second stage inoculum which is used to inoculate the production stage fermenters.

The production stage fermenters are equipped with devices for controlling and maintaining pH at a predetermined level and for continuous metered addition of nutrient. They are charged with a medium containing soybean flour, glucose, ammonium sulfate, and potassium phosphate, sterilized, and the pH is adjusted to pH 5.8 – 6.2. The fermenters are inoculated with the second stage inoculum described above and incubated at about 25° C with agitation and aeration while controlling and maintaining the mixture at approximately pH 6.0 by addition of a base, for example, sodium hydroxide, potassium hydroxide or preferably ammonium hydroxide, as required from time to time. Addition of a source of assimilable carbon, preferably glucose, is started when the concentration of the latter in the broth has dropped to about 0.5% wt/vol, normally about 48 hrs after the start of fermentation, and is maintained until the end of the particular run. In this manner a fermentation broth containing about 60 μg/ml of rapamycin as determined by the assay method described above is obtained in 4 — 5 days, when fermentation is stopped.

Filtration of the mycelium, mixing the latter with a water-miscible lower alkanol, preferably methanol, followed by extraction with a halogenated aliphatic hydrocarbon, preferably trichloroethane, and evaporation of the solvents yields a first oily residue. This first oily residue is dissolved in a lower aliphatic ketone, preferably acetone, filtered from insoluble impurities, the filtrate evaporated to yield a second oily residue which is extracted with a water-miscible lower alkanol, preferably methanol, and the latter extract is evaporated to yield crude rapamycin as a third oily residue. This third oily residue is dissolved in a mixture of a lower aliphatic ketone and a lower aliphatic hydrocarbon, preferably acetone-hexane, an absorbent such as charcoal or preferably silica gel is added to adsorb the rapamycin, the latter is eluted from the adsorbate with a similar but more polar solvent mixture, for example a mixture as above but containing a higher proportion of the aliphatic ketone, the eluates are evaporated and the residue is crystallized from diethyl ether, to yield pure crystalline rapamycin. In this manner a total of 45–58% of the rapamycin initially present in the fermentation mixture is recovered as pure crystalline rapamycin.

CHARACTERIZATION

a. Purified rapamycin is a colourless crystalline compound, m.p. 183°–185° C after recrystallization from ether;

b. rapamycin is soluble in ether, chloroform, acetone, methanol and dimethylformamide; very sparingly soluble in hexane and petroleum ether and substantially insoluble in water;

c. rapamycin shows a uniform spot on thin layer plates of silica gel G (E. Merck A. G., Darmstadt) developed with a variety of thin layer chromatography solvent systems; for example, ether-hexane 40:60 (Rf = 0.42), isopropyl alcohol-benzene 15:85 (Rf = 0.5) and ethanol-benzene 20:80 (Rf = 0.43);

d. rapamycin obtained from four successive fermentation batches gave the following values on repeated elemental analyses:

					AVERAGE
C%	67.24,	66.14,	67.26,	66.72.	66.84
H%	8.93,	8.72,	8.92,	8.9.	8.84
N%	1.39,	1.37,	1.28,	1.38.	1.37

e. rapamycin exhibits the following characteristic absorption maxima in its ultraviolet absorption spectrum (95% ethanol):

267 nm ($E_{1cm}^{1\%}$ 417), 277 nm ($E_{1cm}^{1\%}$ 541) and 288 nm ($E_{1cm}^{1\%}$ 416);

f. the infrared absorption spectrum of rapamycin in chloroform is reproduced in FIG. 1 and shows characteristic absorption bands at 3560, 3430, 1730, 1705 and 1630-1610 cm^{-1};

Further infrared absorption bands are characterized by the following data given in reciprocal centimeters with (*s*) denoting a strong, (*m*) denoting a medium, and (*w*) denoting a weak intensity band. This classification is arbitrarily selected in such a manner that a band is denoted as strong (*s*) if its peak absorption is more than 2/3 of the background in the same region; medium (*m*) if its peak is between 1/3 and 2/3 of the background in the same region; and weak (*w*) if its peak is less than ⅓ of the background in the same region.

