COMMONWEALTH OF AUSTRALIA
Patents Act 1952-1969

CONVENTION APPLICATION FOR A PATENT

(1) Here insert (in full) Name or Names of Applicant or Applicants, followed by Address(es).

(2) Here insert Title of Invention.

(3) Here insert number(s) of basic application(s).

(4) Here insert Name of basic Country or Countries, and basic date or dates.

(5) Signatures of Applicant(s) or Seal of Company and Signatures of its Officers as prescribed by its Articles of Association.

TO: THE COMMISSIONER OF PATENTS.

WATERMARK PATENT & TRADEMARK ATTORNEYS
COMMONWEALTH OF AUSTRALIA - Patents Act 1952

DECLARATION IN SUPPORT OF A CONVENTION APPLICATION UNDER PART XVI., FOR A PATENT

In support of the Convention application made under Part XVI. of the Patents Act 1952 by HOECHST AKTIENGESELLSCHAFT D-6230 Frankfurt am Main 80, Federal Republic of Germany

for a patent for an invention entitled:
Di(nitroxyalkyl)amides of pyridine-2,4- and -2,5-dicarboxylic acids, a process
for the preparation thereof, and the use thereof

By We, Ulrich Tergau, Am Dornbusch 3, D-6239 Eppstein/Taunus
Franz Lapice, Sandweg 2, D-6233 Kelkheim (Taunus) / Fed. Rep. of Germany

do solemnly and sincerely declare as follows:

1. We are authorized by HOECHST AKTIENGESELLSCHAFT
   the applicant/s for the patent to make this declaration on its/their behalf.

2. The basic application/s as defined by Section 141 of the Act was/were made
   by HOECHST AKTIENGESELLSCHAFT
   Federal Republic of Germany P 40 01 002.3 of January 16, 1990

3. Ekkehard BAADER, Amselweg 14, D-6240 Königstein/Taunus
   Fed. Rep. of Germany

   is/are the actual inventor/s of the invention and the facts upon which
   HOECHST AKTIENGESELLSCHAFT
   is/are entitled to make the application are as follows:
   The said HOECHST AKTIENGESELLSCHAFT
   is/are the assignee/s of the said inventor/s

4. The basic application/s referred to in paragraph 2 of this Declaration was/were the
   first application/s made in a Convention country in respect of the invention the
   subject of the application.


HOECHST AKTIENGESELLSCHAFT

ppa. Tergau i.V. Lapice

To the Commissioner of Patents
1. The use of di(nitroxyalkyl)amides of pyridine-2,4- and -2,5-dicarboxylic acids, of the formula I

\[
\begin{align*}
\text{O}_2\text{NO} &- \text{R} - \text{HNOC} - & \text{N} &- \text{CONH} - \text{R} - \text{ONO}_2 \\
\end{align*}
\]

in which
R is linear or branched C\textsubscript{1}-C\textsubscript{4}-alkanediyl,
and the physiologically tolerated salts, for the preparation of pharmaceuticals which inhibit proline hydroxylase and lysine hydroxylase.

3. A compound of the formula I

\[
\begin{align*}
\text{O}_2\text{NO} &- \text{R} - \text{HNOC} - & \text{N} &- \text{CONH} - \text{R} - \text{ONO}_2 \\
\end{align*}
\]
in which
R is methylene, propylene or butylene,
and the physiologically tolerated salts.

7. A compound as claimed in claim 3 or 4 for inhibiting proline hydroxylase and lysine hydroxylase.

8. A compound as claimed in one or more of claims 1 to 4 for use as fibrosuppressants and immunosuppressants.

10. The use of compounds of the formula I or the salts thereof as claimed in one or more of claims 1 to 4 for influencing the metabolism of collagen and collagen-like substances and the biosynthesis of Clq.
Complete Specification for the invention entitled:

DI(NITROXYALKYL)AMIDES OF PYRIDINE-2,4- AND -2,5-DICARBOXYLIC ACIDS, A PROCESS FOR THE PREPARATION THEREOF, AND THE USE THEREOF

The following statement is a full description of this invention, including the best method of performing it known to:
Di(nitroxyalkyl)amides of pyridine-2,4- and -2,5-dicarboxylic acids, a process for the preparation thereof, and the use thereof.

