



Industria y Comercio
SUPERINTENDENCIA

Radicación: 8571881 00000002 Folios: 60
Fecha (MM/AA): 2003-11-26 13:56:00
Remite: DII PCI-PRIME S/S FASE N°1 POR OPOSICION
Dependencia: 2020 DIVISION DE NUEVAS CREACIONES

FÓRMULARIO UNICO DE OPOSICION 2000-12

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OPOSICION A:

- | | |
|---|---|
| ☒ Patente de Invención | ☐ Marca Colectiva |
| ☐ Patente de Modelo de Utilidad | ☐ Marca de Certificación |
| ☐ Diseño Industrial | ☐ Lema Comercial |
| ☐ Esquema de Trazado para Circuitos Integrados | |
| ☐ Marcas de Productos o Servicios | |

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SOLICITUD PUBLICADA

Número del expediente: 02-091-881
Solicitante: Novartis A.G.
Apoderado: Jimena Escobar Uribe
Título de la nueva creación o denominación del signo:
"NOVEDOSO USO MÉDICO DE INHIBIDORES DE SINTASA DE
ALDOSTERONA SOLOS O EN COMBINACIÓN CON ANTAGONISTAS
DEL RECEPTOR AT-1."
Gaceta: 531 de 29/08/03, Página 402 Número de Publicación 12

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OPOSICION PRESENTADA POR:

OPOSITOR

Nombre: Tecnoquímicas S.A.
Dirección: Calle 23 No. 7-39
Teléfono: (092) 882.55.55
Fax: (092) 883.88.89
Domicilio: Cali

IDENTIFICACION

C.C. ☐ NIT ☒
C.E. ☐ Otro ☐
Número: 890 300 466-5

REPRESENTANTE APODERADO (74)

Nombre: José Luis Reyes Villamizar
Dirección: Cra. 17 No. 88-23, Of. 205
Teléfono: 621.26.31
E-mail: reyes_abogados@cablenet.co
Fax: 621.25.42
Domicilio: Bogotá, D.C.

IDENTIFICACION

C.C. ☒ NIT ☐
C.E. ☐ Otro ☐

Número: 79'152.473 de Usaquén
TP 44655

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FUNDAMENTOS DE LA OPOSICION:

Causal: Incumplimiento de los requisitos de patentabilidad (Título II, Capítulo I, Decisión 486 de 2000). Se violan los Arts. 14 (las patentes de invención deben ser de producto o de procedimiento) y 18 (una invención tiene nivel inventivo si no es obvia para una persona versada en la materia).

Justificación: La solicitud pretendida, busca obtener exclusiva sobre el USO de composiciones farmacéuticas y MÉTODOS DE TRATAMIENTO, lo cual no es permitido por nuestra legislación, y así lo fuera deberían cumplir necesariamente con los requisitos materiales de patentabilidad, en especial, para este caso, con los de Nivel Inventivo y Aplicación Industrial.

La combinación de un inhibidor de AT1 con un diurético, pretendida en la solicitud, hacía parte del estado de la técnica para la fecha de la solicitud, y carecía por ello de novedad teniendo en cuenta los documentos que se reseñan en los anexos.

5 Comprobante de pago No. 03-85-922. (Oposición) Fecha 25 Noviembre
03 85 922 (Prórroga) Fecha 25 Noviembre

6 Anexos:

- ☒ Comprobante de pago de la tasa
- ☒ Poderes, si fuere el caso
- ☒ Pruebas de los fundamentos de la oposición
- ☐ Documentos que acrediten el legítimo interés, si fuere el caso
- ☒ Certificado de existencia y representación legal de las partes, cuando sean personas jurídicas
- ☒ Copia de la oposición para el traslado al solicitante.

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NOMBRE: JOSÉ LUIS REYES VILLAMIZAR

FIRMA: 

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Señor

SUPERINTENDENTE DE INDUSTRIA Y COMERCIO

E. S. D.

REF: EXPEDIENTE 02-091-881
TÍTULO SOLICITADO: "NOVEDOSO USO MÉDICO DE INHIBIDORES DE SINTASA DE ALDOSTERONA SOLOS O EN COMBINACIÓN CON ANTAGONISTAS DEL RECEPTOR AT-1"
PUBLICACIÓN: Gaceta 531 de 29-08-2003, número de publicación 12
SOLICITANTE: NOVARTIS AG
APODERADO: JIMENA ESCOBAR URIBE
TRÁMITE: PRESENTACIÓN DE OPOSICIÓN

JOSÉ LUIS REYES VILLAMIZAR, mayor de edad, identificado como aparece al pie de mi firma, abogado en ejercicio, titular de la tarjeta profesional número 44655 del Consejo Superior de la Judicatura, obrando en calidad de apoderado de la sociedad Tecnoquímicas S.A., en ejercicio de lo dispuesto por el artículo 42 de la Decisión 486 de 2000, muy respetuosamente me permito formular la siguiente

I. PETICIÓN

Sírvase negar el beneficio patentario a la solicitud contenida en el expediente 02-091-881, por no cumplir con lo dispuesto por los artículos 14, 16, 18 y 20 de la Decisión 486 de 2000, con base en los fundamentos que se expondrán a continuación.

II. LEGÍTIMO INTERÉS

Asiste a **TECNOQUÍMICAS S.A.** legítimo interés para presentar esta oposición, pues la eventual concesión de la solicitud restringirla injustificadamente el empleo de ciertas sustancias y composiciones farmacéuticas carentes de nivel inventivo, en detrimento de sus legítimos intereses comerciales y de la libre competencia.

III. FUNDAMENTOS DE LA OPOSICIÓN

3.1. Las patentes pueden concederse sobre productos y procedimientos, no sobre usos ni métodos de tratamiento.

Aunque lo expuesto en el título es bien sabido por el Despacho, al punto de haber basado varias decisiones sobre patentes en ese argumento, me permito hacer notar que según el artículo 14 de la Decisión 486 de 2000, las patentes sólo pueden concederse sobre PRODUCTOS O PROCEDIMIENTOS –siempre que estos, además, sean nuevos, tengan nivel inventivo y sean susceptibles de aplicación industrial-.

De igual manera, el artículo 51 ibidem señala que el alcance de la protección conferida por la patente estará determinado por EL TENOR de las reivindicaciones.

Una simple lectura de las reivindicaciones 1 a 7 permite establecer que el solicitante pretende sin más, y sin que sea dado hacer interpretaciones extensivas, obtener exclusividad sobre el USO de composiciones farmacéuticas y principios activos, pretensión inaceptable de acuerdo con las normas citadas.

De igual manera, hago notar que respecto de estas mismas reivindicaciones el USO pretendido tiene el agravante de serlo como parte de MÉTODOS DE TRATAMIENTO para toda suerte de patologías de diversa índole, posibilidad ésta igualmente prohibida por las normas andinas vigentes.

Por último, me permito reiterar que aún si los usos o métodos terapéuticos fueran permitidos en nuestro medio, éstos deberían cumplir necesariamente con los requisitos materiales de patentabilidad, en especial, para este caso, con los de Nivel Inventivo y Aplicación Industrial.

Aunque a la ausencia del primero nos referiremos más adelante, cabe desde ahora anotar que el segundo resulta de imposible cumplimiento, en tanto la *reproducción industrial* del uso separado, combinado o secuencial de medicamentos para tratar ciertas patologías es del resorte de la profesión médica y no de las actividades industriales.

En el mismo sentido expuesto, me permito hacer notar que las reivindicaciones 9 y 10 son inaceptables por pretender expresamente el patentamiento de un método de tratamiento.

3.2. Carencia de Novedad y Nivel Inventivo de las Composiciones Pretendidas

Aunque más adelante volveremos sobre las partes componentes del uso pretendido en las reivindicaciones 1 a 7, es necesario demostrar ahora por qué la composición revelada en la reivindicación independiente número 8 tampoco puede ser objeto de privilegio de patente.

En efecto, allí se reivindica una composición farmacéutica que comprende: i) un inhibidor de sintasa de aldosterona o una sal del mismo farmacéuticamente aceptable, solo o en combinación con ii) un antagonista de receptor de AT1 o un antagonista de receptor AT1 combinado con diurético o, en cada caso, una sal del mismo farmacéuticamente aceptable y iii) un vehículo farmacéuticamente aceptable, para la prevención de, retraso del progreso de, tratamiento de enfermedades o condiciones divididas en tres grupos (Hipertensión, Aterosclerosis y Disfunción Endotelial, incluyendo especies diversas).

Sea lo primero indicar que antes de la fecha de prioridad invocada en la presente solicitud de patente se conocían individualmente los inhibidores de sintasa aldosterona –se acompañan monografías tomadas del Merck Index y las bases de datos de FDA para algunos de los citados-, los inhibidores de AT1 combinados con diuréticos, y el uso de esa clase de composiciones en el tratamiento de enfermedades como la hipertensión, insuficiencia renal, aterosclerosis, resistencia a la insulina y enfermedades cardíacas, entre otras.

Como demostración llamo la atención a lo revelado en la base de datos Electronic Orange Book de la FDA (www.fda.gov/cder/ob), en donde consta la aprobación, desde marzo 6 de 1998, de la combinación un inhibidor de AT1 (Valsartan) y un diurético (Hidroclorotiazida) bajo el nombre comercial DIOVAN HCT. (Cabe recordar que la fecha de prioridad de la solicitud que nos ocupa es abril 12 de 2000)

De lo anterior resulta claro que la combinación de un inhibidor de AT1 con un diurético, tal como es descrita en la reivindicación 8 estaba comprendida en el estado de la técnica para la fecha de la solicitud, y carece por ello de novedad. Ahora bien, si se parte de la discutible tesis según la cual sólo las revelaciones textuales afectan la novedad, cabe preguntarse entonces si el hecho de formular fármacos conocidos, de familias conocidas y de acción compatible, en presencia de auxiliares conocidos y para fines igualmente conocidos representa un "salto

técnico", o constituye, por el contrario, una obvia yuxtaposición que aplicaría de manera evidente una persona versada en la materia. La respuesta es la segunda.

De otro lado, llamo la atención del Despacho a lo revelado en la Reivindicación 4 de la presente solicitud de patente, pues en ella se expresa que: *"... en donde el inhibidor de sintasa de aldosterona se selecciona del grupo que consiste de anastrozol, fadrozol (incluyendo el (+) -enantiómero del mismo) y exemestano, o, en cada caso donde sea aplicable, una sal del mismo farmacéuticamente aceptable"*, puesto que los compuestos allí mencionados **NO son Inhibidores de la sintasa de aldosterona, son inhibidores de la aromatasa no esteroidea** y son ampliamente usados como agentes farmacéuticos "antineoplásicos". Entonces cabe preguntarse si a estos compuestos se les está atribuyendo un nuevo uso, posibilidad inaceptable en Colombia como lo ha sostenido ese despacho, o si lo que se presenta es una incongruencia en tal reivindicación.

Ahora bien, como se ha dicho hasta ahora, resulta clara la comprensión en el estado de la técnica de las dos familias de fármacos que hacen parte de la composición pretendida, al igual que de los agentes preferidos que se revelan en la solicitud, y desde luego, de los auxiliares y vehículos empleados en su elaboración. A este conocimiento debe sumarse el hecho de existir diversas revelaciones sobre su empleo separado o combinado para el tratamiento de las mismas patologías, hecho que no sólo demuestra la predecibilidad de su efecto sinérgico o complementario, sino muy especialmente la compatibilidad demostrada de su empleo combinado.

Hechas las anteriores consideraciones, es del caso revisar brevemente algunos documentos técnicos y patentes que anticipan por sí mismos y sin necesidad de combinación el objeto pretendido y le restan, en consecuencia, cualquier asomo de Novedad y Nivel Inventivo. Veamos:

1. Artículo "Regression of Hypertrophy after Myocardial Infarction is Produced by the Chronic Blockade of Angiotensin Type 1 Receptor in Rats" (1996)

En este documento se revela que el tratamiento crónico con el receptor antagonista AT1 E-4177 ha demostrado efectos biodinámicos y bioquímicos en ratas con falla congestiva cardíaca. (Ver texto completo anexo).

2. Patente US 5.252.565 (1993-10-12)

Esta patente revela compuestos inhibidores de sintasa de aldosterona y su empleo para el tratamiento del hiperaldosteronismo y patologías derivadas, en particular hipertensión, edema, retención de sodio, no sólo cuando dichas patologías resultan de un desorden corporal, sino cuando son inducidas por la ingestión de otros medicamentos.

3. Patente US 5.906.987 (1999-05-25)

Este documento anticipa composiciones farmacéuticas que comprenden la combinación de cantidades efectivas de compuestos para el tratamiento de fallas cardiovasculares, hipertensión, impotencia y osteoporosis.

4. Publicación Internacional PCT/WO98/03180 (1998-01-29)

El documento reseñado revela el empleo de compuestos inhibidores de la sintasa aldosterona—entre ellos anastrozol, androstenediona, exemestano, etc.—, para el tratamiento de la fibrosis y otras patologías.

5. Patente US 5.972.921 (1999-10-26)

Esta patente revela un método para tratar patologías urinarias, mediante la administración de cantidades efectivas de inhibidores de la sintasa aldosterona, entre ellos anastrozol, fadrozol, letrozol, etc, sus sales o esteroisómeros o sales ácidas de adición de los mismos.

6. Publicación PCT/WO99/45779 (1999-09-15)

Esta anterioridad revela composiciones que comprenden Eposartán, una de sus sales, hidratos o solvatos, en forma particulada, un proceso para su producción y su empleo para bloquear receptores de la Angiotensina II y tratar hipertensión, falla cardíaca congestiva y problemas renales.

IV. SOLICITUD DE PLAZO EXTRA PARA SUSTENTAR LA OPOSICIÓN

En desarrollo de lo dispuesto por el artículo 42, inciso 2º de la Decisión 486, solicito comedidamente a su Despacho el plazo adicional de sesenta días para sustentar la presente oposición. Para los anteriores efectos, me permito anexar la constancia de pago correspondiente.

V. PRUEBAS

Ruego que se tengan como tales, sin perjuicio de aquellas que puedan presentarse al vencimiento de la prórroga solicitada y de aquellas que el Despacho considere oficiosamente, las siguientes:

- o Monografías tomadas del Merck Index, 13a Edición Digital
- o Texto íntegro del Artículo "Regression of Hypertrophy alter Myocardial Infarction is Produced by the Chronic Blockade of Angiotensin Type 1 Receptor in Rats"
- o Apartes de la Patente US 5.252.565
- o Apartes de la Patente US 5.906.987
- o Apartes de la Publicación Internacional PCT/WO98/03180
- o Apartes de la Patente US 5.972.921

VI. ANEXOS

Para efectos del trámite correspondiente, me permito anexar al presente escrito los siguientes anexos

1. Poder para actuar.
2. Certificado de existencia y representación legal de Tecnoquímicas S.A.
3. Constancia de pago de las tasas de tramitación de oposición y la prórroga solicitada.
4. Copia para correr traslado al solicitante.
5. Los mencionadas en el acápite de pruebas

VII. NOTIFICACIONES

El suscrito recibirá notificaciones en la secretaría de su Despacho o en mi oficina localizada en la Carrera 17 No. 88-23, Oficina 205, Fax (1) 621.25.42 de esta ciudad. Tecnoquímicas S.A. las recibirá en la secretaría de su Despacho o en su sede localizada en la Calle 23 # 7-39, de la ciudad de Cali.

Señor Superintendente,

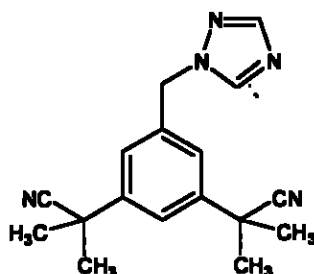


JOSÉ LUIS REYES VILLAMIZAR

C.C. 79'152.473 de Usaquén
T.P. 44655 de C.S.J.

Anastrozole

Search Monographs



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CambridgeSoft

Monograph Number: 632

Title: Anastrozole

CAS Registry Number: 120511-73-1

CAS Name: $\alpha,\alpha,\alpha',\alpha'$ -Tetramethyl-5-(1*H*-1,2,4-triazol-1-ylmethyl)-1,3-benzenediacetonitrile

Additional Names: 2,2'-[5-(1*H*-1,2,4-triazol-1-ylmethyl)-1,3-phenylene]di(2-methylpropionitrile)

Manufacturers' Codes: ZD-1033; ICI-D-1033

Trademarks: Arimidex (Zeneca)

Molecular Formula: C₁₇H₁₉N₅

Molecular Weight: 293.37.

Percent Composition: C 69.60%, H 6.53%, N 23.87%

Literature References: Aromatase inhibitor. Prepn: P. N. Edwards, M. S. Large, EP 296749; *idem*, US 4935437 (1989, 1990 both to ICI). Reviews of pharmacology, clinical pharmacokinetics: P. V. Plourde *et al.*, *Breast Cancer Res. Treat.* 30, 103-111 (1994); *idem et al.*, *J. Steroid Biochem. Molec. Biol.* 53, 175-179 (1995). Clinical trial in advanced breast cancer: A. U. Buzdar *et al.*, *Cancer* 79, 730 (1997).

Properties: Crystals from ethyl acetate/cyclohexane, mp 81-82°.

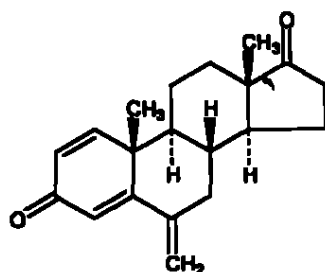
Melting point: mp 81-82°

Therap-Cat: Antineoplastic.

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Exem stane

Search Monographs



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CambridgeSoft

Monograph Number: 3944

Title: Exemestane

CAS Registry Number: 107868-30-4

CAS Name: 6-Methyleneandrosta-1,4-diene-3,17-dione

Manufacturers' Codes: FCE-24304

Trademarks: Aromasin (Pharmacia & Upjohn)

Molecular Formula: C₂₀H₂₄O₂

Molecular Weight: 286.40

Percent Composition: C 81.04%, H 8.16%, O 10.80%

Literature References: Irreversible aromatase inhibitor. Prepn: E. Di Salle *et al.*, DE 3622841; F. Buzzetti *et al.*, US 4808616 (1987, 1989 both to Farmitalia Carlo Erba). Pharmacology: D. Giudici *et al.*, *J. Steroid Biochem.* 30, 391 (1988). HPLC determin in plasma: M. Breda *et al.*, *J. Chromatog.* 620, 225 (1993).

Clinical pharmacology: T. R. J. Evans *et al.*, *Cancer Res.* 52, 5933 (1992). Clinical evaluation in breast cancer: N. Zilembo *et al.*, *Brit. J. Cancer* 72, 1007 (1995).

Properties: mp 188-191°. uv max: 247 nm (ε 13750).

Melting point: mp 188-191°

Absorption maximum: uv max: 247 nm (ε 13750)

Therap-Cat: Antineoplastic (hormonal).


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exemestane

Trade Name(s):
Aromasin

Type of Drug:

Exemestane belongs to a general group of drugs called hormone or hormone antagonists. An antagonist blocks the effect of a hormone. It is used to treat advanced breast cancer in postmenopausal women whose cancer no longer responds to tamoxifen therapy.

How Drug Works:

Exemestane prevents the body's adrenal glands and ovaries from making estrogen. Other steroid hormones are not affected.

How Drug Is Given:

Exemestane is given as a pill once a day after eating a meal. The dose is standard for everyone. Keep the pills in a tightly closed container away from heat and moisture and out of the reach of children and pets.

How Should I Take This Drug?

Take this drug exactly as directed by your doctor. If you do not understand the instructions, ask your doctor or nurse to explain them to you. This drug can be given at different strengths depending on the type of cancer being treated. Dosage may vary depending on your weight and your type of cancer.

Read the following information. If you do not understand it or if any of it causes you special concern, check with your doctor.

Before taking this drug, tell your doctor:

- If you are trying to become pregnant, are pregnant, or breastfeeding. This drug may cause birth defects if either the male or female is taking it at the time of conception or during pregnancy. Men and women who are taking this drug need to use some kind of birth control. However, do not use oral contraceptives ("the pill") without checking with your doctor.
- If you think you may want to have children in the future. Many chemotherapy drugs can cause sterility.
- If you have any of the following medical problems: chickenpox or exposure to chickenpox, gout, heart disease, congestive heart failure, shingles, kidney stones, liver disease, or other forms of cancer.
- If you are taking any other prescription or over-the-counter drugs, including vitamins and herbals.

Should I avoid any other medications, foods, alcohol, and/or activities?

Your prescription and nonprescription medicines may interact with other drugs, causing harm. Certain foods or alcohol can also interact with drug products. Never begin taking a new medicine—prescription or nonprescription—without asking your doctor or nurse if it will interact with alcohol, food, or other medicines. Some drug products can cause drowsiness and affect activities such as driving.

Precautions:

It is important to keep taking the drug even though you are feeling well.

You may feel depressed or have difficulty sleeping while taking this medicine. Talk to your doctor or nurse about ways to manage these side effects to make you feel better.

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taking this drug.

- Most of the following side effects probably will not occur.
- Your doctor or nurse will want to discuss specific care instructions with you.
- They can help you understand these side effects and help you deal with them.

Side Effects:**More common side effects:**

- Tiredness (fatigue)

Less common side effects:

- Hot flashes
- Pain at tumor site
- Nausea
- Depression
- Difficulty sleeping

Rare side effects:

- Increased appetite
- Weight gain
- Pain
- Increased sweating

Side effects/symptoms of this drug:

Tell your doctor or nurse if you have severe hot flashes, difficulty sleeping, or feel depressed. There is much that can be done.

Other side effects not listed above can also occur in some patients. Tell your doctor or nurse if you develop any problems.

FDA Approval:

This drug is approved for cancer treatment.

Note. This information was reprinted from the American Cancer Society's Consumers Guide to Cancer Drugs. Copyright © 2004, Jones and Bartlett Publishers. This information may not cover all possible uses, actions, precautions, side effects, or interactions, is not intended as medical advice, and should not be relied upon as a substitute for consultation with your doctor who is familiar with your medical needs. For more information, contact your American Cancer Society at 1-800-ACS-2345.

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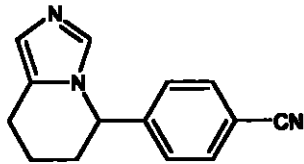


U.S. Food and Drug Administration • Center for Drug Evaluation and Research
FDA Oncology Tools Approval Summary for exemestane for Treatment of advance breast cancer in postmenopausal women whose disease has progressed following tamoxifen therapy.

Generic Drug Name	exemestane
Trade Name	Aromasin
Sponsor or Applicant	Pharmacia & Upjohn Company
Application Number	020753
Supplement Number	000
Supplement Type Code	N
Dosage Form	tablet
General Indication	breast
Line or type of therapy	Second
Specific Indication	Treatment of advance breast cancer in postmenopausal women whose disease has progressed following tamoxifen therapy.
Subpart H-Accelerated Approval?	0
Filing Date	Dec 21 1998
Advisory Committee Date	
Advisory Committee Recommendation	no ODAC
Approval Date	Oct 21 1999
Number of Studies Reviewed	1
Study Comments	Aromasin showed the equivalent efficacy in comparison with megastrol acetate
Study Results	In one comparative study with megastrol acetate, Aromasin showed equal efficacy.
Study Details (Click on NDA or Supplement Number to view details)	NDA 020753 Supplement 000
Follow Up Commitments	

Fadrozol

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CambridgeSoft

Monograph Number: 3958

Title: Fadrozole

CAS Registry Number: 102676-47-1

CAS Name: 4-(5,6,7,8-Tetrahydroimidazo[1,5-a]pyridin-5-yl)benzonitrile

Additional Names: 5-*p*-cyanophenyl-5,6,7,8-tetrahydroimidazo[1,5-a]pyridine

Molecular Formula: C₁₄H₁₃N₃

Molecular Weight: 223.27.

Percent Composition: C 75.31%, H 5.87%, N 18.82%

Literature References: Aromatase inhibitor. Prepn: L. J. Browne, EP 165904; *idem*, US 4617307 (1985, 1986 both to Ciba-Geigy); L. J. Browne *et al.*, *J. Med. Chem.* **34**, 725 (1991). Effects on estrogen biosynthesis: R. E. Steele *et al.*, *Steroids* **50**, 147 (1987). Pharmacology: K. Schieweck *et al.*, *Cancer Res.* **48**, 834 (1988). GC/MS determ in plasma and urine: R. Ackermann, G. Kaiser, *Biomed. Environ. Mass Spect.* **18**, 558 (1989). Pharmacokinetics: G. M. Kochak *et al.*, *J. Clin. Endocrinol. Metabol.* **71**, 1349 (1990). Clinical evaluation in metastatic breast cancer: J. I. Raats *et al.*, *J. Clin. Oncol.* **10**, 111 (1992).

Properties: mp 117-118°.

Melting point: mp 117-118°

Derivative Type: Hydrochloride

CAS Registry Number: 102676-31-3

Manufacturers' Codes: CGS-16949A

Trademarks: Afema (Ciba-Geigy)

Molecular Formula: C₁₄H₁₃N₃.HCl

Molecular Weight: 259.74.

Percent Composition: C 64.74%, H 5.43%, N 16.18%, Cl 13.85%

Properties: Crystals from 2-propanol, mp 231-233°. Sol in water.

Melting point: mp 231-233°

Therap-Cat: Antineoplastic.

FADROZOLE:

<chemical> 4-(5,6,7,8-tetrahydroimidazo(1,5-a)pyridin-5-yl)benzonitrile. A selective aromatase inhibitor effective in the treatment of oestrogen-dependent disease including breast cancer.

Pharmacological action: antineoplastic agent, hormonal, enzyme inhibitors, oestrogen antagonists.