2990 cm^{-1} (m)	1158 cm^{-1} (m)
2955 cm^{-1} (s)	1129 cm^{-1} (s)
2919 cm^{-1} (s)	1080 cm^{-1} (s)
2858 cm^{-1} (s)	1060 cm^{-1} (s)
2815 cm^{-1} (m)	1040 cm^{-1} (m)
1440 cm^{-1} (s)	1020 cm^{-1} (m)
1365 cm^{-1} (m)	978 cm^{-1} (s)
1316 cm^{-1} (m)	905 cm^{-1} (m)
1272 cm^{-1} (m)	888 cm^{-1} (w)
1178 cm^{-1} (s)	866 cm^{-1} (w)

g. the nuclear magnetic resonance spectrum of rapamycin in deuterochloroform is reproduced in FIG. 2;

h. the minimum inhibitory concentration of rapamycin against various microorganism is listed below:

Organisms	Rapamycin: (μg/ml)
Candida albicans (5 strains)	0.02 to 0.1
C. catenulata	< 0.1
C. lipolytica	2.5
C. stellatoidea	< 0.1
C. tropicalis	0.1
C. pseudtropicalis	> 5.0
C. parapsilosis	< 0.1
C. morrera	< 0.1
C. intermedia	< 0.1
M. gypseum	12.5
T. mentagrophytes	> 1000

i. rapamycin exhibits a LD_{50} (i.p., mice) of 597.3 ± 28.1 mg/kg and a LD_{50} (p.o., mice) of >2,500 mg/kg.

In protection studies, mice were infected by intravenous injection of *C. albicans* ATCC 11651. At 1, 4 and 24 hours after infection, mice were administered 10 mg/kg (s. c.) of rapamycin. At this dose 50% of the mice were protected. Treatment with 25 mg/kg (s.c.) offered complete protection. When rapamycin was administered orally, at 10 mg/kg 4 out of 10 mice survived, and at 25 mg/kg complete protection was observed.

A 1% suspension (0.2 ml) of rapamycin in water containing 1.5% polysorbate 80 (Tween 80), when injected intradermally into a rabbit's ear caused no irritation. Similarly, two drops of a 0.5% suspension applied to a rabbit's eye caused no irritation.

The following Examples illustrate further this invention.

EXAMPLE 1

Microorganism

Streptomyces hygroscopicus NRRL 5491 was grown and maintained on oatmeal-tomato paste agar slants (T. G. Pridham, et al., Antibiotic Annual 1956–1957, Medical Encyclopedia Inc., New York, p. 947) and in Roux bottles containing the same medium. Good growth was obtained after 7 days of incubation at 28° C. Spores from one Roux bottle were washed off and suspended into 50 ml of sterile distilled water. This suspension was used to inoculate the first stage inoculum.

The first-stage inoculum medium consisted of Emerson broth [R. L. Emerson et al., J. Bacteriol, 52, 357 (1946)] 0.4%; peptone, 0.4%; sodium chloride, 0.25%; yeast extract, 01.%; and glucose, 1%; pH 7.0; flasks containing the above medium were inoculated with 1% of the spore suspension described above. The inoculated flasks were incubated for 30 hrs. at 28°C on a reciprocating shaker set at 65 r.p.m. (4 inch stroke).

Production stage

The production stage was run in 250-liter New Brunswick fermenters Model F-250, equipped with automatic antifoam addition system and pH recorder-controller. The fermenters were charged with 160 liters of an aqueous production medium (8 KM) consisting of the following constituents:

soluble starch	1.0%
$(NH_4)_2SO_4$	0.5%
K_2HPO_4	0.5%
glucose (Cerelose)	1.5%
$MgSO_4$	0.025%
$ZnSO_4$	0.005%
$MnSO_4$	0.001%
$FeSO_4 . 7H_2O$	0.002%
$CaCO_3$	0.2%
"Blackstrap" molasses	0.5%
hydrolyzed casein (NZ-Case, Sheffield Chemical, Norwich, New York)	0.5%
lard oil (Larex No. 1, Swift Canadian Co., Toronto)	0.2%
pH 7.1 to 7.3	

The fermenters were sterilized at 121° C for 45 minutes, cooled and inoculated with one flask (2% inoculum) of first stage inoculum. Incubation temperature: 28° C; aeration: 0.5 vol/vol/min.; agitation: 250 r. p. m.

