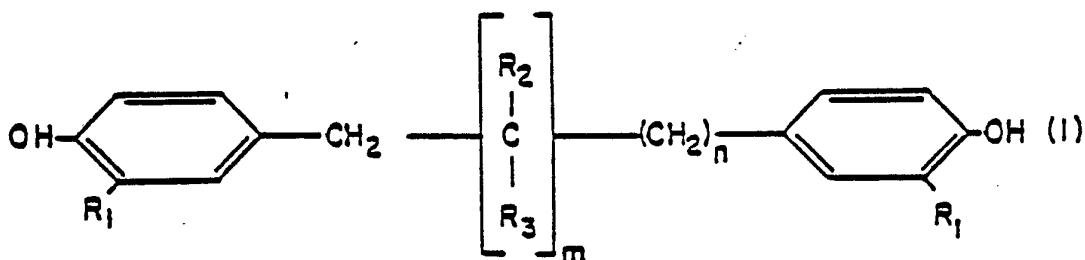




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(54) Title: METHOD FOR INHIBITING AGING**(57) Abstract**

Methods and compositions are provided for retarding aging, i.e., inhibiting damage to human cells caused by toxic byproducts of oxygen metabolism. The anti-aging active ingredient is a compound or mixture of compounds represented by structural formula (I), wherein m is 0, 1 or 2; n is 0 or 1; R₁ is hydrogen, halogen or hydroxy; and R₂ and R₃ each independently represent hydrogen or methyl provided that when m is 0, n is 0 and when m is 1 or 2, n is 1 and R₁ is hydroxy or halogen and that when m is 2, each R₂ can be the same or different and each R₃ can be the same or different or a pharmaceutically acceptable alkali or alkaline earth metal salt thereof.

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METHOD FOR INHIBITING AGINGField of the Invention

This invention relates to methods useful for retarding senescence or aging in mammals. In particular this invention relates both to methods for systemic treatment of
15 humans with compositions comprising hydroxy substituted diphenylalkyls to inhibit damage to cells which causes aging and to methods for topical treatment of humans with compositions comprising hydroxy substituted diphenylalkyls, e.g., to inhibit damage to cells which causes wrinkling/
20 aging of the skin.

Background of the Invention

Actuaries define senescence as the increased likelihood of dying, but the appropriate pathological
25 definition is progressive, multi-system organ atrophy. This is obviously not atrophy of disuse but atrophy of what can be called "cell drop-out". "Cell drop-out" as used herein means the disappearance of cells and consequent shrinkage of the body's organs. Such organ shrinkage is
30 seen in autopsies on the aged. For example, the human brain can decrease from an average weight of 1,500 grams in a young human adult to a 1,000 grams or less in a human of advanced age. A brain that has decreased in size (the

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1 senescent brain) is highly forgetful, is unable to memorize
new information and cannot react quickly to external
stimulae. Shrinkage with age is also found in other human
organs such as the heart, liver, kidneys, lymph nodes,
5 skeletal muscles and vertebrae. Such shrinkages often
correspond to the accumulation of peroxidized, free
radical products seen as brown-yellow age pigment
(lipofuscin). Other corresponding changes that are
associated with aging are wrinkled skin, depleted fat
10 deposits, fewer dermal melanocytes, brittle bones, low
infection resistance, poor exercise tolerance and lack of
reproductive ability.

At the cellular level, senescence means inadequate
DNA repair leading to disordered and/or nonexistent cell
15 replication, loss of mitotic control factors in the
nucleus and cytoplasm including disordered nuclear-
cytoplasmic exchange, and permanent closing of micro-
circulatory capillary beds resulting in focal cell drop-out
and loss of cell and organelle membrane function. This
20 progressive cellular process affects all organs and
tissues throughout the body and its etiology and
pathogenesis must therefore involve a universal and
fundamental aspect of cell physiology.

Aging of the mammalian organism as a whole must be
25 examined at the cellular level because changes of
individual cells affect changes in individual body organs
and changes in individual body organs affect the organism
as a whole. For example, death or dysfunction of a
critical bodily organ, such as the heart, will result in
30 the death of the body as a whole.

In any study on aging two distinct types of cells
must be considered; normally dividing cells and post-

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1 mitotic cells. Normally dividing cells are those of the
skin, hair and gastrointestinal tract, for example. While
thousands of such cells die daily, they are continually
replaced. Their replacement is with nearly exact replicas
5 until the time of aging or senescence begins. This time
begins in the mid-twenties in man. The second cell type,
i.e. post-mitotic cells, are those that make up the heart,
brain and central nervous system, for example. Generally
speaking, post-mitotic cells don't divide or reproduce.
10 Mammals are born with a fixed number of post-mitotic cells
which lose function and die daily throughout a mammal's
life span. Death of a mammal occurs when a critical number
of post-mitotic cells lose function or die in a critical
organ, e.g. the brain.
15 Recent biomedical gerontological research has provided
some theories regarding the metabolic course of events
which leads to the inevitable loss of function,
deterioration, destruction and death of mammalian cells.
One convincing theory relates to mammalian cellular
20 metabolism's reliance on oxygen metabolism. By "oxygen
metabolism" is meant the burning of oxygen in the cells
energy factories (mitochondria) together with foods such
as fatty acids to produce adenosine triphosphate (ATP),
the cell's energy source. Production of ATP occurs
25 continually through enzymatically controlled chemical
reactions. Unfortunately, these chemical reactions are not
100% efficient. For example, in the bacteria, E-coli,
these reactions are only approximately 84% efficient. As
one moves up the scale of evolution, these reactions become
30 increasingly more efficient. In man the reactions are
94±2 % efficient.

More efficient "oxygen metabolism" in man as compared

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1 to other mammals results in a decrease in the burning rate
of oxygen in man compared to other mammals, a decrease in
the burning rate of fatty acids and carbohydrates, a
decrease in the formation of toxic oxygen byproducts and
5 a decrease in the temperature set-point of the body to
below that of other mammals.

Even though the relative amount of byproducts produced
by "oxygen metabolism" in man is less than the amount of
such byproducts produced in other mammals, such byproducts
10 are highly toxic. Examples of these toxic byproducts are
the superoxide (O_2^-) and hydroxyl ($\cdot OH$) radicals. To
illustrate the extreme toxicity of these radicals consider
the following: When superoxide is mixed with water,
concentrated hydrogen peroxide (an oxidative byproduct) is
15 formed. Concentrated hydrogen peroxide poured onto the
skin will damage it within a few minutes to a necrotic,
sickly white appearance. The same chemical reactions occur
inside mammalian, oxygen-burning cells. Although
protective enzymes destroy hydrogen peroxide continually,
20 such defenses are not 100% efficient. Thus, chemical
destruction of cells can result. Since man consumes many
pounds of oxygen daily, even a slight inefficiency in the
use of oxygen resulting in the O_2^- or $\cdot OH$ radicals and
oxidative byproducts is significant. As used herein
25 "toxic byproducts of oxygen metabolism" includes radicals
such as superoxide (O_2^-) and hydroxyl ($\cdot OH$) as well as
oxidative products produced by such radicals such as
hydrogen peroxide and the like.