Chemical name: Benzonitrile, 4-(5,6,7,8-tetrahydroimidazo(1,5-a)pyridin-5-yl)-

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Regression of Hypertrophy After Myocardial Infarction is Produced by the Chronic Blockade of Angiotensin Type 1 Receptor in Rats

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N. MAKINO, T. HATA, M. SUGANO, I. M. C. DIXON AND T. YANAGA. Regression of Hypertrophy After Myocardial Infarction is Produced by the Chronic Blockade of Angiotensin Type 1 Receptor in Rats. *Journal of Molecular and Cellular Cardiology* (1996) 28, 507-517. The efficacy of angiotensin converting enzyme (ACE) inhibitors is well known to prevent the formation of angiotensin II (Ang II) by these agents. The objective of the present study was to evaluate the hemodynamic, biochemical, and morphological responses to Ang II receptor blockade with E-4177, 3-[(2'-carboxyphenyl-4-yl) methyl]-2-cyclopropyl-7-methyl 3H-imidazo[4,5-b] pyridine, in rats with a healing myocardial infarction that had been induced by the surgical occlusion of the left main coronary artery. The left ventricular weight increased 8 and 12 weeks after infarction in comparison to that in sham-operated rats. Among the rats with experimental infarction, treatment with E-4177 significantly decreased the left ventricular weight. Although the infarct size was not affected by E-4177, its administration ameliorated the elevated end-diastolic pressure and reduced the systolic pressure. The effects of this agent on the levels of Ang II type 1 (AT1) receptor mRNA and ACE mRNA were evaluated in the non-infarcted myocardium by reverse transcriptase polymerase chain reaction and binding assays. Treatment with E-4177 reduced both the elevated AT1 mRNA and the number of Ang II receptors, but not the ACE mRNA or ACE activity. While the receptor affinity remained unchanged with this agent, the collagen concentration was decreased. On the other hand, the depressed Na⁺/Ca²⁺ exchange activity was restored in the non-infarcted myocardium at 8 and 12 weeks after injury to the level seen in the sham-operated rats. These findings suggest that the AT1 receptor antagonist, E-4177, has a beneficial effect on the hemodynamics in spite of the lack of any improvement in the infarct size. These observations may be partly attributed to the prevention of angiotensin II formation during the period of post-infarction healing.

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Key Words: Myocardial infarction; Angiotensin II receptor; Angiotensin type 1 receptor; Angiotensin converting enzyme; Collagen; Na⁺/Ca²⁺ exchange.

Introduction

The rat model of chronic myocardial infarction (MI) induced by a ligation of the coronary artery produces a profound alteration in the ventricular

architecture that is characterized by the early expansion of the infarcted area (Weisman *et al.*, 1988), dilatation (Pfeffer, 1991), and hypertrophy of the non-infarcted left ventricle (LV) (Oliver *et al.*, 1991). Such myocardial hypertrophy exhibits

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characteristics which are produced by a combination of pressure and volume overload (Grossman *et al.*, 1975).

This cardiac remodeling process can be reduced by the prolonged administration of a hemodynamically effective angiotensin converting enzyme (ACE) inhibitor such as captopril (Pfeffer *et al.*, 1985), lisinopril (Wollert *et al.*, 1994), or enalapril (Hirsch *et al.*, 1992). The ultimate result is an improvement in long-term survival (Wollert *et al.*, 1994). The effects of ACE inhibitors in heart failure are generally believed to result from the inhibition of either circulating or local (cardiac) angiotensin II (Ang II) formation (Dzau, 1989). Clinical trials have provided convincing evidence that this principle also applies to patients with a large MI (Pfeffer *et al.*, 1992). The mechanism of action of ACE inhibition is generally attributed to a reduction of afterload (Pfeffer and Braunwald, 1990).

Recent studies suggest that the renin-angiotensin system plays an important role both in the development of cardiac hypertrophy (Baker *et al.*, 1992; Makino *et al.*, 1993), and in the production of myocardial fibrosis (Brilla *et al.*, 1990). There is evidence that cardiac hypertrophy is associated with the induction of gene expression for ACE (Schunkert *et al.*, 1990), angiotensinogen (Baker *et al.*, 1992), and Ang II receptors (Suzuki *et al.*, 1993), as well as the increased local synthesis of Ang II within the ventricular myocardium (Sadoshima *et al.*, 1993a). The levels of mRNA or protein for angiotensinogen (Lindpaintner *et al.*, 1993), cardiac ACE (Hirsch *et al.*, 1991), and Ang II receptors (Meggs *et al.*, 1993) are increased in rat hearts that have undergone remodeling after experiment MI.

Ang II is now recognized as a potent growth factor for myocytes (Baker *et al.*, 1992) and is mitogenic for cardiac fibroblasts in cell culture (Sadoshima and Izumo, 1993b). Sadoshima *et al.* (1995) demonstrated that Ang II, via stimulation of Ang II receptors, activates the protein kinase cascade in myocytes. The Ca^{2+} -dependent signalling mechanism is considered to be essential for this Ang II-induced activation.

Two Ang II receptor subtypes, the AT1 and AT2 receptors, have been identified by using receptor subtype-specific, non-peptide antagonists (Wong *et al.*, 1990). Both AT1 and AT2 receptor expression increase in response to the development of cardiac hypertrophy (Suzuki *et al.*, 1993). The expression of the AT1 and the AT2 receptors is upregulated exclusively in myocytes (Meggs *et al.*, 1993). Nio *et al.* (1995) reported that the administration of an AT1 receptor antagonist is effective in reducing the

increased expression of Ang II receptor subtypes induced by MI. It is therefore important to determine whether exposure to an AT1 receptor antagonist may reduce the degree of myocardial damage during the healing stage of MI. Accordingly, the present study was designed to evaluate the hemodynamic, biochemical, and morphological responses to a newly developed AT1 receptor antagonist, E-4177, (Okunishi *et al.*, 1993) in rats after MI.

Materials and methods

Experimental infarction

Male Wistar rats weighing 250–300 g were obtained from Charles River Inc. (Atsugi, Kanagawa, Japan) and housed in our animal facility for at least 3 days before the operation. A coronary artery ligation was performed as described previously (Dixon *et al.*, 1992). Briefly, the rats were anesthetized with 50 mg/kg of pentobarbital i.p. and intubated for mechanical respiratory control. The left coronary artery was ligated 2–3 mm distal to its aortic origin with a 4-0 silk suture, and the heart was repositioned within the chest. A total of 123 rats were operated on, 89 animals underwent the coronary artery ligation while 34 rats had the sham operation. The overall survival rate was 64%: 26 rats died within the first 48 h while six rats died 48 h after the coronary artery ligation. In the sham-operated animals, the suture was loosely tied so as not to obstruct the coronary flow.

The animals were allowed free access to standard rat chow (0.5% sodium). Drinking water was provided *ad libitum*.

The administration of E-4177

The AT1 receptor antagonist E-4177, 3-[(2'-carboxyphenyl-4-yl) methyl]-2-cyclopropyl-7-methyl 3H-imidazo[4,5-b]pyridine, was kindly provided by the Eisai Co., Ltd, Japan. Thirty-two of the surviving rats in rats with coronary artery ligation were treated with E-4177. This drug was dissolved in the drinking water and the concentration was adjusted to achieve a daily intake of 10 mg/kg body weight from the second day after the coronary artery ligation. A pilot study demonstrated that this dose effectively reduced the systolic blood pressure of two kidneys in 1 clip-Goldblatt hypertensive rats (Mori *et al.*, 1994). The other 28 rats did not receive E-4177.

Hemodynamic studies

The animals were anesthetized with an injection of pentobarbital sodium (50 mg/kg, i.p.) 4, 8, or 12 weeks after surgery. The trachea was intubated to maintain adequate ventilation. The right carotid artery was exposed, and a microtip pressure transducer (model SPR-246, Miller Instruments, Houston, TX, USA) was introduced through a proximal arteriotomy. The catheter was advanced carefully through the lumen of the carotid artery until the tip of the transducer entered the LV cavity. The catheter was secured with a silk ligature around the artery, and readings were taken from a Dynograph recorder (Model CP-842G, Nihon Kohden Co. Ltd., Tokyo, Japan). The LV pressure, heart rate, rate of contraction ($+dP/dt$), and rate of relaxation ($-dP/dt$) were all recorded.

Tissue preparation

All animals were killed by decapitation. After the heart was rapidly excised, it was weighed and washed extensively with normal saline to remove all contaminating blood. The hearts were frozen in liquid nitrogen and kept for up to 2 weeks at -80°C until the assays were performed. The atria and the right ventricle were dissected from the LV. The infarct scar and border zone were separated from the viable LV myocardium to determine the collagen concentration and the ACE activity. In sham-operated animals, the corresponding parts of the LV were used for those assays. Only the viable myocardium, representing the inferior wall and interventricular septum, was used to measure the binding of AngII receptor and the sarcolemmal $\text{Na}^+/\text{Ca}^{2+}$ exchange as well as for the molecular biology studies.

Determination of infarct size

The LV was fixed in 10% formalin and embedded in paraffin. Six transverse slices were cut from the apex to the base. A 5- μm section was cut from each slice and mounted. The sections were stained with Masson's trichrome and hematoxylin and eosin to distinguish clearly between fibrous scar and non-infarcted tissue (Hirsch *et al.*, 1991). Normal and infarcted areas were drawn on to a superimposed clear plastic sheet, and the respective areas were determined using the planimetric method. The difference between the two areas were then used to determine the extent of MI, expressed as the

percentage of the left ventricular surface area (Wolpert *et al.*, 1994). Only rats with infarcts greater than 10% of the left ventricular circumference were included in the present study.

Biochemical measurements

The ACE activity in heart tissue was determined using a fluorometric assay modified from that described by Cushman and Cheung (1971). This assay measures the generation of His-Leu from a Hip-His-Leu substrate. Each tissue was homogenized immediately and incubated for 10 min at 37°C (at pH 7.50) in the presence of Hip-His-Leu. The reaction was stopped by the addition of NaOH at pH 8.5. The His-Leu product was then tagged with 0.1% phthalaldehyde and quantitated fluorometrically at an excitation wavelength of 386 nm and emission wavelength of 436 nm.

The myocardial collagen content was measured by the hydroxyproline concentration of tissue, as described by Bergman and Loxley (1961). After the heart was dried for 24 h, the specimens were hydrolysed in 6N hydrochloric acid solution at 100°C . Then p-dimethylanilino-benzaldehyde (Ehrlich's reagent) was dissolved in buffer at pH 7.0 and then was added to form a complex with hydroxyproline. The concentration of hydroxyproline was measured by a spectrophotometric analysis at a wavelength of 558 nm. The collagen content was estimated by multiplying the hydroxyproline content by a factor of 8.2 (Mukherjee and Sen, 1991). The concentration of collagen was expressed as the mg of collagen per gram of wet weight.

To determine the $\text{Na}^+/\text{Ca}^{2+}$ exchange activity, the sarcolemmal membranes were prepared from the viable LV tissue of sham-operated and experimental animals according to the method of Pitts (1979). The Na^+ -dependent Ca^{2+} uptake was measured by a method described in detail elsewhere (Dixon *et al.*, 1992). The protein concentrations of the homogenate samples and the membranes were determined by the method of Lowry *et al.* (1951).

Molecular biology studies

Homogenized viable LV tissues were isolated by a modification of the acid guanidine thiocyanate technique (RNAzol, Cinna/Biotech, Houston, TX, USA). After RNA was pelleted by ultracentrifugation, it was resuspended in 0.2 M sodium acetate at pH 5.5 and rocked at 4°C for 1 h, then

precipitated in 2 volumes ethanol. The precipitated RNA was dissolved in water and the amount was quantified by absorbance at 260 nm, in duplicate measurements. The relative mRNA levels were then compared with the total amount of RNA applied.

The reverse transcriptase polymerase chain reaction (RT-PCR) method was used to detect ACE and Ang II receptor mRNA in LV tissue of sham-operated and experimental rats (Suzuki *et al.*, 1993). Briefly, 1 µg of total RNA was reverse transcribed into cDNA and then amplified by an RTPCR kit (Gibco Life Technologies, Gaithersburg, MD, USA). A non-radiolabeled system (Gibco Life Technologies, Gaithersburg, MD, USA) was used to label the PCR product with biotinylated 14-dCTP. The amplification profile involved denaturation at 95°C for 1 min, annealing at 64°C for 1 min, and extension at 72°C for 1 min. The PCR product from each primer set consists of different base pairs (bp), depending on the primers used. The PCR products are designed not to overlap in size. The ranges of the cycles that were exponential in each mRNA and the PCR cycles for quantitating each mRNA were next determined. The sense and anti-sense primers were synthesized by model 352 and 394 DNA/RNA synthesizers (Applied Biosystems, Foster City, CA, USA).

For the AT1-receptor (Murphy *et al.*, 1991) the sense primer was 5'-GCCAAAGTCACCTGCATCAT-3'; the anti-sense primer was 5'-AATTTTTCCTCCAG-AAAGCC-3'; the PCR product size was 494 bp, there were 26 PCR cycles in mRNA. For ACE, the data were: sense: 5'-GACTGGTCCAACATCTATG-3'; antisense: 5'-ATGAAGCTGACGAAGTACCT-3'; size: 739 bp; 28 cycles. As an internal control for input RNA, a PCR oligonucleotide primer for glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) was chosen at 349 bp from the rat (Fort *et al.*, 1985); sense: 5'-CATGGTCT-ACATGTTCCAGT-3', anti-sense: 5'-GGCTAAGCAG-TTGGTGGTGC-3'; 12 cycles.

After the completion of PCR, 2 µl of each PCR product from the same animal was applied to the same well of a 12.5% polyacrylamide gel (Daitchi-Kagaku Pharm., Tokyo, Japan). Electrophoresis was performed at a constant 20 mA for 90 min in 25 mM Tris, and 0.2 M glycine. Following electrophoresis, the PCR products were transferred electrophoretically to a nylon membrane (Stratagene, La Jolla, Ca, USA) in 0.2 × TBE at 0.4 mA/cm² for 90 min. A semi-dry electroblotting apparatus (Advantec, Tokyo, Japan) was used. The blots were analysed and visualized with a blot detection kit (Millipore, Bedford, MA, USA). The density of each PCR band was analysed with a densitometer (model

620, Japan Bio Rad). The amount of mRNA was described as the ratio to GAPDH.

Ang II receptor assay

Ventricular membranes from rat hearts were prepared using a modification of the method of Baker *et al.* (1984). A tissue homogenate was prepared with 0.25 M sucrose and 25 mM Tris (pH 7.5), containing 0.5 mM ethylenediaminetetraacetic acid (EDTA), 0.5 mM phenylmethylsulfonyl fluoride (PMSF), 10 mg/l bacitracin, 4 mg/ml leupeptin, 4 mg/ml pepstatin, and 40 U/ml trasylol. A Polytron homogenizer was used twice for 30 s each at half-maximal speed. The homogenate was sedimented twice at 10 000 × g for 20 min, and the supernatant was centrifuged at 45 000 × g for 30 min. The pellet was resuspended in 0.6 M KCl and 30 mM histidine (pH 7.0), again containing 0.5 mM EDTA, 0.5 mM PMSF, 10 mg/l bacitracin, 4 mg/ml leupeptin, 4 mg/ml pepstatin, and 40 U/ml trasylol. This was then resedimented at 45 000 × g for 30 min. The pellets obtained from the final centrifugation were washed three times and resuspended in 25 mM Tris (pH 7.5), containing 10 mM MgCl₂, 0.5 mM PMSF, 10 mg/l bacitracin, 4 mg/ml leupeptin, 4 mg/ml pepstatin, and 40 U/ml trasylol; a hand-driven glass/glass homogenizer was used. All centrifugations were performed at 4°C. The membrane preparations were immediately frozen in liquid nitrogen and held in aliquots at -80°C until use. The yield was approximately 1 mg of protein per gram of heart.

Membrane protein (120–140 mg) was incubated with increasing concentrations of the radiolabeled Ang II antagonist [¹²⁵I]-[Sar¹,Ile⁸] Ang II (specific activity; 2 200 Ci/mmol; New England Nuclear, Boston, MA, USA). The incubation mixture was composed of the assay buffer containing [¹²⁵I]-[Sar¹,Ile⁸] Ang II and a competing unlabeled Ang II antagonist. The assay buffer was 25 mM Tris (pH 7.5), containing 10 mM MgCl₂, 2 g/l bovine serum albumin, 10 mg/l bacitracin, 0.5 mM PMSF, and 1 mg/ml of each peptidase inhibitor antipain, phosphoramidon, leupeptin, pepstatin and amastatin (Rogg *et al.*, 1990). The reaction was initiated by the addition of the membrane protein aliquot and then continued for 60 min at 37°C. The saturation isotherms demonstrated increasing concentrations of [¹²⁵I]-[Sar¹,Ile⁸] Ang II between 0.1 and 2 nM.

The binding assays were done in either duplicate or triplicate. Specific binding was defined as the portion of the total counts displayed by 1 nM [Sar¹,Ile⁸] Ang II. At ligand concentrations equivalent to

Table 1 Characteristics of sham-operated rats and rats having experimental MI at 4, 8, and 12 weeks after induction of MI

	4 weeks		8 weeks		12 weeks	
	Sham	Experiment	Sham	Experiment	Sham	Experiment
BW (g)	390±10	413±8	405±9	557±7*	511±9	592±8*
Heart weight (g)	0.83±0.06	0.96±0.04	0.80±0.03	1.24±0.06*	1.00±0.08	1.34±0.07*
LV weight (g)	0.01±0.02	0.67±0.02	0.68±0.03	0.88±0.04*	0.76±0.05*	0.96±0.05*
RV weight (g)	0.22±0.01	0.29±0.01*	0.22±0.02	0.36±0.02*	0.24±0.01	0.38±0.02*
LV to BW (10 ⁻³)	1.56±0.08	1.62±0.10	1.68±0.12	1.58±0.09	1.49±0.08	1.62±0.10*
RV to BW (10 ⁻³)	0.56±0.03	0.72±0.04*	0.54±0.02	0.65±0.03*	0.46±0.02	0.64±0.03*

Values are means±S.E. of six experiments. BW, body weight; LV, left ventricle; RV, right ventricle; **P*<0.05 compared with sham-operated rats at same period.

the affinity of the receptor for the radioligand, specific binding averaged 85%.

Statistical method

The data are given as the mean±S.E.M. Comparisons among three or more groups were made by one-way ANOVA followed by Dunnett's modified *t*-test. A value of *P*<0.05 was considered to be statistically significant.

Results

The left coronary artery occlusion resulted in ischemia in the LV free wall and thereafter caused MI. The regions of the interventricular septum and the RV were not made ischemic by the left coronary artery occlusion.

Table 1 shows the characteristics of experimental MI and sham-operated rats after a ligation of the left coronary artery. The RV weight and the RV weight to body weight ratio in the MI group were significantly increased at 4, 8 and 12 weeks compared with those in the sham-operated animals. In the MI group, the LV weight increased at 8 and 12 weeks, and the LV to body weight ratio increased 12 weeks after operation compared with the findings of the sham-operated rats. The size of the infarct, as determined by the planimetric measurements, did not differ for any of the three stages of healing (Table 2). The heart rate significantly decreased at 12 weeks in the MI rats compared with the sham-operated animals. Among hemodynamic measurements, the LV end-diastolic pressure was significantly different between the sham-operated and experimental rats. The positive and negative *dp/dt* of the LV pressure, representing the rate of

contraction and relaxation, respectively, revealed reductions in the MI rats at all three stages.

Table 3 shows the general and hemodynamic characteristics 12 weeks after the MI induction in the rats treated with E-4177 and untreated animals. Treatment with E-4177 significantly decreased the LV weight, RV weight, and the LV to body weight ratio. On the other hand, the infarct size was similar in the untreated and treated rats. Hemodynamic examinations revealed that the LV systolic and end-diastolic pressures decreased significantly while the rates of contraction and relaxation significantly increased in the treated group compared with the untreated MI rats.

The collagen concentration and ACE activity were examined in two myocardial specimens: viable tissue and scar tissue. Table 4 shows the collagen concentration and ACE activity in the heart tissue from the sham-operated rats, MI rats, and MI rats treated with E-4177. The collagen concentration of the untreated MI group was significantly increased in both viable and scar tissues at 4, 8 and 12 weeks post-operatively compared with the concentrations in the sham-operated group. The administration of E-4177 significantly decreased the collagen concentration in viable LV at all three stages of healing compared with the concentration in the untreated MI group. This agent decreased the collagen concentration in scar tissue at 12 weeks but not at 4 or 8 weeks after MI. The ACE activity was significantly increased in the viable tissue at 4, 8 and 12 weeks post-operatively compared with the sham-operated myocardium. However, the ACE activity was unaltered in the viable and scar tissues by E-4177 at all stages.

The results of Ang II receptor binding assays are shown in Figure 1. The maximum number of Ang II receptors significantly increased in the viable myocardium of the untreated MI rats at 4 to 12 weeks after experimental MI compared with the numbers of Ang II receptors in sham-operated rats. With

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Table 2 Myocardial infarction size and hemodynamic parameters in sham-operated rats and rats having experimental MI at 4, 8, and 12 weeks after induction of MI

	4 weeks		8 weeks		12 weeks	
	Sham	Experiment	Sham	Experiment	Sham	Experiment
MI size (%)	—	26±4	—	24±2	—	23±3
Heart rate (bpm)	422±26	410±18	376±19	366±20	360±18	324±14*
LVEDP (mmHg)	2.0±0.17	11.2±0.62*	3.41±0.19	14.0±0.53*	3.7±0.20	16.2±0.61
LVSP (mmHg)	136±7.4	126±8.8	134±6.9	112±4.9	134±7.1	102±4.1*
+dP/dt (mmHg/s)	6254±342	4021±201*	5820±239	4420±289*	5730±392	3982±260*
−dP/dt (mmHg/s)	5351±171	4023±260*	5530±261	3945±258*	5420±331	3264±249*

Values are means ± s.e. of six experiments. LVEDP, LV end-diastolic pressure; LVSP, LV systolic pressure; +dP/dt, rate of contraction; −dP/dt, rate of relaxation. *P<0.05 compared with sham-operated rats at same period.

Table 3 General and hemodynamic characteristics of rat treated and untreated with E-4177 at 12 weeks after induction of MI

	Untreated (n=6)	E-4177 (n=6)
BW (g)	577±10	571±8
Heart weight (g)	1.42±0.06	1.28±0.09*
LV weight (g)	0.93±0.05	0.76±0.04*
RV weight (g)	0.49±0.02	0.52±0.01
LV to BW (10 ^{−3})	1.61±0.06	1.33±0.09*
RV to BW (10 ^{−3})	0.85±0.03	0.91±0.05
MI size (%)	22±3	21±2
Heart rate (bpm)	324±14	332±13
LVSP (mmHg)	132±4.1	101±4.8*
LVEDP (mmHg)	16.2±0.5	13.8±0.6*
+dP/dt (mmHg/s)	3982±269	4456±274*
−dP/dt (mmHg/s)	3264±249	3879±235*

Values are means ± s.e. MI, myocardial infarction; BW, body weight; LV, left ventricle; RV, right ventricle; LVEDP, LV end-diastolic pressure; LVSP, LV systolic pressure; +dP/dt, rate of contraction; −dP/dt, rate of relaxation. *P<0.05 compared with untreated rats.

Table 4 Collagen concentrations and angiotensin converting enzyme activity (ACE) in scar and viable myocardial tissues from rats at 4, 8, and 12 weeks after induction of MI

	4 weeks		8 weeks		12 weeks	
	Scar	Viable	Scar	Viable	Scar	Viable
Collagen (mg/g wet weight)						
Sham	1.9±0.10	1.6±0.09	1.8±0.08	1.7±0.12	1.8±0.08	1.7±0.08
MI	7.8±0.23	5.1±0.21*	8.9±0.61	5.2±0.22*	10.6±0.53	5.6±0.1*
MI+E-4177	7.6±0.30	1.9±0.05†	8.0±0.41	4.0±0.12*†	7.8±0.47†	3.4±0.12*†
ACE activity (nmol/min/g)						
Sham	27.1±1.1	25±1.3	26.4±2.0	28±1.8	30.6±2.4	27±1.5
MI	109±6.8	58±3.1*	104±6.8	62±2.9*	163±7.2	46±3.7*
MI+E-4177	91±6.9	51±3.5*	96±3.0	58±1.7*	148±8.8	41±2.0*

Samples in sham-operated rats were myocardium obtained from the interventricular septum or the free wall of the left ventricle, which were correlated with the viable tissues or the scar, respectively. In the presence of MI. Values are means ± s.e. of six experiments. E-4177 (10 mg/kg/day) was administered to same rats with MI. MI: myocardial infarction. *P<0.05 compared with sham-operated rats. †P<0.05 compared with untreated MI rats.

E-4177 treatment, the maximum number of Ang II receptors significantly decreased at 4, 8, and 12 weeks compared with the number in the untreated group. The dissociation constant in these assays did

not differ between the control and the experimental groups.

The sarcolemmal Na⁺/Ca²⁺ exchange activity was also examined in the viable myocardium of

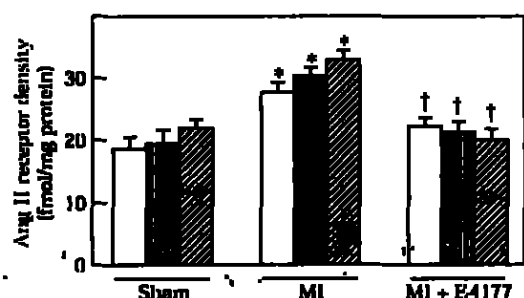


Figure 1 Results of the [^{125} I]-[Ser¹, Ile⁸] angiotensin II binding assay in heart membranes isolated from viable myocardium at 4 (□), 8 (▨) and 12 (■) weeks. The values are the mean $\pm$ S.E. for six rats in each experimental group. * $P < 0.05$ v sham-operated rats. † $P < 0.05$ v untreated infarct rats (MI).

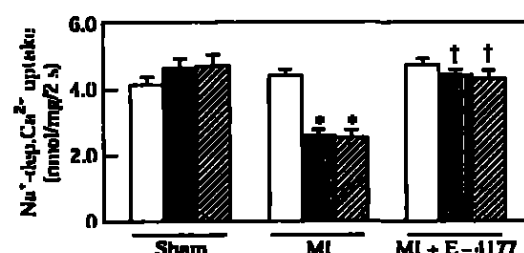


Figure 2 Na⁺/Ca²⁺ exchange activity in rat hearts isolated from the sham-operated and infarcted rats at 4 (□), 8 (▨) and 12 (■) weeks. The Na⁺/Ca²⁺ exchange activity, represented as the sarcolemmal Na⁺-dependent Ca²⁺ uptake at 20 μ M Ca²⁺ for 2 s was measured. The values are the mean $\pm$ S.E. for six rats in each experimental group. * $P < 0.05$ v sham-operated rats. † $P < 0.05$ v untreated infarct rats (MI).

sham-operated and experimental rats at 4, 8, and 12 weeks post-operatively (Fig. 2). The Na⁺/Ca²⁺ exchange activity, represented as the sarcolemmal Na⁺-dependent Ca²⁺ uptake at 20 μ M Ca²⁺ for 2 s, significantly decreased in the untreated MI group at 8 and 12 weeks. Treatment with E-4177 inhibited the depression of Na⁺/Ca²⁺ exchange activity in the viable myocardium.

