COMPOSITIONS AND METHODS FOR TREATING AND PREVENTING BONE DISEASES USING TOCOTRIENOLS

TECHNICAL FIELD OF THE INVENTION

This invention relates to the treatment and prevention of bone diseases using tocotrienols. Specifically, this invention relates to the use of and compositions comprising individual tocotrienols, mixtures of tocotrienols and mixtures of one or more tocotrienols with other bone-enhancing substances. The compositions and methods of this invention are particularly well suited for treating and preventing osteoporosis and other degenerative bone disorders, such as Paget's disease and Gaucher's disease.

BACKGROUND OF THE INVENTION

Osteoporosis is a progressive disorder characterized by a decreased bone mass and increased susceptibility to fractures. Osteoporosis is a major cause of morbidity in postmenopausal women and older men. Although there are a large number of risk factors for the development of osteoporosis, these risk factors mediate their effects through modulation of bone remodeling, bone metabolism and bone resumption (W.B. Ershler et al., Dev. Comp. Immunol., 21(6), pp. 487-99 (1997)).

The goal of osteoporosis pharmacological interventions is to increase bone mass by inhibiting bone resorption and/or enhancing bone formation. Bone resorption is associated with increased osteoclastic activity. Recent studies have suggested that increased secretion of bone active cytokines, such as interleukin-6 (IL-6), from osteoblasts and stromal cells play a pivotal role in increasing osteoclastic activity and the pathogenesis of osteoporosis (G.S. Kim et al., J. Bone Miner. Res., 12(6), pp. 896-902 (1997); E. Sporeno et al., Blood, 87(11), pp. 4510-19 (1996)). IL-6 is also believed to enhance osteoclast recruitment and bone resorption (E. Bornefalk et al., J. Bone Miner. Res., 12(2), pp. 228-33 (1997)). Besides its osteoclastogenic properties, IL-6 also appears to promote differentiation of committed osteoblastic cells towards a more mature phenotype (T. Bellido et al., Endocrinology, 138(9), pp. 3666-76 (1997)). A number of well-recognized bone-enhancing substances influence IL-6 production. For example, estrogen is known to be important in regulating the expression of IL-6 in bone marrow cells (H.K. Vaananen and P.L. Harkonen, Maturitas, 23 Suppl, pp. S65-9 (1996)), calcium concentration in plasma affects IL-6 secretion from mononuclear

blood cells through a direct calcium sensing mechanism (E. Bornefalk et al., supra) and bisphosphonates appear to inhibit IL-6 production by osteoblasts (N. Giuliani et al., Scand. J. Rhematol., 27(l), pp. 3841 (1998)).

Primary preventive therapy against osteoporosis often involves behavioral and dietary modifications. Secondary prevention in women undergoing menopause often involves estrogen replacement therapy (J.Y. Reginstar et al., Rev. Rhum. Ed. Fr., 61(10), pp. 1555S-64S (1994)). In addition to estrogen, there are a number of other agents being used to prevent and treat osteoporosis and other degenerative bone disorders. These prophylactic and therapeutic options for osteoporosis can generally be divided into twosub sets: those that inhibit bone resorption and those that stimulate bone formation. Included in the first category are calcium (including calcium salts), anti-estrogens (such as tamoxifen), environmental estrogens (such as coumestrol, methoxychlor, bisphenol A and zeranol), vitamin D (including its analogs and metabolites), gonadal and anabolic steroids, calcitonin, flavanoids (such as pentahydroxyflavone, naringenin, hesperetin tangeretin, nobiletin, quercetin, apigenin, the isoflavones genestein and daidzein and the isoflavone derivative, ipriflavone), tyrosine kinase inhibitors (such as herbimycin), protein kinase C modulators (such as phorbol esters) and bisphosphonates (such as alendronate, risedronate and etidronate). Some of the agents in this first category also fall into the second, but the most widely used agent to stimulate bone formation is fluoride (usually in the form of sodium fluoride). Synthetic parathyroid hormone, vitamin K (including K1and K2) and cyclic bisphosphonates are also being used as bone-enhancing agents (C. Genera, et al., Drug Serf., 11 (3), pp. 179-95 (1994) and P. Weber, Int. J. Vita. Nut. Res., 67(5), pp. 350-56 (1997)).

Despite the existence of numerous prophylactic and therapeutic options, osteoporosis and other bone diseases remain a serious health threat. Accordingly, there is still a well recognized and unmet need for new agents to prevent and treat osteoporosis and other bone diseases.

SUMMARY OF THE INVENTION

The present invention satisfies the need for new prophylactic and therapeutic agents effective in the prevention and treatment of osteoporosis and other bone diseases.

One embodiment of this invention provides a method for preventing osteoporosis and other bone diseases in a patient comprising the step of administering to the patient a prophylactically effective amount of a composition comprising a tocotrienol, a mixture of

tocotrienols or a combination of one or more tocotrienols with one or more additional bone-enhancing substances.

