P/00/001
Section 29

AUSTRALIA
Patents Act 1990

656829

PATENT REQUEST : STANDARD PATENT

We, being the person(s) identified below as the Applicant, request the grant of a patent to the person identified below as the Nominated Person, for an invention described in the accompanying standard complete specification.

Applicant: Wisconsin Alumni Research Foundation
Address: 514 North Walnut Street, Madison, Wisconsin 53705, USA

Nominated Person: as above
Address: as above

Invention Title: 26, 27-DIMETHYLENE-1 ALPHA, 25-DIHYDROXYVITAMIN D_2 AND 26,27-DIHYDROXYVITAMIN D_2 AND METHODS FOR PREPARING SAME

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BASIC CONVENTION APPLICATION(S) DETAILS

Applicants' Name: Hector Floyd DE LUCA and Naoshi NAKAGAWA
Application Number: 07/813,852
Country: United States of America
Code: US
Date of Application: 26 December 1991

Dated ..... 22 December 1992

CARTER SMITH & BEADLE
Patent Attorneys for the Applicant/s

Our Ref: #11958 BMH:SM

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Section 29(1)
Regulation 3.1(2)

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NOTICE OF ENTITLEMENT

I/We, WISCONSIN ALUMNI RESEARCH FOUNDATION, 614 North Walnut Street, Madison, Wisconsin 53705 USA being the applicant in respect of Application No. , state the following:-

The person(s) nominated for the grant of the patent:

has entitlement from the actual inventor(s) by Assignment

Must be completed for all convention applications

The person(s) nominated for the grant of the patent:

~~is/are the applicant(s) of the basic application(s) listed on the patent request form~~

~~or~~

has entitlement from the applicant(s) of the basic application(s) listed on the patent request form by assignment.

The basic application(s) listed on the request form:

is/are the first application(s) made in a Convention country in respect of the invention

By ..... (Signature) ..... OCT 19, 1992 (Date)

John R. Pike, Managing Director

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(54) Title
26, 27-DIMETHYLENE-1 ALPHA, 25-DIHYDROXYVITAMIN D2 AND 26,27-DIHYDROXYVITAMIN D2 AND METHODS FOR PREPARING SAME

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(56) Prior Art Documents
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WO 91/12239

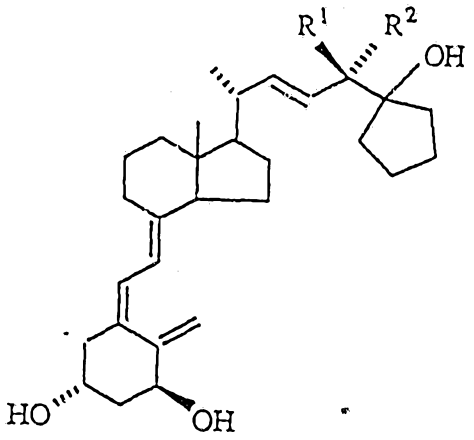
(57)

The present invention provides novel compounds exhibiting a desired, and highly advantageous, pattern of biological activity. These compounds are characterized by a marked intestinal calcium transport activity, as compared to that of 1α, 25-dihydroxyvitamin D_3, while exhibiting much lower activity than 1α,25-dihydroxyvitamin D_3 in their ability to mobilize calcium from bone. Hence, these compounds are highly specific in their calcemic activity. Their preferential activity on intestinal calcium transport and reduced calcium mobilizing activity in bone allows the in vivo administration of these compounds for the treatment of metabolic bone diseases where bone loss is a major concern. Because of their preferential calcemic activity, these compounds would be preferred therapeutic agents for the treatment of diseases where bone formation is desired, such as osteoporosis, osteomalacia and renal osteodystrophy.

CLAIM

1. A compound having the formula

.../2



where R^1 and R^2 may be hydrogen or methyl with the proviso that when R^1 is hydrogen R^2 cannot be hydrogen and when R^1 is methyl R^2 cannot be methyl.

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Regulation 3.2

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COMPLETE SPECIFICATION

FOR A STANDARD PATENT

ORIGINAL

Name of Applicant: WISCONSIN ALUMNI RESEARCH FOUNDATION

Actual Inventor(s): Hector Floyd DE LUCA and Naoshi NAKAGAWA

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Invention Title: 26, 27-DIMETHYLENE-1 ALPHA, 25-DIHYDROXYVITAMIN D_2 AND 26,27-DIHYDROXYVITAMIN D_2 AND METHODS FOR PREPARING SAME

The following statement is a full description of this invention, including the best method of performing it known to me/us:

Background of the Invention

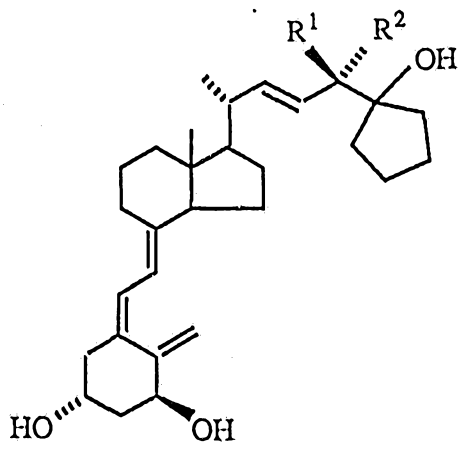
With the discovery of 1α,25-dihydroxyvitamin D_3 as the active form of the vitamin has come an intense investigation of analogs of this hormonal form of vitamin D with the intent of finding analogs that have selective activity. By now, several compounds have been discovered which carry out the differentiative role of 1,25-dihydroxyvitamin D_3 while having little or no calcium activity. Additionally, other compounds have been found that have minimal activities in the mobilization of calcium from bone while having significant activities in stimulating intestinal calcium transport. Modification of the vitamin D side chain by lengthening it at the 24-carbon has resulted in loss of calcium activity and either an enhancement or undisturbed differentiative activity. Placing the 24-methyl of 1α,25-dihydroxyvitamin D_2 in the epi-configuration appears to diminish activity in the mobilization of calcium from bone. On the other hand, increased hydrophobicity on the 26- and 27-carbons seems to increase the total activity of the vitamin D compounds provided the 25-hydroxyl is present.

Summary of the Invention

The present invention provides novel compounds exhibiting a desired, and highly advantageous, pattern of biological activity. These compounds are characterized by a marked intestinal calcium transport activity, as compared to that of 1α, 25-dihydroxyvitamin D_3, while exhibiting much lower activity than 1α,25-dihydroxyvitamin D_3 in their ability to mobilize calcium from bone. Hence, these compounds are highly specific in their calcemic activity. Their preferential activity on intestinal calcium transport and reduced calcium

mobilizing activity in bone allows the in vivo administration of these compounds for the treatment of metabolic bone diseases where bone loss is a major concern. Because of their preferential calcemic activity, these compounds would be preferred therapeutic agents for the treatment of diseases where bone formation is desired, such as osteoporosis, osteomalacia and renal osteodystrophy.

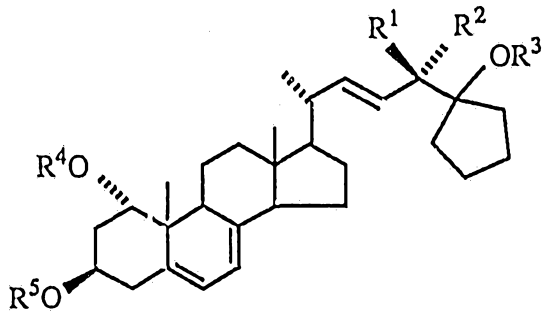
Structurally, the key feature of the compounds having these desirable biological attributes is that they are analogs of 1,25-dihydroxyvitamin D_2 in which a cyclopentane ring is introduced onto the 25 carbon of the side chain of 1α,25-dihydroxyvitamin D_2 and its 24-epimer. Thus, the compounds of this type are characterized by the following general structure:



where R^1 and R^2 may be hydrogen or methyl with the proviso that when R^1 is hydrogen R^2 cannot be hydrogen and when R^1 is methyl R^2 cannot be methyl. The present invention, therefore, provides novel compounds showing preferential activity on intestinal calcium transport and reduced calcium mobilizing activity in bone, and are useful for the treatment of metabolic bone disease, such

as osteoporosis, where bone loss is a major concern. More specifically, the compounds are 26,27-dimethylene-1α,25-dihydroxyvitamin D_2 and 26,27-dimethylene-24-epi-1α,25-dihydroxyvitamin D_2.

This invention also provides novel intermediate compounds formed during the synthesis of the end products. Structurally, the intermediate compounds are characterized by the following general structure:



where R^1 and R^2 may be hydrogen or methyl with the proviso that when R^1 is hydrogen R^2 cannot be hydrogen and when R^1 is methyl R^2 cannot be methyl, and R^3, R^4 and R^5 may be hydrogen or a hydroxy-protecting group.

Brief Description of the Drawings

Fig. 1 is a graph illustrating the percent HL-60 cell differentiation as a function of the concentration of 26,27-dimethylene-1α,25-dihydroxyvitamin D_2, its 24-epimer and 1α,25-dihydroxyvitamin D_3; and

Fig. 2 is a graph illustrating the relative activity of 26,27-dimethylene-1α,25-dihydroxyvitamin D_2, its 24-epimer and 1α,25-dihydroxyvitamin D_3 in binding to the 1,25-dihydroxyvitamin D pig intestinal nuclear receptor.