Compounds which inhibit the enzymes proline hydroxylase and lysine hydroxylase bring about a very selective inhibition of collagen biosynthesis by influencing the collagen-specific hydroxylation reactions. In the course thereof, protein-bound proline or lysine is hydroxylated by the enzymes proline hydroxylase or lysine hydroxylase. If this reaction is suppressed by inhibitors, the result is an insufficiently hydroxylated collagen molecule which is unable to function and can be released by the cells into the extracellular space only in a small amount. Moreover, the insufficiently hydroxylated collagen cannot be incorporated in the collagen matrix and very readily undergoes proteolytic degradation. The consequence of these effects is an overall reduction in the amount of collagen deposited outside the cells.

It is known that the inhibition of proline hydroxylase by known inhibitors such as a,a'-dipyridyl results in inhibition of Clq biosynthesis by macrophages (W. Müller et al., FEBS Lett. 90 (1978), 218; Immunobiology 155 (1978), 47). This leads to the classical pathway of complement activation becoming inoperative. Thus, inhibitors of proline hydroxylase also act as immunosuppressants, for example in immune complex diseases.

It is known that the enzyme proline hydroxylase is efficiently inhibited by pyridine-2,4- and -2,5-dicarboxylic acids (K. Majamaa et al., Eur. J. Biochem. 138 (1984) 239-245). These compounds are, however, effective inhibitors in cell culture only in very high concentrations (Tschank, G. et al., Biochem. J. 238 (1987) 625-633).
DE-A 34 32 094 gives a description of diesters of pyridine-2,4- and -2,5-dicarboxylic acids with 1-6 carbon atoms in the ester alkyl moiety as pharmaceuticals for inhibiting proline hydroxylase and lysine hydroxylase.

These lower alkyl diesters have the disadvantage, however, that in the body they are too rapidly cleaved to the acids and do not reach their site of action in the cell in sufficiently high concentration and thus are poorly suited for possible administration as pharmaceuticals.

DE-A 37 03 959, DE-A 37 03 962 and DE-A 37 03 963 describe in a general form mixed ester/amides, higher alkyl diesters and diamides of pyridine-2,4- and -2,5-dicarboxylic acids, which are effective inhibitors of collagen biosynthesis in animal models.

Thus, DE-A 37 03 959 describes, inter alia, the synthesis of N,N'-bis(2-methoxyethyl)pyridine-2,4-dicarboxamide and N,N'-bis(3-isopropoxypropyl)pyridine-2,4-dicarboxamide.

German Patent Applications P 38 26 471.4 and P 38 28 140.6 propose an improved process for the preparation of N,N'-bis(2-methoxyethyl)pyridine-2,4-dicarboxamide. German Patent Application P 3924093.2 proposes new N,N'-bis(alkoxyalkyl)pyridine-2,4-dicarboxamides.

Both pyridine-2,4- and -2,5-dicarboxamides (Hirakata et al., J. pharm. Soc. Japan 77 (1957) 219 and Haring et al., Helv. 37 (1954) 147, 153) and pyridine-2,4- and -2,5-dicarboxhydrazides (Itai et al., Bl. nation. hyg. Labor. Tokyo, 74 (1956) 115, 117 and Shinohara et al., Chem. High Polymers Japan, 15 (1958) 839) have already been disclosed as agents for tuberculosis.