Another embodiment of this invention provides a method for treating osteoporosis and other bone diseases in a patient comprising the step of administering to the patient a therapeutically effective amount of a composition comprising a tocotrienol, a mixture of tocotrienols or a combination of one or more tocotrienols with one or more additional bone-enhancing substances.

Yet another embodiment of this invention provides novel compositions comprising a combination of one or more tocotrienols with one or more additional bone-enhancing substances that are especially well suited for treating and preventing osteoporosis and other bone diseases.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures IA and 1B show the effects of γ-tocotrienol (GT301) on superoxide production in human peripheral blood neutrophils.

DETAILED DESCRIPTION OF THE INVENTION

As used herein, the following definitions apply (unless expressly noted to the contrary):

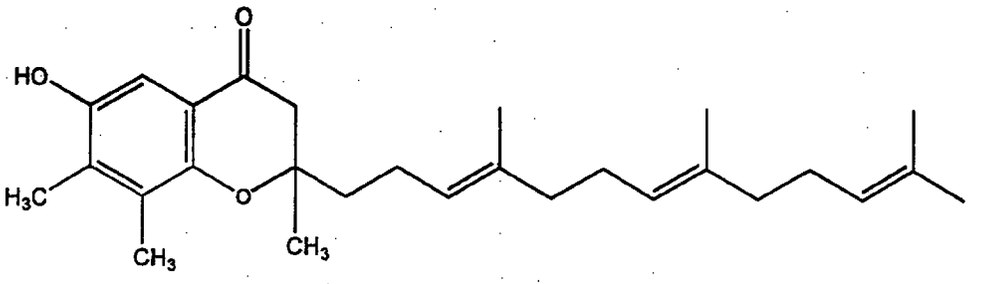
"Bone-enhancing substances" refers to agents that have a beneficial effect on bone condition. Preferably, these bone-enhancing substances inhibit bone resorption and/or stimulate new bone formation and more preferably, these bone-enhancing substances are selected from the individual bone-enhancing agents referred to herein. The markers and tests for measuring these activities are well established in the art (see, for example, M. Gambacciani et al., Calcif. Tissue Int., 6 1 S 1, pp. S 15-18 (1997)).

"Composition' as used herein refers to a preparation for administration via any acceptable route known to those of ordinary skill in the art. Such routes include, but are not limited to oral, nasal, inhalation, parenteral, and topical administration. "Composition" encompasses pharmaceutical compositions as well as dietary supplements, foodstuffs, food additives and the like.

"Patient" refers to a warm-blooded mammal and preferably, a human. Patients in need of prophylactic therapy to prevent osteoporosis and other bone diseases (including Paget's disease and Gaucher's disease) are those patients possessing one or more risk factors for the development of osteoporosis (including post-menopausal women, older men (i.e.,

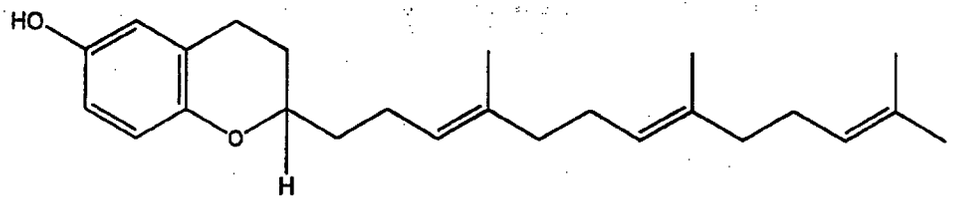
greater than about 50 years old) and patients with inadequate calcium intake) or risk factors associated with other bone diseases. Patients in need of treatment for osteoporosis or other bone diseases are those patients possessing one or more symptoms of osteoporosis, including a decrease in bone mass and an increased susceptibility to bone fractures or symptoms of other bone diseases (e.g., Paget's disease, i.e., pain and deformities due to enlargement of skeletal segments).

"P_{18} tocotrienol" refers to a tocotrienol having the formula



P_{18} tocotrienol and P_{18} are trademarks of Bionutrics, Inc. (Phoenix, Arizona).

"P_{25} tocotrienol" refers to the tocotrienol 3,4-dihydro-2-(4,8,12-trimethyltrideca-3'(E),7'(E), 11"-trienyl)-2H-1-benzopyran-6-ol) which has the formula:



This compound is also known as didesmethyl-tocotrienol in some of the literature cited herein. P_{25} tocotrienol and P_{25} are trademarks of Bionutrics, Inc. (Phoenix, Arizona).

"Prophylactically effective amount" refers to an amount of active ingredient sufficient to prevent onset of one or more of the clinical aspects of osteoporosis or other bone diseases or to prevent one or more of the symptoms of osteoporosis or other bone diseases in a patient having one or more risk factors associated with osteoporosis or other bone diseases.