The relative expressions of ACE mRNA and AT1 mRNA normalized by GAPDH, were determined by RT-PCR (Figs 3 and 4). The AT1 mRNA levels increased in the scar and viable MI tissues at 12 weeks compared with the mRNA expression in the sham-operated rats. The administration of E-4177 decreased the AT1 mRNA levels in the scar and viable MI tissues (Fig. 3). However, after treatment with E-4177 the increased expression of ACE mRNA was not affected in the viable tissue, but was significantly decreased in the scar tissue (Fig. 4).

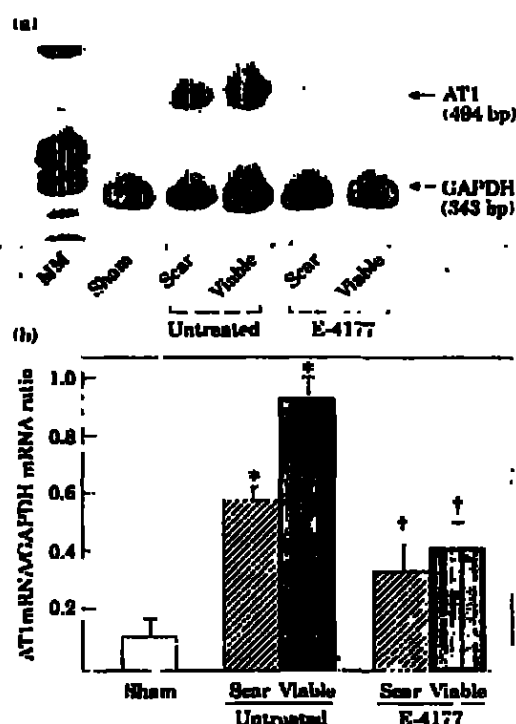


Figure 3 The detection of AT1 mRNA (a) by RT-PCR in the myocardium from the sham-operated, the untreated infarcted rats, and infarcted rats with E-4177. Samples from untreated and infarcted rats were taken 12 weeks after coronary artery ligation and were divided into scar and viable tissues. GAPDH was used as an internal control. M.M. was a molecular weight marker. The bar graph (b) shows the AT1 mRNA expression on the basis of data by RT-PCR. Values are the mean $\pm$ S.E.M. ($n = 6$). * $P < 0.05$ v sham-operated rats, † $P < 0.05$ v untreated scar or viable tissue.

Discussion

The present study demonstrated that chronic treatment with the AT1 receptor antagonist E-4177 had both hemodynamic and biochemical effects on rats with congestive heart failure after MI. Other data also demonstrate that ACE inhibitors are effective in treating congestive heart failure and LV dysfunction without overt heart failure (Pfeffer *et al.*, 1985; Hirsch *et al.*, 1992; Wollert *et al.*, 1994). These effects are usually considered to prevent the formation of Ang II, because the renin-Ang II system is activated after acute MI. Several investigators have studied the effects of Ang II receptor antagonists on the regression of cardiac hypertrophy (Kojima *et al.*, 1994; Ruzicka *et al.*, 1994). In experimental and clinical studies of congestive heart failure, the administration of low doses of AT1 receptor antagonist, losartan, produced vasodilation (Gottlieb *et*

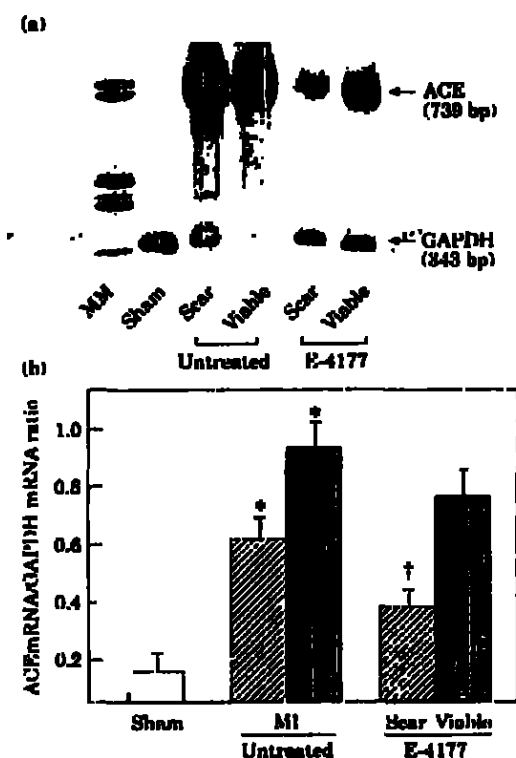


Figure 4 The detection of ACE mRNA (a) by RT-PCR in the myocardium from the sham-operated, untreated infarcted rats, and infarcted rats with E-4177. The samples from untreated and infarcted rats were taken 12 weeks after coronary artery ligation and were divided into scar and viable tissues. GAPDH was used as an internal control. M.M. was a molecular weight marker. The bar graph (b) shows the ACE mRNA expression on the basis of data by RT-PCR. The values are the mean $\pm$ S.E.M. ($n=6$). * $P<0.05$ v sham-operated rats, † $P<0.05$ v untreated scar or viable tissue.

et al., 1993) and improved cardiac pump performance (Capasso *et al.*, 1994).

The LV weight in the present study significantly increased at 8 and 12 weeks after MI compared with the weights in the sham-operated rats. These observations suggest that the reactive hypertrophy in the non-infarcted myocardium was produced in the LV. While the RV weight significantly increased after MI compared with the weights in the sham-operated rats. These findings are consistent with previous observations in which cardiac hypertrophy has been shown to develop after MI (Pfeffer and Braunwald, 1990; Olivetti *et al.*, 1991). Anversa *et al.* (1991) described that LV myocyte hypertrophy is accomplished by increases in the myocytes diameter and length, whereas the expansion of RV myocytes

is the result of an enlargement of the cellular diameter with no change in the cell length. Thus, the alterations in the myocyte diameter and length are consistent with the cellular shape changes characteristic of a combination of pressure and volume overload stress on the infarcted LV and the pressure overload stress on the unaffected RV (Pfeffer and Braunwald, 1990; Olivetti *et al.*, 1991; Anversa *et al.*, 1991). The coronary-ligated rat model is characterized by a stable compensated low-output state, regional blood flow redistribution, renal sodium avidity, and increased mortality (Pfeffer *et al.*, 1985). The depressed cardiac pump performance in our study was characterized by an increase in the LV end-diastolic pressure and a reduction in the peak rates of pressure increase and decay.

The results of the present study thus demonstrate a significant depression in the $\text{Na}^+/\text{Ca}^{2+}$ exchange in the sarcolemmal vesicles isolated from rat hearts of rats at 4, 8, and 12 weeks after MI. The sarcolemmal $\text{Na}^+/\text{Ca}^{2+}$ exchange is suggested to participate in the efflux of Ca^{2+} from the cytosolic space and therefore maintains the diastolic cytosolic Ca^{2+} concentration below 10^{-6} M. Accordingly, the depressed relaxation of the myocardium at 4–12 weeks of myocardial infarction may be explained partly on the basis of the observed decrease in the $\text{Na}^+/\text{Ca}^{2+}$ exchange activity (Dixon *et al.*, 1992). On the other hand, Ang II, generated locally in the myocardium after myocardial infarction, is considered to cause an impairment of diastolic relaxation (Schunkert *et al.*, 1990). Although the exact mechanism for the improvement of $\text{Na}^+/\text{Ca}^{2+}$ exchange by an AT1 blockade is unknown, Ang II raises the cytosolic Ca^{2+} concentration in the myocardium (Jindpalntner and Ganten, 1991) and activates the protein kinase C through the AT1 receptor signaling mechanism (Schunkert *et al.*, 1995). The AT1 blockade used in this study may ameliorate the Ang II-activated signal transduction in the viable non-infarcted myocardium.

As seen with another AT1 receptor antagonist, losartan (Capasso *et al.*, 1994), this type of drug reduces the LV end-diastolic pressure and improves the myocardial contractility state of the heart, possibly by preventing the positive inotropic effect of Ang II. The prevention of the effect of Ang II on the cardiac coronary vasculature may affect the myocardial performance by altering the coronary flow dynamics. However, Raya *et al.* (1991) reported that AT1 receptor blockade had not either early or late effects on cardiac function in rats following MI, despite the reduction in cardiac filling pressures. Although our results indicated an im-

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provement in the hemodynamic profiles in the treated animals, the size of the infarcted portion did not differ significantly in the animals treated with the AT1 receptor antagonist v untreated infarct rats.

Evidence indicates that the renin-angiotensin system may be important in modulating the adaptive growth pattern in the cardiac hypertrophy produced by a pressure overload (Baker *et al.*, 1992; Sadoshima *et al.*, 1995). Because ACE is involved in the formation of Ang II and the breakdown of bradykinin (Mimran *et al.*, 1980), the latter peptide, a potent vasodilator may contribute to the acute or chronic hypotensive effects of ACE inhibitors in the development of cardiac hypertrophy. AT1 receptor blockade has been shown to lack any bradykinin-potentiating effect (Wong *et al.*, 1990). Although ACE inhibitors, such as captopril, ameliorate the injurious effects of MI (Pfeffer *et al.*, 1985; Lindpaintner and Ganten, 1991), undesirable side effects can occur from the therapeutic use of ACE inhibitors as an influence of kinins, such as bradykinin or substance P (Mimran *et al.*, 1980). Thus, AT1 receptor antagonists have a potential advantage compared with ACE inhibitors. The antagonist, E-4177 used here is AT1 specific, as is losartan, and it has no effect on ACE activity (Okunishi *et al.*, 1993). E-4177 inhibited competitively the specific binding of [¹²⁵I]-[Sar¹, Ile⁶]Ang II and this agent for IC₅₀ is twice as potent as losartan in human arteries and three times as potent in rabbit aortic strips (Okunishi *et al.*, 1993). We selected the dose of E-4177 of 10 mg/kg/day, to reduce the blood pressure. In addition, E-4177 reduced the collagen concentration in scar tissue and the viable myocardium after 12 weeks of MI.

Recent studies using *in situ* hybridization and immunofluorescent techniques have shown the localization of angiotensin, renin, ACE, Ang I, and Ang II in cardiac myocytes and fibroblasts in neonatal rat cell cultures, thus supporting the view that the intracardiac renin-angiotensin system is capable of synthesizing biologically active peptides (Dostal *et al.*, 1992a). Ang II can stimulate directly the proliferative growth of neonatal rat cardiac fibroblasts in culture (Baker *et al.*, 1992; Sadoshima and Izumo, 1993b). The present study makes it clear that Ang II has an important growth-promoting role in the heart. Whether the biologically active peptide involved in the hypertrophic response in the non-infarcted myocardium was generated locally or produced in the peripheral circulation could not be determined in this investigation. It has been postulated that the heart may act as an autocrine, paracrine, or even an intracrine organ

(Lindpaintner and Ganten, 1991). The prevention of non-infarct hypertrophy by a specific AT1 receptor antagonist suggests that an intact intracrine renin-angiotensin system is therefore not an important pathway in the rat myocardium.

We evaluated the gene expression of ACE and AT1 receptors in rat hearts with MI and the effects of the Ang II receptor antagonist. Our findings indicate that MI increases mRNA and protein levels of AT1 receptors in the non-infarcted viable portion. In the present study, the ACE activity and the density of Ang II receptor showed relatively small changes despite the marked increases in their mRNA levels. These observations may be explained by the stimulation in the turnover rate for each protein in the viable myocardium and those protein activities appear to demonstrate relatively few changes. In addition, since the gene expression by the PT-PCR method depends on the number of cycles of PCR, the mRNA expression levels obtained in this study might be somewhat exaggerated and may therefore appear to demonstrate a marked increase in the viable myocardium. The administration of the AT1 receptor antagonist reduced effectively the increased gene expression of the AT1 receptor in scar tissue and viable myocardium, whereas it did not affect the increased gene expression of ACE although this agent did decrease the amount of ACE mRNA in scar tissue. Meggs *et al.* (1993) reported that acute MI enhanced the message of the Ang II receptor in the surviving myocytes. Our data on the AT1 receptor mRNA levels correlated with those results, although we did not isolate the cardiac myocytes from myocardial tissue. Recently, Sadoshima and Izumo (1993b) characterized the direct effect of Ang II on cultured myocytes and non-myocytes from neonatal rats in which the Ang II-induced hypertrophy response of myocytes and mitogenic response of fibroblasts were mediated primarily by the AT1 receptor. They also demonstrated that an AT1 receptor antagonist losartan fully blocked the induction of immediate-early gene (*c-fos*, *c-jun* and *c-myc*) expression in myocytes and fibroblasts, while this agent inhibited the late gene (α -actin and atrial natriuretic factor) and growth factor gene (angiotensin and transforming growth factor- β 1) expressions. Based on the above findings, it is considered therefore that viable myocytes are a target of Ang II, which appears to play a role in the structural remodeling after MI.

Since the size of the infarction did not differ significantly between the animals treated chronically with the AT1 receptor antagonist and the untreated animals, we cannot comment on the effectiveness of Ang II receptor antagonists as ther-

apy for MI. However, this study did demonstrate, for the first time, the ability of the AT1 receptor antagonist, E-4177, to inhibit the increase in Ang II receptor expression in non-infarcted myocardium during the healing period of MI. Although the AT1 receptor antagonist could not completely normalize the increased expression of Ang II receptors in the non-infarcted tissue, the inhibitory effect appears to be due to either a direct suppression of the autocrine and/or the paracrine action of Ang II, or to be secondary to the hemodynamic changes.

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References

- ANVERSA PD, SONNENBLICK EH. 1991. Ischemic cardiomyopathy: pathophysiologic mechanisms. *Prog Cardiovasc Dis* 33: 49-70.
- BAKER KM, BAKER GW, DOSTAL DE. 1992. Cardiac actions of angiotensin II: role of an intracardiac renin-angiotensin system. *Ann Rev Physiol* 54: 227-241.
- BERKMAN I, LUXLEY R. 1961. Two improved and simplified methods for the spectrophotometric determination of hydroxyproline. *Anal Chem* 35: 1961-1965.
- BRILLA CG, PUCK R, TAN LB, JANICKI JS, WINKER KT. 1990. Remodeling of the rat right and left ventricles in experimental hypertension. *Circ Res* 67: 1355-1364.
- CAPASSO JM, LI P, MURAN L, HANMAN MV, ANVERSA P. 1994. Efficacy of angiotensin-converting enzyme inhibition and AT1 receptor blockade on cardiac pump performance after myocardial infarction in rats. *J Cardiovasc Pharmacol* 23: 584-593.
- CUSHMAN DW, CHUNG HS. 1971. Concentrations of angiotensin-converting enzyme in tissue of the rat. *Biochim Biophys Acta* 250: 261-265.
- DIXON IMC, HATA T, DHALLA NS. 1992. Sarcoplasmic calcium transport on congestive heart failure due to myocardial infarction in rats. *Am J Physiol* 262: H1387-H1394.
- DOSTAL DE, ROTHMAN KN, CHIRNIN AI, COOPER GR, BAKER KM. 1992a. Intracardiac detection of angiotensinogen and renin: a localized renin-angiotensin system in neonatal rat heart. *Am J Physiol* 263: C838-C850.
- DZAU VJ. 1989. Short- and long-term determinations of cardiovascular function and therapy: contributions of circulating and tissue renin-angiotensin systems. *J Cardiovasc Pharmacol* 14(Suppl. 4): S1-S5.
- FORT PI, MARTY I, PICHACZYK M, SAMBUTY SEI, DANI CH, JEANTHON PH, BLANCHARD JM. 1985. Various rat adult tissues express only one major mRNA species from the glyceraldehyde-3-phosphate-dehydrogenase multigenic family. *Nucl Acids Res* 13: 1431-1442.
- GORTIJA SS, DIXON IMC, FLACK E, KOSTIS J, LEVIN TB, LERJARRET T, DICKINSON M. 1993. Hemodynamic and neurohormonal effects of angiotensin II antagonist losartan in patients with congestive heart failure. *Circulation* 88: 1602-1609.
- GROSSMAN WD, JONES D, McLAURIN LP. 1975. Wall stress and patients with hypertrophy in the human left ventricle. *J Clin Invest* 56: 58-64.
- HIRSH AT, TALANOFF CE, SCHUNKERT H, PAUL M, DZAU VJ. 1991. Tissue specific activation of cardiac angiotensin converting enzyme in experimental heart failure. *Circ Res* 69: 475-482.
- HIRSH AT, TALANOFF CE, SMITH AD, SCHUNKERT H, INGEFINGER JR, DZAU VJ. 1992. Differential effects of captopril and enalapril on tissue renin-angiotensin systems in experimental heart failure. *Circulation* 86: 1566-1574.
- KOJIMA M, SHIOJIMA I, YAMAZAKI T, KOMURO I, YUNZING Z, YING W, MIZUKI T, UHKE K, TOMI K, KAIKAWAKI T, NAGAI R, YAZAKI Y. 1994. Angiotensin II receptor antagonist TCV-116 induces regression of hypertensive left ventricular hypertrophy in vivo and inhibits the intracellular signaling pathway of stretch-mediated cardiomyocyte hypertrophy in vitro. *Circulation* 89: 2204-2211.
- LINDPAINTNER K, GANTEN D. 1991. The cardiac renin-angiotensin system. An appraisal of present experimental and clinical evidence. *Circ Res* 68: 905-921.
- LINDPAINTNER K, LIU W, NIEDERMAIER N, JIST H, GANTEN D, DRXELER H. 1993. Selective activation of cardiac angiotensinogen gene expression in post-infarction ventricular remodeling in the rat. *J Mol Cell Cardiol* 25: 133-143.
- LOWRY OH, ROSEBROUGH NJ, FARR AL, RANDALL RJ. 1951. Protein measurement with the Folin phenol reagent. *J Biol Chem* 193: 265-275.
- MAKINO N, MATSUI H, MASUTOMO K, HATA T, YANAGA T. 1993. Effects of angiotensin converting enzyme inhibitor on regression in cardiac hypertrophy. *Mol Cell Biochem* 119: 23-28.
- MERIN LG, COOPER GJ, HUANG H, CHENG P, LI P, CAPASSO JM, HANLEY CJ, ANVERSA P. 1993. Regulation of angiotensin II receptors on ventricular myocytes after myocardial infarction in rats. *Circ Res* 72: 1149-1162.
- MIMRAN A, TARAGHETTA R, LAROCHE B. 1980. The anti-hypertensive effect of captopril: evidence of an influence of kinins. *Hypertension* 2: 732-737.
- MORI N, ISHIHARA H, MATSUYUKA T, SAITO I. 1994. Anti-hypertensive, anti-myocardial hypertrophic and renal protective effects of E4177, an angiotensin II AT1 receptor antagonist, in rat (Abstract). *Can J Physiol Pharmacol* 72 (Suppl. 1): 131.
- MUKHERJEE D, SHIN S. 1991. Alteration of collagen phenotypes in ischemic cardiomyopathy. 88: 1141-1146.
- MURPHY TJ, ALEXANDER RW, GREENBLUM KK, RINGH MS, BASTRIN KE. 1991. Isolation of a cDNA encoding the vascular type-1 angiotensin II receptor. *Nature (London)* 351: 233-235.
- OKUNISHI H, SONO K, OKA Y, KOBAYASHI T, KAWAMOTO T, ISHIHARA H, MORI N, MIYAZAKI M. 1993. In vitro pharmacology of a novel non-peptide angiotensin II receptor antagonist, E-4177. *Jpn J Pharmacol* 62: 239-244.
- OLIVETTI G, CAPASSO JM, MERIN LG, SONNENBLICK EH, ANVERSA P. 1991. Cellular basis of chronic ventricular remodeling after myocardial infarction in rats. *Circ Res* 68: 856-869.

- PERPETER MA, PERPETER JM, STRIMLING C, FINN P, 1985. Survival after an experimental myocardial infarction: beneficial effects of long-term therapy with captopril. *Circulation* 72: 406-412.
- PERPETER MA, BRAUNWALD E, 1990. Ventricular remodeling after myocardial infarction: experimental observations and clinical implications. *Circulation* 81: 1161-1172.
- PERPETER JM, 1991. Progressive ventricular dilation in experimental myocardial infarction and its attenuation by angiotensin-converting enzyme inhibition. *Am J Cardiol* 68: 17D-25D.
- PERPETER MA, BRAUNWALD E, MOYR LA, BASTA L, BROWN J, CHINNY TE, DAVIS BR, GRITMAN EM, GORDMAN S, FLAKEN GC, 1992. Effect of captopril on myocardial mortality and morbidity in patients with left ventricular dysfunction after myocardial infarction: results of the Survival and Ventricular Enlargement trial: the SAVE investigation. *N Engl J Med* 327: 669-677.
- PITTS BJR, 1979. Stoichiometry of sodium-calcium exchange in cardiac sarcolemmal vesicles. *J Biol Chem* 254: 6232-6235.
- RAYA TE, FINKEL SJ, LEE RW, 1991. Hemodynamic effects of direct angiotensin II blockade compared to converting enzyme inhibition in rat model for heart failure. *Am J Hypertens* 4: 334S-340S.
- ROCK H, SCHMID A, DE GASPARO M, 1990. Identification and characterization of angiotensin II receptor subtypes in rabbit ventricular myocardium. *Biochem Biophys Res Commun* 173: 416-422.
- RIZACKA M, YUAN B, LINDEN FHH, 1994. Effects of enalapril versus losartan on regression of volume overload-induced cardiac hypertrophy in rats. *Circulation* 90: 484-491.
- SADOSHIMA J, XU Y, SLAYTER HS, IZUMO S, 1993a. Autocrine release of angiotensin II mediates stretch-induced hypertrophy of cardiac myocytes in vivo. *Cell* 75: 977-984.
- SADOSHIMA J, IZUMO S, 1993b. Molecular characterization of angiotensin II induced hypertrophy of cardiac myocytes and hyperplasia of cardiac fibroblast: Critical role of the AT1 receptor subtype. *Circ Res* 73: 413-423.
- SADOSHIMA J, QIU Z, MORGAN JP, IZUMO S, 1995. Angiotensin II and other hypertrophic stimuli mediated by G protein-coupled receptors activate tyrosin kinase, mitogen-activated protein kinase, and 80-kD S6 kinase in cardiac myocytes: the critical role of Ca^{2+} -dependent signaling. *Circ Res* 76: 1-15.
- SCHUNKER H, DZAU VJ, TANG SS, HIRASHI AT, APSTEIN CS, LONIGLI H, 1990. Increased rat cardiac angiotensin converting enzyme activity and mRNA expression in pressure overload left ventricular hypertrophy. Effect on coronary resistance, contractility, and relaxation. *J Clin Invest* 86: 1913-1921.
- SCHUNKER H, SADOSHIMA J, CORNELIUS T, KAGAWA Y, WUNDERLICH ET, IZUMO R, RUDOLPH G, LONIGLI BH, 1995. Angiotensin II-induced growth responses in isolated adult rat hearts. Evidence for load-independent induction of cardiac protein synthesis by angiotensin II. *Circ Res* 76: 480-497.
- SUZUKI J, MATSUIBARA H, URAKAMI M, INADA M, 1993. Rat angiotensin II (type 1A) receptor mRNA regulation and subtype expression in myocardial growth and hypertrophy. *Circ Res* 73: 439-447.
- WEISMAN HF, BUSH DE, MANNIS JA, WEISBERG ML, HRAJY B, 1988. Cellular mechanisms of myocardial infarct expansion. *Circulation* 78: 186-201.
- WILLIAMS KC, STUBBS R, VON BRUNN B, DRUCKER H, 1994. Survival after myocardial infarction in the rat: role of tissue angiotensin converting enzyme inhibition. *Circulation* 90: 2457-2467.
- WONG PC, HART SD, ZARPAR AM, CHIU AT, ARDELY RJ, SMITH RD, TIMMERMAN PBMWM, 1990. Functional studies of nonpeptide angiotensin II receptor subtype-specific ligands: losartan (All-1) and PD123177 (All-2). *J Pharmacol Exp Ther* 255: 584-592.

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[54] HALOETHYL-SUBSTITUTED STEROID
ENZYME INHIBITORS

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abandoned.

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532/631

[58] Field of Search 532/632, 631, 613, 650,
532/526; 514/177, 178, 182

[56] References Cited

U.S. PATENT DOCUMENTS

3,121,079 2/1964 Oberster et al. 532/642
3,138,623 6/1964 Bergstrom 532/646
3,207,733 9/1964 Bowers et al. 532/642

3,284,448 11/1966 Cross 532/632

FOREIGN PATENT DOCUMENTS

0434571 6/1991 European Pat. Off. .

OTHER PUBLICATIONS

Marcotte, et al. "Synthesis and Evaluation of 10 β -Sub-
stituted 4-Estrene-3,17-Diones as Inhibitors of Human
Placental Microsomal Aromatase." *Saravali*, 39(3), Mar.
1982, pp. 325-344.

Marcotte et al., *Biochemistry*, 21, 2773 (1982).

Shih et al., *J. Chem. Soc., Chem. Commun.*, 1987, 213.

Childers et al., *J. Chem. Soc., Chem. Commun.*, 1987,
320.

Childers et al., *J. Org. Chem.*, 53, 5947 (1988).

Morrison and Boyd, "Organic Chemistry" (Boston,
Allyn and Bacon 1979) pp. 564, 568, 569.

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[57]

ABSTRACT

The present invention is directed to a group of com-
pounds which are haloethyl substituted steroidal en-
zyme inhibitors. These compounds are useful as aroma-
tase, 19-hydroxylase, and aldosterone biosynthesis in-
hibitors and they are prepared from the corresponding
epoxide.

12 Claims, No Drawings.

HALOETHYL-SUBSTITUTED STEROID ENZYME INHIBITORS

The present application is a continuation-in-part of U.S. Ser. No. 303,191, filed Apr. 2, 1990 now abandoned.

BACKGROUND OF THE INVENTION

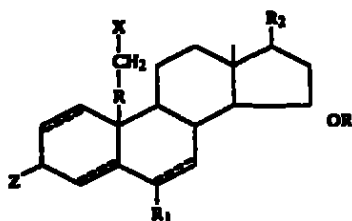
The estrogen hormones, estrone and estradiol, which are involved in many physiological processes, are formed from cholesterol via several enzymatic steps. The enzyme aromatase is the final rate limiting enzyme in the nonreversible conversion of the androgen hormones, testosterone and androstenedione, to the estrogen hormones, estradiol and estrone. Compounds such as aromatase inhibitors may thus regulate or inhibit androgen to estrogen conversion, and have therapeutic utility in treating clinical conditions potentiated by the presence of estrogens.