JP 53/28175 (78/28175) describes N,N'-bis(2-nitroxyethyl)pyridine-2,4- and -2,5-dicarboxamides as substances with a vasodilator action.
It has now been found, surprisingly, that di(nitroxy-alkyl)amides of pyridine-2,4- and -2,5-dicarboxylic acids, of the formula I

\[
\text{O}_2\text{NO-}R\text{-HNOC---CONH-R-ON0}_2
\]  

(I)

in which

5  
R is C₁⁻⁻⁻-alkanediyl,  
and the physiologically tolerated salts, effectively inhibit lysine hydroxylase and proline hydroxylase in animal models.

Accordingly, the invention relates to a) the use of compounds of the formula I

\[
\text{O}_2\text{NO-}R\text{-HNOC---CONH-R-ON0}_2
\]  

(I)

in which

5  
R is C₁⁻⁻⁻-alkanediyl,  
and the physiologically tolerated salts, for the preparation of a pharmaceutical which inhibits proline hydroxylase and lysine hydroxylase.

The invention additionally relates to b) the compounds of the formula I  
in which

5  
R is methylene, propylene or butylene,  
and the physiologically tolerated salts, for use as pharmaceuticals.
The invention additionally relates to c) the compounds of the formula I in which
\[ R \text{ is methylene, propylene or butylene,} \]
and the physiologically tolerated salts thereof.

The invention particularly relates to the compounds of the formula I defined in a), b) and c) for use as fibro-suppressants and immunosuppressants and for the inhibition of proline hydroxylase and lysine hydroxylase and for influencing the metabolism of collagen and collagen-like substances and the biosynthesis of C1q.

All the said alkyl radicals with more than 2 carbon atoms can be both straight-chain and branched.

The invention additionally relates to a process for the preparation of compounds of the formula I, which comprises reacting a compound of the formula II

\[
\begin{align*}
\text{O} \\
\text{Y-C} \\
\text{N} \\
\text{C-Y} \\
\text{O}
\end{align*}
\]

(II)

with a compound of the formula III

\[
\text{H}_2\text{N-R-ONO}_2
\]

(III)

where R has the meaning specified for formula I, and Y is halogen, hydroxyl or C1-C4-alkoxy, or forms together with the carbonyl group an active ester or a mixed anhydride, or comprises nitrating a compound of the formula IV
in which
R is as defined above, and subsequently converting the
reaction products where appropriate into their physiologically tolerated salts.

The preparation of compounds of the formula I and the
preparation of those starting substances required for
this which cannot be bought is described in detail
hereinafter.

The compounds according to the invention are prepared
most straightforwardly by the two components, the pyri-
dine derivative of the formula (II) and the amine of the
formula (III), being mixed in equimolar amounts or with
an up to about 5-fold excess of III and reacted at
temperatures between \(-30\) and \(150^\circ\text{C}\), preferably at 20 to
\(100^\circ\text{C}\), until the reaction is complete. The completion of
the reaction can be determined, for example, by thin-
layer chromatography. One variant of this process com-
prises using a suitable solvent such as diethyl ether or
dimethoxyethane or tetrahydrofuran, chlorinated hydro-
carbons such as methylene chloride, chloroform, tri-
or tetrachloroethylene, benzene, toluene or else polar
solvents such as dimethylformamide, acetone, alcohols
such as methanol or ethanol or dimethyl sulfoxide. It is
also possible in this case to use an excess of amine of
the formula (III), which can be up to about 5-fold
amounts. The temperatures for this reaction are between
room temperature and the boiling point of the solvent,
with temperatures in the range from room temperature to
\(130^\circ\text{C}\) being particularly preferred.

The reaction can likewise be carried out via a mixed
anhydride such as ethyl chloroformate or via an activated ester such as paranitrophenyl ester (Y = ClCH₂-COO or NO₂-C₆H₄-O). Appropriate methods are described in the literature.

It is also possible, where appropriate, for the reaction to be carried out in the presence of bases. Examples of suitable additional bases are carbonates or bicarbonates such as sodium or potassium carbonate or sodium or potassium bicarbonate, or tertiary amines such as triethylamine, tributylamine, ethyldiisopropylamine or heterocyclic amines such as N-alkylmorpholine, pyridine, quinoline or dialkylanilines.