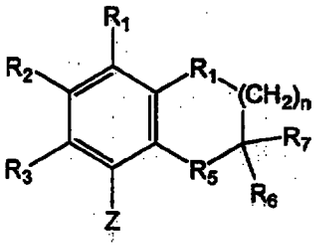
"Prophylactically acceptable means" refers to means effective to impart a prophylactic effect.

"Tocotrienol". when used to refer to a specific compound, refers to the compound 3,4-dihydro-2-methyl-2-(4.8,12-trimethyltrideca-3'(E), 7'(E,), 11-trienyl)-2H-benzopyran-6-ol.

When used generally to describe the class of compounds, the definition set forth below will apply.

"Tocotrienol', when used in the general sense, refers to compounds possessing the following three structural characteristics: (1) a hydrogen donor group (or a group that can be hydrolyzed to a hydrogen donor group) attached to an aromatic ring system; (2) a side chain attached to the aromatic ring system comprising one or more isopreniod or isoprenoid-like units and (3) a methylene unit or a functional group having at least one lone pair of electrons positioned adjacent to the atom to which the side chain is attached to the aromatic zing system, said electrons being conjugated to the aromatic ring system (preferably CH_2 C=O, CHOH, O, S or NH). Specific isoprenoid-like units include truncated isoprenoids and truncated or full-length isoprenoids that may be or may not be partially saturated and are optionally substituted with OH, NH2 and C_1 -C_6 branched or unbranched alkyl or alkoxy. Preferred tocotrienols for use in the methods of this invention are those which are naturally occurring (including tocotrienol, α-, β-, γ-, δ-tocotrienol, P_{25}, and P_{18}) and may be used individually or in combination. These naturally occurring tocotrienols may be conveniently isolated from biological materials or synthesized from commercially available starting material. Preferably, the tocotrienols for use in the methods of this invention are obtained from biological materials that have been stabilized and extracted, such as by the processes described in PCT publication WO 91/17985 (the entire disclosure of which is incorporated by reference herein). Examples of preferred biological materials. tocotrienols and methods for obtaining tocotrienols synthetically and from biological materials are referred to in co-owned US patent 5,591,772 and PCT publication WO 91/17985 (the entire disclosures of which are incorporated by reference herein). Preferred biological materials from which the some of the preferred tocotrienols useful in the compositions and methods of this invention may be obtained include those from conifers. legumes, asteraceae, poaceae and palmae and more specifically, tocotrienol-rich extracts from stabilized brans (especially, stabilized rice bran), psyllium seed, barley, pine nut, sunflower, peanut, palm fruit, millet, avocado, juniper berries, cedar leaves, mango, pine needles, rubber tree leaves, tomato and amaranth. In addition to the tocotrienols described above, the term "tocotrienol" also includes the farnesylated tetrahydro-naphthalenols and monocyclic tocotrienol analogs described (both generically and specifically) in US patents 5,204.373 and 5.393,776.

Specific preferred tocotrienols of this invention include those of formula (I):



I

wherein

R_1 and R_3 are each independently selected from the group consisting of H, halogen, OH, OCH_3 and C_1-C_6, branched or unbranched alkyl (preferably, H, halogen and C_1 -C_3 branched or unbranched alkyl and more preferably, H and methyl);

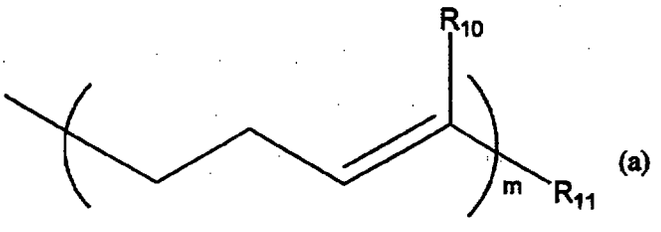
R_2 is selected from the group consisting of halogens (preferably, chlorine) and hydrogen donor group selected from the group consisting of OH, NHR_8, CO_2Y or $C(R_8)_2CO_2H$ And C_1-C_8 branched or unbranched alkyl substituted with OH, NHR_8, $C0_2Y$ or $C(R_8)_2CH_2H$ (preferably, OH and C_1-C_3 branched or unbranched alkyl substituted with OH and more preferably, OH);

R_4 is selected from the group consisting of O, NH, CH-R_9, C=O and CH-OH (preferably O, CH_2 and C=O);

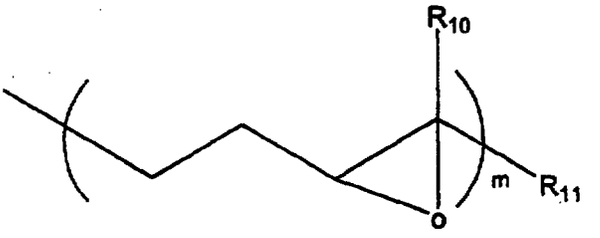
R_2 is selected from the group consisting of CH_2, C=O, CHOH, O, S and NH (preferably, O, CH_2 and C=O and more preferably, O and C=O);