A titre of ca. 20 μg/ml, determined by microbiological assay on agar plates seeded with *Candida albicans*, was reached in 5 days. The fermentation was stopped.

Extraction and isolation of the antibiotic was performed by one of the following methods:

EXTRACTION

a. The fermentation mixture was extracted twice with 1 v/v of n-butanol. The combined butanol extracts were washed with 1 v/v of water, dried with anhydrous sodium sulfate and evaporated to dryness under reduced pressure to yield a residue. The oily residue was extracted three times with 2 litres of methanol. The combined methanol extracts were passed through diatomaceous earth ("Celite") and evaporated to dryness to yield an oily residue containing crude rapamycin.

b. The fermentation mixture was filtered over diatomaceous earth ("Celite"). The filtrate was extracted twice with 1 v/v of ethyl acetate. The ethyl acetate

extracts were washed with 1 volume of water, dried with anhydrous sodium sulfate and evaporated under reduced pressure to dryness. The residue was extracted twice with 1 liter of methanol. The methanol extracts were evaporated under reduced pressure to yield an oily residue containing crude rapamycin.

c. The mycelium obtained as described under section (b) was washed with 1 to 2 volumes of water. The washed mycelium was extracted three times with 5 volumes of methanol per weight of wet mycelium each time. The methanolic extracts were pooled and concentrated under reduced pressure to a small volume of an aqueous phase containing approximately 10% v/v of methanol. This aqueous phase was extracted three times with 1 vol. of methylene chloride; the methylene chloride extracts were combined, dried with anhydrous sodium sulfate and evaporated to yield an oily residue.

The oily residue was diluted with 1 volume of petroleum ether, and 30% w/v of charcoal (Darco G60) was added. The mixture was stirred for half an hour and filtered. The charcoal, which retained substantially all of the product, was washed twice with one volume of petroleum ether. The charcoal was eluted three times with 5 vol. (based on the weight of the charcoal) of a mixture of methylene chloride and ether (50:50). The methylene chloride-ether extracts were evaporated to dryness and the residue dissolved in a small amount of ether. The crude product was obtained by precipitation from the ether solution with cold petroleum ether.

Alternatively, the oily residue obtained by any of the extraction procedures described above was diluted with 1 vol. of hexane and passed through a preparative column of silica gel G. The product was absorbed on the column. The silica gel G containing absorbed product was washed with several volumes of hexane and 50:50 hexane-ether mixtures. The product was eluted from the column with ether. The ether eluant was evaporated to a small volume and crude product obtained by precipitation from the ether solution with cold petroleum ether.

Purification

The aforementioned crude product was purified further by column chromatography on silica gel G Merck (50:1 w/v) in hexane-ether (50:50). The product was eluted from the column with ether. The ether eluate was evaporated to a small volume. Purified rapamycin was precipitated with petroleum ether. The analytical samples were prepared by crystallization from ether, m.p. 183°–185° C.

EXAMPLE 2

Streptomyces hygroscopicius NRRL 5491 is grown and spores are obtained in the same manner as described in Example 1.

First Stage Inoculum

Erlenmeyer flasks (500 ml) are filled with 100 ml of the following medium:

Soybean flour (Archer-Daniels Co., Midland, Mich. "Special X")	= 4%	wt/vol
Glucose (Cerelose)	= 2%	wt/vol
Ammonium sulfate	= 0.3%	wt/vol
Calcium carbonate	= 0.15%	wt/vol
Water to volume, pH 7.1 to 7.3		

The flasks are sterilized at 121° for 35 minutes and cooled to 25°. The flasks are inoculated with 4% (4 ml) of spore suspension described above and incubated on a gyrotary shaker (2 inch stroke) at 240 rpm for 24 hrs at 25° C.

Second Stage Inoculum

Twenty-four liter flat bottom flasks containing 3.2 l of the inoculum medium described above at pH 7.1 – 7.3 are sterilized by autoclaving at 121° for 35 minutes, shaken to resuspend the insoluble material and resterilized for another 45 minutes. The flasks are cooled to 25° and inoculated with 64 ml of first stage inoculum, placed on a reciprocating shaker (4 inches stroke) set at 65 rpm and incubated for 18 hrs at 25° C.