One variant for the preparation of the compounds of the formula I comprises nitration of the corresponding hydroxyalkyldiamides of pyridine-2,4- or -2,5-dicarboxylic acid (IV). This entails adding concentrated nitric acid to the corresponding hydroxyalkyldiamides at reaction temperatures from -20°C to +10°C, preferably at -10°C to -5°C. The reaction time in this case is 10-240 min, preferably 20-90 min. The reaction product is subsequently neutralized where appropriate.

Where appropriate the products can be worked up, for example, by extraction or by chromatography, for example on silica gel. The isolated product can be recrystallized and, where appropriate, reacted with a suitable acid to give a physiologically tolerated salt. Examples of suitable acids are:

- mineral acids such as hydrochloric and hydrobromic acid, and sulfuric, phosphoric, nitric or perchloric acid or organic acids such as formic, acetic, propionic, succinic, glycolic, lactic, malic, tartaric, citric, maleic, fumaric, phenylacetic, benzoic, methanesulfonic, toluenesulfonic, oxalic, 4-aminobenzoic, naphthalene-1,5-disulfonic or ascorbic acid.
Those starting compounds of the formula (III) which cannot be bought can be synthesized by processes known from the literature.

The starting compounds of the formula (II) are obtained, for example, by converting pyridine-2,4- or -2,5-dicarboxylic acid into the corresponding pyridine-2,4- or -2,5-dicarbonyl halide, preferably chloride (by processes known from the literature), preferably in the presence of a catalyst such as dimethylformamide. This acid halide can then be reacted, for example, either with a suitable alcohol, for example paranitrobenzyl alcohol, to give the corresponding active ester, or else with lower alcohols such as methanol or ethanol to give the corresponding esters. It is likewise also possible for the pyridine-2,4- or -2,5-dicarboxylic acid initially to be converted by addition of a suitable carboxylic acid or of a carboxylic ester such as ethyl chloroformate into a mixed anhydride which is then reacted with the amines (III) to give the products according to the invention. An appropriate method is likewise described in the literature.

The starting compounds of the formula (IV) are obtained, for example, by reacting corresponding N,N'-bis(alkoxyalkyl)pyridine-2,4- or -2,5-dicarboxamides, preferably the bis(methoxyalkyl)diamide by processes known from the literature, for example with boron tribromide. The preparation of the bis(alkoxyalkyl)diamides is known and described, for example, in DE-A 3,703,959. This entails reacting a reactive pyridinedicarboxylic acid derivative, for example the pyridinedicarbonyl chloride, with an alkoxyalkylamine.

The compounds of the formula I according to the invention have valuable pharmacological properties and display, in particular, activity as inhibitors of proline hydroxylase and lysine hydroxylase, as fibrosuppressant and immuno-suppressant.
Because of these pharmacological properties, the compounds according to the invention are suitable for the treatment of disturbances of the metabolism of collagen and collagen-like substances and for the treatment of disturbances of the biosynthesis of Clq.

Hence the invention furthermore relates to the use of the compounds of the formula I according to the invention and of the physiologically tolerated salts thereof for the treatment of the abovementioned metabolic disorders.

The compounds can be used as pharmaceuticals either alone or mixed with physiologically tolerated auxiliaries or excipients. They can be administered for this purpose orally in doses of 0.01 - 25.0 mg/kg/day, preferably 0.01 - 5.0 mg/kg/day or parenterally in doses of 0.001 - 5 mg/kg/day, preferably 0.001 - 2.5 mg/kg/day, in particular 0.005 - 1.0 mg/kg/day. The dose can also be increased in severe cases. However, in many cases, lower doses are also sufficient. These data relate to adults weighing about 75 kg.

The invention additionally embraces the use of the compounds according to the invention for the preparation of pharmaceuticals which are employed for the treatment and prophylaxis of the abovementioned metabolic disorders.