R_6 is selected from the group consisting of H and C_1-C_6 branched or unbranched alkyl (preferably, H and C_1-C_3 branched or unbranched alkyl and more preferably, H and methyl);

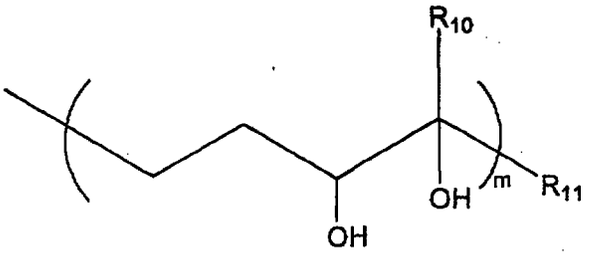
R_7 is selected from the group consisting of isoprenoid and isoprenoid-like side chains, and more preferably from the group consisting of side chains of formulas (a)-(c).



(a)



(b) and



(c)

and also includes combinations of one or more of the repeating units shown in side chains (a)-(c), and, if the repeating unit shown in side chain (a) is present, one or more unsaturated analogs thereof or, if the repeating unit in side chain (c) is present, one or more oxidated analogs thereof (including keto and dione analogs thereof) and wherein each R_{10} is independently selected from the group consisting of H, OH, NH_2 and C_1-C_6 branched or unbranched alkyl or alkoxy and R_{11} is selected from the group consisting of H, C_1-C_6 branched or unbranched alkyl or alkoxy, CH_2OH, CO_2H (and C_1-C_6 alkyl esters thereof) and OH (preferably, R_7 is a side chain of formula (a), wherein R_{10} and R_{11} are each independently selected from the group consisting of H and C_1-C_3 branched or unbranched alkyl and more preferably, H and methyl);

each R_8 and R_9 is independently selected from the group consisting of H and C_1-C_6 branched or unbranched alkyl (preferably, H and C_1-C_3 branched or unbranched alkyl and more preferably, H and methyl);

Y is H or C_1-C_{18} branched or unbranched alkyl (preferably H and C_1-C_6 branched or unbranched alkyl and more preferably, H and C_1-C_4 branched or unbranched alkyl);

Z is selected from the group consisting of H, halogen, OH, CH_2OH, CH_2, OCH_3 and $COCH_3$ (preferably H and CH_3);

n is an integer selected from the group consisting of 0, 1, 2, 3 and 4 (preferably 0 and 1); and

m is an integer selected from the group consisting of 1-30 (preferably 1-20, more preferably 3-10 and most preferably, 3-7).

More preferred tocotrienols of this invention include tocotrienol, α-tocotrienol, tocotrienol, γ-tocotrienol, δ- tocotrienol, P_{18} tocotrienol and P_{25} tocotrienol,

This invention expressly encompasses the prodrug form of tocotrienols. Upon administration to a patient such a prodrug undergoes biotransformation to their active form. Prodrugs include the esterified form of the tocotrienols used in this invention which comprises a carboxylic acid functionality.

The tocotrienols for use in the compositions and methods of this invention may be in their isomerically pure form or be present as mixtures of isomers. For example, the tocotrienols of this invention may exist as the d- or l-isomer or the d,l-racemic mixture. The naturally occurring isomer (usually the d-isomer) and the d,l-racemic mixture are preferred.

"TRF" refers to a tocotrienol rich fraction obtained by the stabilization and extraction of a biological source. TRF typically contains varying amounts of tocotrienol, α-tocotrienol, β-tocotrienol, γ-tocotrienol and δ-tocotrienol and may also contain quantities of P_{18} tocotrienols and P_{25} tocotrienols. Most commonly, TRF will be comprise at least about 25% to about 90% tocotrienols w/w (preferably, at least about 35% to about 90% and more preferably, at least about 50% to about 90%). "TRF_{25}" refers to a TRF comprising a significant weight percentage of P_{25} tocotrienol. Preferably, TRF_{25} comprises at least about 5% P_{25}, more preferably, at least about 10% P_{25}, and even more preferably, at least about 15% P_{25} w/w. An example of the preparation of a specific TRF_{25} is set forth in A. A. Qureshi et al., Nutr. Biochem. 8, pp. 290-98 (1997). TRF_{25} is a preferred component of the compositions and methods described herein. TRF_{25} is a trademark of Bionutrics, Inc. (Phoenix, Arizona). TRF and TRF_{25} may be used in any of the methods and compositions described herein for individual tocotrienols or mixtures thereof.

All documents cited herein are incorporated by reference in their entirety including any drawings.