19-Nordeoxycorticosterone (19-norDOC) is known to induce mineralocorticoid hypertension. In the biosynthetic formation of 19-norsteroids, such as 19-norDOC, the initial step is the adrenal C₁₉ hydroxylation of an appropriate steroid such as deoxycorticosterone (DOC). The inhibition of the biosynthetic formation of 19-norDOC by inhibition of 19-hydroxylation of DOC would thus serve to decrease the level of 19-norDOC present in the animal involved and reduce hypertensive effects attributable to the presence of this material.

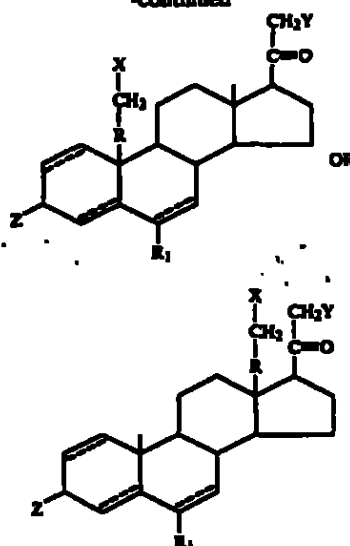
Aldosterone is a steroidal hormone which is synthesized in the zona glomerulosa cells of the adrenal glands. The primary biological function of the compound is the regulation of salt retention. In particular, aldosterone plays a major role in controlling the reabsorption of sodium ions from the kidney filtrates. Thus, a deficiency of the enzyme responsible for the synthesis of aldosterone is a characteristic of patients with a salt-losing syndrome, while primary hyperaldosteronism can result from hyperbiosynthesis of aldosterone as caused by an adrenocortical tumor or the administration of certain drugs. The hyperaldosteronism may involve hypertension, hypokalemia, alkalosis, muscular weakness, polyuria, and polydipsia. Thus, treatment of hyperaldosteronism and the conditions associated with it would be possible by blockage of the enzymatic synthesis of aldosterone.

SUMMARY OF THE INVENTION

The present invention is directed to novel haloethyl-substituted steroidal enzyme inhibitors, their related intermediates, and the process for their preparation. These compounds may be represented by the following formulas:



-continued



wherein

--- represents a single or double bond;

X=Br, Cl, or I;

R=CHOH or C=O;

R₁=H, C₁₋₄ alkyl, =O, or -OH;

R₂=O, -OH, or -O-(C₁₋₄ alkanoyl);

Z=O, =CH₂, -OH, or -O-(C₁₋₄ alkanoyl); and

Y=H, -OH, or -O-(C₁₋₄ alkanoyl), and when

Y=H, -OH, or -O-(C₁₋₄ alkanoyl), Z may not

include -OH, and R₁ may not include =O or -OH.

Examples of C₁₋₄ alkyl include methyl, ethyl, propyl,

isopropyl, butyl, and isobutyl. Examples of C₁₋₄ alkanoyl include formyl, acetyl, propionyl, and butyryl.

When R is CHOH, two optical isomers are possible.

The present invention encompasses the individual pure isomers, or mixtures of the two isomers in any proportion.

The (R) isomer for the haloethyl moiety at C₁₀ is preferred for aromatase activity. In referring to the various compounds above, the entire X-CH₂-R-

group can be considered as the substituent in which case the first two compounds above can be considered as

10-substituted estranes and the third compound a 13-substituted gonane. Alternatively, the R carbon atom

can be considered as corresponding to an angular methyl carbon in which case the three groups of compounds above can be described as 19-(X-CH₂-

substituted) androstanes, 19-(X-CH₂-

substituted) pregnanes and 18-(X-CH₂-

substituted) pregnanes respectively. In this case then, the optical center which results

when R is CHOH can be described as at the 19-position in the first two structures and the 18-position in the third structure.

DETAILED DESCRIPTION OF THE INVENTION

The compounds of the present invention are inhibitors of aromatase, 19-hydroxylase, and aldosterone biosynthesis. As aromatase inhibitors, they are useful in treating hyperestrogenemia. The compounds are useful in controlling abnormally high levels of estrogens, both when the high levels observed are relatively steady, or when there are brief surges of elevated levels occurring as part of cyclical body functions. Both females and

males can be treated, although obviously, the level of estrogen which would be considered high in males would be much lower than the amount considered high in females. These compounds are also useful as anti-fertility agents to prevent ovulation or implantation in females, or to reduce the mating behavior in males where brain aromatization is required for such behavior. These compounds further have value in treating gynecomastia, male infertility resulting from elevated estrogen levels, and hyperestrogenemia, which may precede myocardial infarction. The compounds may also have value in the treatment of breast cancer and various estrogen-induced or estrogen-stimulated disorders, such as benign prostatic hypertrophy and endometrial hyperplasia.

The bioconversion of deoxycorticosterone via a 19-hydroxylase pathway to 19-nordeoxycorticosterone potentiates its mineralocorticoid activity. Mineralocorticoid excess results in a syndrome characterized by hypokalemia, metabolic alkalosis, polydipsia, polyuria, and hypertensive conditions. Increased excretion of 19-nordeoxycorticosterone has been reported for hypertensive patients, including those with primary aldosteronism, Cushing's syndrome, 17 α -hydroxylase deficiency, and individuals with essential hypertension. As 19-hydroxylase inhibitors, these compounds may be useful as antihypertensive agents and for management of edemous conditions often associated with sodium retention and potassium loss.

As inhibitors of aldosterone, these compounds are useful for the treatment of hyperaldosteronism and various conditions wherein a reduction of the excess amount of aldosterone responsible for the condition would be beneficial. Thus, they are useful in the treatment of hyperaldosteronism and any associated hypertension, edema, and sodium retention, whether this is a result of some bodily disorder, or whether it results from the administration of some agent. As a result of their effects on the factors responsible for edema or sodium retention, the indicated compounds would be useful in a method of treatment as diuretic agents.

To achieve their desired effect, the compounds of the present invention may be administered orally, parenterally, for example, intravenously, intraperitoneally, intramuscularly, or subcutaneously, including the injection of the active ingredient directly into tissue or tumor sites, to a patient in need of treatment. The term patient is taken to mean a warm-blooded animal, for example, mammals such as humans, primates, cattle, dogs, cats, horses, sheep, mice, rats, and pigs. These compounds may also be administered in the form of a pharmaceutical preparation, and may further be incorporated into sustained delivery devices. The amount of compound administered will vary over a wide range and be any effective amount. Depending on the patient to be treated, the condition to be treated, and mode of administration, the effective amount of compound administered will vary from about 0.01 to 150 mg/kg of body weight per day, and preferably from about 0.1 to 50 mg/kg body weight per day.

For oral administration, the compounds can be formulated into solid or liquid preparations, such as capsules, pills, tablets, troches, powders, solutions, suspensions, or emulsions. The solid unit dosage forms can be a capsule which can be of the ordinary gelatin type containing the active compound and a carrier, for example, lubricants and inert filler such as lactose, sucrose and corn starch. In another embodiment, an active com-

pound of the invention can be tableted with conventional tablet bases such as lactose, sucrose and corn starch in combination with binders such as acacia, corn starch, or gelatin, disintegrating agents such as potato starch, or alginic acids and a lubricant such as stearic acid or magnesium stearate.

For parenteral administration the compounds may be administered as injectable dosages of a solution or suspension of the compound in a physiological acceptable diluent with a pharmaceutical carrier which can be a sterile liquid such as water-in-oil with or without the addition of a surfactant and other pharmaceutically acceptable adjuvants. Illustrative of oils which can be employed in these preparations are those of petroleum, animal, vegetable or synthetic origin, for example, peanut oil, soybean oil, and mineral oil. In general, water, saline, aqueous dextrose and related sugar solutions, ethanol and glycols, such as propylene glycol or polyethylene glycol are preferred liquid carriers, particularly for injectable solutions.

The compounds can be administered in the form of a cutaneous patch, a depot injection, or implant preparation which can be formulated in such a manner as to permit a sustained release of the active ingredient. The active ingredient can be compressed into pellets or small cylinders and implanted subcutaneously or intramuscularly as depot injections or implants. Implants may employ inert materials such as biodegradable polymers and synthetic silicones, for example, Silastic (®), silicone rubber manufactured by Dow Corning Corporation. Further information on suitable pharmaceutical carriers and formulation techniques are found in standard texts such as *Remington's Pharmaceutical Sciences*, Mack Publishing Company, Easton, Pa.

Inhibition of aromatase activity is demonstrated by using laboratory methods similar to procedures described in U.S. Pat. No. 4,322,416, and as published in Johnston et al., *Endocrinology* 115:776, 1984, and Burkhardt et al., *Steroids* 45:357, 1985.

In this assay, the inhibitor is preincubated with enzyme prior to assaying for activity in the presence of high substrate levels. A time-related decrease in enzyme activity can be indicative of irreversible binding of the inhibitor with the enzyme.

In the time-dependent assay, an amount of the enzyme inhibitor in 100 μ l of the assay buffer described above which will provide assay concentrations which are usually between 1 nM and 10 μ M are added to 35 ml centrifuge tubes containing 600 μ l of the NADPH generating system. The preincubation is started by the addition of 700 μ l of of aromatase preparation, usually 50-800 μ g of microsomal protein per ml of assay buffer. These preparations are mixed using a vortex mixer and incubated for 0, 5, 10, or 20 minutes at 25° C. Then 100 μ l of androstenedione (~6.8 μ M) containing 1 β -³H androstenedione is added in assay buffer to provide an assay concentration of substrate (0.50 μ M) which is at least ten times the K_m of androstenedione (0.04 μ M). Following vortexing, the enzyme incubation is continued for 10 minutes before being terminated by the addition of chloroform. The amount of radioactivity in the aqueous fraction is determined by scintillation procedures. The enzymatic activity for each concentration of inhibitor at each time period of preincubation is calculated as a percent of the respective vehicle control which is arbitrarily set at 100%. Therefore, the relative enzyme inhibition is expressed as a percentage: (100

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US005906987A

United States Patent [19]
Chwalisz et al.

[11] **Patent Number:** **5,906,987**
[45] **Date of Patent:** **May 25, 1999**

[54] **TREATMENT OF MALE CLIMACTERIC DISORDERS WITH NITRIC OXIDE SYNTHASE SUBSTRATES AND/OR DONORS, IN COMBINATION WITH ANDROGENS AND/OR AROMATASE INHIBITORS**

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[21] **Appl. No.:** **08/812,912**

[22] **Filed:** **Mar. 10, 1997**

[51] **Int. Cl.⁶** **A61K 31/36; A61K 31/535;**
A61K 31/34; A61K 31/195; A61K 31/13

[52] **U.S. Cl.** **514/177; 514/178; 514/236.2;**
514/470; 514/565; 514/668

[58] **Field of Search** **514/177, 178,**
514/236.2, 470, 565, 668

[56] **References Cited**

U.S. PATENT DOCUMENTS

5,260,276 **11/1993** **Cody et al.** **514/14**
5,378,715 **1/1995** **Stein et al.** **514/329**

FOREIGN PATENT DOCUMENTS

93/10144 **5/1993** **WIPO.**
93/25580 **12/1993** **WIPO.**
95/11537 **1/1995** **WIPO.**
95/12408 **1/1995** **WIPO.**
95/13812 **5/1995** **WIPO.**
WO95/13800 **5/1995** **WIPO.**

OTHER PUBLICATIONS

Garfield et al., "Reversal of Preeclampsia Symptoms Induced in Rats by Nitric Oxide Inhibition With L-Arginine, Steroid Hormones and an Endothelin Antagonist," Soc. Gynecol. Invest. Abst. P384 (1994).

Chwalisz et al., "Estradiol Inhibits the Onapristone-Induced Preterm Parturition in Guinea Pigs by Blocking Cervical Ripening," J. Soc. Gynecol. Invest., vol. 2, No. 2, Mar. 1995, P101.

Chwalisz et al., "Role of Progesterone During Pregnancy: Models of Parturition and Preeclampsia," Z. Geburtshilfe Perinatol, vol. 198, No. 5-5, pp. 170-180, 1994.

Richard et al., "Vivo Evidence of an Endothelin-Induced Vasopressor Tone After Inhibition of Nitric Oxide Synthesis in Rats," Circulation, vol. 91, No. 3, pp. 771-775, Feb. 1995.

Yallampalli et al., "Inhibition of Nitric Oxide Synthesis in Rats During Pregnancy Produces Signs Similar to Those of Preeclampsia," Am J Obstet. Gynecol, vol. 169, No. 5, pp. 1316-1320, 1993.

Yallampalli et al., "Uterine Contractile Responses to Endothelin-1 and Endothelin Receptors are Elevated During Labor," Biol Reprod, vol. 51, No. 4, pp. 640-645, 1994.

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[57]

ABSTRACT

The symptoms of climacterium in male mammals, e.g., hypertension, cardiovascular disease and osteoporosis, are ameliorated by the administration to an afflicted individual one or both of a nitric oxide substrate and/or nitric acid donor, in combination with an androgen, an aromatase inhibitor or both, wherein the circulating levels of testosterone in the afflicted individual are increased.

30 Claims, 2 Drawing Sheets

proteinuria—the classical triad of preeclampsia). These symptoms are related to the decrease in vascular resistance and placental perfusion. Preeclampsia is a well known model of atherosclerosis as the decrease in placental perfusion is accompanied by increased fibrin deposition in placental vessels and increased thrombus formation (Roberts et al., 1989). Thus, nitric oxide substrates and/or donors alone or in combination with estrogen and progesterone are particularly efficacious for hormone replacement therapy to prevent climacteric symptoms (climacterium) such as atherosclerosis, hypertension, hot flashes, etc. in women.

EP 0441 119 A2 discloses the use of L-arginine in the treatment of hypertension and other vascular disorders. It suggests that the mechanism by which L-arginine is effective for this purpose is because it may be the physiological precursor of "the most powerful endothelial-derived relaxing factor, nitric oxide." The use of L-arginine in combination with other pharmaceutically active agents is not discussed in this publication.

SUMMARY OF THE INVENTION

In a method aspect, this invention relates to a method of treating climacterium (climacteric symptoms) in a male mammal, comprising administering to a patient in need of such treatment an effective amount of

(a) at least one of a nitric oxide synthase substrate, a nitric oxide donor, or a compound which stimulates nitric oxide synthesis in target cells, and

(b) an androgen or an aromatase inhibitor, or both.

In a product aspect, this invention relates to pharmaceutical compositions comprising an admixture of effective amounts of

(a) at least one of a nitric oxide synthase substrate, a nitric oxide donor, or a compound which stimulates nitric oxide synthesis in target cells, and

(b) an androgen or an aromatase inhibitor, or both.

It is thus an object of the invention to provide a method for the prevention and treatment of climacterium (climacteric symptoms), including cardiovascular disease, hypertension, impotence and osteoporosis, in male mammals, e.g., humans, with a nitric oxide substrate and/or donor, in combination with an androgen and/or an aromatase inhibitor, which method provides an increase in endogenous testosterone levels.

It is a further object to provide a method for hormone replacement therapy (HRT) in male mammals, e.g., humans, with a nitric oxide substrate and/or donor, in combination with an androgen and/or an aromatase inhibitor, which method provides an increase in testosterone levels.

A further object is the provision of pharmaceutical compositions useful in practicing the methods of this invention.

Upon further study of the specification and appended claims, further objects and advantages of this invention will become apparent to those skilled in the art.

BRIEF DESCRIPTION OF THE DRAWINGS

Various other objects, features and attendant advantages of the present invention will be more fully appreciated as the same becomes better understood when considered in conjunction with the accompanying drawings, wherein:

FIG. 1 shows the effect of testosterone on blood pressure in L-NAME-treated rats post-partum. The control group (●) received only the vehicles. The testosterone-only group (■) was treated with 100 µg/rat/day, s.c., starting on day 2 postpartum. The L-NAME only group (Δ) was treated with

50 mg/rat/day, s.c., continuous infusion, starting on day 17 of pregnancy. The L-NAME+testosterone group (▼) was treated with 50 mg/rat/day of L-NAME, s.c., continuous infusion, starting on day 17 of pregnancy, and with 100 µg/rat/day of testosterone, s.c., starting on day 2 postpartum.

FIG. 2 shows the effect of 5α-dihydrotestosterone on blood pressure in L-NAME-treated rats post-partum. The control group (●) received only the vehicles. The dihydrotestosterone-only group (■) was treated with 100 µg/rat/day, s.c., starting on day 2 postpartum. The L-NAME only group (Δ) was treated with 50 mg/rat/day, s.c., continuous infusion, starting on day 17 of pregnancy. The L-NAME+dihydrotestosterone group (▼) was treated with 50 mg/rat/day of L-NAME, s.c., continuous infusion, starting on day 17 of pregnancy, and with 100 µg/rat/day of dihydrotestosterone, s.c., starting on day 2 post-partum.

DETAILED DISCLOSURE

The methods and pharmaceutical compositions of this invention treat climacterium (climacteric symptoms) in an aging male mammal, e.g., in a male human, who is manifesting the symptoms thereof or who is a high risk candidate for doing so, e.g., based on increased blood pressure. The invention employs either or both of a nitric oxide synthase substrate (e.g., L-arginine) and a nitric oxide donor (e.g., sodium nitroprusside or glyceryl trinitrate), and/or a compound which stimulates nitric oxide synthesis in the target cells, in combination with one or more of an androgen (e.g., testosterone or testosterone ester) and an aromatase inhibitor (e.g., tamoxifen).

During our research on these various conditions, it was unexpectedly found that androgens, e.g., testosterone and 5α-dihydrotestosterone (DHT), partially compensate for the L-NAME-induced increase in blood pressure in post-partum female rats. This is particularly surprising in view of the fact that both androgens slightly increased blood pressure in rats which did not receive L-NAME. These results indicate that androgens, similarly to progestins, induce compensatory mechanisms when nitric oxide synthesis is blocked or reduced. These results strongly suggest that androgens, as well as compounds such as aromatase inhibitors, which increase the endogenous androgen levels in male mammals, modulate nitric oxide synthesis or activity, as do estrogens and progestins. These results further implicate the physiological role of testosterone in the development of pathological conditions occurring during aging in male mammals, e.g., humans, including cardiovascular disease, dementia, etc.

Because these abnormal conditions of aging in male mammals, e.g., humans, are produced by or aggravated by subnormal nitric oxide synthesis, both nitric oxide synthase substrates, e.g., L-arginine, and nitric oxide donors, e.g., sodium nitroprusside, nitroglycerin, glycerin trinitrate, SIN-1, isosorbide mononitrate, isosorbide dinitrate and diethylenetriamine/NO (DETA/NO), are useful for ameliorating the symptoms thereof and, in one aspect of the method of this invention, a combination of both are employed. In addition, or instead, compounds which stimulate nitric oxide synthesis in the target cells, e.g., epithelial and/or brain nitric oxide synthase (eNOS and hNOS) stimulators, can be employed.

An additional effect is achieved when an androgenic agent is administered concurrently with the nitric oxide substrate and/or nitric oxide donor. Thus, an androgen (e.g., testosterone or testosterone ester) and/or an aromatase inhibitor (e.g.,

climacterium, treatment with nitric oxide substrates and/or nitric oxide donors in combination with androgens and/or aromatase inhibitors will have tremendous advantages for climacterium therapy.

The results of these experiments on post-partum rats indicate that androgens, similarly to progestins, induce compensatory mechanisms when nitric oxide synthesis is blocked or reduced. Thus, since the blood pressure-increasing effects of L-NAME are partially compensated by androgens, it can be concluded that administration of a nitric oxide donor or a nitric oxide substrate may have greater effects when an nitric oxide substrate or donor are combined with an androgen or an aromatase inhibitor. These effects are not mediated by estrogens, since DHT cannot be converted to estradiol. These results strongly suggest that androgens, as well as compounds such as aromatase inhibitors, which increase the endogenous androgen levels in male mammals, modulate nitric oxide synthesis or activity, as do estrogens and progestins.

Without wishing to be bound by mechanism, a direct effect on the blood vessels is proposed. These results further implicate the physiological role of testosterone in the development of pathological conditions occurring during aging in male mammals, e.g., humans, including, e.g., cardiovascular disease, hypertension, osteoporosis, impotence, dementia, etc., as well as under circumstances wherein the normal testosterone-producing tissue is not functional, e.g., after orchiectomy.

The method of treatment employed in this invention can be employed for the treatment of hypertension, as an adjuvant in thrombotic disorders, and hemorrhage, etc., following the dosage regime described herein.

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention in its fullest extent. The preferred specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the disclosure in any way whatsoever.

The entire disclosure of all applications, patents and publications, cited above and below are hereby incorporated by reference.

EXAMPLES

Example 1

Treatment of Climacterium (Climacteric Symptoms)

To a human male (ca 50 years; 80-120 kg) displaying the signs of male climacterium symptoms, e.g., high blood pressure, osteoporosis, etc., administer 0.5-40 g of L-arginine per os daily, with an androgen, e.g., testosterone, 1 to 10 mg/day transdermally until the symptoms are ameliorated. Thereafter, administer 0.5 to 10 g of L-arginine and 1-10 mg/day, preferably 4 mg/day, of testosterone daily.

Example 2

Treatment of Climacterium (Climacteric Symptoms)

To a male comparable to and displaying the same symptoms as Example 1, administer daily 2x5 mg of nitroglycerine per os in place of L-arginine.

Example 3

Treatment of Climacterium (Climacteric Symptoms)

To a male similar to and displaying the same symptoms as Example 1, administer daily 5-10 mg of nitroglycerine

transdermally with an androgen, testosterone, 1-10 mg per day transdermally.

Example 4

Hormone Replacement Therapy

To a male similar to and displaying the same symptoms as Example 1, administer daily 0.5-20 g of L-arginine in combination with an aromatase inhibitor in amounts sufficient to raise the endogenous testosterone levels by at least 30%, e.g., stamestane at 10-200 mg daily.

Example 5

Hormone Replacement Therapy

To a male after orchidectomy, administer L-arginine 0.5 to 20 g daily and/or a nitric oxide donor, e.g., nitroglycerine at 2x2.5 mg daily with one or more of the following: an androgen, e.g., transdermal testosterone at 4 mg/day or testosterone propionate at 250 mg i.m. 2-3 times/week, and/or an aromatase inhibitor, e.g., stamestane at 20-200 mg/day. The sex steroids and/or aromatase inhibitor are to be given either continuously with L-arginine and/or a nitric oxide donor, or sequentially.

The preceding examples can be repeated with similar success by substituting the generically or specifically described reactants and/or operating conditions of this invention for those used in the preceding examples.

From the foregoing description, one skilled in the art can easily ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions.

REFERENCES

- Barbieri, R. L. The bladder in menopause: Lower urinary tract dysfunction during the climacteric. *Curr. Problems Obstet. Gynecol. Fertil.* 1994; 17(6):196-228.
- Eli, G. and Berman, A. Estrogen effects on the urethra: beneficial effects in women with genuine stress incontinence. *Obstet. Gynecol.* 1993; 48(7):509-517.
- Sartori, M. G., Barakat, E. C., Girad, M. J., Goncalves, W. J., Sartori, J. P., de Lima, G. R. Menopausal genuine stress urinary incontinence treated with conjugated estrogens plus progestogens. *Int. J. Gynecol. Obstet.* 1995; 49(2):165-169.
- Cardozo, L. D. and Kelleher, C. J. Sex hormones, the menopause and urinary problems. *Gynecol. Endocrinol.* 1995; 9(1):75-84.
- Cardozo, L. and Kelleher, C. Sex hormones and the female lower urinary tract. *Physiotherapy* 1994; 80:135-138.
- Brandeis, G. H. and Resnick, N. M. Pharmacotherapy of urinary incontinence in the elderly. *Drug Therapy* 1992; 22:93-102.
- Furchgott, R. F. and Zawadzki, J. V. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 1980; 288:373-376.
- Moncada, S., Palmer, R. M. G. and Higgs, E. A. Nitric oxide; physiology, pathophysiology and pharmacology. *Pharmacol. Rev.* 1991; 43:109-142.
- Ignarro, L. J. Physiological significance of Nitric oxide. *Seminars in Perinatology* 1991; 15:20-26.
- Ehrun, L., Adolfsson, J. and Wilund, N. P. Nitric oxide synthase activity in the human urogenital tract. *Urol. Res.* 1994; 22:287-290.

11. Andersson, K. E. and Pernow, K. Nitric oxide synthase and nitric oxide mediated effects in lower urinary tract smooth muscles. *World J. Urol.* 1994; 12:274-280.
12. Smet, P. J., Lidyane, K. A., Jonavicius, J., Marshall, V. R. Distribution of NADPH-diaphorase-positive nerves supplying the human urinary bladder. *J. Autonomic Nervous System* 1994; 47:109-113.
13. Lee, J. G., Wein, A. J., Levin, R. M. Comparative pharmacology of the male and female rabbit bladder neck and urethra: Involvement of nitric oxide. *Pharmacology* 10
14. Chwalisz, K. and Garfield, R. E. Role of progesterone during pregnancy: Models of parturition and preeclampsia. *Z. Geburtsh. u. Perinat.* 198;170-180.

What is claimed is:

1. A method of treating climacteric symptoms in a male mammal, comprising administering to a patient in need of such treatment an effective amount of
- (a) at least one of a nitric oxide synthase substrate, a nitric oxide donor, or a compound which stimulates nitric oxide synthesis in target cells,
- (b) an androgen or an aromatase inhibitor, or both.
2. A method of claim 1, wherein the mammal is a human male suffering from symptoms of androgen deprivation.
3. A method of claim 1, wherein the mammal is a human male who has exhibited a need for or is a candidate for hormone replacement therapy.
4. A method of claim 1, wherein the mammal is a human male and a nitric oxide synthase substrate is administered thereto.
5. A method of claim 4, wherein the nitric oxide substrate is L-arginine.
6. A method of claim 1, wherein the mammal is a human male and a nitric oxide donor is administered therein.
7. A method of claim 6, wherein the nitric oxide donor is sodium nitroprusside, nitroglycerin, glyceryltrinitrate, SIN-1, isosorbid mononitrate or isosorbid dinitrate.
8. A method of claim 6, wherein the nitric oxide donor is administered orally.
9. A method of claim 6, wherein the nitric oxide donor is administered transdermally as a patch.
10. A method of claim 1, wherein the androgen is testosterone or a testosterone ester.
11. A method of claim 10, wherein the androgen is administered orally.
12. A method of claim 10, wherein the androgen is administered transdermally as a patch.
13. A method of claim 10, wherein the androgen is administered intramuscularly or subcutaneously.
14. A method of claim 1, wherein the mammal is a human male and an aromatase inhibitor is administered thereto.