Detailed Description of the Invention

As used in the description and in the claims, the term hydroxy-protecting group signifies any group

commonly used for the temporary protection of hydroxy functions, such as for example, alkoxycarbonyl, acyl, alkylsilyl, and alkoxyalkyl groups, and a protected hydroxy group is a hydroxy function derivatized by such a protecting group. Alkoxycarbonyl protecting groups are groupings such as methoxycarbonyl, ethoxycarbonyl, propoxycarbonyl, isopropoxycarbonyl, butoxycarbonyl, isobutoxycarbonyl, tert-butoxycarbonyl, benzyloxycarbonyl or allyloxycarbonyl. The term 'acyl' signifies an alkanoyl group of 1 to 6 carbons, in all of its isomeric forms, or a carboxyalkanoyl group of 1 to 6 carbons, such as an oxalyl, malonyl, succinyl, glutaryl group, or a aromatic acyl group such as benzoyl, or a halo, nitro or alkyl substituted benzoyl group. The word 'alkyl' as used in the description or the claims, denotes a straight-chain or branched alkyl radical of 1 to 10 carbons, in all its isomeric forms. Alkoxyalkyl protecting groups are groupings such a methoxymethyl, ethoxymethyl, methoxyethoxymethyl, or tetrahydrofuranyl and tetrahydropyranyl. Preferred alkylsilyl-protecting groups are trimethylsilyl, triethylsilyl, t-butyldimethylsilyl, and analogous alkylated silyl radicals.

The present invention is more specifically described by the following examples, which are meant to be illustrative only of the process of synthesis and of the novel compounds, both end products and intermediates, obtainable thereby. In these examples, specific compounds identified by Arabic numerals (e.g. compounds 1, 2, 3, ... etc.) refer to the structures so numbered in the process schematics. Additionally examples are provided which are illustrative of the distinctive biological characteristics of the new compounds, such characteristics serving as a basis for the application of these compounds in the treatment of metabolic bone disease.

Preparation of Compounds

General Procedures

Ultraviolet (UV) absorption spectra were recorded with a Perkin-Elmer Lambda 3b UV-VIS spectrophotometer. Nuclear magnetic resonance (NMR) spectra were recorded at 500 or 400 MHz with Bruker AM-500 multinuclear or AM-400 wide bore multinuclear spectrometers in the solvents noted. Chemical shifts (δ) are reported downfield from internal Me_4Si (δ0.00) or $CHCl_3$ (δ7.24). Low- and high-resolution mass spectra were recorded at 70 eV (unless otherwise stated) on a Kratos MS-50 TC instrument equipped with a Kratos DS-55 Data System. High resolution data were obtained by peak matching. Samples were introduced into the ion source maintained at 120-250°C via a direct-insertion probe. Silica gel 60 (Merck, 230-400 mesh) was used for column chromatography. High performance liquid chromatography (HPLC) was performed using a Waters Associates Liquid chromatograph equipped with a model 6000A solvent delivery system, a Model U6K Universal injector and a model 450 variable wavelength detector. Zorbax Sil Dupont column (4 x 6 mm x 25 cm) was used. Solvent system: 15% 2-propanol in n-hexane. Tetrahydrofuran (THF) was distilled from sodium benzophenone ketyl. Other solvents were purified by standard methods. Reactions involving vitamin D compounds were carried out under a nitrogen atmosphere with magnetic stirring.

Example 1 - Synthesis of 26,27-Dimethylene-1α,25-Dihdyroxyvitamin D_2 (compound 11a) and its 24-epimer (compound 11b) (Process Schemes I and II)

In the synthesis described herein and in Schemes I and II, the following abbreviations are employed:

DHP: 2,3-dihydropyran

PPTS: pyridinium p-toluenesulfonate

THP: 2-tetrahydropyranyl

THF: tetrahydrofuran
Ts: p-toluenesulfonyl
DMAP: 4-dimethylaminopyridine
DMF: N,N-dimethylformamide
Ph: phenyl
mCPBA: m-chloroperbenzoic acid
TES: triethylsilyl
TBAF: tetrabutylammonium fluoride

The synthesis of compounds 11a and 11b may be summarized as follows:

The synthesis of side chain sulfones 7a,b started from (R)- or (S)- methyl 3-hydroxy-2-methylpropanate. The hydroxy group was protected to provide 2-tetrahydropyranyl (THP) ester 1. The ester 1 was converted into cyclopentanol 2 by an action of 1,4-dibromomagnesiobutane. The THP protecting group was removed to give diol 3. The primary hydroxy group of the diol was converted to the corresponding p-toluenesulfonate 4. The p-toluenesulfonate 4 was converted into phenylsulfide 5 on treatment with thiophenol. The sulfide was oxidized with peracid to sulfone 6. The tertiary hydroxy group was protected as triethylsilyl (TES) ether to give protected sulfone 7.

The sulfone 7 was condensed with aldehyde 9, after deprotonation with lithium diethylamide. The resulting hydroxy sulfone was acetylated, and then submitted to reductive elimination by sodium-amalgam to give (E)-olefin 8. The protective groups of 25- and 3β-hydroxy groups were removed to give provitamin 10. Photo- and thermoisomerization of provitamin 10, followed by deprotection of the 1α-hydroxy group yielded Vitamin D derivative 11.

It should be noted that in the present description and in scheme II, compound 9 is a known compound. Compound 9 may be prepared in accordance with PCT Patent Application No. WO88/07545.