The invention furthermore relates to pharmaceuticals which contain one or more compounds of the formula I according to the invention and/or the physiologically tolerated salts thereof.

The pharmaceuticals are prepared by processes which are known per se and familiar to those skilled in the art. The pharmacologically active compounds (= active substance) according to the invention are employed as pharmaceuticals either as such or, preferably, in combination with suitable pharmaceutical auxiliaries or
excipients in the form of tablets, coated tablets, capsules, suppositories, emulsions, suspensions or solutions, in which the content of active substance is up to about 95%, advantageously between 10 and 75%.

Examples of suitable auxiliaries or excipients for the desired pharmaceutical formulation are, besides solvents, gel-formers, suppository bases, tablet auxiliaries and other active substance vehicles, also antioxidants, dispersing agents, emulsifiers, antifoam agents, flavor correctives, preservatives, solubilizers and colorants.

The active substances can be administered orally, parenterally or rectally.

The active compounds are mixed with the additives suitable for this purpose, such as excipients, stabilizers or inert diluents, and converted by customary methods into suitable dosage forms such as tablets, coated tablets, hard gelatin capsules, aqueous alcoholic or oily suspensions or aqueous or oily solutions.

Examples of inert excipients which can be used are gum arabic, magnesia, magnesium carbonate, potassium phosphate, lactose, glucose or starch, especially corn starch. This preparation can be carried out both as dry and as wet granules. Examples of suitable oily excipients or solvents are vegetable or animal oils, such as sunflower oil or fish liver oil.

For subcutaneous or intravenous administration, the active compounds are converted into solution, suspension or emulsion, if desired with the substances suitable for this purpose, such as solubilizers, emulsifiers or other auxiliaries. Examples of suitable solvents are physiological saline or alcohols, for example ethanol, propanol, glycerol, as well as sugar solutions such as glucose or mannitol solutions, or else a mixture of the various solvents mentioned.
The invention is explained in detail hereinafter by means of examples.

Precursor 1:

**bis-N,N’-(Methoxyethyl)amide of pyridine-2,4-dicarboxylic acid**

![Chemical Structure](image)

3 g of pyridine-2,4-dicarboxylic acid are introduced into 50 ml of toluene and 1 ml of DMF, and 2.7 ml of thionyl chloride are added dropwise to the solution. The mixture is heated until no further evolution of gas is evident (about 2.5 h). The mixture is cooled, 5 ml of toluene are distilled out, and 4.6 ml of 2-methoxyethylamine and 5 ml of triethylamine are added dropwise to the solution. The solution is stirred at room temperature for 4 h and then evaporated, the residue is taken up in water and extracted 4 x with methylene chloride. The combined organic phases are dried over magnesium sulfate and evaporated. The crude product is chromatographed on silica gel (solvent ethyl acetate).

Melting point: 42 - 44°C

\[ ^1H-NMR (CDCl_3): \delta = 1.2 \ (3H, \ tr); \ 3.3-3.8 \ (12H, \ qu. \ and \ m); \ 7.9 \ (1H, \ m); \ 8.4-8.5 \ (1H, \ m); \ 8.7-8.8 \ (1H, \ m); \]
Precursor 2:

bis-N,N'-(2-Hydroxyethyl)amide of pyridine-2,4-dicarboxylic acid

\[
\text{CONH-CH}_2\text{-CH}_2\text{-OH} \quad \text{CONH-CH}_2\text{-CH}_2\text{-OH}
\]

0.5 g of bis-(N,N'-(2-methoxyethyl)amide of pyridine-2,4-dicarboxylic acid (precursor 1) is dissolved in 10 ml of dichloromethane and, at -78°C, boron tribromide (11 ml, 1 molar solution in dichloromethane) is added dropwise. After addition is complete, allow to reach room temperature and then stir for 3 hours. Pour into 100 ml of saturated bicarbonate solution and extract 3 x with ethyl acetate. The combined organic solvents are dried with magnesium sulfate and evaporated. The crude product is chromatographed on silica gel.