An example of cell damage caused by the byproducts
30 from oxygen reactions, i.e. by toxic byproducts of oxygen
metabolism, is damage to a cell's genetic material, DNA.
It is known that approximately 7,000 DNA "hits" by toxic

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1 byproducts of oxygen metabolism occur daily in man.
Fortunately, most of these are repaired enzymatically by
the cells. It is known, however, that some cells that are
not repaired may become cancerous.

5 Of the two previously described cell types, i.e. the
normally dividing cells and post-mitotic cells, normally
dividing cells are able to reproduce and replace damaged
cells. Post-mitotic cells, on the other hand, while they
are sometimes repaired after bombardment with toxic oxygen
10 byproducts of oxygen metabolism, most often such
bombardment reduces their function, damages their DNA
and/or kills them outright. This is a steady-state, linear
aging process until damage to DNA and cellular defensive
enzymes becomes so great that the aging process acquires an
15 endogenous, accelerating character.

The long term, lifetime effects of this endogenous
toxic chemical bombardment can also be exemplified in the
wrinkling and hardening of the skin and arteries with age.
The skin and arteries consist of supportive material
20 called collagen and elastin. Collagen is the major
protein of the white fibers of connective tissue, cartilage
and bone. Elastin, or elastic tissue, is the major
connective tissue protein of elastic structures such as
the large blood vessels and the skin. Elastin enables
25 these structures to stretch, and then resume their original
shape and size.

Collagen and elastin contain fibers internally linked
together by chemical bonds called a "imide bonds". It is
theorized that mammalian aging involves the oxidation of
30 these imide bonds to "amide bonds". In the skin and
arterial collagen and elastin of mammals, as more and more
amide bonds are formed, the collagen and elastin fibers

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1 become increasingly less elastic and flexible. In man, it
is known that these fibers harden at a rate of
approximately 7% per decade after the age of maturity
(approximately mid-twenties). This means that the arterial
5 -vascular system has a theoretical life span of
approximately 140 years before becoming 100% rigid.

Free radical pathology mechanisms seem to be involved
at key points in the etiology and pathogenesis of cancer,
occlusive atherosclerosis and wrinkling of the skin. Free
10 radical pathology ensues largely from free radical and
oxidation products which negatively affect cell membranes,
collagen, elastin, immune functions, micro-circulation,
nucleic acids and regulatory proteins. Scientific studies
have shown that this mechanism progresses from inciting
15 factors to free radical pathologic reactions to products
to damage to organelles, cells and tissues resulting in
aging and disease. Aging seems to simultaneously affect
all cells, tissues and organs throughout the body in an
insidious, progressive pattern whose pathogenesis and
20 etiology therefore are thought to involve a fundamental
and universal aspect of cell physiology.

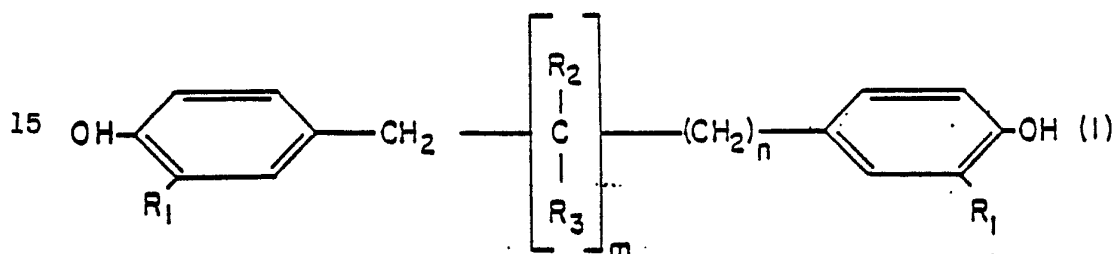
The clinical significance of toxic free radicals and
oxidative agents generated endogenously in living cells
has been documented in various scientific, medical
25 publications. An association between these toxic agents
and aging has been observed.

It is therefore desired that methods be provided for
inhibiting aging caused by free radicals and oxidative
agents produced in living cells. Such methods desirably
30 include systemic treatment of humans to inhibit aging of
the entire body as well as topical treatment, e.g., to
inhibit wrinkling/aging of the skin.

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1 Summary of the Invention

The present invention is directed to methods and compositions useful for retarding aging in humans, i.e., inhibiting damage to human cells caused by toxic byproducts of oxygen metabolism. One embodiment of the present invention comprises administering to a human a daily dosage of from about 0.5 mg per kilogram of body weight to less than about 2 mg per kilogram of body weight of a pharmaceutically acceptable composition comprising a compound or mixture of compounds represented by the following structural formula:



wherein

m is 0, 1 or 2;

20 n is 0 or 1;

R1 is hydrogen, halogen or hydroxy; and

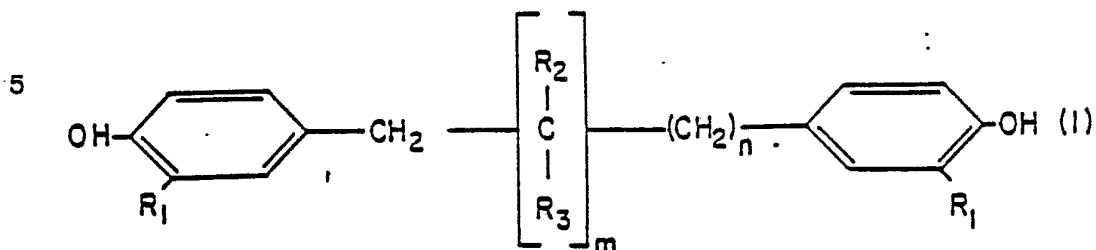
R2 and R3 each independently represents hydrogen or methyl provided that when m is 0, n is 0 and when m is 1 or 2, n is 1 and R1 is hydroxy or halogen and that when m is 25 2, each R2 can be the same or different and each R3 can be the same or different or a pharmaceutically acceptable alkali or alkaline earth metal salt thereof.

Another embodiment of this invention is directed to methods and compositions for inhibiting wrinkling of the skin in a human by topically administering to the skin of 30 such a human a pharmaceutically acceptable composition containing an effective amount of a compound or mixture of

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1 compounds represented by the following structural formula to inhibit wrinkling:



wherein

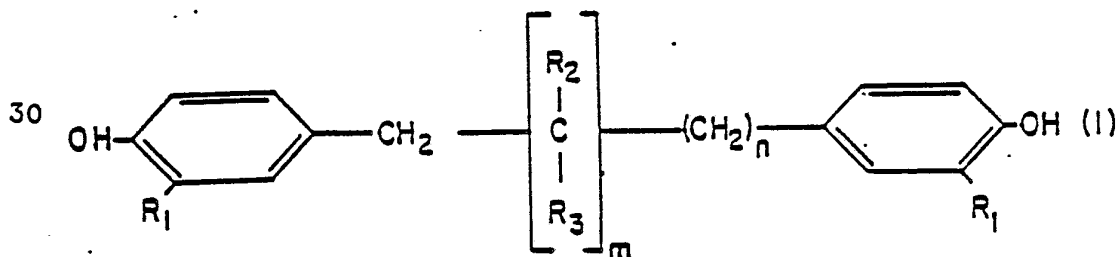
10 m is 0, 1, or 2;

n is 0 or 1;

R1 is hydrogen, halogen or hydroxy; and

R2 and R3 each independently represents hydrogen or methyl provided that when m is 0, n is 0 and when m is 1 or 15 2, n is 1 and R1 is hydroxy or halogen and that when m is 2, each R2 can be the same or different and each R3 can be the same or different or a pharmaceutically acceptable alkali or alkaline earth metal salt thereof.