Without wishing to be bound to any particular theory, it appears that tocotrienols may be useful in preventing and treating osteoporosis and other bone disorders due to their unique combination of antioxidant and antiinflammatory properties. As opposed to conventional therapeutics that target a single mechanism, tocotrienols target multiple mechanisms leading to and propagating osteoporosis and other bone disorders. Tocotrienols inhibit the production

of a variety of cytokines (including IL-6) which have been linked to the genesis and histology of osteoporosis and other bone disorders. Furthermore, tocotrienols mediate the levels of superoxide and, perhaps, nitric oxide production. These factors have been linked to the process of osteoclastic bone resorption (P.M. Gallop et al., Connect. Tissue Res. 29(2) pp. 153-61 (1993) and D. M. Evans and S. H. Ralston, J. Bone Miner. Res., 11 (3), pp. 3 0005 (1996)). In particular, the bone-enhancing effects of ipriflavone have been attributed at least in part to the ability of one of its main metabolites (7-isopropoxy-3-(4-hydroxyphenyl)-4H-1-benzopyran- 4-one) to inhibit liposaccharide (LPS) induced nitric oxide release (S. Koncz and E.J. Horvath, Acta Physiol. Hung., 84(3), pp. 223-38 (1996)). Tocotrienols are also associated with the reduction of advanced glycation endproducts (AGEs). The production of AGEs have recently been linked to osteoclast induced bone resorption (T. Myata et al., J. Am. Soc. Nephral., 8(2), pp. 260-70 (1997)). As a result of their unique combination of properties, tocotrienols are capable of combating the root causes and reversing the degenerative effects of osteoporosis and other bone diseases.

Compositions of this invention are prepared by combining one or more tocotrienols with an acceptable carrier. For pharmaceutical compositions of this invention, the carrier must be pharmaceutically acceptable (i.e., a carrier which is non-toxic to the patient at the administered level and which does not destroy the activity of the active component(s) of the composition). Acceptable carriers, including pharmaceutically acceptable carriers, are well known to those of ordinary skill in the art.

The compositions of this invention may be used or administered by any therapeutically or prophylactically acceptable means to a patient in need of osteoporosis treatment or prophylaxis. For example, pharmaceutical compositions of this invention may be administered orally, nasally, topically, transdermally or parenterally. These compositions may be formulated so as to impart a time-released benefit. Oral compositions may take the form of tablets, capsules, caplets, emulsions, liposomes, suspensions, powders and the like. Topical compositions include, but are not limited to, gels, lotions and creams. Parenteral compositions take the form of sterile solutions and emulsions and the like. Intravenous compositions include, but are not limited to, sterile solutions. The preferred route of administration is oral.

Dosage levels and requirements are well-recognized in the art and may be chosen by those of ordinary skill in the art from publicly available sources. Typically, dosage levels

will range between about 0.1 and about 10,000 mg of a tocotrienol or mixture of tocotrienols per dose. Preferably, the range is between about 0.1 and about 5,000 mg (more preferable, between 0.1 and 1000 mg) of active ingredient per dose. Continuous dosing may be required over a period of time to obtain maximum benefit. Specific dosage and treatment regimens will depend upon factors such as the patient's overall health status, the severity and course of the patient's disorder or disposition thereto and the judgment of the treating physician. Higher or lower doses may be employed as needed.

Tocotrienols and mixtures thereof may be used in combination with bone-enhancing agents in the methods described herein. These additional therapeutic and prophylactic agents may be administered separately from the tocotrienols or mixtures thereof, or they may be formulated together in a single dosage form. Such combination therapy advantageously utilizes lower dosages of the other bone-enhancing agents which may reduce or avoid possible toxicity incurred when those agents are used as monotherapies or which may create an additive or synergistic effect. In addition to conventional agents used in the treatment and prevention of osteoporosis, the tocotrienols used in the methods of this invention may be also used in conjunction with any conventional anti-inflammatory or anti-oxidative agent. These conventional agents preferably possess independent boneenhancing properties. In a particularly preferred embodiment, one or more tocotrienols may be used in conjunction with one or more of the following bone-enhancing substances: calcium (including calcium salts), estrogen. anti-estrogens (such as tamoxifen), environmental estrogens (such as coumestrol, methoxychlor, bisphenol A and zeranol), vitamin D (including its analogs and metabolites, such as calciferol and 1-alpha hydroxycholecalciferol), gonadal and anabolic steroids. calcitonin. flavanoids (such as pentahydroxyflavone, naringenin, hesperetin, tangeretin, nobiletin. quercetin. apigenin. the isoflavones genestein and daidzein and the isoflavone derivative. ipriflavone (including metabolites thereof), tyrosine kinase inhibitors (such as herbimycin), protein kinase C modulators (such as the phorbol esters), bisphosphonates (including cyclic bisphosphonates and alendronate, risedronate and etidronate), fluorides (such as sodium fluoride), synthetic parathymid hormone, vitamin K (including K I and K2), other cytokine mediators (such as IL- I inhibitors like probucol and lipoic acid, IL-6 inhibitors. IL- 11 inhibitors, TNF inhibitors, INF-gamma inhibitors, tocopherols (such as α--tocopherol (Vitamin E)) and selenium. Preferred combinations include one or more

tocotrienols with one or more agents selected from calcium (including calcium salts), flavanoids (more preferably, ipriflavone), vitamin D, vitamin K, lipoic acid and tocopherols.