\(^1\text{H-NMR (CDCl}_3\text{)}: \delta = 1.5-2.2 (4H, m); 3.4 (4H, m); 3.6 (4H, m); 7.9-8.0 (1H, m); 8.4-8.5 (1H, m); 8.7-8.8 (1H, m)

Example 1

N,N'-Di(2-nitroxyethyl)amide of pyridine-2,4-dicarboxylic acid

\[
\text{CONH-CH}_2\text{-CH}_2\text{-ONO}_2 \quad \text{CONH-CH}_2\text{-CH}_2\text{-ONO}_2
\]

1 g of di(2-hydroxyethyl)amide of pyridine-2,4-dicarboxylic acid (precursor 2) is added at -10°C to -5°C
to 5 ml of concentrated nitric acid. The mixture is allowed to warm to 2°C and then stirred for 40 minutes. It is poured into ice/water and neutralized with sodium carbonate. The solution is extracted three times with dichloromethane, and the organic phases are dried over magnesium sulfate and evaporated. The residue crystallizes from ether.

Yield: 750 mg
Melting point: 85 - 88°C
1. The use of di(nitroxyalkyl)amides of pyridine-2,4- and -2,5-dicarboxylic acids, of the formula I

\[ \text{O}_2\text{NO-R-HNOC-} \quad \text{CONH-R-ONO}_2 \]  

(I)

in which

R is linear or branched C\textsubscript{1}-C\textsubscript{4}-alkanediyl, and the physiologically tolerated salts, for the preparation of pharmaceuticals which inhibit proline hydroxylase and lysine hydroxylase.

2. The use as claimed in claim 1, wherein

R is ethylene or propylene.

3. A compound of the formula I

\[ \text{O}_2\text{NO-R-HNOC-} \quad \text{CONH-R-ONO}_2 \]  

(I)

in which

R is methylene, propylene or butylene, and the physiologically tolerated salts.

4. A compound of the formula I as claimed in claim 3, wherein

R is methylene or n-propylene.

5. A compound as claimed in claim 3 or 4, for use as a pharmaceutical.

6. A process for the preparation of compounds of the
formula I as claimed in claim 3, which comprises reacting a compound of the formula II

\[
\text{\text{\text{\text{\text{II}})}}
\]

with a compound of the formula III

\[
\text{H}_2\text{N-\text{R-ONO}_2} (\text{III})
\]

where R has the meanings specified in claim 3, and Y is halogen, hydroxyl or C\text{I}-C\text{I}-alkoxy, or forms together with the carbonyl group an active ester or a mixed anhydride, or comprises nitrating a compound of the formula IV

\[
\text{\text{\text{\text{\text{IV}})}}
\]

in which R is as defined in claim 3, and subsequently converting the reaction products where appropriate into their physiologically tolerated salts.

7. A compound as claimed in claim 3 or 4 for inhibiting proline hydroxylase and lysine hydroxylase.

8. A compound as claimed in one or more of claims 1 to 4 for use as fibrosuppressants and immunosuppressants.

9. A pharmaceutical containing a compound of the formula I as claimed in claim 3 or 4 with tolerated pharmaceutical vehicles.
10. The use of compounds of the formula I or the salts thereof as claimed in one or more of claims 1 to 4 for influencing the metabolism of collagen and collagen-like substances and the biosynthesis of Clq.

11. The use of compounds of the formula I or the salts thereof as claimed in one or more of claims 1 to 4 for the treatment of disturbances of the metabolism of collagen and collagen-like substances and of the biosynthesis of Clq.

12. A process for the preparation of pharmaceuticals for influencing the metabolism of collagen and collagen-like substances and the biosynthesis of Clq, which comprises adding a compound of the formula I or the salts thereof as claimed in one or more of claims 1 to 4 to the pharmaceutical.

DATED this 14th day of January 1991.

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