In yet other embodiments of the present invention 20 there are disclosed methods and compositions for inhibiting the formation of amide bonds in human collagen and elastin. One such method includes topically administering to the skin of such a human a pharmaceutically acceptable composition comprising an amount of a compound or mixture 25 of compounds represented by the following structural formula sufficient to inhibit amide bond formation in human collagen or elastin:



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1 wherein

m is 0, 1 or 2;

n is 0 or 1;

R1 is hydrogen, halogen or hydroxy; and

5 R2 and R3 each independently represents hydrogen or methyl provided that when m is 0, n is 0 and when m is 1 or 2, n is 1 and R1 is hydroxy or halogen and that when m is 2, each R2 can be the same or different and each R3 can be the same or different or a pharmaceutically acceptable
10 alkali or alkaline earth metal salt thereof.

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1 Brief Description of the Drawings

These are the features, aspects and advantages of the present invention will be more fully understood when considered with respect to the following detailed
5 description, appended claims, and accompanying drawings, wherein:

Fig. 1 is a schematic representation of two collagen fibers (R) or of two elastin fibers (R) lying side by side, showing a reversible imide bond linking them together;

10 Fig. 2 is a schematic representation of two collagen fibers (R) or two elastin fibers (R) joined together by an amide bond which is further oxidized to a permanent amide bond;

Fig. 3 is a graph showing the results of a double
15 blind study set forth in Example 6, wherein a composition containing 2% NDGA by weight was applied topically to the skin of the foreheads of human volunteers; and

Fig. 4 is a graph showing the results of a pharmacokinetic time course study wherein single NDGA
20 doses of 50 mg, 75 mg, 100 mg and 200 mg were administered to human volunteers in a double blind study.

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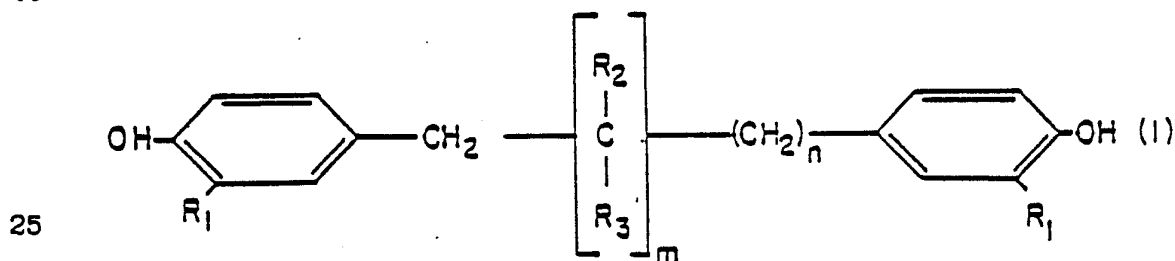
1 Detailed Description

The invention relates to the use of pharmaceutically acceptable compositions which include effective amounts of hydroxy diphenylalkyl derivatives as the active ingredient for retarding the degenerative process of aging in mammals, particularly in man.

The term, "aging" as used herein generally means any damage to the cells caused by toxic byproducts of oxygen metabolism. Such aging can, for example, result from partial damage or complete destruction of cells or from the conversion of imide bonds to amide bonds in human collagen and/or elastin resulting in hardening of the arteries or skin wrinkling, etc. Thus, the phrases "inhibiting aging" or "retarding aging" mean inhibiting or retarding damage to cells caused by toxic byproducts of oxygen metabolism.

The hydroxy diphenylalkyl derivatives useful in practice of principles of this invention may be represented by the following structural formula:

20



25

wherein

m is 0, 1 or 2;

n is 0 or 1;

R1 is hydrogen, halogen or hydroxy; and

30

R2 and R3 each independently represent hydrogen or methyl provided that when m is 0, n is 0 and when m is 1 or 2, n is 1 and R1 is hydroxy or halogen.

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and that when m is 2, each R2 can be the same or different and each R3 can be the same or different.

5

Pharmaceutically acceptable salts of the compounds of formula (1) are also useful in practice of this invention. Furthermore, mixtures of compounds of formula (1) including the pharmaceutically acceptable salts may be used in accordance with practice of principles of this invention.

10

Preferred compounds of the formula (1) are bis (p-hydroxy-phenyl) methane and 1,4-bis (3,4-dihydroxyphenyl) - 2,3-dimethylbutane (nordihydroguaiaretic acid or NDGA).

15

If "m" is 3 or greater and/or if "n" is 2 or greater, the compound will not be transported as readily as desired through the mitochondrial membranes. It is thought that the use of other antioxidant substances such as ethoxyquin, butylated hydroxytoluene (BHT), 2-mercaptoethylamine and methionine in place of the compounds of formula (1) is not desired. This is because the aforementioned antioxidants do not have as desirable a lipophilicity or zwitterion effect as do the compounds of formula (1). Therefore the compounds of formula (1) provided in accordance with this invention are transported more readily through the mitochondrial membranes than are the aforementioned antioxidants.

20

25

Preferably R1 is a halogen or hydroxy. Having a halogen or hydroxy at R1 tends to draw electron density to the R1 or halogen moiety. This reduces electron density over the entire phenyl ring, and in turn, reduces electron density on the hydroxy group adjacent to R1. The hydroxy adjacent R1 then becomes a relatively better electron "sink" or free radical electron acceptor which provides a highly deactivated phenol group which in turn tends to

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1 increase the efficiency of the molecule in scavenging
cell-damaging radicals such as hydroxyl ($\cdot\text{OH}$) radicals and
other toxic byproducts of cell oxygen metabolism.

5 An important feature of the hydroxy diphenylalkyl
derivatives set forth as being useful in accordance with
practice of this invention, is that they have two highly
deactivated phenol groups per molecule. None of the other
known antioxidant substances previously disclosed by the
literature to be useful as age retarding substances in
10 various species have this feature.