15. A method of claim 1, wherein the mammal is a human male and the amount of the nitric oxide synthase substrate, nitric oxide donor or both administered is effective to raise the blood level of circulating L-arginine in a male to whom the composition is administered to at least about 10-50 nmole above the normally 50-100 nmole circulating levels and/or to raise the nitric oxide donor level to about 1-1000 nmolar.

16. A method of claim 1, wherein the mammal is a human male and the amount of an androgen, an aromatase inhibitor or both administered is effective to raise blood serum total testosterone level to between about 100 and about 600 mg/dl.

17. A method of claim 1, wherein components (a) and (b) are administered sequentially.

18. A method of claim 1, wherein components (a) and (b) are administered simultaneously.

19. A method of claim 1, wherein the male mammal is a human suffering from or at risk of developing symptoms of osteoporosis.

20. A method of claim 1, wherein the male mammal is a human suffering from or at risk of developing hypertension.

21. A method of claim 1, wherein the male mammal is a human suffering from or at risk of developing cardiovascular disease.

22. A pharmaceutical composition comprising an admixture of effective amounts of

(a) at least one of a nitric oxide synthase substrate, a nitric oxide donor, or a compound which stimulates nitric oxide synthesis in target cells,

(b) an androgen or an aromatase inhibitor or both.

23. A composition of claim 22, wherein (a) comprises a nitric oxide synthesis substrate.

24. A composition of claim 23, wherein the nitric oxide synthesis substrate is L-arginine.

25. A composition of claim 22, wherein (a) is a nitric oxide donor.

26. A composition of claim 25, wherein the nitric oxide donor is sodium nitroprusside, nitroglycerin, glyceryltrinitrate, SIN-1, isosorbid mononitrate or isosorbid dinitrate.

27. A composition of claim 22, wherein (b) is an androgen.

28. A composition of claim 22, wherein the androgen is testosterone or testosterone propionate.

29. A composition of claim 22, wherein (b) is an aromatase inhibitor.

30. A composition of claim 29, wherein the aromatase inhibitor is toremifene.

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶: A61K 31/565, 31/57, 31/135, 31/158, 31/105, 31/166, 31/122	A2	(11) International Publication Number: WO 98/03180 (43) International Publication Date: 29 January 1998 (29.01.98)
(21) International Application Number: PCT/GB97/01973 (22) International Filing Date: 22 July 1997 (22.07.97) (30) Priority Data: 9615348.1 22 July 1996 (22.07.96) GB 9701600.0 27 January 1997 (27.01.97) GB (71) Applicant (for all designated States except US): THE VICTORIA UNIVERSITY OF MANCHESTER [GB/GB]; Oxford Road, Manchester M13 9PL (GB). (72) Inventors; and (75) Inventors/Applicants (for US only): FERGUSON, Mark, William, James [GB/GB]; 13 Peel Moot Road, Heaton Moor, Stockport, Cheshire SK4 4PL (GB). ASHCROFT, Gillian, Sarah [GB/GB]; 3 Canal Bank, Anley Bridge, Wigan WN6 9AN (GB). (74) Agent: ATKINSON, Peter, Birch; Marks & Clark, Sunnex House, 83-85 Mosley Street, Manchester M2 3LG (GB).		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Burundian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TO). Published <i>Without international search report and to be republished upon receipt of that report.</i>
(54) Title: USE OF SEX STEROID FUNCTION MODULATORS TO TREAT WOUNDS AND FIBROTIC DISORDERS (57) Abstract The present application relates to the use of compounds that influence the sex hormone system for the treatment of wounds and/or fibrotic disorders. Preferred compounds for use in such treatments are steroid hormones and especially the oestrogens.		

9. The use of a compound as claimed in claim 2 wherein said compound is a promoter of an androgenic activity.

10. The use of a compound as claimed in claim 9 wherein said compound is an androgen hormone or androgen receptor agonist selected from testosterone, dihydrotestosterone, 5 α -androstanediol, testosterone undecanoate, testosterone enanthate, testosterone esters, testosterone propionate, mesterolone, danazol and gestrinone.

11. The use of a compound as claimed in claim 9 wherein said compound is one of an inhibitor of androgen or androgen receptor agonist breakdown (such as aminoglutethamide) or a modulator of the activity of luteinising hormone, follicle stimulating hormone or chorionic gonadotrophin.

12. The use of a compound as claimed in claim 2 wherein said compound is an anabolic steroid.

13. The use of a compound according to claim 12 wherein the anabolic steroid is nandrolone or stanozolol.

14. The use of a compound as claimed in claim 1 wherein said compound is one which serves to inhibit fibrosis.

15. The use of a compound as claimed in claim 14 wherein said compound is a promoter of progesterone activity.

16. The use of a compound as claimed in claim 15 wherein said compound is progesterone or a progesterone receptor agonist selected from the group of compounds consisting of allyloestrenol, desogestrel, dydrogesterone, ethynodiol diacetate,

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gestodene, gestranol hexanoate, hydroxyprogesterone hexanoate, levonorgestrel, megestrol acetate, medroxyprogesterone acetate, norethisterone, norethisterone acetate, norethisterone enanthate, norgestimate or norgesterel.

17. The use of a compound as claimed in claim 15 wherein the compound is one of an inhibitor of progesterone or progesterone receptor agonist breakdown or a modulator of the activity of luteinising hormone, follicle stimulating hormone or chorionic gonadotrophin.

18. The use of a compound as claimed in claim 14 wherein said compound is an inhibitor of oestrogenic activity.

19. The use of a compound as claimed in claim 18 wherein said inhibitor of oestrogenic activity is an oestrogen receptor antagonist and is one of the group consisting of tamoxifen, clomiphene citrate or cyclofenil.

20. The use of a compound as claimed in claim 19 wherein the compound is tamoxifen.

21. The use of a compound as claimed in claim 18 wherein said inhibitor of oestrogenic activity is an inhibitor of oestrogen production selected from the group consisting of anastrozole, 4-hydroxy androstenedione, exemestane, oestrone-3-O-sulphate, fadrazole hydrochloride or formestane) or a phytoestrogen.

22. The use of a compound as claimed in claim 14 wherein said compound is an inhibitor of androgenic activity.

23. The use of a compound as claimed in claim 22 wherein said compound is an androgen receptor antagonist



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United States Patent [19]
Santti et al.

[11] **Patent Number:** **5,972,921**
[45] **Date of Patent:** **Oct. 26, 1999**

[54] **USE OF AN AROMATASE INHIBITOR IN THE TREATMENT OF DECREASED ANDROGEN TO ESTROGEN RATIO AND DETRUSOR URETHRAL SPHINCTER DYSYNERGIA IN MEN**

[75] **Inventors:** Risto Santti, Naantali; Antti Telo, Iittainen; Tomi Streng, Turku; Kalle Halonen, Rusko; Lauri Kangas, Raisio; Risto Lammintausta, Turku, all of Finland

[73] **Assignee:** Hormon Medical Oy Ltd., Turku, Finland

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[51] **Int. Cl.⁸** A61K 31/36; A61K 31/44; A61K 31/445; A61K 31/41

[52] **U.S. Cl.** 514/177; 514/179; 514/300; 514/318; 514/383

[58] **Field of Search** 514/383, 300, 514/318, 177, 179

[56] **References Cited**

FOREIGN PATENT DOCUMENTS

WO 94/13645 6/1994 WIPO.

OTHER PUBLICATIONS

Allen R. Glass, MD, "Gynecomastia," 23 *Clinical Andrology* 825-837 (Dec. 1994).

Huma de Lignieres, "Transdermal Dihydrotestosterone Treatment of Andropause," 25 *Ann Med* 235-241 (1993).

Albert Radlmaier et al., "Estrogen Reduction by Aromatase Inhibition for Benign Prostatic Hyperplasia," 29 *The Prostate* 199-209 (1996).

Joseph E. Osterling et al., "Aromatase Inhibition in the Dog," 139 *The Journal of Urology* 832-839 (Apr. 1988).

Heinz Schilcher, "Miktionsbeeinflussende Mittel zur Behandlung der BPH," *Phytotherapie in der Urologie* 69-93 (1992).

Primary Examiner—William R. A. Jarvis
Attorney, Agent, or Firm—Adduci, Mastriani & Schaumborg, L.L.P.

[57] **ABSTRACT**

The invention relates to a method for the treatment of detrusor urethral sphincter dysynergia in men, said method comprising administering to the patient an effective amount of an aromatase inhibitor. The invention also concerns a method for the treatment of decreased androgen to estrogen ratio in men, said method comprising administering to the patient an effective amount of an aromatase inhibitor.

8 Claims, 8 Drawing Sheets

TABLE 8

Estrogen and testosterone concentrations (mean (SD)) before and 1 and 2 days after single doses of MPV-2213d as well as the ratio testosterone to estradiol. Mean (SD) is presented. T = testosterone, E = estradiol. The ratio T to E is calculated as T nmol/l/E pmol/l. Day 0 is the pretreatment value at 8 a.m. On days 1 and 2 the hormone concentrations were measured at the same time (at 8 a.m.) to eliminate the diurnal changes of the hormone concentrations.

Dose (mg)	estradiol pmol/l (day 0)	estradiol pmol/l (day 1)	estradiol pmol/l (day 2)	testosterone nmol/l (day 0)	testosterone nmol/l (day 1)	testosterone nmol/l (day 2)
0.03	124 (36.7)	133 (63.9)	124 (67.1)	25 (4.2)	25 (8.1)	25 (6.9)
0.3	97 (20.4)	85 (15.5)	93 (36.7)	17 (4.6)	19 (7.1)	21 (8.1)
3	92 (11.2)	39 (5.5)	89 (10.1)	16 (3.5)	16 (2.1)	20 (5.0)
9	155 (16.1)	66 (2.5)	149 (23.1)	25 (3.0)	39 (2.6)	32 (5.9)
30	159 (32.6)	56 (19.9)	130 (38.6)	23 (1.2)	31 (1.5)	27 (3.1)
100	116 (31.9)	36 (13.4)	58 (12.1)	14 (3.5)	17 (7.0)	21 (5.0)
300	115 (30.0)	33 (12.8)	55 (17.8)	17 (3.3)	24 (4.3)	24 (4.1)
600	101 (43.6)	33 (16.6)	51 (28.6)	18 (5.1)	23 (6.6)	24 (8.7)

Dose (mg)	Ratio T to E (day 0)	Ratio T to E (day 1)	Ratio T to E (day 2)
0.03	0.20	0.18	0.20
0.3	0.18	0.22	0.22
3	0.17	0.41	0.22
9	0.16	0.42	0.22
30	0.14	0.53	0.30
100	0.12	0.47	0.36
300	0.14	0.72	0.43
600	0.17	0.70	0.47

On the basis of the results obtained in the animal model as described above for the study of detrusor urethral sphincter dyssynergia, we suggest that estrogens play an essential role in the development of the muscular dysfunction involved in detrusor urethral sphincter dyssynergia. Experiments in developmentally estrogenized rats with the aromatase inhibitor MPV-2213d show that the estrogen-related neuromuscular changes are at least partially reversible. Experiments in young men (<40 yrs) show that with a single dose of MPV-2213d estradiol concentrations fall dramatically and testosterone concentrations increase slightly enabling thus improvement of DATER.

It will be appreciated that the methods of the present invention can be incorporated in the form of a variety of embodiments, only a few of which are disclosed herein. It will be apparent for the specialist in the field that other embodiments exist and do not depart from the spirit of the invention. Thus, the described embodiments are illustrative and should not be construed as restrictive.

REFERENCES

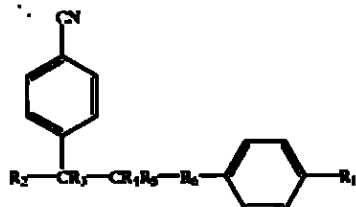
- Hinman, F. and Baumann, F. W. Vesical and urethral damage from voiding dysfunction in boys without neurologic or obstructive disease. *J. Urol.* 109:727, 1973.
- Hoffman, B. F., P. F. Crane, E. Lepeschkin, B. Surawick, and H. C. Herrlich. Comparison of cardiac monophasic action potentials recorded by intracellular and suction electrodes. *Am. J. Physiol.* 196(6):1297-1301, 1959.
- Iosif, C. S., Batra, S., Elk, A. and Amedt. Estrogen receptors in the human female lower urinary tract. *Am. Obstet. Gynecol.* 141:817, 1981.
- Kaplan, S. A., Santarosa, R. P., d'Alisera, P. M., Fay, B. J., Ikeguchi, E. F., Hendricks, J. Klein, L. and Te, A. L.

Pseudodysynergia (contraction of the external phincter during voiding) misdiagnosed as chronic nonbacterial prostatitis and the role of biofeedback as a therapeutic option. *J. of Urol.* 157:2234-2237, 1997.

- Kaplan, S. A., Ikeguchi, E. F., Santarosa, R. P., d'Alisera, P. M., Hendricks, J. Klein, L., Te, A. E. and Miller, M. Etiology of voiding dysfunction in men less than 50 years of age. *Urology* 47:R36-R39, 1996.
- Larsson, B., Andersson, K.-E., Batra, S., Mattiasson, A. and Sjogren, C. Effects of Estradiol on Norepinephrine-Induced Contraction, Alpha Adrenoceptor Number and Norepinephrine Content in the Female Rabbit Urethra. *The Journal of Pharmacology and Experimental Therapeutics* 229:557-563, 1984.
- Letrozole CGS-20267. Aromatase inhibitor. *Drugs of the Future* 19:335-337, 1994.
- Maggi, C. A., P. Santicoli, and A. Meli. The nonstop transvesical cystometrogram in urethane-anesthetized rats. *J. of Pharm. Meth.* 15: 157-167, 1986a.
- Morita, T., Tsuchiya, N., Tsuji, T. and Kondo, S. Changes of Autonomic Receptors Following Castration and Estrogen Administration in the Male Rabbit Urethral Smooth Muscle. *Tohoku J. Exp. Med.* 166:403-405, 1992.
- Oelrich, T. H. The Urethral Sphincter Muscle in the Male. *Am. J. of Anat.* 158:229-246, 1980.
- Prostatic Obstruction. Pathogenesis and treatment. Ed. Christopher R. Chapple. Springer-Verlag 1994.
- Rudy, D. C., Awad, S. A. and Downe, J. W. External sphincter dyssynergia: An abnormal continence reflex. *J. of Urol.* 140:105-110, 1988.
- Smith, P., Heimer, G., Norgren, and Ulmsten, U. Steroid Hormone Receptors in Pelvic Muscles and Ligaments in Women. *Gynecol. Obstet. Invest.* 30:27-30, 1990.
- Strauss, H., Klima, G., Pöschel, S., Horninger, W. and Hartsch, G. Anatomy and Innervation of the Rhabdosphincter of the Male Urethra. *Prostate* 28:24-31, 1996.
- Truett PR, Mueller PH, Bhattacharya AS, Dickers J, Monnet G, White G: Open dose-finding study of a new potent and selective nonsteroidal aromatase inhibitor, CGS 20 267, in healthy male subjects. *J Clin Endocrinol Metab* 77: 319-323, 1993.
- van Asselt, E., J. Groen, and R. van Mastrigt. A comparative study of voiding in rat and guinea pig: simultaneous measurement of flow rate and pressure. *Am. J. of Physiol.* 269 (Regulatory Integrative Comp. Physiol. 38): R98-R103, 1995.
- Vanden Broucke H, Moerckx H, Kuymans J.M.H.: Aromatase inhibitors—mechanism for non-steroidal inhibitors. *Breast Cancer Res Treatm* 30: 43-55, 1994
- Wilson, P. D., Barker, G., Barnard, R. J. and Siddie, N. C. Steroid Hormone Receptors in the Female Urinary Tract. *Urol. Int.* 39:5-8, 1984.
- Yablonsky, F., Savasta, M., Manier, M., Poirer, M., Lacolle, J. Y. and Freustein, C. Autoradiographic Localization of α_1 -Adrenoceptors in the Dog Prostate and Urethra With ^{3} H-Prazosin. *Neurourology and Urodynamics* 10:257-266, 1991.
- We claim:
1. A method for the treatment of (i) detrusor urethral sphincter dyssynergia in men, wherein said detrusor urethral sphincter dyssynergia is urethral dysfunction which is due to decreased androgen to estrogen ratio in a patient, or (ii) decreased androgen to estrogen ratio in men, said method comprising administering to the patient an effective amount of an aromatase inhibitor.

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2. The method according to claim 1 wherein the aromatase inhibitor is a compound of selected from the group consisting of anastrozole, fadrozole, letrozole, vorozole, rugelthimide, stamestane, exemestane, formestane, YM-511 (4-[N-(4-bromobenzyl)-N-(4-cyanophenyl)amino]-4H-1,2,4-triazole), ZD-1033 (arimedex) and NKS-01 (14- α -hydroxyandrost-4-ene-3,6,17-trione); or a compound of formula (I)



wherein R_1 is hydrogen, methyl, methoxy, nitro, amino, cyano, trifluoromethyl, difluoromethyl, monofluoromethyl or halogen; R_2 is a heterocyclic radical selected from 1-imidazolyl, triazolyl, tetrazolyl, pyrazolyl, pyrimidinyl, oxazolyl, thiazolyl, isoxazolyl and isothiazolyl; R_3 is hydrogen or hydroxy; R_4 is hydrogen; R_5 is hydrogen or hydroxy; or R_4 is hydrogen and R_3 and R_5 combined form a bond; or R_3 is hydrogen and R_4 and R_5 combined form $=O$; R_6 is methylene, ethylene, $-\text{CHOH}-$, $-\text{CH}_2\text{CHOH}-$,

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$-\text{CHOH}-\text{CH}_2-$, $-\text{CH}=\text{CH}-$ or $-\text{C}(=\text{O})-$; or R_4 is hydrogen and R_3 and R_6 combined is $-\text{CH}-$ or $-\text{CH}-\text{CH}_2-$; or a stereoisomer, or a non-toxic pharmaceutically acceptable acid addition salt thereof.

3. The method according to claim 2 wherein the aromatase inhibiting compound is 1-[1-(4-cyanophenyl)-3-(4-fluorophenyl)-2-hydroxypropyl]-1,2,4-triazole, a stereoisomer or a non-toxic pharmaceutically acceptable acid addition salt thereof.

4. The method according to claim 3 wherein the aromatase inhibiting compound is 1-[1-(4-cyanophenyl)-3-(4-fluorophenyl)-2-hydroxypropyl]-1,2,4-triazole, diastereoisomer a+d.

5. The method according to claim 1 wherein said detrusor urethral sphincter dysynergia in combination with enlargement of the prostate.

6. The method according to claim 1 wherein said detrusor urethral sphincter dysynergia is not combined enlargement of the prostate.

7. The method according to claim 1 wherein the decreased androgen to estrogen ratio is expressed as symptoms related to the andropause.

8. The method according to claim 7 wherein said symptoms are selected from the group consisting of infertility, obesity, erectile dysfunction, impotence and gynecomastia.

* * * * *

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A01N 43/50	A1	(11) International Publication Number: WO 99/45779 (43) International Publication Date: 16 September 1999 (16.09.99)
(21) International Application Number: PCT/US99/04651 (22) International Filing Date: 11 March 1999 (11.03.99) (30) Priority Data: 60/077,660 11 March 1998 (11.03.98) US (71) Applicant (for all designated States except US): SMITHKLINE BEECHAM CORPORATION [US/US]; One Franklin Plaza, Philadelphia, PA 19103 (US). (72) Inventor; and (73) Inventor/Applicant (for US only): VENKATESH, Gopadi, M. [US/US]; 544 General Learned Road, King of Prussia, PA 19406 (US). (74) Agents: McCARTHY, Mary, E. et al.; SmithKline Beecham Corporation, Corporate Intellectual Property, UW 2220, 709 Swedeland Road, P.O. Box 1539, King of Prussia, PA 19406-0939 (US).		(81) Designated States: AE, AL, AU, BA, BE, BG, BR, CA, CN, CZ, EE, GB, GH, GM, HR, HU, ID, IL, IN, IS, JP, KP, KR, LC, LK, LR, LT, LV, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, SL, TR, TT, UA, US, UZ, VN, YU, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: NOVEL COMPOSITIONS OF EPROSARTAN (57) Abstract This invention relates to a novel composition comprising eprosartan, or a salt, solvate, or hydrate thereof, in particulate form, a process for its production and methods of using the composition to block angiotensin II receptors and to treat hypertension, congestive heart failure and renal failure.		

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What is claimed is:

1. A pharmaceutical composition comprising eprosartan, or a salt, solvate, or hydrate thereof, in particulate form, said composition having a particle size distribution such that the median value of the volume mean diameter is within the range of from 450 to 700 nm.
2. The composition according to claim 1 wherein eprosartan, or a salt, solvate, or hydrate thereof, is eprosartan mesylate.
3. The composition according to claim 1 which is produced by wet milling eprosartan, or a salt, solvate, or hydrate thereof, in the presence of water and pharmaceutically acceptable excipients.
4. The composition wherein the excipient is hydroxypropylmethyl cellulose or mannitol.
5. A process for the preparation of the composition according to claim 1 in which the eprosartan, or a salt, solvate, or hydrate thereof, in particulate form, is produced by milling eprosartan, or a salt, solvate, or hydrate thereof, using a bead mill in the presence of hard zirconium oxide beads, water and hydroxypropylmethyl cellulose, optionally adding one or more pharmaceutically acceptable excipients, spray drying and filling the resulting powder into capsules.
6. A method of blocking angiotensin II receptors which comprises administering to a subject in need thereof an effective amount of the composition according to claim 1.
7. A method of treating hypertension which comprises administering to a subject in need thereof an effective amount of the composition according to claim 1.
8. A method of treating congestive heart failure which comprises administering to a subject in need thereof an effective amount of the composition according to claim 1.
9. A method of treating renal failure which comprises administering to a subject in need thereof an effective amount of the composition according to claim 1.

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10. A method of treating hypertension which comprises administering stepwise or in physical combination the composition according to claim 1 and a second pharmaceutically active compound selected from the group consisting of a diuretic, a calcium channel blocker, a β -adrenoceptor blocker, a renin inhibitor, and an angiotensin converting enzyme inhibitor.
11. The method according to claim 10 wherein the second pharmaceutically active compound is a diuretic.
12. The method according to claim 11 wherein the diuretic is hydrochlorothiazide.
13. The use of a composition according to claim 1 for the treatment of diseases in which blockade of the angiotensin II receptor is indicated.
14. The use of a composition according to claim 1 for the treatment of hypertension.
15. The use of a composition according to claim 1 for the treatment of congestive heart failure.
16. The use of a composition according to claim 1 for the treatment of renal failure.
17. The use of a composition according to claim 1 in combination with a diuretic for the treatment of hypertension.
18. The use according to claim 17 wherein the diuretic is hydrochlorothiazide.



AA 328673
NOTARIA TERCERA
EXPEDIENTE 114

ESCRITURA NUMERO CUATRO MIL QUINIENTOS -
TREINTA Y NUEVE (No.4.539) -----

En la ciudad de Cali, Capital del Depar-
tamento del Valle del Cauca, República de
Colombia, a los VEINTITRES (23) días -
del mes de OCTUBRE del año DOS MIL UNO --

(2.001), ante mí, JORGE ENRIQUE CAICEDO ZAMORANO, Notario

T E R C E R O (3o.) del Círculo de Cali, - - - - -

- - - - -

- - - - -

compareció JUAN MANUEL BARBERI OSPINA mayor de edad, vecino de Cali,
identificado con la cédula de ciudadanía número 14.949.561 de Cali, y dijo: ----:

PRIMERO.- Que obra en su carácter de Representante Legal de en nombre y
representación de TECNOQUIMICAS S.A., sociedad comercial organizada y existente
conforme a las leyes colombianas, con domicilio y sede principal en Cali constituida
mediante escritura pública número 2670 de la Notaría Segunda de Cali de fecha 12 de
junio de 1957, reformada en distintas oportunidades, según consta en el certificado
expedido por la Cámara de Comercio de Cali -----

SEGUNDO: Que en tal carácter, por este público instrumento, concede Poder Especial
pero amplio y suficiente a JOSE LUIS REYES VILLAMIZAR, mayor de edad, vecino de
Bogotá, identificado con C.C. # 79.152.473 c. Isaquén, abogado en ejercicio, portador
de la T.P 44655 del C.S.de la J., domiciliado en Bogotá, D.C., República de Colombia
con dirección carrera 7 No. 88.23 oficina 205, de la misma ciudad, como su representante
con poder especial, amplio y suficiente, para obtener la protección de nuestra propiedad
industrial y contra la competencia desleal, a cuyo efecto lo autorizo, para que nos
represente ante cualesquiera entidad o persona, ejerzan o no jurisdicción, especialmente
ante las del orden administrativo, contencioso-administrativo y judicial, en todo lo
concerniente a los siguientes asuntos: a) Obtención, formulación de observaciones y
oposición a patentes de invención, modelos y dibujos industriales y registro de
licencias de uso de esos derechos; b) Las acciones de oposición, cancelación, nulidad,
medidas cautelares, concesión de licencias y en general los procesos civiles, penales,
administrativos o contencioso-administrativos relacionados con estos derechos u otros de
diferente naturaleza; acciones y procesos que promovamos o se nos promuevan; y c) para

ESTE PAPEL NO TIENE COSTO ALGUNO PARA EL USUARIO

4539
0523

LUZ MERY CORREA
ELABORACION DE ESCRITURAS
Notaría 3ª de Cali

NOTA: Este documento es una copia de la escritura pública original, la cual se encuentra en el expediente 114 de la Notaría Tercera de Cali.

que en todos los asuntos arriba determinados, , tanto en los términos señalados por los artículos 42 y complementarios de la Decisión 486 de la Comunidad Andina como del artículo 70 del C.P.C pueda sustituir este mandato, transigir, conciliar, admitir los hechos del proceso, desistir, cancelar, recibir, renunciar, y ratificar los actos de agentes oficiosos y ceder a título gratuito y/o oneroso, licenciar, inscribir contratos de cesión y de licencia, inscripción de prendas, levantamiento de prendas, inscribir derechos de retención y en general todos los asuntos que se deban tramitar ante la Superintendencia de Industria y Comercio.-----

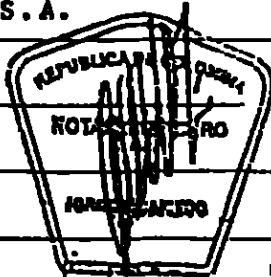
Leído el presente instrumento al otorgante, lo aprobó y en constancia firma conmigo el Notario de todo lo cual doy fe.-----

Se agregan comprobantes. Derechos \$30.000.00 Decreto 1681 de Septiembre 16 de 1996. Reformado mediante Resolución No.5839 de Diciembre 27 del año 2.000 y Decreto 1326 de Julio 06 del año 2.001. Se corrigió en la hoja número AA 3286877. ENMENDADO "OCTUBRE" VALE.