EXAMPLES

In order that this invention be more fully understood, the following examples are set forth. These examples are for the purpose of illustration only and are not to be construed as limiting the scope of the invention in any way.

The methods used for obtaining and purifying tocotrienols and mixtures thereof (including TRF) useful for the methods of this invention are described in the Examples section of US patent 5,591,772. Stabilization of rice bran follows Example 1 of US patent 5,591,772. Purification of TRF, P_{25} tocotrienols and P_{18} tocotrienol follow Examples 2-4 of US patent 5.591,772.

The following specific protocols can be used to produce rice bran oil from which tocotrienols and mixtures thereof can be isolated:

Protocol I: Dry Heat Stabilization

Extruder: Wenger Model X-25

Standard Screw/Barrel Setup

Barrel No.	Standard Port	Screw No.	Standard Port
5	28714-9	5	28320-1
4	28318-1	4	8326-9
3	28371-9	3	28326-1
2	28318-1	2	28326-1
1	28350-1	1	28387-1

Standard Die Setup

Die/Spacer	Measurement	Standard Port
Spacer	0.375	28340-11
Back Plate	0.625	28361-51
Intermediate	0.218	28316-723
Front Plate	0.235	28389-507

Feed Rate:	1000 lbs/hr
Temperature:	170°C at exit die
Pressure:	975-1025 psi
Moisture Food:	12%
Moisture Discharge:	9.6%
Residence Time:	15 seconds
Run Duration:	8 hours
Sample Size:	50 lbs

Protocol II: Dry Heat Followed By Wet Heat Stabilization

Dry Heat Stage: Protocol I

Wet Heat Stage:

Extruder: Anderson 4 inch

Screw Barrel Configuration: Standard Cut Flight

Die Setup:

Diameter: 0.1875 inches

Length: 0.75 inches

Operating Conditions:

Feed Rate: 378 lbs/hr

Shaft Speed: 279 rpm

Steam Injection: 36 lbs/hr (32 psi at #8 hole)

Mechanical Pressure: 750 psi (ast.)

Moisture Feed: 1 1.4%

Discharge Moisture: 15%

Discharge Rate: 450 lbs/hr

Discharge Temp.: 121 °C

Protocol III: Drying/Cooling Procedure

The wet heat stabilized product of protocol II (15% moisture) was discharged onto aluminum trays and placed in a tray oven at 101.1 °C until the moisture content was 8-10% (approximately 1. 5 hrs). The trays were then placed on tray racks and allowed to cool at ambient temperature (approximately 20°C).

Oil to Hexane Ratio:	1:4
No. of Washings:	3
Extraction Temperature	40°C

The hexane was removed from the extract by mild heating (40°C) under a mild vacuum.

Protocol V: Dewaxing

20 lbs of crude oil were refrigerated for 24 hrs at - 15.6°C. The supernatant (containing the dewaxed oil) was decanted from the solidified waxes. The waxes were then centrifuged to removed entrained oil, yielding 0.59 lbs of waxes and 19.41 lbs of dewaxed oil.

Evaluation of Activity of Tocotrienols

Example 1

The effects of tocotrienols (in the form of the TRF mixture and individual tocotrienols) on plasma levels of thromboxane B_2 and platelet factor 4 in chickens were determined. These levels are known to correlate with the levels of inflammatory cytokines, such as IL-6. The following feeding conditions were used:

Each group of six chickens (6-week old female white leghorn chickens) was administered a chick mash control diet or a control diet containing one or more additives. The amount of feed consumed by all groups was comparable to the control group. The feeding period was 4 weeks. The birds were fasted for a period of 14 hours prior to sacrifice.

The chicken mash control diet contained the following ingredients:

Ingredient	Weight (g)
Corn (8.8% protein)	615.0
Soybean Meal	335.0
Corn Oil	10.0
Calcium Carbonate	10.0
Dicalcium Phosphate	20.0
Iodized Salt	5.0
Mineral Mixture	2.5
Vitamin Mixture	2.5

The mineral mixture contained per kg feed: zinc sulfate•H_20, 110 mg; manganese sulfate •$5H_20$, 70 mg; ferric citrate•H_20, 500 mg; copper sulfate•$5H_20$, 16 mg; sodium selenite, 0.2 mg; DL-methionine, 2.5 g; choline chloride (50%), 1 .5 g; ethoxyquin (1,2-dihydro-6-ethoxy-2,2, 4-trimethylquinoline), 125 mg; and thiamine HCl, 1.8 mg. The vitamin mixture contained per kg feed: vitamin A, 1,500 units; vitamin D_3, 400 units; vitamin E, 10 units; riboflavin, 3.6 mg; calcium panthothenate, 10 mg; niacin, 25 mg; pyridoxine HCl, 3 mg; folacin, 0.55 mg; biotin, 0.15 mg; vitamin B_{12}, 0.01 mg; and vitamin K_1, 0.55 mg.