The compounds of formula (1) are known and may
be prepared according to methods disclosed in the litera-
ture from known materials. Such references include
Lieberman et al, Journal of American Chemical Society,
15 Vol. 69, pp 1540 (1947) and U.S. Patent No. 2,644,822
issued 1953 to Pearl, both of which are incorporated
herein by this reference. The pharmaceutically acceptable
salts may be mono- or di-salts and include the alkali
metal salts, in particular, the sodium and potassium salts
20 and the alkaline earth metal salts, such as the magnesium
and calcium salts. These salts may also be prepared by
methods presently known in the art.

The effective age retarding amount of the active
compounds of formula (1) are preferably administered
25 topically or orally either alone or in admixture with
conventional pharmaceutical carriers. The active compounds
of formula (1) may, if desired also be administered in
appropriate pharmaceutically acceptable carriers,
intravenously, subcutaneously, intramuscularly and
30 intracutaneously. They may be administered in such forms
as creams, lotions, tablets, dispersible powders, granules,
capsules, syrups and elixirs. The compositions may contain

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1 one or more conventional adjuvants, such as sweetening agents, flavoring agents, coloring agents and preserving agents.

5 Tablets may contain one or more compounds of the formula (1) in admixture with conventional pharmaceutically acceptable excipients, e.g. inert diluents, such as calcium carbonate, sodium carbonate, lactose, sorbitol and talc, granulating and disintegrating agents, e.g. starch and alginic acid, binding agents, e.g. sorbitol,
10 microcrystalline cellulose, gelatin and acacia, and lubricating agents, e.g. magnesium stearate, stearic acid and talc. The tablets may be uncoated or coated by known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained
15 action over a longer period.

Creams and lotions may contain the active ingredient in admixture with conventional cosmetically (and pharmaceutically) acceptable excipients, e.g. moisturizers and humectants such as uric acid, reticulan, polymucosaccharides, hyaluronic acid and aloe vera. Similarly, oral
20 liquids, e.g. suspensions may contain the active ingredients of formula (1) in admixture with any of the conventional excipients utilized for the preparation of such compositions, e.g. suspending agents (methylcellulose, tragacanth and sodium alginate) and wetting agents (lecithin, polyoxyethylene stearate and polyoxyethylene sorbitan mono-oleate). Capsules may contain the active ingredient of formula (1) alone or admixed with an inert solid diluent, e.g. calcium carbonate, calcium phosphate
25 and kaolin.
30

Tablets, creams, lotions, oral liquids and capsules may all contain various preservatives. Examples of such

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1 preservatives are derivatives of butylated hydroxytoluene
(BHT), butylated hydroxyanisole, methionine, cysteine,
ethoxyquin, ascorbic acid, tocopherol, catalase, superoxide
dismutase, glutathione, glutathione peroxidase,
5 2-mercaptoethylamine, cystamine, benzoic acid, ethyl-o-hydroxybenzoate, ethylenediamine tetraacetic acid and all other mercaptans, thiols and disulfides. The pharmaceutical and cosmetic preparations provided in accordance with practice of principles of this invention may contain
10 up to about 90% of the active ingredient of formula (1) in combination with the above described carriers or adjuvants.

The foregoing recitation of materials to be used in compositions containing compounds of formula (1) is presented for purposes of illustration and not limitation,
15 it being understood that a variety of equivalent materials could also be used if desired.

DOSAGE

The effective oral dosage of the compounds of formula
20 (1) employed for the retardation of aging, e.g. wrinkling of the skin and hardening of the arteries, may vary depending on the particular compound employed and the severity of the condition being treated. However, in general, satisfactory results are obtained when the
25 compounds of formula (1) are administered to humans at a daily dosage of from about 0.5 mg to less than about 2 mg per kilogram (kg) of body weight; preferably given in divided doses two to six times a day, or in sustained release form. When the daily dosage of compounds of
30 formula (1) is less than about 0.5 mg/kg of body weight the effect on preventing cell damage, i.e., inhibiting aging is only marginal. When the daily dosage of compounds

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1 of formula (1) is at about 2 mg/kg or greater the provision
of such compounds becomes uneconomical. Further, such
higher dosages are not required and may have an undesirable
reverse effect, actually speeding up the aging process
5 since the excess may upset the delicate balance of cell
metastasis.

Dosage forms suitable for internal use comprise the
active compound of formula (1) in either acid or salt form
or mixtures thereof in intimate admixture with a solid or
10 liquid which are pharmaceutically acceptable carriers or
diluent. The preferred pharmaceutical compositions from
the standpoint of preparation and ease of administration
are solid compositions, hard-filled capsules or tablets
containing from about 20 to 100 mg of the active ingredient
15 in acid or salt form.

EXAMPLE 1

Tablets suitable for oral administration in accordance
20 with practice of this invention may be prepared by
conventional pharmacological techniques and may contain the
following ingredients. (While Nordihydroguaiaretic acid
(NDGA) is shown in the Examples as the active ingredient,
any compounds or mixture of compounds defined by the
25 formula (1) could be used). Such tablets are useful in the
retardation of biological aging of mammals.

Ingredients	Weight
Nordihydroguaiaretic acid (NDGA)	40 mg.
30 Sorbitol	208 mg.
Magnesium Sterate	2 mg.

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EXAMPLE 2

Dry filled capsules suitable for oral administration in accordance with practice of this invention which may contain the following ingredients are prepared in a conventional manner.

<u>Ingredients</u>	<u>Weight</u>
Nordihydroguaiaretic acid (NDGA)	80 mg.
10 Inert solid diluent used to insure good mechanical flow in filling capsules (lactose, sorbitol, starch, kaolin)	170 mg.

Examples of formulations for topical application provided in accordance with practice of this invention containing NDGA as the active ingredient are shown in examples 3 and 4 below:

EXAMPLE 3

20

<u>Ingredients</u>	<u>Composition % W/W</u>
Nordihydroguaiaretic acid (NDGA)	0.5
Purified water	88.0
Butylene glycol	1.0
25 Octyl Palmitate	1.0
Aloe Vera extract	1.0
Glyceryl Sterate	0.5
Peg-100 Sterate	0.5
Ceteareth-20	0.5
30 reticulan	0.5
Myristyl myristate	0.5

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1	Mucopolysaccharides	0.5
	Jojoba oil	0.5
	Collagen	0.5
	Amino Acids	0.5
5	Paba	0.5
	Vitamin A	0.5
	Vitamin E	0.5
	Allantoin	0.5
	Imidazolidinyl urea	0.5
10	Carbomer 934	0.5
	Vitamin B-5	0.5
	Methyl-propylparaben	0.2
	Tea	0.1
	Vitamin D	0.1
15	Natural fragrance	0.1

EXAMPLE 4

In Example 4 the composition of Part A is formulated separately from the composition of Part B and then Parts A and B are mixed together.