Juan m. Barberi

JUAN MANUEL BARBERI OSPINA

En rep. de TECNOQUIMICAS S.A.



El Notario Tercero de Cali

Dr. JORGE ENRIQUE CAICEDO ZAMORANO

EN JUNIO DE ESTE AÑO SE ELEGIRA JUNTA DIRECTIVA DE LA CAMARA DE COMERCIO. LAS INSCRIPCIONES DE CANDIDATOS DEBEN HACERSE EN LA PRIMERA QUINCENA DE MAYO. PARA INFORMACION DETALLADA DIRIGIRSE A LA SEDE PRINCIPAL O COMUNICARSE AL SIGUIENTE TELEFONO: 8861339

CAMARA DE COMERCIO DE CALI

FECHA: 23 DE MAYO DE 2,002 HORA: 15:50:09 PAGINA 1
0036226328

CERTIFICADO DE EXISTENCIA Y REPRESENTACION

EL SUSCRITO SECRETARIO DE LA CAMARA DE COMERCIO DE CALI

C E R T I F I C A :

NOMBRE:
TECNOQUIMICAS S A

AFILIADO

DOMICILIO: CALI VALLE

DIRECCION COMERCIAL: CL. 23 No. 7 39

DIRECCION NOTIFICACION JUDICIAL: CL. 23 No. 7 39

CIUDAD: CALI

MATRICULA NRO: 007049-04

C E R T I F I C A :

NIT No. 890,300,466 -5

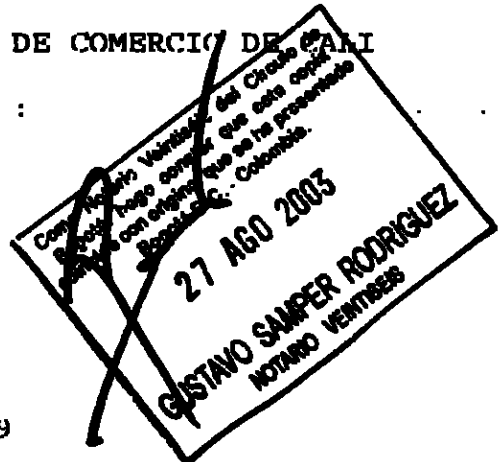
C E R T I F I C A :

CONSTITUCION: POR ESCRITURA NRO. 2670 DEL 12 DE JUNIO DEL AÑO 1957, NOTARIA SEGUNDA CALI INSCRITA EN LA CAMARA DE COMERCIO EL 01 DE JULIO DEL AÑO 1957 BAJO EL NRO. 16627 DEL LIBRO IX, SE CONSTITUYO LA SOCIEDAD DENOMINADA: COMPANIAS TECNOQUIMICAS LIMITADA

C E R T I F I C A :

QUE POR ESCRITURA PUBLICA NRO. 8438 DEL 30 DE DICIEMBRE DEL AÑO 1970, NOTARIA SEGUNDA CALI INSCRITA EN LA CAMARA DE COMERCIO EL 07 DE ENERO DEL AÑO 1971 BAJO EL NRO. 42260 DEL LIBRO IX, LA SOCIEDAD SE TRANSFORMO DE LIMITADA----- EN COMANDITA POR ACCIONES ----- BAJO EL NOMBRE DE: COMPANIAS TECNOQUIMICAS LIMITADA COMANDITA POR ACCIONES (SIC).

C E R T I F I C A :



CAMARA DE COMERCIO DE CALI

FECHA: 23 DE MAYO

DE 2,002

HORA: 15:50:09

PAGINA 2
0036226328

116

QUE POR LA ESCRITURA NRO. 2139 DEL 21
DE ABRIL DEL AÑO 1972, NOTARIA SEGUNDA CALI
INSCRITA EN LA CAMARA DE COMERCIO EL 26 DE ABRIL DEL AÑO
1972 BAJO EL NRO. 00794 DEL LIBRO IX, LA SOCIEDAD CAMBIO SU
NOMBRE DE:
COMPANIAS TECNOQUIMICAS LIMITADA COMANDITA POR ACCIONES
(SIC)
POR EL DE:
TECNOQUIMICAS LIMITADA & CIA S.C.A.

C E R T I F I C A :

QUE POR ESCRITURA PUBLICA NRO. 3255 DEL 30 DE AGOSTO
DEL AÑO 1983, NOTARIA DECIMA DE CALI
INSCRITA EN LA CAMARA DE COMERCIO EL 31 DE AGOSTO DEL AÑO
1983 BAJO EL NRO. 62343 DEL LIBRO IX, LA SOCIEDAD SE TRANS-
FORMO DE COMANDITA POR ACCIONES EN ANONIMA-----
BAJO EL NOMBRE DE:
TECNOQUIMICAS S A

C E R T I F I C A :

REFORMAS:

ESCRITURA No.	FECHA	NOTARIA	FECHA	No. INS	LIBRO
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FECHA: 23 DE MAYO

DE 2,002

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PAGINA 3
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2624	30-06-2000	03 CALI	07-07-2000	04740 IX
3814	15-09-2000	03 CALI	18-09-2000	06381 IX

C E R T I F I C A :

VIGENCIA: QUE LA SOCIEDAD NO SE HALLA DISUELTA Y SU DURACION ES
HASTA EL 01 DE ENERO DEL AÑO 2057

C E R T I F I C A :

OBJETO SOCIAL. LA SOCIEDAD TIENE POR OBJETO: A) LA FABRICACION, EL ENVASADO, LA IMPORTACION, LA EXPORTACION, LA VENTA Y DISTRIBUCION DE PRODUCTOS BIOLOGICOS Y QUIMICOS, MEDICAMENTOS O PRODUCTOS FARMACEUTICOS, PERFUMERIA O EFECTOS DE TOCADOR, PRODUCTOS VETERINARIOS, AGRICOLAS Y ALIMENTOS NATURALES Y/O PRODUCTOS DE CONSUMO POPULAR DE CUALQUIER NATURALEZA Y SERVICIOS EN GENERAL. B) INVERTIR EN SOCIEDADES O EMPRESAS DE CUALQUIER NATURALEZA O ESPECIE DE LA SOCIEDAD MERCANTIL, NACIONAL O EXTRANJERA, QUE TENGAN COMO ACTIVIDAD O NEGOCIO PROPIO DE SU OBJETO LA FABRICACION, DISTRIBUCION Y COMERCIO EN GENERAL DE PRODUCTOS BIOLOGICOS Y QUIMICOS, MEDICAMENTOS O PRODUCTOS FARMACEUTICOS, PERFUMERIA O EFECTOS DE TOCADOR, PRODUCTOS VETERINARIOS, AGRICOLAS, ALIMENTOS NATURALES Y/O PROCESADOS, PRODUCTOS DE CONSUMO POPULAR, SERVICIOS EN GENERAL O LA EXPLOTACION DE ESTABLECIMIENTOS DE COMERCIO DEDICADOS A DICHO GIRO. C) ADQUIRIR ACCIONES, CUOTAS SOCIALES, BONOS CEDULAS, TITULOS U OTROS VALORES EN COMPANIAS DE CUALQUIER NATURALEZA O TIPO LEGAL, DEDICADAS A LA EXPLOTACION DE CUALQUIER ACTIVIDAD ECONOMICA LICITA Y ENAJENARLAS. D) LA EXPLOTACION DEL NEGOCIO DE LA PUBLICIDAD Y EL ESTABLECIMIENTO DE OFICINAS DEDICADAS A ESTE NEGOCIO O A LA CREACION DE ORGANOS DE PUBLICIDAD HABLADOS O ESCRITOS. E) GARANTIZAR LAS OBLIGACIONES PROPIAS DE LOS ACCIONISTAS O DE TERCEROS. EN DESARROLLO DEL OBJETO SOCIAL PODRA OBTENER PRIVILEGIOS INDUSTRIALES, CONCESIONES, PATENTES DE INVENCION, DE MEJORAS O PROCEDIMIENTOS, MARCAS DE FABRICA O DE COMERCIO, YA SEA EN NOMBRE PROPIO O DE TERCEROS, OTORGAR A TERCEROS, DENTRO O FUERA DEL PAIS CONCESIONES O USOS DE

EXPLOTACION SOBRE ESTOS DERECHOS, EJECUTAR TODA CLASE DE CONTRATOS DE COMPRAVENTA, DISTRIBUCION DE MATERIAS PRIMAS, DE PRODUCTOS SEMIELABORADOS O ELABORADOS EN EL MERCADO INTERNO O EN EL EXTERIOR Y EN GENERAL, LA CELEBRACION DE TODO ACTO O CONTRATO SOBRE BIENES MUEBLES O INMUEBLES DE COMERCIO, INDUSTRIALES O DE OTRA INDOLE QUE SE REFIERAN DIRECTAMENTE A LAS ACTIVIDADES QUE PERSIGUE LA SOCIEDAD.

C E R T I F I C A :

ORGANOS DE DIRECCION Y ADMINISTRACION. PARA LOS FINES DE SU DIRECCION, ADMINISTRACION Y REPRESENTACION, LA SOCIEDAD TIENE LOS SIGUIENTES ORGANOS: A) ASAMBLEA GENERAL DE ACCIONISTAS. B) JUNTA DIRECTIVA. C) PRESIDENTE EJECUTIVO. D) TRES VICEPRESIDENTES EJECUTIVOS. E) VICEPRESIDENCIAS ADMINISTRATIVAS.

LA DIRECCION DE LA SOCIEDAD CORRESPONDE, EN PRIMER TERMINO A LA ASAMBLEA GENERAL DE ACCIONISTAS Y EN SEGUNDO LUGAR, A LA JUNTA DIRECTIVA COMO DELEGADA DE AQUELLA.

LA REPRESENTACION LEGAL DE LA SOCIEDAD Y LA GESTION DE LOS NEGOCIOS SOCIALES, ESTARAN A CARGO DEL PRESIDENTE EJECUTIVO Y DE LOS TRES VICEPRESIDENTES EJECUTIVOS.

FUNCIONES DE LA ASAMBLEA DE ACCIONISTAS. ENTRE OTRAS LAS SIGUIENTES: 1) ACORDAR LA FUSION DE LA SOCIEDAD, SU TRANSFORMACION, LA ENAJENACION O EL ARRENDAMIENTO DE LA EMPRESA SOCIAL, LA DISOLUCION O LA PRORROGA Y CUALQUIER REFORMA, AMPLIACION MODIFICACION DEL CONTRATO SOCIAL. 9) AUTORIZAR LA ADQUISICION DE ACCIONES PROPIAS, CON SUJECION A LOS REQUISITOS ESTABLECIDOS POR LA LEY. 10) ADOPTAR EN GENERAL, TODAS LAS MEDIDAS QUE RECLAMEN EL CUMPLIMIENTO DE LOS ESTATUTOS O EL INTERES DE LA SOCIEDAD.

EN LA JUNTA DIRECTIVA SE ENTIENDE DELEGADO EL MAS AMPLIO MANDATO PARA ADMINISTRAR LA SOCIEDAD Y POR CONSIGUIENTE, TENDRA ATRIBUCIONES SUFICIENTES PARA ORDENAR QUE SE EJECUTE O CELEBRE CUALQUIER ACTO O CONTRATO COMPRENDIDO DENTRO DEL OBJETO SOCIAL Y PARA ADOPTAR LAS DETERMINACIONES NECESARIAS EN ORDEN A QUE LA SOCIEDAD CUMPLA SUS FINES Y DE MANERA ESPECIAL, TENDRA LAS SIGUIENTES FUNCIONES ENTRE OTRAS: C) NOMBRAR Y REMOVER LIBREMENTE AL PRESIDENTE EJECUTIVO, A LOS VICEPRESIDENTES EJECUTIVOS Y A LOS VICEPRESIDENTES ADMINISTRATIVOS. E) DISPONER EL ESTABLECIMIENTO O LA CLAUSURA DE SUCURSALES O AGENCIAS, DENTRO O FUERA DEL DOMICILIO SOCIAL. F) FIJAR LAS POLITICAS DE LA SOCIEDAD EN LOS DIFERENTES ORDENES DE SU ACTIVIDAD, ESPECIALMENTE EN MATERIA FINANCIERA, ECONOMICA Y LABORAL; APROBAR PLANES DE INVERSION Y DE PRODUCCION; DECIDIR SOBRE NUEVAS LINEAS DE PRODUCCION Y VENTA, ESTABLECER NORMAS GENERALES EN MATERIA DE PRECIOS Y DE VENTAS DE LOS PRODUCTOS ELABORADOS POR LA SOCIEDAD O DE LOS MATERIALES Y DEMAS ELEMENTOS EN QUE ELLA NEGOCIE Y DICTAR NORMAS Y REGLAMENTOS PARA LA ORGANIZACION Y EL FUNCIONAMIENTO DE TODAS LAS DEPENDENCIAS DE LA SOCIEDAD.

G) DETERMINAR LA APLICACION QUE DEBA DARSE A LAS UTILIDADES QUE, CON EL CARACTER DE RESERVAS DE INVERSION, HAYAN SIDO APROPIADAS POR LA ASAMBLEA DE ACCIONISTAS. H) AUTORIZAR PREVIAMENTE LAS OPERACIONES QUE TENGAN POR OBJETO: 1) ADQUIRIR, HIPOTECAR Y EN CUALQUIER OTRA FORMA GRAVAR O LIMITAR EL DOMINIO DE BIENES RAICES CUYA CUANTIA SEA SUPERIOR A \$100.000.000.00 MONEDA CORRIENTE; 2) CONSTITUIR PRENDA SOBRE BIENES SOCIALES O DARLOS EN ANTICRESIS PARA ACTOS O CONTRATOS CUYA CUANTIA SEA SUPERIOR A \$100.000.000.00 MONEDA CORRIENTE; 3) HACER INVERSIONES EN BIENES DE CAPITAL O EFECTUAR REPARACIONES O MEJORAS DE LOS MISMOS, CUANDO UNAS U OTRAS IMPLIQUEN, EN CADA CASO, COSTOS O DESEMBOLSOS EN CUANTIA SUPERIOR A \$100.000.000.00 MONEDA LEGAL; 4) AUTORIZAR LOS CONTRATOS DE MUTUO QUE PUEDAN CELEBRAR LA SOCIEDAD LOS REPRESENTANTES LEGALES CUANDO SU CUANTIA SEA SUPERIOR A \$100.000.000.00 MONEDA LEGAL COLOMBIANA O A US\$300.000.00 L) AUTORIZAR AL REPRESENTANTE LEGAL PARA QUE SOLICITE, LLEGADO EL CASO, QUE SE ADMITA A LA COMPANIA A LA CELEBRACION DE CONCORDATO PREVENTIVO CON SUS ACREEDORES. M) SERVIR DE ORGANO CONSULTIVO Y ASESOR DE LOS REPRESENTANTES DE LA COMPANIA.

LA ADMINISTRACION INMEDIATA DE LA COMPANIA, SU REPRESENTACION LEGAL Y LA GESTION DE LOS NEGOCIOS SOCIALES ESTARAN A CARGO DEL PRESIDENTE EJECUTIVO Y DE LOS TRES VICEPRESIDENTES EJECUTIVOS, DESIGNADOS POR LA JUNTA DIRECTIVA PARA PERIODOS DE UN AÑO, REELEGIBLES INDEFINIDAMENTE Y REMOVIBLES LIBREMENTE POR ELLA EN CUALQUIER TIEMPO, QUIENES COMO REPRESENTANTES LEGALES PODRAN ACTUAR CONJUNTA O SEPARADAMENTE CON IGUALDAD DE FUNCIONES Y CALIDADES. TODOS LOS EMPLEADOS DE LA COMPANIA CON EXCEPCION DE LOS DESIGNADOS POR LA ASAMBLEA DE ACCIONISTAS Y LOS DEPENDIENTES DEL REVISOR FISCAL, SI LOS HUBIERE, ESTARAN SOMETIDOS EN EL DESEMPEÑO DE SUS CARGOS A LOS REPRESENTANTES LEGALES.

LOS REPRESENTANTES LEGALES SON MANDATARIOS CON REPRESENTACION INVESTIDOS DE FUNCIONES EJECUTIVAS Y ADMINISTRATIVAS Y COMO TALES TIENEN A SU CARGO LA REPRESENTACION LEGAL DE LA COMPANIA, LA GESTION COMERCIAL Y FINANCIERA, LA RESPONSABILIDAD DE LA ACCION ADMINISTRATIVA, LA COORDINACION Y LA SUPERVISION GENERAL DE LA EMPRESA, LAS CUALES CUMPLIRAN CON ARREGLO A LAS NORMAS DE LOS ESTATUTOS Y A LAS DISPOSICIONES LEGALES Y CON SUJECION A LAS ORDENES E INSTRUCCIONES DE LA JUNTA DIRECTIVA.

COMO REPRESENTANTES LEGALES DE LA COMPANIA EN JUICIO Y FUERA DE JUICIO, LOS REPRESENTANTES TIENEN FACULTADES PARA EJECUTAR O CELEBRAR SIN OTRAS LIMITACIONES QUE LAS ESTABLECIDAS EN LOS ESTATUTOS EN CUANTO SE TRATE DE OPERACIONES QUE DEBAN SER PREVIAMENTE AUTORIZADAS POR LA JUNTA DIRECTIVA O POR LA ASAMBLEA DE ACCIONISTAS, TODOS LOS ACTOS O CONTRATOS COMPRENDIDOS DENTRO DEL OBJETO SOCIAL O QUE TENGAN CARACTER SIMPLEMENTE PREPARATORIO, ACCESORIO O COMPLEMENTARIO PARA LA REALIZACION DE LOS FINES QUE PERSIGUE LA SOCIEDAD Y LOS QUE SE RELACIONAN DIRECTAMENTE CON LA EXISTENCIA Y EL FUNCIONAMIENTO DE LA MISMA. LOS REPRESENTANTES QUEDAN INVESTIDOS DE PODERES ESPECIALES PARA TRANSIGIR, ARBITRAR Y COMPROMETER LOS NEGOCIOS SOCIALES.

PROMOVER O COADYUVAR ACCIONES JUDICIALES, ADMINISTRATIVAS O CONTENCIOSO ADMINISTRATIVAS EN QUE LA COMPANIA TENGA INTERES E INTERPONER TODOS LOS RECURSOS QUE SEAN PROCEDENTES CONFORME A LA LEY; DESISTIR DE LAS ACCIONES O RECURSOS QUE INTERPONGAN; NOVAR OBLIGACIONES O CREDITOS; DAR O RECIBIR BIENES EN PAGO; CONSTITUIR APODERADOS JUDICIALES O EXTRAJUDICIALES, DELEGARLES FACULTADES REVOCAR MANDATOS Y SUSTITUCIONES.

PARAGRAFO: LOS REPRESENTANTES LEGALES DE LA SOCIEDAD TIENEN AMPLIAS FACULTADES, SIN NINGUNA LIMITACION Y SIN REQUERIR AUTORIZACION PREVIA DE LA JUNTA DIRECTIVA, PARA ACTUAR EN NOMBRE DE LA SOCIEDAD EN LICITACIONES PUBLICAS, PRIVADAS O CUALQUIER OTRA MODALIDAD DE CONTRATACION, PRESENTAR PROPUESTAS, ACEPTAR ADJUDICACIONES Y CONTRATAR SIN LIMITACION DE CUANTIA ALGUNA ANTE LAS ENTIDADES PUBLICAS, EMPRESAS INDUSTRIALES Y COMERCIALES DEL ESTADO, SOCIEDADES DE ECONOMIA MIXTA, BIEN SEAN ELLAS DEL ORDEN NACIONAL, DISTRITAL, DEPARTAMENTAL O MUNICIPAL.

C E R T I F I C A :

NOMBRAMIENTOS:

DOCUMENTO: ACTA NRO: 393
FECHA: 1993/01/20
ORIGEN: JUNTA DIRECTIVA
FECHA INSCRIPCION: 1993/01/26 NRO. 62203 LIBRO: 09

FUERON NOMBRADOS:

FRANCISCO JOSE BARBERI OSPINA
C.C. 14,445,880
PRESIDENTE EJECUTIVO

JUAN MANUEL BARBERI OSPINA
C.C. 14,949,561
VICEPRESIDENTE EJECUTIVO

JUAN B. DE LOS RIOS GUTIERREZ
C.C. 17,108,621
VICEPRESIDENTE EJECUTIVO

EMILIO SARDI APARICIO
C.C. 14,434,582
VICEPRESIDENTE EJECUTIVO

C E R T I F I C A :

DOCUMENTO: ACTA NRO: 38
FECHA: 1992/02/03
ORIGEN: ASAMBLEA EXTRAORDINARIA DE ACCIONISTAS
FECHA INSCRIPCION: 1992/06/05 NRO. 54016 LIBRO: 09
DOCUMENTO: ACTA NRO: 52

FECHA: 23 DE MAYO

DE 2,002

HORA: 15:50:09

FECHA: 1999/03/26

ORIGEN: ASAMBLEA GENERAL DE ACCIONISTAS

FECHA INSCRIPCION: 1999/05/10 NRO. 03193

LIBRO: 09

DOCUMENTO: ACTA

NRO: 54

FECHA: 2000/03/31

ORIGEN: ASAMBLEA GENERAL DE ACCIONISTAS

FECHA INSCRIPCION: 2000/05/24 NRO. 03622

LIBRO: 09

FUERON NOMBRADOS:

JUNTA DIRECTIVA

PRINCIPALES

SUPLENTES

FRANCISCO JOSE BARBERI O.

GUSTAVO SANCHEZ L.

C.C. 14,445,880

C.C. 16,625,960

JUAN MANUEL BARBERI O.

VICTOR ROSA G.

C.C. 14,949,561

C.C. 6,811,102

JUAN B. DE LOS RIOS G.

CARLOS ALBERTO JIMENEZ

C.C. 17,108,621

C.C. 16,702,695

EMILIO SARDI A.

ERNESTO TRUJILLO PEREZ

C.C. 14,434,582

C.C. 14,984,104

DIEGO QUIJANO REISSNER

ANA MARIA ALVAREZ

C.C. 16,615,708

C.C. 31,170,896

C E R T I F I C A :

DOCUMENTO: ACTA

NRO: 54

FECHA: 2000/03/31

ORIGEN: ASAMBLEA GENERAL DE ACCIONISTAS

FECHA INSCRIPCION: 2000/05/03 NRO. 02989

LIBRO: 09

FUERON NOMBRADOS:

ARTHUR ANDERSEN & CIA. LTDA

NIT. 860,039,453

REVISOR FISCAL

C E R T I F I C A :

DOCUMENTO: DCTO. PRIVADO

NRO: S/N

FECHA: 2000/04/27

FECHA INSCRIPCION: 2000/05/03 NRO. 02990

LIBRO: 09

FECHA: 23 DE MAYO

DE 2,002

HORA: 15:50:09

PAGINA 8

0036226328 172

LA FIRMA ARTHUR ANDERSEN & CIA. LTDA
NOMBRE A:

JUAN CARLOS GUTIERREZ GONZALEZ
C.C. 16,737,040
REVISOR FISCAL PRINCIPAL

C E R T I F I C A :

DOCUMENTO: DCTO. PRIVADO NRO: S/N
FECHA: 2001/09/03
FECHA INSCRIPCION: 2001/10/25 NRO. 06933 LIBRO: 09

LA FIRMA ARTHUR ANDERSEN & CIA. LTDA
NOMBRE A:

GLORIA CRISTINA BLANCO GIRALDO
C.C. 66,827,344
REVISOR FISCAL SUPLENTE

C E R T I F I C A :

QUE EL 25 DE JULIO DEL AÑO 1996 BAJO EL NRO. 00144 DEL
LIBRO V, SE INSCRIBIO EN LA CAMARA DE COMERCIO LA ESCRITURA
NRO 5235 DEL 19 DE JULIO DEL AÑO 1996, NOTARIA DECIMA DE
CALI

MEDIANTE LA CUAL SE LE CONCEDE PODER ESPECIAL, AMPLIO Y
SUFICIENTE A CARLOS ALBERTO QUINTERO, MAYOR DE EDAD, VECINO DE
ESTA CIUDAD, IDENTIFICADO CON LA CEDULA DE CIUDADANIA NRO.
14.440.778 DE CALI, PARA QUE EN NOMBRE Y REPRESENTACION DE LA
SOCIEDAD, CUYA PERSONERIA OSTENTA, EL REPRESENTANTE EN LOS
SIGUIENTES ACTOS: A) PRESENTAR EN LA ADMINISTRACION DE
IMPUESTOS Y ADUANAS NACIONALES O DEL ORGANISMO QUE HAGA SUS
VECES Y FIRMAR EN REPRESENTACION DE LA SOCIEDAD, LAS
DECLARACIONES DEL IMPUESTO SOBRE LA RENTA Y COMPLEMENTARIOS QUE
CORRESPONDA A LA SOCIEDAD, Y TODAS LAS DEMAS DECLARACIONES QUE
LA SOCIEDAD DEBA PRESENTAR ANTE DICHA ENTIDAD, TALES COMO LA DE
VENTAS, RETENCION EN LA FUENTE, TIMBRE Y CUALESQUIER OTRA
DECLARACION QUE POR LEY LA SOCIEDAD ESTE OBLIGADA A PRESENTAR.
B) PARA PRESENTAR Y SUSCRIBIR TODOS LOS DOCUMENTOS QUE OBLIGUEN
A TECNOQUIMICAS S.A., COMO DECLARANTE DEL IMPUESTO DE INDUSTRIA
Y COMERCIO Y EL COMPLEMENTARIO DE AVISOS Y TABLEROS EN LOS
MUNICIPIOS EN QUE LA SOCIEDAD ESTE OBLIGADA AL PAGO DE LOS
CITADOS IMPUESTOS, PUDIENDO IGUALMENTE NOTIFICARSE DE TODAS LAS
ACTUACIONES, ACTOS, DILIGENCIAS O GESTIONES RELACIONADAS O QUE
SE DERIVEN DE LOS MENCIONADOS TRIBUTOS. C) PARA PRESENTAR Y
SUSCRIBIR TODOS LOS DOCUMENTOS QUE OBLIGUEN A TECNOQUIMICAS S.A.
COMO DECLARANTE DEL IMPUESTO PREDIAL Y COMPLEMENTARIOS EN LOS
MUNICIPIOS DONDE TENGA BIENES INMUEBLES, PUDIENDO IGUALMENTE
NOTIFICARSE DE TODAS LAS ACTUACIONES, ACTOS, DILIGENCIAS O
GESTIONES RELACIONADAS O QUE SE DERIVEN DEL CITADO IMPUESTO. D)

SISTEMA DE INFORMACION NACIONAL EMPRESARIAL

FECHA: 23 DE MAYO DE 2,002 HORA: 15:50:09

PARA DAR RESPUESTA A LOS REQUERIMIENTOS ORDINARIOS Y A LAS SOLICITUDES DE INFORMACION EN RELACION CON LOS IMPUESTOS ADMINISTRADOS POR LA DIRECCION DE IMPUESTOS Y ADUANAS NACIONALES Y POR LAS DEPENDENCIAS MUNICIPALES QUE ADMINISTREN LOS IMPUESTOS DE CARACTER TERRITORIAL. E) Y EN GENERAL, PARA QUE ASUMA LA PERSONERIA EN NOMBRE DE TECNOQUIMICAS S.A., SIEMPRE QUE LO ESTIME CONVENIENTE, DE MANERA QUE EN NINGUN CASO LA SOCIEDAD QUEDE SIN REPRESENTACION EN LOS ASUNTOS RELACIONADOS CON LAS FACULTADES OTORGADAS.