Results are reported as mean ± standard deviation. Percentages of control are reported in parentheses. The following results were obtained:

EXPERIMENT 1

Diet	Thromboxane B_2 (mg/100 ml)	Platelet Factor 4 (mg/ml)
1) Control diet + 5% corn oil	16.7 ± 1.69 (100.0)	7.2 ± 0.48 (100.0)
2) Control diet + 5% corn oil + waxes (50 ppm)	15.8 ± 1.29 (94.6)	7.5 ± 0.42 (104.2)
3) control diet + 5% corn oil + TRF (50 ppm)	12.4 ± 1.42 (74.3)	5.7 ± 0.64 (79.2)

Significant decreases of thromboxane B_2 and platelet factor 4 plasma levels were observed in the chickens fed a control diet supplemented with TRF.

EXPERIMENT 2

Diet	Thromboxane B_2 (pg/ml)	Platelet Factor 4 (ng/ml)
1) Control Diet	25.84 ± 0.84 (100.00)	13.9 ± 0.49 (100.00)
2) Control Diet + TRF (50 ppm)	20.55 ± 0.95 (79.53)	9.67± 0.43 (73.87)
3) Control Diet + α-tocotrienol (50 ppm)	21.63 ± 0.89 (83.71)	8.78 ± 0.72 (67.07)
4) Control Diet + γ-tocotrienol (50 ppm)	19.22 ± 0.78 (74.38)	8.36 ± 0.79 (62.87)
5) Control Diet + δ-tocotrienol (50 ppm)	18.65 ± 0.99 (72.17)	8.23 ± 0.77 (62.87)
6) Control Diet + desmethyl-tocotrienol (50 ppm)	16.74 ± 1.62 (64.78)	7.76 ± 1.67 (59.28)
7) Control Diet + P_{25} tocotrienol (50 ppm)	16.42 ± 1.36 (63.54)	7.27 ± 0.74 (55.54)
8) Control Diet + Geraniol (100 ppm)	25.46 ± 1.52 (98.32)	12.38 ± 1.26 (94.57)

9) Control Diet + Lovastatin (100 ppm)	24.89 ± 0.88 (96.32)	12.24 ± 1.49 (93.51)
10) Control Diet + Geraniol (50 ppm) + Lovastatin (50 ppm)	24.95 ± 0.97 (96.56)	12.19 ± 1.15 (93.12)

Significant decreases of thromboxane B_2 and platelet factor 4 plasma levels were observed in the chickens fed a control diet supplemented with TRF and individual tocotrienols.

Example 2

The effects of tocotrienols (in the form of the TRF mixture and individual tocotrienols) on plasma levels of thromboxane B_2 and platelet factor 4 in swine were determined. These levels are known to correlate with the levels of inflammatory cytokines (including IL-6). The following feeding conditions were used:

Each group of three swine (5-month old swine carrying Lpd^s and Lpu^1 mutant alleles) were administered a control diet or a control diet supplemented with one or more additives. After a 12 hour fast, plasma samples were taken at 42 days from the start of the feeding period.

The swine control diet contained the following ingredients:

Ingredient	Weight Percentage
Corn (9.3% protein)	78.37
Soybean Meal (44.0% protein)	15.42
Lard	3.00
Calcium Carbonate	0.95
Dicalcium Phosphate	0.96
Mineral Mixture	0.30
Vitamin Mixture	1.00

The mineral mixture contained per kg feed: zinc sulfate•H20, 110 mg; manganese sulfate •$5H_20$, 70 mg; ferric citrate•H_20, 500 mg; copper sulfate•$5H_20$, 16 mg; sodium selenite, 0.2 mg; DL-methionine, 2.5 g; choline chloride (50%), 1.5 g; ethoxyquin (1,2-dihydro-6-ethoxy-2,2,4-trimethyquinoline), 125 mg; and thiamine HCl, 1.8 mg. The vitamin mixture contained per kg feed: vitamin A, 1,500 units; vitamin D_3, 400 units; vitamin E, 10 units; riboflavin, 3.6 mg; calcium panthothenate, 10 mg; niacin, 25 mg; pyridoxine HCl, 3 mg; folacin, 0.55 mg; biotin, 0. 15 mg; vitamin B_{12}, 0.01mg; and vitamin K_1, 0.55 mg.

The gain in body weight in all groups was comparable to the control.

Results are reported as mean ± standard deviation. Percentages of control are reported in parentheses. The following results were obtained:

Diet	Thromboxane B_2 (pg/100 ml)	Platelet Factor 4 (ng/100ml)
1) Control Diet	75.93 ± 1.45 (100.00)	24.12 ± 1.75 (100.00)
2) Control Diet + TRF (50 ppm)	64.55 ± 1.18 (95.23)	20.32 ± 1.70 (84.30)
3) Control Diet + γ-tocotrienol (50 ppm)	62.54 ± 1.39 (92.25)	20.65 ± 1.15 (85.28)
4) Control Diet + desmethyl-tocotrienol (50 ppm)	60.48 ± 1.46 (87.66)	19.85 ± 1.27 (82.59)
5) Control Diet + P_{25} tocotrienol (50 ppm)	57.03 ± 1.95 (83.02)	19.15 ± 1.45 (79.36)

Significant decreases of thromboxane B_2 and platelet factor 4 plasma levels were observed in the chickens fed a control diet supplemented with TRF and individual tocotrienols.