	<u>Ingredients</u>	<u>Part A</u>	<u>% W/W</u>
	Nordihydroguaiaretic acid (NDGA)		2.0
25	Incroquant BTQ		1.0
	Crodacol CS-50		0.5
	Super Sterol Ester		0.5
	Crodamol PMP		1.0
	Polawax		1.5
30	Crosilk liquid		5.0

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1	<u>Ingredients</u>	<u>Part B</u>	<u>% W/W</u>
	Volatile Silicone 03314		0.5
	Water deionized		87.0
	GG Germaben II		1.0
5			

The ingredients listed under Part A of Example 4 are manufactured and distributed under their respective trademarks by Croda, Inc., New York, New York. "Volatile Silicone 03314" is manufactured and distributed by SWS
10 Silicones Corporation, Adrian, Michigan and "GG Germaben II" is manufactured and distributed by Sutton Laboratories, Inc., Chatham, New Jersey.

Preferred compositions for topical administration
15 contain from about 0.5% to about 20% by weight of the active ingredient of formula (1) in either acid or salt form. Since the compounds of formula (1) are active by themselves in retarding aging, i.e., in inhibiting damage to cells, they are contemplated as the only "active
20 ingredient" in the compositions provided in accordance with practice of principles of this invention. More preferably the compositions comprise from about 2% to about 5%. If the composition contains less than about 0.5% it will be only marginally effective and at greater than about
25 20% the economics would not be as favorable as desired and such high concentrations could possibly produce detrimental side effects. However, compositions for topical application where the active ingredient is in concentrations of from .001% to 90% by weight are
30 contemplated.

Without being bound by theory, it is thought that the compositions described above when administered orally or

1 topically, as described, inhibit oxidation of imide bonds
between collagen or elastin fibers to amide bonds. This
can be seen by referring to Fig. 1 where a pair of collagen
fibers (or a pair of elastin fibers) 10 and 12 are shown
5 schematically lying adjacent to each other. The fibers owe
their elasticity to reversible bonds that bind them
together chemically. The two active sites that provide the
bonds are the -CHO and -NH₂ moieties. The fibers are shown
bonded together by the reversible imide bond 14 at the
10 bottom of Fig. 1. This imide bonding is caused by the
uptake and giving off of water (H₂O) which is called
hydration and dehydration. Because the imide bonds open
and close continuously the collagen (or elastin) fibers
glide freely past one another. This biochemical effect
15 provides elasticity to the skin and arteries.

Turning to Fig. 2 (at the top) there are shown (for
purposes of illustration) the same two fibers 10 and 12
initially bonded together by the imide bond 14. The bond
is shown encircled at 16 at the bottom of Fig. 2 after it
20 has been oxidized to the amide bond.

Humans stop growing and maturing after 20-30 years.
During aging, the skin and arteries of man and other
mammals become increasingly inelastic. This inelasticity
is largely the result of the oxidation of the imide bond
25 to the amide bond as is shown in Fig. 2. The amide bond is
permanent and results in a permanent rigid linking between
collagen and elastin fibers.

EXAMPLE 5

30

As previously indicated, the compounds of formula (1)
are useful because they possess pharmacological activity

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1 in mammals including humans. In one aspect of practice of
techniques of this invention, the compounds of formula (1)
are useful in retarding the aging phenomena of skin
wrinkling. In this example, twenty four (24) Uppsala mice
5 were fed standard lab chow (provided by Anticimax of
Södertälje, Sweden) containing 0.5 weight% NDGA ad libitum
from the twelfth to thirty-sixth month of their lifespan.
Six (6) control mice were fed ad libitum the same lab chow
as the aforementioned 24 mice except that the chow did not
10 contain NDGA.

The NDGA fed mice were visually more youthful and
healthy at 26 months of age than control mice. The last
remaining control mouse showed significant hair loss,
pigmentation loss, poor eyesight and difficulty in movement
15 as compared with the four NDGA fed mice that were also
still living at this stage of the experiment.

Especially apparent was the greater skin wrinkling in
control mice versus NDGA fed mice. This increased wrinkl-
ing is indicative of collagen and elastin hardening and
20 cross-linking via the conversion of imide to amide bonds
as discussed above.

EXAMPLE 6

25 Turning to Fig. 3, the results of a double-blind time
study using a composition containing 2% by weight NDGA can
be seen. This composition was the same as the composition
set forth in Example 3 with the exception that the percent-
age of water was reduced to 86.5%. Starting at time zero,
10 milliliters of this cream was vigorously applied topic-
30 ally onto the skin of the forehead of a volunteer. Measure-
ments were taken at different intervals using non-invasive,

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1 reflective, near-infrared spectroscopy. The methods of
non-invasive, reflective, near infrared spectroscopy used
in this example are set forth in detail in R. Lippman,
"Rapid in vivo Quantification and Comparision of
5 Hydroperoxides and Oxidized Collagen in Aging Mice, Rabbits
and Man", Experimental Gerontology, Vol.. 20, pp. 1-5, 1985,
which is incorporated herein by this reference. Results
showed that lipid hydroperoxides in the other skin layers
and microvascularization decreased from about 190 to 151
10 micromole/cm² skin after about one hour. The
pharmacokinetics of topical NDGA application showed a
gradual return to a normal level of 190 micromole/cm² level
after approximately 4 hours. This experiment showed that
facial skin and skin unsaturated lipids are preserved, and
15 damage is prevented by applying NDGA according to the
procedure of this example.

EXAMPLE 7

20 Glial cells from a human brain were cultivated by
monolayering on Petri dishes. Cellular ATP production
was measured using bioluminescent methods (luciferin,
luciferase and photomultiplier detection). NDGA was added
to various cultures to a level of 0.5% w/v and an increase
25 in steady state ATP production was observed.

EXAMPLE 8

Mitrochondria from a human liver were freshly isolated
30 and maintained in a culture environment so that normal
respiration and ATP production occurred. Special chemical
probes, e.g. luminol-carnitine derivatives, were pipetted

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1 into the mitochondria culture media. Uptake of the probes
occurred in the mitochondrial inner membrane where oxygen
metabolism and toxic oxygen byproducts are produced. The
probes emitted chemiluminescent light in proportion to
5 superoxide (O_2^-) and hydroxyl radicals ($\cdot OH$). This light
was measured by a photomultiplier, and the results were
quantified and recorded. NDGA added to the mitochondria in
latter experiments significantly reduced the steady state
light, and therefore, NDGA significantly reduced toxic
10 oxygen byproducts and increased mitochondrial metabolic
efficiency. Increased lifespan of the human mitochondria
was also observed to occur.

EXAMPLE 9

15

Turning to Fig. 4 a graph of results of a
pharmacokinetic time-course (double-blind) study of single
doses of NDGA given to human volunteers at time zero is
shown. Single doses of NDGA of 50 mg, 75 mg, 100 mg and
20 200 mg were given to volunteers who had fasted at least
12 hours (overnight). Placebos were given to two (2) other
volunteers. Using the above referenced non-invasive
technique called reflective, near-infrared spectroscopy,
changes in the microvascular concentrations of lipid
25 hydroperoxides were monitored during 8 hour test periods.
The graph of Fig. 4 plots the concentration of
hydroperoxides in the skin in micro mole/cm² on the
vertical axis (as measured by the near-infrared
spectroscopy technique) vs. time in hours on the horizontal
30 axis. Subjects received single doses of NDGA and placebos
orally, respectively.