(R)-Methyl 3-(2-tetrahydropyranyl) oxy-2-methylpropanoate 1a. To a mixture of (R)-(-)-methyl 3-hydroxy-2-methylpropanoate (Aldrich; 4.94g, 41.8mmol) and 2,3-dihydropyran (4.22g, 50.2mmol) in dichloromethane (100mL) was added pyridinium p-toluenesulfonate (525mg, 2.08mmol) in one portion, and the mixture was stirred at ambient temperature for 2.25hr. To the mixture 2,3-dihydropyran (1.05g, 12.5mmol) was added and the mixture was stirred at ambient temperature for 30min. The mixture was poured into brine, and the organic layer was separated. The aqueous layer was extracted with diethyl ether, and the combined organic solutions were washed with brine, and dried over sodium sulfate. Filtration and concentration gave 14.83g of an oily material, which was purified by column chromatography (silica gel 75g, ethyl acetate in n-hexane, 5-10%) to give 8.20g (97.0%) of 1a, as a colorless oil.

^{1}H-NMR δ ($CDCl_3$, 500MHz); 1.12, 1.13 (3H, two d, J=7.0Hz), 1.38-1.85 (6H), 2.21 (1H, m), 3.32-3.91 (4H), 3.63 (3H, br s), 4.54 (1H, dd, J=12.0 and 2.9Hz)

(S)-Methyl 3-(2-tetrahydropyranyl) oxy-2-methylpropanoate 1b. In the same manner as for 1a, (S)-(-)-methyl 3-hydroxy-2-methylpropanoate (Aldrich; 4.96g, 42.0mmol) was converted into 8.38g (98.7%) of 1b, as a colorless oil, which showed virtually the same ^{1}H-NMR spectrum as 1a.

(R)-1-[1-(2-Tetrahydropyranyl) oxy-2-propyl]-1-cyclopentanol 2a. To an ice-cooled and stirred solution of 1,4-bisbromomagnesiobutane (prepared from 1.24g of magnesium turnings and 5.03g of 1,4-dibromobutane in 55mL of tetrahydrofuran) was added a solution of 1a (4.0g, 19.8mmol) in diethyl ether (30mL) dropwise at 0-25°C under nitrogen over 70min. The mixture was stirred at ambient temperature for 2hr,

and then quenched by the addition of ammonium chloride solution. The organic layer was separated, and the aqueous layer was extracted with diethyl ether. The combined organic layers were washed with brine, and dried over sodium sulfate. Filtration and concentration gave 5.98g of an oily material, which was purified by column chromatography (silica gel 60g, ethyl acetate in n-hexane, 2.5-20%) to give 3.58g (79.3%) of 2a, as a colorless oil.

^{1}H-NMR δ ($CDCl_3$, 500MHz); 1.00, 1.02 (3H, two d, J=7.3Hz), 1.38-1.93 (15H), 3.04 (1H, d, J=16.2Hz), 3.35 (0.5H, dd, J=9.8 and 4.9Hz), 3.42-3.56 (1.5H), 3.73-3.85 (1.5H), 3.97 (0.5H, dd, J=9.6 and 4.7Hz), 4.55 (1H, d, J=14.9Hz)

(S)-1-[1-(2-Tetrahydropyranyl) oxy-2-propyl]-1-cyclopentanol 2b.

In the same manner as for 2a, 1b (3.0g. 14.8mmol) was converted into 3.58g (quantitative) of 2b, as a colorless oil, which showed virtually the same ^{1}H-NMR spectrum as 2a.

(R)-1-(1-Hydroxy-2-propyl)-1-cyclopentanol 3a. A mixture of 2a (3.42g, 15.0mmol) and pyridinium p-toluenesulfonate (188mg) in ethanol (100mL) was heated at 40-50°C with stirring for 10 hr. The mixture was diluted with toluene and a small amount of triethylamine was added to the mixture. After evaporation of ethanol, the residue was poured into brine and extracted with ethyl acetate until none of the product remained the in aqueous layer. The combined organic layers were washed with brine, and dried over sodium sulfate. Filtration and concentration gave 2.53g of an oily material, which was purified by column chromatography (silica gel 20g,

ethyl acetate in n-hexane, 20-50%) to give 1.88g (86.9%) of 3a, as a colorless oil.

^{1}H-NMR δ ($CDCl_3$, 500MHz); 0.97 (3H, d, J=7.2Hz), 1.38-1.86 (9H), 3.44 (1H, br s), 3.60 (1H, dd, J=9.7 and 4.8Hz), 3.81 (1H, d, J=8.0Hz), 3.93 (1H, br s)

(S)-1-(1-Hydroxy-2-propyl)-1-cyclopentanol 3b. In the same manner as for 3a, 2b (3.41g, 14.9mmol) was converted into 1.98g (92.1%) of 3b, as a colorless oil, which showed the same ^{1}H-NMR spectrum as 3a.

(R)-1-(1-p-Toluenesulfonyloxy-2-propyl)-1-cyclopentanol 4a. A mixture of 3a (1.79g, 12.4mmol), pyridine (5mL), and p-toluenesulfonyl chloride (4.26g, 22.3mmol) in dichloromethane (40mL) was stirred below 10°C for 2 days. The reaction mixture was poured into copper (II) sulfate solution and extracted with diethyl ether. The combined organic layers were washed with copper (II) sulfate solution, water, sodium bicarbonate solution and brine, and dried over sodium sulfate. Filtration and concentration gave 6.27g of an oily material, which was purified by column chromatography (silica gel 60g, ethyl acetate in n-hexane, 5-33%) to give 3.74g (quantitative) of 4a as a colorless oil.