Example 3

The effects of γ-tocotrienol on the release of superoxide in human peripheral blood neutrophils were determined. Superoxide amplifies the inflammatory response thereby increasing bone resorption.

The neutrophils tested were isolated by density centrifugation on Ficoll-Hypaque gradients using conventional methods (see E. Serbinova et al., Free Rad. Bio. and Med., 10, pp. 263-75 (1991)). The neutrophils were then placed in a 96-well plate. γ-Tocotrienol and phorbol myristate acetate were added to the wells at the same time. The secretion of superoxide was measured as the superoxide dismutase-inhibitable reduction of ferricytochrome C. The results of this study are displayed in Figure 1.

The amount of released superoxide was reduced from 19.7 nmole ($5x10^5$ cells/hour) in the control to 8.0 and 0.0 nmole at γ-tocotrienol concentrations of 10^{-6} and 10^{-5}, respectively.

Example 4

The following models can be used to evaluate the efficacy of tocotrienols for the prevention and treatment of osteoporosis and other bone diseases:

R. Civitelli et al., Calcif. Tissue Int., 56, pp. 215-19 (1995) describe a rat model to measure bone biomechanics, impact strength and mineral bone density.

K. Notoya et al., Calcif Tissue Int. 58(2), pp. 88-94 (1996) describe a rat model to measure bone restoration in immobilized rats following unilateral sciatic neurectomy.

N. Giuliani et al., supra, describe an in vitro model for measuring the production of IL-6 in the human osteoblastic cell line, MG-63.

Although certain embodiments and examples have been used to describe the present invention, it will be apparent to those skilled in the art that changes to the embodiments and examples shown may be made without departing from the scope and spirit of the invention.

Other embodiments are within the following claims.



CLAIMS

What is claimed is:

1. A composition comprising one or more tocotrienols and one or more bone enhancing agents selected from the group consisting of calcium, calcium salts, ipriflavone, vitamin D and vitamin K.

2. The composition according to claim 1, wherein the bone-enhancing agent is ipriflavone.

3. The composition according to claim 1, wherein the composition comprises TRF or TRF_{25}.

4. A dietary supplement comprising the composition according to claim 1 or 3.

5. A foodstuff comprising the composition according to claim 1 or 3.

6. A pharmaceutical composition comprising the composition according to claim 1 or 3.

7. A method for preventing osteoporosis or other bone diseases in a patient comprising the step of administering to the patient a prophylactically effective amount of a composition comprising one or more tocotrienols.

8. The method according to claim 7, wherein the composition comprises TFR or TRF_{25}.

9. The method according to claim 7 or 8, wherein the composition comprises one or more additional bone enhancing substances.

10. The method according to claim 9, wherein the additional bone-enhancing substance is selected from the group consisting of bone resorption inhibitors and bone formation stimulators.

11. The method according to claim 10, wherein the additional bone-enhancing substance is selected from the group consisting of calcium, estrogen, anti-estrogens, environmental estrogens, vitamin D, gonadal and anabolic steroids, calcitonin, flavanoids, tyrosine kinase inhibitors, protein kinase C modulators, bisphosphonates, fluorides, synthetic parathyroid hormone, vitamin K, cytokine mediators and selenium.

12. The method according to claim 10, wherein the additional bone-enhancing substance is selected from the group consisting of calcium salts, estrogen, tamoxifen, coumestrol, methoxychlor, bisphenol A, zeranol, calciferol, 1-alphahydroxycholecalciferol, pentahydroxyflavone, naringenin, hesperetin, tangeretin, nobiletin, quercetin, apigenin, genestein and daidzein, ipriflavone, herbimycin, phorbol esters, cyclic bisphosphonates, alendronate, risedronate, etidronate, sodium fluoride, vitamins KI and K2, probucol, lipoic acid and α-tocopherol.

13. The method according to claim 10, wherein the additional bone-enhancing substance is selected from the group consisting of calcium salts, ipriflavone, genestein, vitamin D, vitamin K, lipoic acid and tocopherols.

14. The method according to claim 7, wherein the composition comprises one or more tocotrienols selected from the group consisting of tocotrienol, α-tocotrienol, β-tocotrienol, γ-tocotrienol, δ-tocotrienol, P_{18} tocotrienol and P_{25} tocotrienol.

15. The method according to claim 7, wherein the composition is administered orally.

16. The method according to claim 7, wherein the composition comprises between about 0.1 and about 5000 mg of tocotrienol/dose.

17. The method according to claim 7, further comprising the step of administering to the patient one or more additional bone-enhancing substances.