The results, as can be seen in Fig. 4, show that NDGA

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1 was absorbed into the blood stream after about 1 to 2 hours
upon oral administration into an empty stomach. NDGA
reduced levels of microvascular lipid hydroperoxides at
least 15% during the 4 to 8 hour study periods. A nearly
5 flat line at approximately 150 micromole/cm² (not shown
on the graph) was recorded for the two volunteers who
received placebos. It is concluded that NDGA is an
effective neutralizer of toxic byproducts of oxygen
metabolism, i.e. lipid hydroperoxides, when taken at
10 regular intervals of at least 6 hours, 3 times daily.

The above descriptions of exemplary embodiments of
the methods and compositions for retarding aging, i.e.,
inhibiting damage to cells in mammals caused by toxic
byproducts of oxygen metabolism, are for illustrative
15 purposes. Because of variations which will be apparent to
those skilled in the art, the present invention is not
intended to be limited to the particular embodiments
described above. The scope of the invention is defined in
the following claims.

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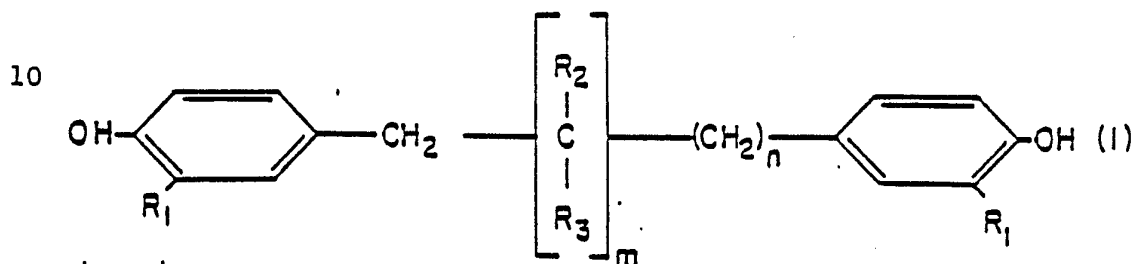
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1 What is claimed:

1. A method for retarding aging in humans by administering to a human a daily dosage of from about 0.5 mg per kilogram of body weight to less than about 2 mg per 5 kilogram of body weight of a pharmaceutically acceptable composition comprising a compound or a mixture of compounds represented by the following structural formula:



wherein

15 m is 0, 1 or 2;

n is 0 or 1;

R1 is hydrogen, halogen or hydroxy; and

20 R2 and R3 each independently represents hydrogen or methyl provided that when m is 0, n is 0 and when m is 1 or 2, n is 1 and R1 is hydroxy or halogen and that when m is 2, each R2 can be the same or different and each R3 can be the same or different or

25 a pharmaceutically acceptable alkali or alkaline earth metal salt thereof.

2. The method according to claim 1 wherein the compound is nordihydroguaiaretic acid.

30 3. The method according to claim 1 wherein the compound is bis (p-hydroxyphenyl) methane.

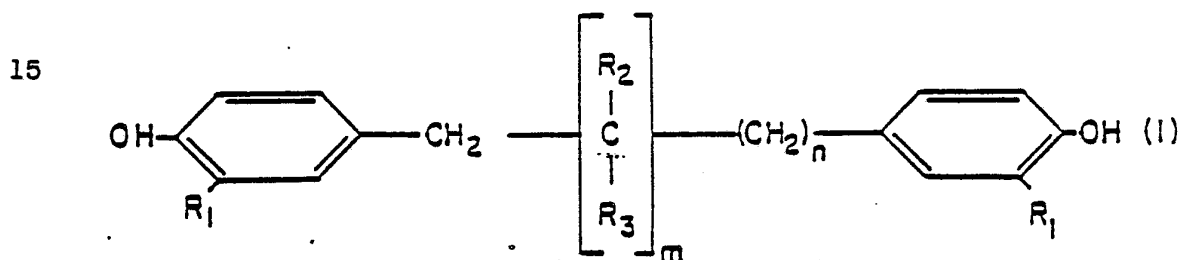
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1 4. The method according to claim 1 wherein the composition is administered orally.

5 5. The method according to claim 1 wherein the composition is administered by a means selected from the group consisting of intravenously, subcutaneously, intramuscularly and intracutaneously.

10 6. A pharmaceutical composition in solid dosage form for retarding aging in humans consisting essentially of a compound or mixture of compounds represented by the following structural formula as the active ingredient:



20 wherein

m is 0, 1 or 2;

n is 0 or 1;

R1 is hydrogen, halogen or hydroxy; and

25 R2 and R3 each independently represents hydrogen or methyl provided that when m is 0, n is 0 and when m is 1 or 2, n is 1 and R1 is hydroxy or halogen and that when m is 2, each R2 can be the same or different and each R3 can be the same or different or

30 a pharmaceutically acceptable alkali or alkaline earth metal salt thereof, wherein from about 20 mg to 100 mg of said active ingredient is present in the composition.

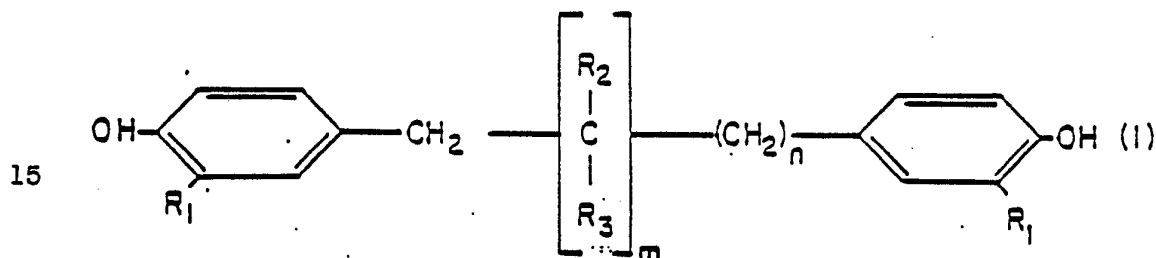
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1 7. A composition as is claimed in claim 6 wherein
the compound is nordihydroguaiaretic acid.

5 8. A composition as is claimed in claim 6 wherein
the compound is bis (p-hydroxyphenyl) methane.

9. A composition for topical administration to the
skin of a human containing an effective amount of a
compound or mixture of compounds represented by the
10 following structural formula to inhibit skin wrinkling:



wherein

m is 0, 1 or 2;

n is 0 or 1;

20 R1 is hydrogen, halogen or hydroxy; and

25 R2 and R3 each independently represents hydrogen or
methyl provided that when m is 0, n is 0 and when
m is 1 or 2, n is 1 and R1 is hydroxy or halogen
and that when m is 2, each R2 can be the same or
different and each R3 can be the same or
different or

a pharmaceutically acceptable alkali or alkaline earth
metal salt thereof.

30 10. A composition as is claimed in claim 9 wherein
the compound is nordihydroguaiaretic acid.