^{1}H-NMR δ ($CDCl_3$, 500MHz); 0.95 (3H, d, J=6.8Hz), 1.28-1.92 (9H), 2.42 (3H, s), 3.91 (1H, dd, J=9.6 and 7.8Hz), 4.18 (1H, dd, J=9.6 and 4.6Hz), 7.32 (2H, d, J=8.0Hz), 7.75 (2H, d, J=8.0Hz)

(S)-1-(1-p-Toluenesulfonyloxy-2-propyl)-1-cyclopentanol 4b. In the same manner as for 4a, 3b (1.92g, 13.3mmol) was converted into 3.46g (87.2%) of 4b, as a colorless oil, which showed the same ^{1}H-NMR spectrum as 4a.

(S)-1-(1-Benzenesulfenyl-2-propyl)-1-cyclopentanol 5a.
To a mixture of 4a (3.65g, 11.9mmol) and triethylamine (2.5mL) in N,N-dimethylformamide (18mL) was added thiophenol (1.8mL) in one portion. The mixture was stirred at ambient temperature overnight. The mixture was poured into water, and extracted with diethyl ether. The combined organic layers were washed with sodium bicarbonate solution and brine, and dried over sodium sulfate. Filtration and concentration gave 3.26g of an oily material, which was purified by column chromatography (silica gel 40g, ethyl acetate in n-hexane, 2.5-20%) to give 2.06g (73.3%) of 5a, as a pale yellow oil.

^{1}H-NMR δ ($CDCl_3$, 500MHz); 1.24 (3H, d, J=6.3Hz), 1.53 (1H, br s), 1.56-2.04 (9H), 2.85 (1H, dd, J=12.9 and 10.2Hz), 3.44 (1H, dd. J=12.9 and 2.8Hz), 7.28 (1H, t, J=8.0Hz), 7.39 (2H, t, J=8.0Hz), 7.46 (2H, d, J=8.0Hz)

(R)-1-(1-Benzenesulfenyl-2-propyl)-1-cyclopentanol 5b.
In the same manner as for 5a, 4b (3.65g, 11.9mmol) was converted into 2.23g (82.8%) of 5b, as a pale yellow oil, which showed the same ^{1}H-NMR spectrum as 5a.

(S)-1-(1-Benzenesulfonyl-2-propyl)-1-cyclopentanol 6a.
To a stirred and ice-cooled mixture of 5a (2.06g, 8.71mmol) in dichloromethane (19mL) and saturated sodium bicarbonate solution (28mL) was added m-chloroperbenzoic acid (85%, 4.24g, 20.9mmol) portionwise. The mixture was stirred in an ice bath for 50min. An excess amount of peracid was decomposed with sodium thiosulfate solution in the presence of a small amount of potassium iodide. The organic layer was separated, and the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with sodium bicarbonate solution and brine, and dried

over sodium sulfate. Filtration and concentration gave 3.16g of an oily material, which was purified by column chromatography (silica gel 32g, ethyl acetate in n-hexane, 20-33%) to give 2.43g (quantitative) of 6a, as a colorless oil.

^{1}H-NMR δ ($CDCl_3$, 400MHz); 1.17 (3H, d, J=6.8Hz), 1.46-1.86 (8H), 2.18 (1H, m), 3.00 (1H, dd, J=14.5 and 9.0Hz), 3.41 (1H, br d, J=14.5Hz), 7.57 (2H, t, J=7.3Hz), 7.65 (1H, t, J=7.3Hz), 7.92 (2H, d, J=7.3Hz)

(R)-1-(1-Benzenesulfonyl-2-propyl)-1-cyclopentanol 6b. In the same manner as for 6a, 5b (2.23g, 9.43mmol) was converted into 2.34g (92.5%) of 6b, as a colorless oil, which showed the same ^{1}H-MMR spectrum as 6a.

(S)-1-(1-Benzenesulfonyl-2-propyl)-1-triethylsiloxycyclopentane 7a. To a solution of 6a (2.40g, 8.94mmol) and imidazole (1.22g, 17.9mmol) in N,N-dimethylformamide (20mL) was added chlorotriethylsilane (2.2mL, 13.1mmol) in one portion. The mixture was stirred at ambient temperature for 3d. The mixture was poured into ice water, and extracted with diethyl ether. The combined organic layers were washed with water and brine, and dried over sodium sulfate. Filtration and concentration gave 4.24g of an oily material, which was purified by column chromatography (silica gel 40g, ethyl acetate in n-hexane, 10%) to give 3.67g (quantitative) of 7a, as a colorless oil.