Production Stage

The production stage is run in 250 liter New Brunswick fermenters Model F-250 equipped with automatic antifoam addition system and pH recorder-controller. The fermenters are charged with 160 liters of an aqueous production medium consisting of the following constituents:

Soybean flour (Archer-Daniels Co., Midland, Mich., "Special X")	= 3%	wt/vol
Glucose (Cerelose)	= 2%	wt/vol
Ammonium sulfate	= 0.1%	wt/vol
Potassium phosphate (monobasic)	= 0.5%	wt/vol
Antifoaming Agent ("DF-143 - PX" Mazer Chemicals, Inc., Gurnee, Ill.,)	= 0.05%	wt/vol

The fermenters are sterilized at 121° C for 30 minutes, cooled, and the pH is adjusted to 5.8 to 6.2 with ammonium hydroxide. They are then inoculated with one flask (2%) of second stage inoculum and fermentation is allowed to proceed at 25° C, with aeration at 0.25 v/v/min and agitation at 200 rpm.

The pH of the fermentation broth starts to drop at 30 – 35 hours and is controlled at 6.0 until the end of fermentation by the automatic, on demand, addition of ammonium hydroxide. At about 48 hrs. of propagation the glucose concentration in the broth drops to about 0.5%, and continous addition of 40% glucose solution is started at a rate of 3.75% of fermentation mixture volume per day and continued until the end of fermentation. A titer of about 60 μg/ml. determined by microbiological assay on agar plates seeded with Candida albicans is reached in 4 to 5 days. The fermentation is stopped at this point.

Extraction and isolation of the antibiotic is performed by the following procedure:

The fermentation mixture is filtered over diatomaceous earth (Celite) to recover the mycelium. A typical 400 liter batch obtained from three fermenters yields about 60 kg of wet mycelium. The wet mycelium is mixed with 1 vol/wt of methanol by agitation and the mixture is extracted twice with 2 vol of trichloroethane (methyl chloroform), yielding about 250 liters of trichloroethane extract containing about 22 – 24 g of rapamcyin. The trichloroethane extract is evaporated to dryness under reduced pressure to yield 1 to 1.4 kg of oily residue. This residue is added slowly with agitation to 5 vols of acetone and the resulting precipitate is separated by filtration. The acetone solution is evaporated to dryness under reduced pressure to yield an oily residue. This oily residue is extracted twoce with 2 and 1 vols of methanol respectively. The combined methanol extracts are filtered and the remaining oil is discarded. The methanol extract containing rapamycin is

evaporated to dryness under reduced pressure to yield 200 to 300 g of oily residue. This residue is dissolved in 5 v/wt of 15% acetone in hexane. To this solution of the oily residue, silica gel (Merck) is added in an amount equal to twice the weight of the oily residue and the mixture is stirred for about one hr. The mixture is filtered on a sintered glass funnel and the filitrate rejected. The silica gel containing rapamycin is washed with several volumes of 15% acetone in hexane. The washed silica gel is eluted with 30% acetone in hexane. The eluant is evaportated to dryness to yield about 60 to 150 g of dry residue. The dry residue is dissolved in ether and pure rapamycin is separated by crystallization. A typical run containing about 24 g of crude rapamycin yields between 10 and 14 g of pure crystalline product.

We claim:

1. A pharmaceutical composition which comprises a pharmaceutically acceptable carrier and an antifungally effective amount of the antibiotic rapamycin which

a. is a colourless, crystalline compound with a melting point of 183° to 185° C, after recrystallization from ether;

b. is soluble in ether, chloroform, acetone, methanol and dimethylformamide, very sparingly soluble in hexane and petroleum ether and substantially insoluble in water;

c. shows a uniform spot on thin layer plates of silica gel;

d. has a characteristic elemental analysis of about C, 66.84%; H, 8.84% N, 1.37%;