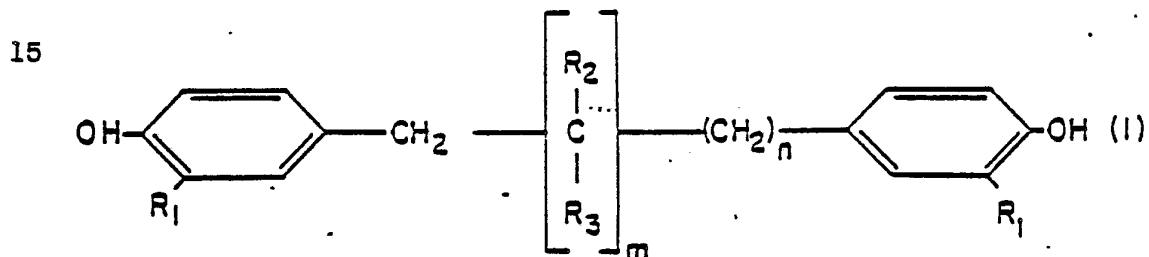
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- 28 -

11. A composition as is claimed in claim 9 wherein the compound is bis (p-hydroxyphenyl) methane.

12. A composition as is claimed in claim 10 wherein the composition contains from about 0.5% to about 20% by weight of nordihydroguaiaretic acid compared to the total weight of the composition.

13. A composition for topical administration to the skin of a human consisting essentially of an effective amount of a compound or mixture of compounds represented by the following structural formula as the active ingredient to inhibit skin wrinkling:



20 wherein

m is 0, 1 or 2;

n is 0 or 1;

R1 is hydrogen, halogen or hydroxy; and

25 R2 and R3 each independently represents hydrogen or methyl provided that when m is 0, n is 0 and when m is 1 or 2, n is 1 and R1 is hydroxy or halogen and that when m is 2, each R2 can be the same or different and each R3 can be the same or different or

30 a pharmaceutically acceptable alkali or alkaline earth metal salt thereof.

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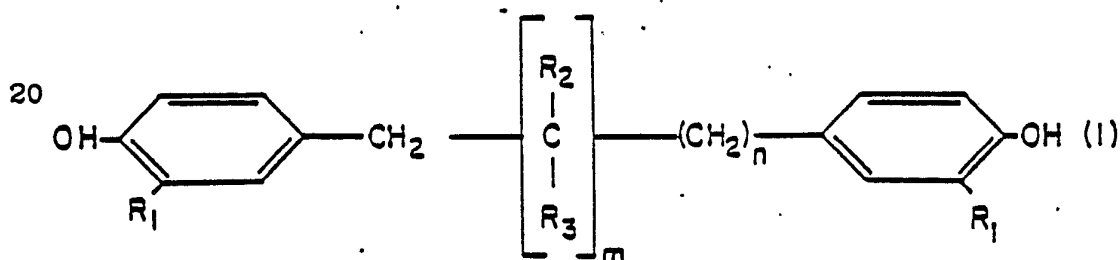
- 29 -

1 14. A composition as is claimed in claim 13 wherein
the compound is nordihydroguaiaretic acid.

15. A composition as is claimed in claim 13 wherein
5 the compound is bis (p-hydroxyphenyl) methane.

16. A composition as is claimed in claim 14 wherein
the composition contains from about 0.5% to about 20% by
weight of nordihydroguaiaretic acid compared to the total
10 weight of the composition.

17. A method for inhibiting wrinkling of the skin of
a human by topically administering to the skin of such a
human a pharmaceutically acceptable composition consisting
15 essentially of an effective amount of a compound or
mixture of compounds represented by the following
structural formula to inhibit wrinkling:



wherein

25 m is 0, 1 or 2;

n is 0 or 1;

R1 is hydrogen, halogen or hydroxy; and

30 R2 and R3 each independently represents hydrogen or
methyl provided that when m is 0, n is 0 and when
m is 1 or 2, n is 1 and R1 is hydroxy or halogen
and that when m is 2, each R2 can be the same or
different and each R3 can be the same of

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1 different or
a pharmaceutically acceptable alkali or alkaline earth
metal salt thereof.

5 18. The method according to claim 17 wherein the
compound is nordihydroguaiaretic acid.

10 19. The method according to claim 17 wherein the
compound is bis (p-hydroxyphenyl) methane.

15 20. The method according to claim 18 wherein the
composition contains from about 0.5% to about 20% by weight
nordihydroguaiaretic acid compared to the total weight of
the composition.

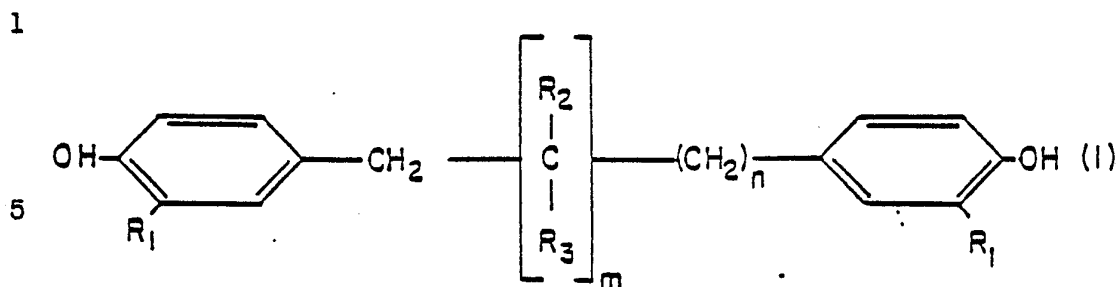
20 21. The method according to claim 17 wherein the
composition is rubbed daily onto the skin of the human
in the area where wrinkling is to be inhibited.

25 22. The method according to claim 20 wherein the
composition is rubbed daily the skin of the human in the
area where wrinkling is to be inhibited.

30 23. A method for inhibiting the formation of amide
bonds in human collagen and elastin by topically
administering to the skin of such a human a
pharmaceutically acceptable composition comprising a
amount of a compound or mixture of compounds represented
by the following structural formula sufficient to inhibit
amide bond formation in human collagen or elastin:

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wherein

m is 0, 1 or 2;

10 n is 0 or 1;

R1 is hydrogen, halogen or hydroxy; and

15 R2 and R3 each independently represents hydrogen or methyl provided that when m is 0, n is 0 and when m is 1 or 2, n is 1 and R1 is hydroxy or halogen and that when m is 2, each R2 can be the same or different and each R3 can be the same or different or

a pharmaceutically acceptable alkali or alkaline earth metal salt thereof.

20

24. The method according to claim 23 wherein the compound is nordihydroquairctic acid.

25. The method according to claim 23 wherein the 25 compound is bis (p-hydroxyphenyl) methane.

26. The method according to claim 24 wherein the composition contains from about 0.5% to about 20% by weight nordihydroquairctic acid compared to the total weight of 30 the composition.

27. The method according to claim 23 wherein the composition is rubbed daily onto the skin of the human

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1 in the area where amide bond formation is to be inhibited.