^{1}H-NMR δ ($CDCl_3$, 500MHz); 0.52 (6H, q, J=7.9Hz), 0.88 (9H, t, J=7.9Hz), 1.11 (3H, d, J=6.7Hz), 1.35-1.74 (8H), 2.06 (1H, m), 2.90 (1H, dd, J=14.4 and 9.7Hz), 3.42 (1H, d, J=14.4Hz), 7.56 (2H, t, J=7.5Hz), 7.64 (1H, t, J=7.5Hz), 7.91 (2H, d, J=7.5Hz)

(R)-1-(1-Benzenesulionyl-2-propyl)-1-triethylsiloxycyclopentane 7b. In the same manner as for 7a, 6b (2.20g, 8.19mmol) was converted into 3.19g (quantitative) of 7b, as a colorless oil, which showed the same ^{1}H-NMR spectrum as 7a.

(22E,24R)-26,27-Dimethylene-1α, 3β-bis(methoxycarbonyloxy)-25-triethylsiloxyergosta-5,7,22-triene 8a. To a stirred solution of 7a (1.0g, 2.61mmol) in tetrahydrofuran (30mL) was added a solution of lithium diethylamide (prepared from 0.44mL of diethylamine and 2.5mL of 1.6N n-butyllithium in 7mL of tetrahydrofuran; 6.6mL) dropwise at -50-60°C under nitrogen. The mixture was stirred at -50-60°C for 1hr, and then cooled to -78°C. To the mixture was added a solution of (20S) -1α,3ß- bis(methoxycarbonyloxy)-20-methylpregna-5,7-dien-21-al 9 (670mg, 1.45mmol) in tetrahydrofuran (20mL) dropwise over a period of 50min. The mixture was stirred for 50min, and then quenched by the addition of saturated ammonium chloride solution and ethyl acetate. The organic layer was separated and the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, and dried over sodium sulfate. Filtration and concentration gave 2.97g of a residue. The residue was dissolved in dichloromethane (20mL) and to the solution was added 4-dimethylaminopyridine (2.12g, 17.4mmol) and acetic anhydride (1.3mL, 13.8mmol), and the mixture was stirred at ambient temperature overnight. The mixture was poured into a mixture of ice and ethyl acetate, and the organic layer was separated. The aqueous layer was extracted with ethyl acetate, and the combined organic layers were washed with brine and dried over sodium sulfate. Filtration and concentration gave 2.37g of a residue. The residue was dissolved in a mixture of tetrahydrofuran (30mL) and methanol (30mL) and the

solution was stirred at -40 to -30°C. To the solution was added sodium bicarbonate (2.27g) and 5% sodium amalgam (pulverized and washed with tetrahydrofuran; 10.72g). The mixture was stirred at -40 to -30°C for 2.5hr. The supernatant was filtered through a pad of Celite and the solids was washed with ethyl acetate. The combined organic solution was poured into a cold mixture of diluted hydrochloric acid and ethyl acetate, and the organic layer was separated. The aqueous layer was extracted with ethyl acetate and the combined organic layers were washed with brine, and dried over sodium sulfate. Filtration and concentration gave 2.75g of a residue, which was purified by column chromatography (silica gel 50g, ethyl acetate in n-hexane, 5-50%) to give 648mg (65.2% from 9) of 8a, as white solids.

^{1}H-NMR δ ($CDCl_3$, 500MHz); 3.77 (3H, s), 3.78 (3H, s), 4.84 (1H, br s), 4.89 (1H, m), 5.20 (1H, dd, J=15.2 and 8.5Hz), 5.30 (1H, dd, J=15.2 and 8.6Hz), 5.36 (1H, m), 5.67 (1H, m)

(22E,24S)-26,27-Dimethylene-1α,3β-bis(methoxycarbonyloxy)-25-triethylsiloxyergosta-5,7,22-triene 8b. In the same manner as for 8a, 7b (1.0g, 2.61mmol) was converted into 803mg (67.4% form 9) of 8b, as white solids.

^{1}H-NMR δ ($CDCl_3$, 500MHz); 3.77 (3H, s), 3.79 (3H, s), 4.84 (1H, br s), 4.90 (1H, m), 5.21 (1H, dd, J=15.3 and 8.3Hz), 5.30 (1H, dd, J=15.3 and 8.4Hz), 5.37 (1H, m), 5.68 (1H, m)

(22E,24R)-26,27-Dimethylene-1α-methoxycarbonyloxyergosta-5,7,22-triene-3β,25-diol 10a. To a solution of 8a (597mg, 0.871mmol) in tetrahydrofuran (12mL) was added 1M solution of tetrabutylammonium fluoride in tetrahydrofuran (4.4mL), and the mixture was stirred at ambient temperature overnight. The mixture was poured into cold brine, and extracted with ethyl acetate. The combined organic layers were washed with sodium bicarbonate solution and brine, and dried over sodium sulfate. Filtration and evaporation gave 0.78g of a residue. To the residue was added methanol (50mL) and potassium carbonate (0.5g) and the mixture was stirred in a cold room (at 8°C) overnight. The mixture was poured into cold brine and extracted with ethyl acetate.

The combined organic layers were washed with brine, and dried over sodium sulfate. Filtration and concentration gave 1.54g of a residue, which was purified by column chromatography (silica gel 30g, ethyl acetate in n-hexane, 20-80%) to give 313mg (70.1%) of 10a, as white solids.