e. exhibits the following characteristic absorption maxima in its ultraviolet absorption spectrum (95% ethanol): 267 nm ($E_{1cm}^{1\%}$ 417), 277 nm ($E_{1cm}^{1\%}$ 541) and 288 nm ($E_{1cm}^{1\%}$ 416);

f. has a characteristic infrared absorption spectrum shown in accompanying FIG. 1;

g. has a characteristic nuclear magnetic resonance spectrum as shown in accompanying FIG. 2;

h. has a minimum inhibitory concentration of 0.02 to 0.1 μg/ml against *Candida albacans;* and

i. exhibits a LD_{50} (i.p., mice) of 597.3 ± 28.1 mg/kg and a LD_{50} (p.o., mice) of >2,500 mg/kg.

2. A method of inhibiting the growth of pathogenic fungi in a mammal which comprises administering to said mammal an antifungally effective amount of the antibiotic rapamycin which

a. is a colourless, crystalline compound with a melting point of 183° to 185° C, after recrystallization from ether;

b. is soluble in ether, chloroform, acetone, methanol and dimethylformamide, very sparingly soluble in hexane and petroleum ether and substantially insoluble in water;

c. shows a uniform spot on thin layer plates of silica gel;

d. has a characteristic elemental analysis of about C, 66.84%; H, 8.84%; N, 1.37%;

e. exhibits the following characteristic absorption maxima in its ultraviolet absorption spectrum (95% ethanol); 267 nm ($E_{1cm}^{1\%}$ 417), 277 nm ($E_{1cm}^{1\%}$ 541) and 288 nm ($E_{1cm}^{1\%}$ 416);

f. has a characteristic infrared absorption spectrum shown in accompanying FIG. 1;

g. has a characteristic nuclear magnetic resonance spectrum as shown in accompanying FIG. 2;

h. has a minimum inhibitory concentration of 0.02 to 0.1 μg/ml against *Candida albicans;* and

i. exhibits a LD_{50} (i.p., mice) of 597.3 ± 28.1 mg/kg and a LD_{50} (p.o., mice) of >2,500 mg/kg.

* * * * *

Industria y Comercio
SUPERINTENDENCIA

DIVISIÓN DE NUEVAS CREACIONES

RADICACIÓN EXPEDIENTE No 07-089192

EL EXTRACTO DE LA PRESENTE SOLICITUD DE **PATENTE DE INVENCIÓN**

FUE PUBLICADO EN LA GACETA DE PROPIEDAD INDUSTRIAL No **584** DE

FECHA **31** DE **DICIEMBRE DE 2007** EL TÉRMINO HÁBIL SEÑALADO POR

LA LEY EXPIRA EL **31** DE **MARZO** DEL **2008.**

NOTA: Dentro del plazo de los seis (6) meses siguientes a partir de la fecha de publicación en la gaceta de Propiedad Industrial, deberá solicitar y cancelar el examen de patentabilidad so pena de que la solicitud caiga en abandono, de acuerdo con lo establecido en el Artículo 44 de la Decisión 486/2000.

SE PRESENTARON OPOSICIONES POR PARTE DE TERCEROS.

SI ____X_____ NO__ _ _ _____

Industria y Comercio
SUPERINTENDENCIA

1259
128

2020
Bogotá, D.C.,

Oficio N°: 5476

Doctor:
Álvaro Correa Ordóñez
Apoderado

ASUNTO:	Radicación PCT N°	07-89192
	Trámite	011
	Evento	379
	Actuación	440
	Folios	010

Con fundamento en lo previsto por el artículo 43 de la Decisión 486 de la Comisión de la Comunidad Andina, se le comunica que la oposición presentada por la doctora Eliana Isaura Moreno Bohórquez, en su calidad de apoderada de Gynopharm S.A., reúne los requisitos formales exigidos por la ley al momento de su presentación.

En cumplimiento de lo dispuesto en la citada norma, se le concede el término de sesenta (60) días hábiles siguientes a la fecha de notificación del presente oficio, para que haga valer sus argumentaciones y presente pruebas.

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 CESPEDES DE VERGEL
Jefe de la División de Nuevas Creaciones.

El anterior oficio fue notificado por fijación en lista de fecha, 25 ABR 2008.

202021

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