C E R T I F I C A :

QUE EL 02 DE AGOSTO DEL AÑO 1999 BAJO EL NRO. 00323 DEL LIBRO V, SE INSCRIBIO EN LA CAMARA DE COMERCIO LA ESCRITURA NRO 2719 DEL 15 DE JULIO DEL AÑO 1999, NOTARIA TERCERA CALI

MEDIANTE LA CUAL SE CONCEDE PODER ESPECIAL, PERO AMPLIO Y SUFICIENTE A SAMUEL GOMEZ UPEGUI, MAYOR DE EDAD, VECINO DE CALI, IDENTIFICADO CON LA CEDULA DE CIUDADANIA NRO. 16.633.490, GERENTE DEL DISTRITO DE VENTAS DEL OCCIDENTE COLOMBIANO DE LA SOCIEDAD, CON SEDE EN CALI, UBICADO EN LA CALLE 23 NRO. 4-66, PARA EL EJERCICIO DE LAS SIGUIENTES FACULTADES: 1) PARA QUE EN NOMBRE DE TECNOQUIMICAS S.A. Y EN ASUNTOS QUE TENGA QUE VER CON EL DISTRITO DE VENTAS PROMUEVA ACCIONES EN NOMBRE DE ESTA ANTE ENTIDADES PUBLICAS, FUNCIONARIOS O EMPLEADOS NACIONALES, DEPARTAMENTALES Y MUNICIPALES DEL ORDEN JUDICIAL, ADMINISTRATIVO O POLICIVO, YA SEA EN PROCESOS, ACTUACIONES, SIMPLES ACTOS O DILIGENCIAS Y GESTIONES DE CARACTER LABORAL, CIVIL, COMERCIAL Y POLICIVO, CON FACULTADES DE DESISTIMIENTO, ALLANAMIENTO Y CONCILIACION, EN LOS QUE EL ESTABLECIMIENTO ESTE VINCULADO COMO DEMANDANTE, DEMANDADO O TERCERO INTERVINIENTE. PARAGRAFO: LA FACULTAD DE CONCILIACION, DESISTIMIENTO Y ALLANAMIENTO SE LIMITA HASTA LA SUMA EQUIVALENTE A CINCUENTA (50) SALARIOS MINIMOS LEGALES MENSUALES. 2) PARA QUE OTORQUE PODER Y CONSTITUYA MANDATARIOS PARA INICIAR LAS ACCIONES CIVILES, COMERCIALES Y PENALES TENDIENTES A LA RECUPERACION DE LA CARTERA, SIN LIMITACION DE CUANTIA, CON EXPRESA FACULTAD PARA ASISTIR EN NOMBRE DE LA SOCIEDAD A LAS AUDIENCIAS DE CONCILIACION, CON AUTORIZACION PARA TRANSIGIR, DESISTIR, CONCILIAR O COMPROMETER A LA SOCIEDAD EN TODOS LOS PROCESOS, GESTIONES JUDICIALES, ADMINISTRATIVAS O RECLAMACIONES EXTRAPROCESO EN QUE INTERVENGA EN ASUNTOS DEL ESTABLECIMIENTO CON LAS LIMITACIONES PREVISTAS EN EL PARAGRAFO DEL NUMERAL ANTERIOR. 3) PARA SUSCRIBIR LOS CONTRATOS DE TRABAJO Y DARLOS POR TERMINADO DEL PERSONAL VINCULADO O QUE SE VINCULE AL ESTABLECIMIENTO DE COMERCIO. 4) PARA CONSTITUIR APODERADO O APODERADOS EN DESARROLLO DE LA REPRESENTACION LABORAL, SI ASI LO CONSIDERA NECESARIO, TENIENDO SUMA DILIGENCIA PARA QUE TECNOQUIMICAS S.A. EN NINGUN CASO QUEDE SIN LA DEBIDA REPRESENTACION EN LA SALVAGUARDIA DE SUS DERECHOS Y OBLIGACIONES LABORALES. 5) PARA QUE EN DESARROLLO DE LAS ACTIVIDADES DEL ESTABLECIMIENTO DE COMERCIO EJERZA LAS SIGUIENTES FACULTADES ESPECIALES: A) COMPRAR Y PERMUTAR TODA CLASE DE BIENES MUEBLES, CUYA FINALIDAD SEA NECESARIA PARA EL MEJOR DESEMPEÑO DE LAS ACTIVIDADES DEL ESTABLECIMIENTO, HASTA

POR LA SUMA QUE INDIQUE LOS MANUALES Y PROCEDIMIENTOS INTERNOS DE LA SOCIEDAD. PARAGRAFO: ES ENTENDIDO QUE EL APODERADO EN SU CONDICION DE GERENTE, ESTA FACULTADO PARA REALIZAR LA VENTA, SIN LIMITACION DE CUANTIA, DE LOS ARTICULOS PROPIOS DE LA ACTIVIDAD COMERCIAL DEL DISTRITO DE VENTAS. B) MANEJAR LAS CUENTAS CORRIENTES CON FACULTADES PARA GIRAR CHEQUES HASTA POR LA CUANTIA QUE DETERMINEN LOS MANUALES Y PROCEDIMIENTOS INTERNOS DE LA SOCIEDAD. C) OTORGAR CREDITOS EN DESARROLLO DE LAS VENTAS DE LOS ARTICULOS PROPIOS DE LA ACTIVIDAD COMERCIAL DEL DISTRITO DE VENTAS, DE ACUERDO CON LO QUE DETERMINEN LOS MANUALES Y PROCEDIMIENTOS INTERNOS DE LA SOCIEDAD. D) RECIBIR A NOMBRE DE LA SOCIEDAD CUALQUIER SUMA DE DINERO, EXPIDIENDO LOS COMPROBANTES EN QUE CONSTE EL PAGO.

C E R T I F I C A :

QUE EL 05 DE OCTUBRE DEL AÑO 1999 BAJO EL NRO. 00356 DEL LIBRO V, SE INSCRIBIO EN LA CAMARA DE COMERCIO LA ESCRITURA NRO 3703 DEL 17 DE SEPTIEMBRE DEL AÑO 1999, NOTARIA TERCERA CALI

MEDIANTE LA CUAL SE CONCEDE PODER ESPECIAL, PERO AMPLIO Y SUFICIENTE AL SENOR A PIO CLAUDIO GUTIERREZ BOTERO, MAYOR DE EDAD, VECINO DE MEDELLIN, IDENTIFICADO CON LA CEDULA DE CIUDADANIA NRO. 8.238.529 DE MEDELLIN, GERENTE DEL DISTRITO DE VENTAS DE LA REGIONAL DE ANTIOQUIA DE LA SOCIEDAD, CON SEDE EN MEDELLIN, PARA EL EJERCICIO DE LAS SIGUIENTES FACULTADES:

1) PARA QUE EN NOMBRE DE TECNOQUIMICAS S.A. Y EN ASUNTOS QUE TENGA QUE VER CON EL DISTRITO DE VENTAS PROMUEVA ACCIONES EN NOMBRE DE ESTA ANTE ENTIDADES PUBLICAS, FUNCIONARIOS O EMPLEADOS NACIONALES, DEPARTAMENTALES Y MUNICIPALES DEL ORDEN JUDICIAL, ADMINISTRATIVO O POLICIVO YA SEA EN PROCESOS, ACTUACIONES, SIMPLES ACTOS O DILIGENCIAS Y GESTIONES DE CARACTER LABORAL, CIVIL, COMERCIAL Y POLICIVO, CON FACULTADES DE DESISTIMIENTO, ALLANAMIENTO Y CONCILIACION, EN LOS QUE EL ESTABLECIMIENTO ESTE VINCULADO COMO DEMANDANTE, DEMANDADO O TERCERO INTERVINIENTE.

PARAGRAFO: LA FACULTAD DE CONCILIACION, DESISTIMIENTO Y ALLANAMIENTO SE LIMITA HASTA LA SUMA EQUIVALENTE A CINCUENTA (50) SALARIOS MINIMOS LEGALES MENSUALES. 2) PARA QUE OTORQUE PODER Y CONSTITUYA MANDATARIOS PARA INICIAR LAS ACCIONES CIVILES, COMERCIALES Y PENALES TENDIENTES A LA RECUPERACION DE LA CARTERA, SIN LIMITACION DE CUANTIA, CON EXPRESAS FACULTADES PARA ASISTIR EN NOMBRE DE LA SOCIEDAD A LAS AUDIENCIAS DE CONCILIACION, CON AUTORIZACION PARA TRANSIGIR, DESISTIR, CONCILIAR O COMPROMETER A LA SOCIEDAD EN TODOS LOS PROCESOS, GESTIONES JUDICIALES, ADMINISTRATIVAS O RECLAMACIONES EXTRAPROCESO EN QUE INTERVENGA EN ASUNTOS DEL ESTABLECIMIENTO CON LAS LIMITACIONES PREVISTAS EN EL PARAGRAFO DEL NUMERAL ANTERIOR. 3) PARA SUSCRIBIR LOS CONTRATOS DE TRABAJO Y DARLOS POR TERMINADO DEL PERSONAL VINCULADO O QUE SE VINCULE AL ESTABLECIMIENTO DE COMERCIO. 4) PARA CONSTITUIR APODERADO O APODERADOS EN DESARROLLO DE LA REPRESENTACION LABORAL, SI ASI LO CONSIDERA NECESARIO, TENIENDO SUMA DILIGENCIA PARA QUE

TECNOQUIMICAS S.A. EN NINGUN CASO QUEDE SIN LA DEBIDA REPRESENTACION EN LA SALVAGUARDIA DE SUS DERECHOS Y OBLIGACIONES LABORALES. 5) PARA QUE EN DESARROLLO DE LAS ACTIVIDADES DEL ESTABLECIMIENTO DE COMERCIO EJERZA LAS SIGUIENTES FACULTADES ESPECIALES: A) COMPRAR Y PERMUTAR TODA CLASE DE BIENES MUEBLES CUYA FINALIDAD SEA NECESARIA PARA EL MEJOR DESEMPEÑO DE LAS ACTIVIDADES DEL ESTABLECIMIENTO, HASTA POR LA SUMA QUE INDIQUE LOS MANUALES Y PROCEDIMIENTOS INTERNOS DE LA SOCIEDAD. PARAGRAFO: ES ENTENDIDO QUE EL APODERADO EN SU CONDICION DE GERENTE, ESTA FACULTADO PARA REALIZAR LA VENTA, SIN LIMITACION DE CUANTIA, DE LOS ARTICULO PROPIOS DE LA ACTIVIDAD COMERCIAL DEL DISTRITO DE VENTAS. B) MANEJAR LAS CUENTAS CORRIENTES CON FACULTADES PARA GIRAR CHEQUES HASTA POR LA CUANTIA QUE DETERMINEN LOS MANUALES Y PROCEDIMIENTOS INTERNOS DE LA SOCIEDAD. C) OTORGAR CREDITOS EN DESARROLLO DE LAS VENTAS DE LOS ARTICULOS PROPIOS DE LA ACTIVIDAD COMERCIAL DEL DISTRITO DE VENTAS, DE ACUERDO CON LO QUE DETERMINE LOS MANUALES Y PROCEDIMIENTO INTERNOS DE LA SOCIEDAD. D) RECIBIR A NOMBRE DE LA SOCIEDAD CUALQUIER SUMA DE DINERO, EXPIDIENDO LOS COMPROBANTES EN QUE CONSTE EL PAGO.

C E R T I F I C A : .

QUE EL 06 DE SEPTIEMBRE DEL AÑO 2001 BAJO EL NRO. 00213 DEL LIBRO V, SE INSCRIBIO EN LA CAMARA DE COMERCIO LA ESCRITURA NRO 2873 DEL 10 DE JULIO DEL AÑO 2001, NOTARIA TERCERA CALI

MEDIANTE LA CUAL SE CONCEDE PODER ESPECIAL, AMPLIO Y SUFICIENTE A MARIA TERESA CANON DE GUZMAN, IDENTIFICADA CON LA CEDULA DE CIUDADANIA NRO. 31.238.104 DE CALI, CARLOS ARTURO PENA NIETO IDENTIFICADO CON LA CEDULA DE CIUDADANIA NRO. 19.483.439 DE BOGOTA Y ANGELA MARIA CORREA CARDONA IDENTIFICADA CON LA CEDULA DE CIUDADANIA No.66.835.555 DE CALI, LA PRIMERA COMO PRINCIPAL Y EL SEGUNDO COMO PRIMER SUPLENTE Y LA TERCERA COMO SEGUNDA SUPLENTE, PARA QUE REPRESENTEN A LA SOCIEDAD EN TODOS LOS ASUNTOS CAMBIARIOS, CON FACULTAD PARA FIRMAR DECLARACIONES DE CAMBIOS SEA POR CREDITOS EN MONEDA EXTRANJERA, POR MOVIMIENTOS DE LAS CUENTAS CORRIENTES DE COMPENSACION, IMPORTACIONES DE BIENES Y SERVICIOS, TRANSFERENCIAS Y OTROS CONCEPTOS, REGISTRO DE PRESTAMO EN MONEDA EXTRANJERA OTORGADOS A LA EMPRESA EN SU CONDICION DE RESIDENTE Y TODOS AQUELLOS DOCUMENTOS O DECLARACIONES QUE DEBAN HACERSE ANTE EL BANCO DE LA REPUBLICA U OTRAS ENTIDADES GUBERNAMENTALES RESPECTO DE OPERACIONES EN EL MERCADO CAMBIARIO. LOS APODERADOS SUSCRIBIRAN TODOS LOS DOCUMENTOS Y REALIZAR TODOS LOS ACTOS QUE OBLIGUEN A TECNOQUIMICAS S.A. ANTE LAS AUTORIDADES CAMBIARIAS, PUDIENDO IGUALMENTE NOTIFICARSE DE TODAS LAS ACTUACIONES, ACTOS ADMINISTRATIVOS, DILIGENCIAS O GESTIONES RELACIONADAS O QUE SE DERIVEN DE DICHA ACTIVIDAD Y EN GENERAL PARA QUE ASUMA LA PERSONERIA A NOMBRE DE TECNOQUIMICAS S.A., SIEMPRE QUE LE ESTIMEN CONVENIENTE, DE MANERA QUE EN NINGUN CASO QUEDE SIN LA DEBIDA REPRESENTACION EN LA SALVAGUARDIA DE SUS DERECHOS Y CUMPLIMIENTO DE OBLIGACIONES DE CARACTER CAMBIARIO Y EN LOS ASUNTOS CONCERNIENTES PARA LAS MATERIAS DE QUE TRATA ESTE

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PODER. LOS APODERADOS SE CENIRAN RIGUROSAMENTE A LOS TERMINOS EXPRESADOS EN ESTE MANDATO QUE SE OTORGA POR TERMINO INDEFINIDO Y CUALQUIER NOTIFICACION O REVOCATORIA SE HARA EXPRESAMENTE POR ESCRITURA PUBLICA.

C E R T I F I C A :

QUE EL 13 DE FEBRERO DEL AÑO 2002 BAJO EL NRO. 00298 DEL LIBRO V, SE INSCRIBIO EN LA CAMARA DE COMERCIO LA ESCRITURA NRO 307 DEL 31 DE ENERO DEL AÑO 2002, NOTARIA TERCERA CALI

MEDIANTE LA CUAL SE LE CONCEDE PODER ESPECIAL, AMPLIO Y SUFICIENTE A JORGE ROBERTO OBANDO RODRIGUEZ, MAYOR DE EDAD, VECINO DE ESTA CIUDAD, IDENTIFICADO CON LA CEDULA DE CIUDADANIA NRO. 14.960.183 DE CALI, PARA QUE EN NOMBRE Y REPRESENTACION DE LA SOCIEDAD, CUYA PERSONERIA OSTENTA: 1o.) PROMUEVA O DESISTA DE ACCIONES ANTE CORPORACIONES; ENTIDADES O FUNCIONARIOS DEL ORDEN JUDICIAL O GUBERNATIVO, YA SEA EN PROCESOS, ACTUACIONES O ACTOS SIMPLES, DILIGENCIAS O GESTIONES EN QUE TECNOQUIMICAS S.A. TENGA INTERES COMO ACTOR, DEMANDANDO O TERCERO INTERVINIENTE EN RELACION CON LOS ASUNTOS DE CARACTER LABORAL, POLICIVO, PENAL O DE FISCALIA, EN QUE LA EMPRESA SEA PARTE ACTIVA O PASIVA, CON CAPACIDAD PARA NOTIFICARSE Y CONTESTAR Y HACERSE PARTE DE ELLOS EN NOMBRE DE TECNOQUIMICAS S.A. 2o.) SUSCRIBA Y DE POR TERMINADO LOS CONTRATOS DE TRABAJO DEL PERSONAL VINCULADO A LA EMPRESA. 3o.) CONSTITUYA APODERADOS EN DESARROLLO DE ESTA REPRESENTACION LABORAL, REVOQUE Y SUSTITUYA DICHOS PODERES CUANDO LO ESTIME PROCEDENTE. 4o.) TRANSIGA, CONCILIE, DESISTA Y COMPROMETA A LA EMPRESA EN TODOS LOS TRAMITES ADMINISTRATIVOS Y PROCESOS JUDICIALES, DE CARACTER LABORAL, POLICIVO, PENAL O DE FISCALIA EN LOS QUE LA EMPRESA SEA PARTE DEMANDANTE, DEMANDADA O TERCERO INTERVINIENTE. PARAGRAFO. LA FACULTAD PARA TRANSIGIR O CONCILIAR SE LIMITA A LA SUMA DE QUINIENTOS (500) SALARIOS LEGALES MENSUALES. 5o.) OTORQUE PODERES Y CONSTITUYA MANDATARIOS PARA INICIAR LAS ACCIONES JUDICIALES DE CARACTER CIVIL, COMERCIAL Y PENAL TENDIENTES A LA RECUPERACION DE LOS CREDITOS A FAVOR DE LA SOCIEDAD, BIEN SEA REPRESENTADOS EN FACTURAS, CHEQUES, TITULOS VALORES O CUALQUIER OTRO DOCUMENTO REPRESENTATIVO DE CREDITO, HASTA POR SUMA EQUIVALENTE A QUINIENTOS (500) SALARIOS MINIMOS LEGALES. MENSUALES, CON EXPRESAS FACULTADES PARA ASISTIR EN NOMBRE DE TECNOQUIMICAS S.A. A LAS AUDIENCIAS DE CONCILIACION QUE PROGRAMEN LOS JUZGADOS CIVILES MUNICIPALES Y DEL CIRCUITO, CON AUTORIZACION PARA TRANSIGIR, DESISTIR CONCILIAR O COMPROMETER A LA SOCIEDAD HASTA POR LA CUANTIA INDICADA. 6o. REPRESENTA A TECNOQUIMICAS S.A. DENTRO DE LOS TRAMITES DE CONCORDATOS, PROCESOS DE REESTRUCTURACION ECONOMICA PREVISTO EN LA LEY 550 DE 1999 Y LAS NORMAS QUE LA ADICIONEN O MODIFIQUEN Y LIQUIDACIONES OBLIGATORIAS QUE SE TRAMITEN ANTE LA SUPERINTENDENCIA DE SOCIEDADES O ANTE LAS ENTIDADES PERTINENTES CUANDO SE TRATE DE OTRA CLASE DE ACREEDORES O ANTE LOS JUZGADOS CIVILES, CON EXPRESAS FACULTADES PARA TRANSIGIR, DESISTIR,

CONCILIAR O COMPROMETER, NOMBRAR APODERADOS, PRESENTAR LOS CREDITOS, APORTAR LAS PRUEBAS QUE ESTIME NECESARIAS, ASISTIR A LAS AUDIENCIAS PRELIMINARES Y DEFINITIVAS, ACORDAR FORMULAS DE PAGO, RECIBIR Y EN GENERAL PARA EJERCER TODAS AQUELLAS GESTIONES NECESARIAS PARA OBTENER EL RECONOCIMIENTO Y PAGO DE LOS CREDITOS PRESENTADOS. LAS FACULTADES AQUI ESTABLECIDAS ESTAN LIMITADAS PARA LOS CREDITOS HASTA POR CUANTIA DE QUINIENTOS (500) SALARIOS MINIMOS LEGALES MENSUALES. PARAGRAFO. IGUALMENTE QUEDA FACULTADO PARA ACEPTAR Y REPRESENTAR A TECNOQUIMICAS CUANDO LA EMPRESA SEA NOMBRADA EN JUNTA DE ACREEDORES O EN ORGANOS EQUIVALENTES O SIMILARES, EN LOS PROCESOS QUE TRAMITEN ANTE LA SUPERINTENDENCIA DE SOCIEDADES O ANTE LAS ENTIDADES PERTINENTES CUANDO SE TRATE DE OTRA CLASE DE DEUDORES. 7o.) REPRESENTA A TECNOQUIMICAS S.A. EN LOS PROCEDIMIENTOS DE CONCILIACION QUE LOS DEUDORES DE LA SOCIEDAD CONVOQUEN ANTE LAS ENTIDADES FACULTADAS PARA ELLO CON EL FIN OBTENER UN ACUERDO EN EL PAGO DE LAS ACREENCIAS, CON EXPRESAS FACULTADES PARA TRANSIGIR, DESISTIR, CONCILIAR O COMPROMETER Y RECIBIR, FACULTADES QUE SE LIMITAN HASTA POR LA SUMA DE QUINIENTOS (500) SALARIOS MINIMOS LEGALES MENSUALES. 8o.) REVISE LOS EXPEDIENTES DE LOS PROCESOS JUDICIALES DE CARACTER EJECUTIVO EN QUE LA SOCIEDAD SEA PARTE DEMANDANTE, EN TODO EL TERRITORIO NACIONAL, PARA LA RECUPERACION DE SUS ACREENCIAS. 9o.) APRUEBE Y SUSCRIBA ACUERDOS PRIVADOS DE PAGO Y CONCILIACIONES DE OBLIGACIONES A FAVOR DE TECNOQUIMICAS POR LA VENTA DE SUS PRODUCTOS HASTA POR LA SUMA DE (500) SALARIOS MINIMOS LEGALES MENSUALES.