^{1}H-NMR δ ($CDCl_3$, 500MHz); 0.62 (3H, s), 1.00 (3H, s), 1.02 (3H, d, J=6.7Hz), 1.03 (3H, d, J=6.2Hz), 3.78 (3H, s), 3.99 (1H, br), 4.82 (1H, br s), 5.33 (2H, m), 5.36 (1H, m), 5.66 (1H, m)

(22E,24S)-26,27-Dimethylene-1α-methoxycarbonyloxyergosta-5,7,22-triene-3β,25-diol 10b. In the same manner as for 10a, 8b (588mg, 0.858mmol) was converted into 302mg (68.7%) of 10b, as white solids.

^{1}H-NMR δ ($CDCl_3$, 500MHz); 0.63 (3H, s), 1.01 (3H, s), 1.03 (3H, d, J=6.8Hz), 1.04 (3H, d, J=6.5Hz), 3.78 (3H, s), 4.00 (1H, m), 4.82 (1H, br s), 5.34 (1H, dd, J=15.4 and 8.2Hz), 5.37 (1H, m), 5.40 (1H, dd, J=15.4 and 7.4Hz), 5.67 (1H, m)

(5Z,7E,22E,24R)-26,27-Dimethylene-9,10-secoergosta-5,7,10(19),22-tetraene-1α,3β,25-triol 11a. A stirred and ice-cooled solution of 10a (103mg, 0.201mmol) in a mixture of diethyl ether (100mL) and benzene (20mL) was irradiated with medium pressure mercury lamp for 30min under nitrogen. The mixture was concentrated under reduced pressure and the residue was dissolved in benzene (20mL) and left to stand at ambient temperature for 15d under nitrogen covered with aluminum foil. The mixture was concentrated under reduced pressure, and the residue was treated with 1% lithium hydroxide hydrate solution in methanol (5mL) at ambient temperature for 1hr under nitrogen. The mixture was poured into ice water, and extracted with ethyl acetate. The combined organic layers were washed with brine, and dried over sodium sulfate. Filtration and concentration under reduced pressure gave an oily material, which was purified by column chromatography (silica gel 10g, ethyl acetate in n-hexane. 33-80%) to give 37.6mg (36.5%) of 10a and 27.4mg (30.0%, 47.2% based on recovery of 10a) of 11b, as white solids.

UV (ethanol); λ max 266nm, λ min 228nm MS (EI) m/z 454 (M^+), 436 (M^+-H2O), 418 (M^+-2H_2O), 400 (M^+-3H_2O), 370 ($M^++1-C_5H_9O$), 352 ($M^++1-C_5H_9O-H_2O$), 334 ($M^++1-C_5H_9O-2H_2O$), 285 ($M^++1-C_9H_{12}O_2-H_2O$), 269 ($M^+-C_{11}H_{19}O-H_2O$), 152 ($C_9H_{12}O_2$), 85 (base peak, C_5H_9O)
HRMS m/z; Found 454.3434, Calcd. for $C_{30}H_{46}O_3$ 454.3447

^1H-NMR δ ($CDCl_3$, 500MHz); 0.55 (3H, s), 1.01 (3H, d, J=6.9Hz), 1.02 (3H, d, J=6.4Hz), 4.23 (1H, br s), 4.43 (1H, m), 4.99 (1H, s), 5.36-5.42 (3H), 6.01 (1H, d, J=11.3Hz), 6.38 (1H, d, J=11.3Hz)
HPLC T_R (min); 12.0

(5Z,7E,22E,24S)-26,27-Dimethylene-9,10-secoergosta-5,7,10(19),22tetraene-1α,3β,25-triol 11b. In the same manner as for 11a, 10b (100mg, 0.195mmol) was converted into 23.5mg (26.5%, 55.4% based on recovery of 10b) of 11b, as white solids, and 52.2mg (52.2%) of 10b was recovered.

U V (ethanol); λmax 266nm, λmin 228nm MS (EI) m/z; 454 (M^+), 436 (M^+-H_2O), 418 (M^+-2H_2O), 352 ($M^++1-C_5H_2O-H_2O$), 334 ($M^++1-C_5H_9O-2H_2O$), 285 ($M^++1-C_9H_{12}O_2-H_2O$), 269 ($M^+-C_{11}H_{19}O-H_2O$), 152 ($C_9H_{12}O_2$), 85 (base peak, C_5H_9O)
HRMS m/z; Found 454.3472, Calcd. for $C_{30}H_{46}O_3$ 454.3447

^{1}H-NMR δ ($CDCl_3$, 500MHz); 0.56 (3H, s), 1.03 (6H, d, J=7.0Hz), 4.23 (1H, br s), 4.43 (1H, m), 5.00 (1H, s), 5.32 (1H, s), 5.35 (1H, dd, J=15.4 and 7.6Hz), 5.38 (1H, dd, J=15.4 and 6.8Hz), 6.01 (1H, d, J=11.3Hz), 6. 38 (1H, d, J=11.3Hz)
HPLC T_R (min); 12.1

Biological Activity of 26,27-Dimethylene-1α,25-Dihydroxyvitamin D_2 (Compound 11b) and its 24-epimer (compound 11a).