28. The method according to claim 26 wherein the
composition is rubbed daily onto the skin of the human
5 in the area where wrinkling is to be inhibited.

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Fig. 1

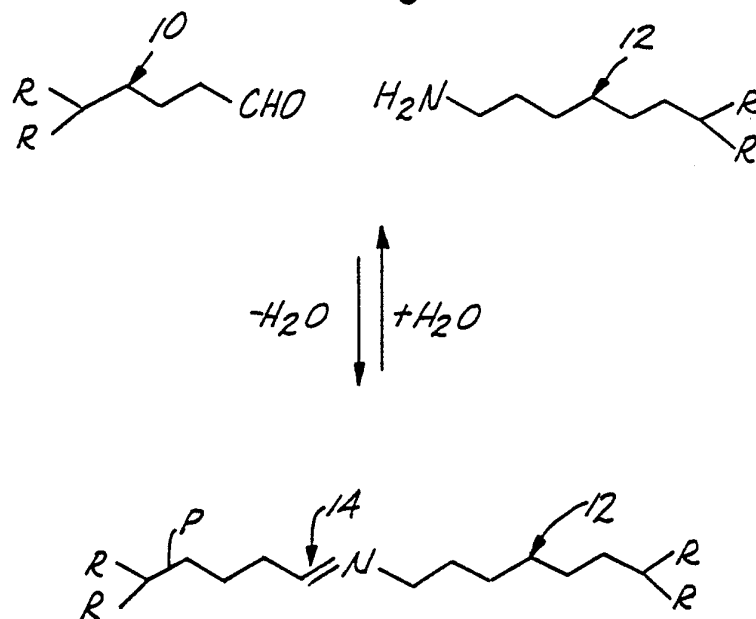
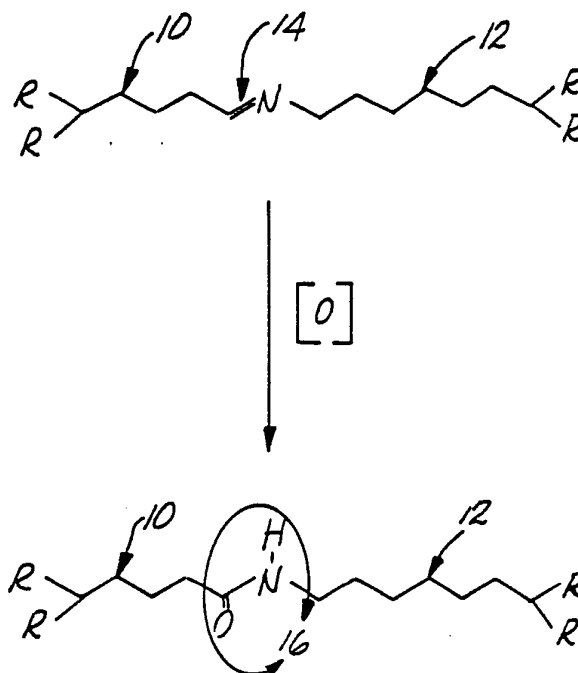


Fig. 2



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Fig. 3

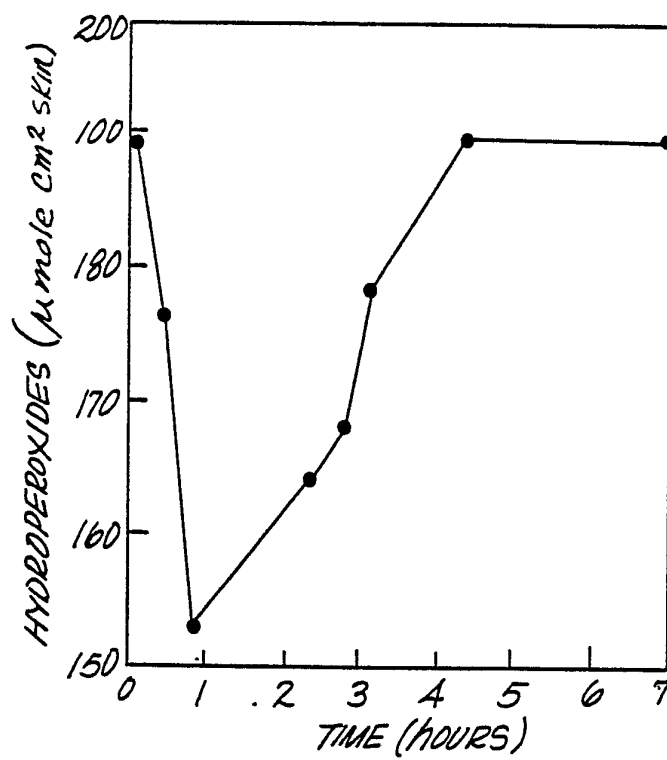
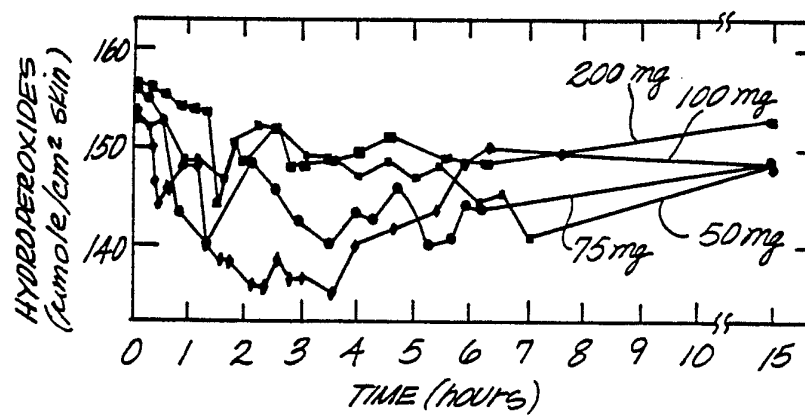


Fig. 4



INTERNATIONAL SEARCH REPORT

International Application No PCT/US87/01039

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ³		
According to International Patent Classification (IPC) or to both National Classification and IPC IPC(4): A61K 31/05 U.S. CL: 514/731		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁴		
Classification System	Classification Symbols	
U.S.	514/731	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁵		
III. DOCUMENTS CONSIDERED TO BE RELEVANT ¹⁴		
Category ⁶	Citation of Document, ¹⁶ with indication, where appropriate, of the relevant passages ¹⁷	Relevant to Claim No. ¹⁸
A	US, A, 2,644,822 (PEARL) 07 July 1953. See the entire document.	1-28
A	US, A, 3,934,034 (MANNING) 20 January 1976. See the entire document.	1-28
A	US, A, 4,530,844 (SMERBECK ET AL) 23 July 1985. See the entire document.	1-28
¹⁵ * Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. "&" document member of the same patent family		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search ² 04 August 1987	Date of Mailing of this International Search Report ³ 12 AUG 1987	
International Searching Authority ¹ ISA/US	Signature of Authorized Officer ²⁰ Stanley Friedman	