C E R T I F I C A :

QUE EL 13 DE FEBRERO DEL AÑO 2002 BAJO EL NRO. 00300 DEL LIBRO V, SE INSCRIBIO EN LA CAMARA DE COMERCIO LA ESCRITURA NRO 309 DEL 31 DE ENERO DEL AÑO 2002, NOTARIA TERCERA CALI

MEDIANTE LA CUAL SE LE CONCEDE PODER ESPECIAL, PERO AMPLIO Y SUFICIENTE A DANIEL AMEZQUITA, MAYOR DE EDAD, VECINO DE BARRANQUILLA, IDENTIFICADO CON LA CEDULA DE CIUDADANIA NRO. 8.681.962 DE BARRANQUILLA, GERENTE DEL DISTRITO DE VENTAS DE LA REGIONAL DE LA ZONA ATLANTICA DE LA SOCIEDAD, CON SEDE EN BARRANQUILLA, PARA EL EJERCICIO DE LA SIGUIENTES FACULTADES: 1) PARA QUE EN NOMBRE DE TECNOQUIMICAS S.A. Y EN ASUNTOS QUE TENGA QUE VER CON EL DISTRITO DE VENTAS SE NOTIFIQUE Y PROMUEVA ACCIONES EN NOMBRE DE ESTA ANTE ENTIDADES PUBLICAS, FUNCIONARIOS O EMPLEADOS NACIONALES, DEPARTAMENTALES Y MUNICIPALES DEL ORDEN JUDICIAL, ADMINISTRATIVO POLICIVO, PENAL O DE FISCALIA, YA SEA EN PROCESOS, ACTUACIONES, SIMPLES ACTOS O DILIGENCIAS Y GESTIONES, CON FACULTADES DE DESISTIMIENTO, ALLANAMIENTO Y CONCILIACION, EN LOS QUE EL ESTABLECIMIENTO ESTE VINCULADO COMO DEMANDANTE, DEMANDADO O TERCERO INTERVINIENTE. PARAGRAFO: LA FACULTAD DE CONCILIACION, DESISTIMIENTO Y ALLANAMIENTO SE LIMITA HASTA LA SUMA EQUIVALENTE A CINCUENTA (50) SALARIOS MINIMOS LEGALES MENSUALES. 2) PARA QUE OTORQUE PODER Y CONSTITUYA MANDATARIOS PARA INICIAR LAS ACCIONES CIVILES, COMERCIALES Y PENALES TENDIENTES A LA RECUPERACION DE

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LA CARTERA, SIN LIMITACION DE CUANTIA, CON EXPRESAS FACULTADES PARA ASISTIR EN NOMBRE DE LA SOCIEDAD A LAS AUDIENCIAS DE CONCILIACION, CON AUTORIZACION PARA TRANSIGIR, DESISTIR, CONCILIAR O COMPROMETER A LA SOCIEDAD EN TODOS LOS PROCESOS, GESTIONES JUDICIALES, ADMINISTRATIVAS O RECLAMACIONES EXTRA PROCESO EN QUE INTERVENGA EN ASUNTOS RELACIONADOS CON EL ESTABLECIMIENTO DE COMERCIO CON LAS LIMITACIONES PREVISTAS EN EL PARAGRAFO DEL NUMERAL ANTERIOR. 3) PARA QUE OTORQUE PODER Y CONSTITUYA MANDATARIOS PARA HACERSE PARTE EN LOS PROCESOS CONCORDATARIOS, DE LIQUIDACION OBLIGATORIA, O REESTRUCTURACION ECONOMICA QUE SE TRAMITEN ANTE LA SUPERINTENDENCIA DE SOCIEDADES O ANTE OTRAS SUPERINTENDENCIAS O ENTIDADES CUANDO SE TRATE DE OTRA CLASE DE DEUDORES, TENDIENTES A LA RECUPERACION DE LA CARTERA, SIN LIMITACION DE CUANTIA, CON EXPRESAS FACULTADES PARA ASISTIR EN NOMBRE DE LA SOCIEDAD A LAS DIVERSAS AUDIENCIAS CON AUTORIZACION PARA TRANSIGIR, DESISTIR, CONCILIAR O COMPROMETER A LA SOCIEDAD EN TODOS LOS PROCESOS, GESTIONES JUDICIALES, ADMINISTRATIVAS O RECLAMACIONES EXTRA PROCESO EN QUE INTERVENGA EN ASUNTOS DEL ESTABLECIMIENTO CON LAS LIMITACIONES PREVISTAS EN EL PARAGRAFO DEL NUMERAL PRIMERO. 4) PARA SUSCRIBIR LOS CONTRATOS DE TRABAJO Y DARLOS POR TERMINADO DEL PERSONAL VINCULADO O QUE SE VENTILE AL ESTABLECIMIENTO DE COMERCIO. 5) PARA CONSTITUIR APODERADO O APODERADOS EN DESARROLLO DE LA REPRESENTACION LABORAL, SI ASI LO CONSIDERA NECESARIO, TENIENDO SUMA DILIGENCIA PARA QUE TECNOQUIMICAS S.A. EN NINGUN CASO QUEDE SIN LA DEBIDA REPRESENTACION EN LA SALVAGUARDIA DE SUS DERECHOS Y OBLIGACIONES LABORALES. 6) PARA QUE EN DESARROLLO DE LAS ACTIVIDADES DEL ESTABLECIMIENTO DE COMERCIO EJERZA LAS SIGUIENTES FACULTADES ESPECIALES: A) COMPRAR Y PERMUTAR TODA CLASE DE BIENES MUEBLES CUYA FINALIDAD SEA NECESARIA PARA EL MEJOR DESEMPEÑO DE LAS ACTIVIDADES DEL ESTABLECIMIENTO, HASTA POR LA SUMA QUE INDIQUE LOS MANUALES Y PROCEDIMIENTOS INTERNOS DE LA SOCIEDAD. PARAGRAFO: ES ENTENDIDO QUE EL APODERADO, EN SU CONDICION DE GERENTE, ESTA FACULTADO PARA REALIZAR LA VENTA, SIN LIMITACION DE CUANTIA, DE LOS ARTICULOS PROPIOS DE LA ACTIVIDAD COMERCIAL DEL DISTRITO DE VENTAS. B) MANEJAR LAS CUENTAS CORRIENTES CON FACULTADES PARA GIRAR CHEQUES HASTA POR LA CUANTIA QUE DETERMINEN LOS MANUALES Y PROCEDIMIENTOS INTERNOS DE LA SOCIEDAD. C) OTORGAR CREDITOS EN DESARROLLO DE LAS VENTAS DE LOS ARTICULOS PROPIOS DE LA ACTIVIDAD COMERCIAL DEL DISTRITO DE VENTAS, DE ACUERDO CON LO QUE DETERMINEN LOS MANUALES Y PROCEDIMIENTOS INTERNOS DE LA SOCIEDAD. D) RECIBIR A NOMBRE DE LA SOCIEDAD CUALQUIER SUMA DE DINERO, EXPIDIENDO LOS COMPROBANTES EN QUE CONSTE EL PAGO.

C E R T I F I C A :

QUE EL 13 DE FEBRERO DEL AÑO 2002 BAJO EL NRO. 00301 DEL LIBRO V, SE INSCRIBIO EN LA CAMARA DE COMERCIO LA ESCRITURA NRO 310 DEL 31 DE ENERO DEL AÑO 2002, NOTARIA TERCERA CALI

MEDIANTE LA CUAL SE LE CONCEDE PODER ESPECIAL, PERO AMPLIO Y SUFICIENTE A CARLOS ORLANDO MUTIS, MAYOR DE EDAD, VECINO DE BUCARAMANGA, IDENTIFICADO CON LA CEDULA DE CIUDADANIA No.

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91.200.418 DE BUCARAMANGA, GERENTE DEL DISTRITO DE VENTAS DE LA REGIONAL ORIENTE DE LA SOCIEDAD, CON SEDE EN BUCARAMANGA, PARA EL EJERCICIO DE LAS SIGUIENTES FACULTADES: 1) PARA QUE EN NOMBRE DE TECNOQUIMICAS S.A. Y EN ASUNTOS QUE TENGA QUE VER CON EL DISTRITO DE VENTAS SE NOTIFIQUE Y PROMUEVA ACCIONES EN NOMBRE DE ESTA ANTE ENTIDADES PUBLICAS, FUNCIONARIOS O EMPLEADOS NACIONALES, DEPARTAMENTALES Y MUNICIPALES DEL ORDEN JUDICIAL, ADMINISTRATIVO POLICIVO, PENAL O DE FISCALIA, YA SEA EN PROCESOS, ACTUACIONES, SIMPLES ACTOS O DILIGENCIAS Y GESTIONES, CON FACULTADES DE DESISTIMIENTO, ALLANAMIENTO, Y CONCILIACION, EN LOS QUE EL ESTABLECIMIENTO ESTE VINCULADO COMO DEMANDANTE, DEMANDADO O TERCERO INTERVINIENTE. PARAGRAFO: LA FACULTAD DE CONCILIACION, DESISTIMIENTO Y ALLANAMIENTO SE LIMITA HASTA LA SUMA EQUIVALENTE A CINCUENTA (50) SALARIOS MINIMOS LEGALES MENSUALES. 2) PARA QUE OTORQUE PODER Y CONSTITUYA MANDATARIOS PARA INICIAR LAS ACCIONES CIVILES, COMERCIALES Y PENALES TENDIENTES A LA RECUPERACION DE LA CARTERA, SIN LIMITACION DE CUANTIA, CON EXPRESAS FACULTADES PARA ASISTIR EN NOMBRE DE LA SOCIEDAD A LAS AUDIENCIAS DE CONCILIACION, CON AUTORIZACION PARA TRANSIGIR, DESISTIR, CONCILIAR O COMPROMETER A LA SOCIEDAD EN TODOS LOS PROCESOS, GESTIONES JUDICIALES, ADMINISTRATIVAS O RECLAMACIONES EXTRA PROCESO EN QUE INTERVENGA EN ASUNTOS RELACIONADOS CON EL ESTABLECIMIENTO DE COMERCIO CON LAS LIMITACIONES PREVISTAS EN EL PARAGRAFO DEL NUMERAL ANTERIOR. 3) PARA QUE OTORQUE PODER Y CONSTITUYA MANDATARIOS PARA HACERSE PARTE EN LOS PROCESOS CONCORDATARIOS, DE LIQUIDACION OBLIGATORIA, O REESTRUCTURACION ECONOMICA QUE SE TRAMITEN ANTE LA SUPERINTENDENCIA DE SOCIEDADES O ANTE OTRAS SUPERINTENDENCIAS O ENTIDADES CUANDO SE TRATE DE OTRA CLASE DE DEUDORES, TENDIENTES A LA RECUPERACION DE LA CARTERA, SIN LIMITACION DE CUANTIA, CON EXPRESAS FACULTADES PARA ASISTIR EN NOMBRE DE LA SOCIEDAD A LAS DIVERSAS AUDIENCIAS CON AUTORIZACION PARA TRANSIGIR, DESISTIR, CONCILIAR O COMPROMETER A LA SOCIEDAD EN TODOS LOS PROCESOS, GESTIONES JUDICIALES, ADMINISTRATIVAS O RECLAMACIONES EXTRA PROCESO EN QUE INTERVENGA EN ASUNTOS DEL ESTABLECIMIENTO CON LAS LIMITACIONES PREVISTAS EN EL PARAGRAFO DEL NUMERAL PRIMERO. 4) PARA SUSCRIBIR LOS CONTRATOS DE TRABAJO Y DARLOS POR TERMINADO DEL PERSONAL VINCULADO O QUE SE VINCULE AL ESTABLECIMIENTO DE COMERCIO. 5) PARA CONSTITUIR APODERADO O APODERADOS EN DESARROLLO DE LA REPRESENTACION LABORAL, SI ASI LO CONSIDERA NECESARIO, TENIENDO SUMA DILIGENCIA PARA QUE TECNOQUIMICAS S.A. EN NINGUN CASO QUEDE SIN LA DEBIDA REPRESENTACION EN LA SALVAGUARDIA DE SUS DERECHOS Y OBLIGACIONES LABORALES. 6) PARA QUE EN DESARROLLO DE LAS ACTIVIDADES DEL ESTABLECIMIENTO DE COMERCIO EJERZA LAS SIGUIENTES FACULTADES ESPECIALES: A) COMPRAR Y PERMUTAR TODA CLASE DE BIENES MUEBLES CUYA FINALIDAD SEA NECESARIA PARA EL MEJOR DESEMPEÑO DE LAS ACTIVIDADES DEL ESTABLECIMIENTO, HASTA POR LA SUMA QUE INDIQUE LOS MANUALES Y PROCEDIMIENTOS INTERNOS DE LA SOCIEDAD. PARAGRAFO: ES ENTENDIDO QUE EL APODERADO, EN SU CONDICION DE GERENTE, ESTA FACULTADO PARA REALIZAR LA VENTA, SIN LIMITACION DE CUANTIA, DE LOS ARTICULOS PROPIOS DE LA ACTIVIDAD COMERCIAL DEL DISTRITO DE VENTAS. B) MANEJAR LAS CUENTAS CORRIENTES CON FACULTADES PARA

GIRAR CHEQUES HASTA POR LA CUANTIA QUE DETERMINEN LOS MANUALES Y PROCEDIMIENTOS INTERNOS DE LA SOCIEDAD. C) OTORGAR CREDITOS EN DESARROLLO DE LAS VENTAS DE LOS ARTICULOS PROPIOS DE LA ACTIVIDAD COMERCIAL DEL DISTRITO DE VENTAS, DE ACUERDO CON LO QUE DETERMINEN LOS MANUALES Y PROCEDIMIENTOS INTERNOS DE LA SOCIEDAD. D) RECIBIR A NOMBRE DE LA SOCIEDAD CUALQUIER SUMA DE DINERO, EXPIDIENDO LOS COMPROBANTES EN QUE CONSTE EL PAGO.

C E R T I F I C A :

QUE EL 04 DE MARZO DEL AÑO 2002 BAJO EL NRO. 00118 DEL LIBRO V, SE INSCRIBIO EN LA CAMARA DE COMERCIO LA ESCRITURA NRO 308 DEL 31 DE ENERO DEL AÑO 2002, NOTARIA TERCERA CALI

MEDIANTE LA CUAL SE CONCEDE PODER ESPECIAL, PERO AMPLIO Y SUFICIENTE A HERNANDO ANGULO MOYANO, MAYOR DE EDAD, VECINO DE BOGOTA, IDENTIFICADO CON LA CEDULA DE CIUDADANIA NRO. 19.322.139 DE BOGOTA, GERENTE DEL DISTRITO DE VENTAS DE LA REGIONAL CUNDINAMARCA DE LA SOCIEDAD, CON SEDE EN BOGOTA, PARA EL EJERCICIO DE LAS SIGUIENTES FACULTADES: 1) PARA QUE EN NOMBRE DE TECNOQUIMICAS S.A. Y EN ASUNTOS QUE TENGA QUE VER CON EL DISTRITO DE VENTAS SE NOTIFIQUE Y PROMUEVA ACCIONES EN NOMBRE DE ESTA ANTE ENTIDADES PUBLICAS, FUNCIONARIOS O EMPLEADOS NACIONALES, DEPARTAMENTALES Y MUNICIPALES DEL ORDEN JUDICIAL, ADMINISTRATIVO POLICIVO, PENAL O DE FISCALIA, YA SEA EN PROCESOS, ACTUACIONES, SIMPLES ACTOS O DILIGENCIAS Y GESTIONES, CON FACULTADES DE DESISTIMIENTO, ALLANAMIENTO Y CONCILIACION, EN LOS QUE EL ESTABLECIMIENTO ESTE VINCULADO COMO DEMANDANTE, DEMANDADO O TERCERO INTERVINIENTE.

PARAGRAFO: LA FACULTAD DE CONCILIACION, DESISTIMIENTO Y ALLANAMIENTO SE LIMITA HASTA LA SUMA EQUIVALENTE A CINCUENTA (50) SALARIOS MINIMOS LEGALES MENSUALES. 2) PARA QUE OTORQUE PODER Y CONSTITUYA MANDATARIOS PARA INICIAR LAS ACCIONES CIVILES, COMERCIALES Y PENALES TENDIENTES A LA RECUPERACION DE LA CARTERA, SIN LIMITACION DE CUANTIA, CON EXPRESAS FACULTADES PARA ASISTIR EN NOMBRE DE LA SOCIEDAD A LAS AUDIENCIAS DE CONCILIACION, CON AUTORIZACION PARA TRANSIGIR, DESISTIR, CONCILIAR O COMPROMETER A LA SOCIEDAD EN TODOS LOS PROCESOS, GESTIONES JUDICIALES, ADMINISTRATIVAS O RECLAMACIONES EXTRA PROCESO EN QUE INTERVENGA EN ASUNTOS RELACIONADOS CON EL ESTABLECIMIENTO DE COMERCIO CON LAS LIMITACIONES PREVISTAS EN EL PARAGRAFO DEL NUMERAL ANTERIOR. 3) PARA QUE OTORQUE PODER Y CONSTITUYA MANDATARIOS PARA HACERSE PARTE EN LOS PROCESOS CONCORDATARIOS, DE LIQUIDACION OBLIGATORIA, O REESTRUCTURACION ECONOMICA QUE SE TRAMITEN ANTE LA SUPERINTENDENCIA DE SOCIEDADES O ANTE OTRAS SUPERINTENDENCIAS O ENTIDADES CUANDO SE TRATE DE OTRA CLASE DE DEUDORES, TENDIENTES A LA RECUPERACION DE LA CARTERA, SIN LIMITACION DE CUANTIA, CON EXPRESAS FACULTADES PARA ASISTIR EN NOMBRE DE LA SOCIEDAD A LAS DIVERSAS AUDIENCIAS CON AUTORIZACION PARA TRANSIGIR, DESISTIR, CONCILIAR O COMPROMETER A LA SOCIEDAD EN TODOS LOS PROCESOS, GESTIONES JUDICIALES, ADMINISTRATIVAS O RECLAMACIONES EXTRA PROCESO EN QUE INTERVENGA EN ASUNTOS DEL ESTABLECIMIENTO CON LAS

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LIMITACIONES PREVISTAS EN EL PARAGRAFO DEL NUMERAL PRIMERO. 4) PARA SUSCRIBIR LOS CONTRATOS DE TRABAJO Y DARLOS POR TERMINADO DEL PERSONAL VINCULADO O QUE SE VINCULE AL ESTABLECIMIENTO DE COMERCIO. 5) PARA CONSTITUIR APODERADO O APODERADOS EN DESARROLLO DE LA REPRESENTACION LABORAL, SI ASI LO CONSIDERA NECESARIO, TENIENDO SUMA DILIGENCIA PARA QUE TECNOQUIMICAS S.A. EN NINGUN CASO QUEDE SIN LA DEBIDA REPRESENTACION EN LA SALVAGUARDIA DE SUS DERECHOS Y OBLIGACIONES LABORALES. 6) PARA QUE EN DESARROLLO DE LAS ACTIVIDADES DEL ESTABLECIMIENTO DE COMERCIO EJERZA LAS SIGUIENTES FACULTADES ESPECIALES: A) COMPRAR Y PERMUTAR TODA CLASE DE BIENES MUEBLES CUYA FINALIDAD SEA NECESARIA PARA EL MEJOR DESEMPEÑO DE LAS ACTIVIDADES DEL ESTABLECIMIENTO, HASTA POR LA SUMA QUE INDIQUE LOS MANUALES Y PROCEDIMIENTOS INTERNOS DE LA SOCIEDAD. PARAGRAFO: ES ENTENDIDO QUE EL APODERADO, EN SU CONDICION DE GERENTE, ESTA FACULTADO PARA REALIZAR LA VENTA, SIN LIMITACION DE CUANTIA, DE LOS ARTICULOS PROPIOS DE LA ACTIVIDAD COMERCIAL DEL DISTRITO DE VENTAS. B) MANEJAR LAS CUENTAS CORRIENTES CON FACULTADES PARA GIRAR CHEQUES HASTA POR LA CUANTIA QUE DETERMINEN LOS MANUALES Y PROCEDIMIENTOS INTERNOS DE LA SOCIEDAD. C) OTORGAR CREDITOS EN DESARROLLO DE LAS VENTAS DE LOS ARTICULOS PROPIOS DE LA ACTIVIDAD COMERCIAL DEL DISTRITO DE VENTAS, DE ACUERDO CON LO QUE DETERMINEN LOS MANUALES Y PROCEDIMIENTOS INTERNOS DE LA SOCIEDAD. D) RECIBIR A NOMBRE DE LA SOCIEDAD CUALQUIER SUMA DE DINERO, EXPIDIENDO LOS COMPROBANTES EN QUE CONSTE EL PAGO.

C E R T I F I C A :

CAPITAL:	NRO. DE ACCIONES	VALOR NOMINAL
AUTORIZADO		
\$27,200,000,000.00	54,400,000	\$500.00
SUSCRITO		
\$27,123,804,000.00	54,247,608	\$500.00
PAGADO		
\$27,123,804,000.00	54,247,608	\$500.00

C E R T I F I C A :

QUE EL 27 DE MARZO DE 2001 BAJO EL NRO.1979 DEL LIBRO IX, SE INSCRIBIO EN LA CAMARA DE COMERCIO UN DOCUMENTO PRIVADO DE FECHA MARZO 21 DE 2001, EN LA CUAL CONSTA LA SITUACION DE CONTROL EJERCIDA POR LA SOCIEDAD TECNOQUIMICAS S.A.

MATRIZ: TECNOQUIMICAS S.A.
DOMICILIO: SANTIAGO DE CALI.
NACIONALIDAD: COLOMBIANA

ACTIVIDAD: LAS ACTIVIDADES DE TECNOQUIMICAS CORRESPONDEN A LA PRODUCCION Y COMERCIALIZACION DE PRODUCTOS BIOLÓGICOS, QUÍMICOS, MEDICAMENTOS Y PRODUCTOS FARMACÉUTICOS, PANALES, PRODUCTOS VETERINARIO Y AGRÍCOLAS, ALIMENTOS, COSMÉTICOS Y DE TOCADOR Y PRODUCTOS DE ASEO PARA EL HOGAR.

SUBORDINADA: ADHESIVOS INTERNACIONALES S.A.-ADHINTER S.A.

SISTEMA DE INFORMACION NACIONAL EMPRESARIAL

FECHA: 23 DE MAYO

DE 2,002

HORA: 15:50:09

C E R T I F I C A :

QUE NO FIGURAN OTRAS INSCRIPCIONES QUE MODIFIQUEN TOTAL O PARCIALMENTE EL PRESENTE CERTIFICADO

LOS ACTOS DE REGISTRO QUEDAN EN FIRME CINCO (5) DIAS HABILES DESPUES DE LA FECHA DE SU INSCRIPCION, SIEMPRE Y CUANDO, DENTRO DE DICHO TERMINO NO SEAN OBJETO DE RECURSOS EN LA VIA GUBERNATIVA.

C E R T I F I C A :

DADO EN CALI

A LOS 23 DIAS DEL MES DE MAYO

DE 2,002 HORA: 15:50:09

LA INFORMACION SUMINISTRADA EN LA PRESENTE CERTIFICACION, ES RESPONSABILIDAD EXCLUSIVA DE LA CAMARA DE ORIGEN.

DE CONFORMIDAD CON EL DECRETO 2150 DE 1.995 Y LA AUTORIZACION IMPARTIDA POR LA SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO LA FIRMA MECANICA QUE APARECE A CONTINUACION TIENE PLENA VALIDEZ PARA TODOS LOS EFECTOS LEGALES.

EL SECRETARIO

123456

 2B

FECHA: 23 DE MAYO

DE 2,002

HORA: 15:50:09

DOMICILIO: PALMIRA. VALLE DEL CAUCA
NACIONALIDAD: COLOMBIANA

ACTIVIDAD: LAS ACTIVIDADES DE ADHINTER S.A. SE DESARROLLAN EXCLUSIVAMENTE EN LA ZONA FRANCA DEL PACIFICO CORRESPONDEN A LA PRODUCCION Y COMERCIALIZACION EN EL MERCADO NACIONAL E INTERNACIONAL DE ADHESIVOS MEDICINALES TALES COMO CURAS, VENDITAS, ESPARADRAPOS, PARCHES, VENDAS; ADHESIVOS PARA USO EN OFICINA TALES COMO CINTAS, PEGANTES, PAPELES ADHESIVOS Y CINTAS ADHESIVAS PARA USO INDUSTRIAL.

PRESUPUESTO DE CONTROL: TECNOQUIMICAS S.A., TIENE SUSCRITAS EN LA SOCIEDAD ADHESIVOS INTERNACIONALES S.A. ADHINTER S.A., LA CANTIDAD DE DOS MILLONES CUATROCIENTOS TREINTA MIL (2.430.000) ACCIONES ORDINARIAS DE VALOR NOMINAL DE CIENTO PESOS (\$100.00) SOBRE UN TOTAL DE TRES MILLONES (3.000.000) DE ACCIONES ORDINARIAS SUSCRITAS QUE REPRESENTA UNA PARTICIPACION DIRECTA DEL 81% SOBRE EL TOTAL DEL CAPITAL SUSCRITO Y PAGADO DE LA SOCIEDAD.

C E R T I F I C A :

QUE A SU NOMBRE FIGURA MATRICULADO EN LA CAMARA DE COMERCIO BAJO EL NRO.007050-02 UN ESTABLECIMIENTO DE COMERCIO DENOMINADO: TECNOQUIMICAS
UBICADO EN LA:
CL. 23 No. 7 39
DE CALI
Y RENOVÓ POR EL AÑO 2002.



C E R T I F I C A :

QUE A SU NOMBRE FIGURA MATRICULADO EN LA CAMARA DE COMERCIO BAJO EL NRO.311234-02 UN ESTABLECIMIENTO DE COMERCIO DENOMINADO: TECNOQUIMICAS
UBICADO EN LA:
CR. 7 No. 22 122
DE CALI
Y RENOVÓ POR EL AÑO 2002.

C E R T I F I C A :

QUE A SU NOMBRE FIGURA MATRICULADO EN LA CAMARA DE COMERCIO BAJO EL NRO.311235-02 UN ESTABLECIMIENTO DE COMERCIO DENOMINADO: TECNOQUIMICAS
UBICADO EN LA:
CL. 23 No. 4 66
DE CALI
Y RENOVÓ POR EL AÑO 2002.

C E R T I F I C A :

RENOVACION MATRICULA: QUE LA SOCIEDAD EFECTUO LA RENOVACION DE SU MATRICULA MERCANTIL EL 27 DE MARZO DE 2002.

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--- SUPERINTENDENCIA Y CONTROL ---
Radicación : 25971851 UNIFORME Folios: 63
Fecha (IMP): 2003-11-26 13:56:00
Tramite : N11 PCI-PR1EN1 379 PASA N1C1 400 UNIFORME
Dependencia: 2020 DIVISION DE NUEVAS OPERACIONES

NIT : 800176089-2

RECIBO OFICIAL DE CAJA : 03 - 85,922
FECHA : NOVIEMBRE 25 DE 2003

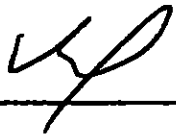
***** CONSIGNACION *****

DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PAGO	Vr. PAGO
JUAN PABLO REYES	CONSIGNACION	BANCO POPULAR	050-00110-6	5053506	25/11/2003	32,000.00
JUAN PABLO REYES	CONSIGNACION	BANCO POPULAR	050-00110-6	16388384	25/11/2003	54,000.00
JUAN PABLO REYES	CONSIGNACION	BANCO POPULAR	050-00110-6	16388385	25/11/2003	272,000.00
JUAN PABLO REYES	CONSIGNACION	BANCO POPULAR	050-00110-6	16388386	25/11/2003	544,000.00

***** CONCEPTO *****

CANT.	RENTISTICO	CONCEPTO	TOTAL CONCEPTO
1	50005-01-04	PRESENTACION DE OBSERVACIONES	
12		MARCAS Y LEYAS COMERCIALES	212,000.00
		TOTAL :	\$ 212,000.00

SOM: DOSCIENTOS DOCE MIL PESOS

RESPONSABLE : 

RECIBO DE CAJA APLICADO AL EXPEDIENTE No. _____

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RECEPCION DE PAGOS Y CONCEPTOS
Modificacion : 02091881 00000002 Folios: 63
Fecha (FIRM): 2003-11-25 13:06:30
Itemite : 011 PCI-PRIMEI 3/1 FASE MCI 400 IMPOSICION
Dependencia: 2000 DIVISION DE NUEVAS CREACIONES

NIT : 800176089-2

RECIBO OFICIAL DE CAJA : 03 - 85,927
FECHA : NOVIEMBRE 25 DE 2003

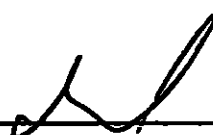
***** CONSIGNACION *****

DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PGO	Vr. PAGO
JUAN PABLO REYES	CONSIGNACION	BANCO POPULAR	050-00110-6	5053506	25/11/2003	32,000.00
JUAN PABLO REYES	CONSIGNACION	BANCO POPULAR	050-00110-6	16388384	25/11/2003	54,000.00
JUAN PABLO REYES	CONSIGNACION	BANCO POPULAR	050-00110-6	16388385	25/11/2003	272,000.00
JUAN PABLO REYES	CONSIGNACION	BANCO POPULAR	050-00110-6	16388386	25/11/2003	544,000.00

***** CONCEPTO *****

CANT.	RENTISTICO	CONCEPTO	TOTAL CONCEPTO
1	50005-01-08	PRORROGA DE TERMINOS	
		31 PRORROGA DE TERMINOS	60,000.00
		TOTAL :	\$ 60,000.00

SON: SESENTA MIL PESOS

RESPONSABLE : 

RECIBO DE CAJA APLICADO AL EXPEDIENTE No. _____