Example 2 - Calcemic Activity

Weanling male rats obtained from the Holtzman Company were fed a low calcium (0.02%), 0.3% phosphorus, vitamin D-deficient diet for three weeks. After this time, the animals were severely hypocalcemic. They were then implanted with Alzet minipumps that delivered approximately 13μL of solution per day which contained the indicated dose in Table 1 dissolved in 5% ethanol, 95% propylene glycol. After 7 days the rats were killed and the duodena were used for determination of intestinal calcium transport by the everted intestinal

sac technique (Martin & DeLuca, 1967) and serum calcium (bone calcium mobilization). The tests were made against 1,25-dihydroxyvitamin D_3 and are reported in Table 1.

TABLE 1

INTESTINAL CALCIUM TRANSPORT AND BONE CALCIUM MOBILING ACTIVITIES OF 26,27-DIMETHYENE-1α,25-DIHYDROXYVITAMIN D_2 AND 26,27-DIMETHYENE-24-EPI-1α,25-DIHYDROXYVITAMIN D_2

Treatment	Doses (pmoles/ day)	Intestinal Calcium Transport Serosal/ Mucosal	Bone Calcium Mobilization (Serum Calcium) (mg/100 ml)
None	0	3.0 ± 0.2	4.2 ± 0.2
1,25-$(OH)_2D_3$	130	8.5 ± 0.9	5.7 ± 0.3
Dimethylen-1,25-$(OH)_2D_2$	130	5.2 ± 0.4	4.4 ± 0.1
	325	6.6 ± 0.6	5.8 ± 0.1
Dimethylen-24-epi-1,25-$(OH))_2D_2$	130	6.3 ± 0.3	4.4 ± 0.1
	325	7.8 ± 0.1	5.1 ± 0.4

The results show that the dimethylene-1,25-dihydroxy-vitamin D_2 and the dimethylene-24-epi-1,25-dihydroxy-vitamin D_2 are both less active than 1,25-dihydroxy-vitamin D_3 in both the mobilization of calcium from bone and intestinal calcium transport. However, both of the 26,27-dimethylene-D_2 compounds have highly significant intestinal calcium transport activity. The amount of bone calcium mobilizing activity is considerably less than 1,25-dihydroxyvitamin D_3, and in the case of the 24-epi-compound, it is considerably less active in this regard. These compounds, therefore, by showing preferential activity on intestinal calcium transport and reduced calcium mobilizing activity in bone suggest that they

disease where bone loss is a major issue, such as osteoporosis, osteomalacia and renal osteodystrophy.

Example 3 - Measurement of Differentiation in HL-60 Cells

The measurement of differentiation in HL-60 cells (human leukemia cells) was carried out according to the general procedures described by DeLuca et al., U. S. Patent 4,717,721. As shown in Table 2, degree of differentiation is assessed by a standard assay, namely, NBT reduction, and results are expressed as the percent of differentiated cells produced in response to treatment with various concentrations of vitamin D compounds.

TABLE 2

Differentiation Activity in HL-60 Cells in Culture

Compound	Concentration (molar)	% Cells Showing Differentiation NBT Reduction
$1,25\text{-}(OH)_2D_3$	1×10^{-7}	86 ± 3
	1×10^{-8}	61 ± 3
	1×10^{-9}	38 ± 4
26,27-Dimethylen-$1,25\text{-}(OH)_2D_2$	1×10^{-7}	89 ± 3
	5×10^{-8}	73 ± 4
	1×10^{-8}	59 ± 4
	5×10^{-9}	36 ± 4
	1×10^{-9}	23 ± 2
26,27-Dimethylen-24-epi-$1,25\text{-}(OH)_2D_2$	1×10^{-7}	90 ± 3
	5×10^{-8}	77 ± 2
	1×10^{-8}	63 ± 4
	5×10^{-9}	44 ± 4
	1×10^{-9}	21 ± 2

[a]Standard error of the mean of 3-4 determinations.

The results of this assay is shown in Table 2. It is evident that the novel analogs (compounds 11a and

11b) are about equally as active as 1,25-$(OH)_2D_3$ itself in causing differentiation of HL-60 cells in culture.

The results shown in Figure 1 illustrate that these compounds when added to cultures of human HL-60 cells cause their differentiation into monocytes and show activity approximately equal to 1α,25-dihydroxyvitamin D_3. Figure 2 shows both 26,27-dimethylene- 1,25-dihydroxyvitamin D_2 and its 24-epimer are at least equal in activity to 1,25-$(OH)_2D_3$ in binding to the 1,25-$(OH)_2D$ pig intestinal nuclear receptor.

Because these compounds are at least as active as 1,25-$(OH)_2D_3$ in differentiation, receptor binding, and approximately equal in intestinal calcium transport activity but are very much less active in mobilizing bone calcium, they would appear to be ideal for treatment of diseases where bone formation is desired.

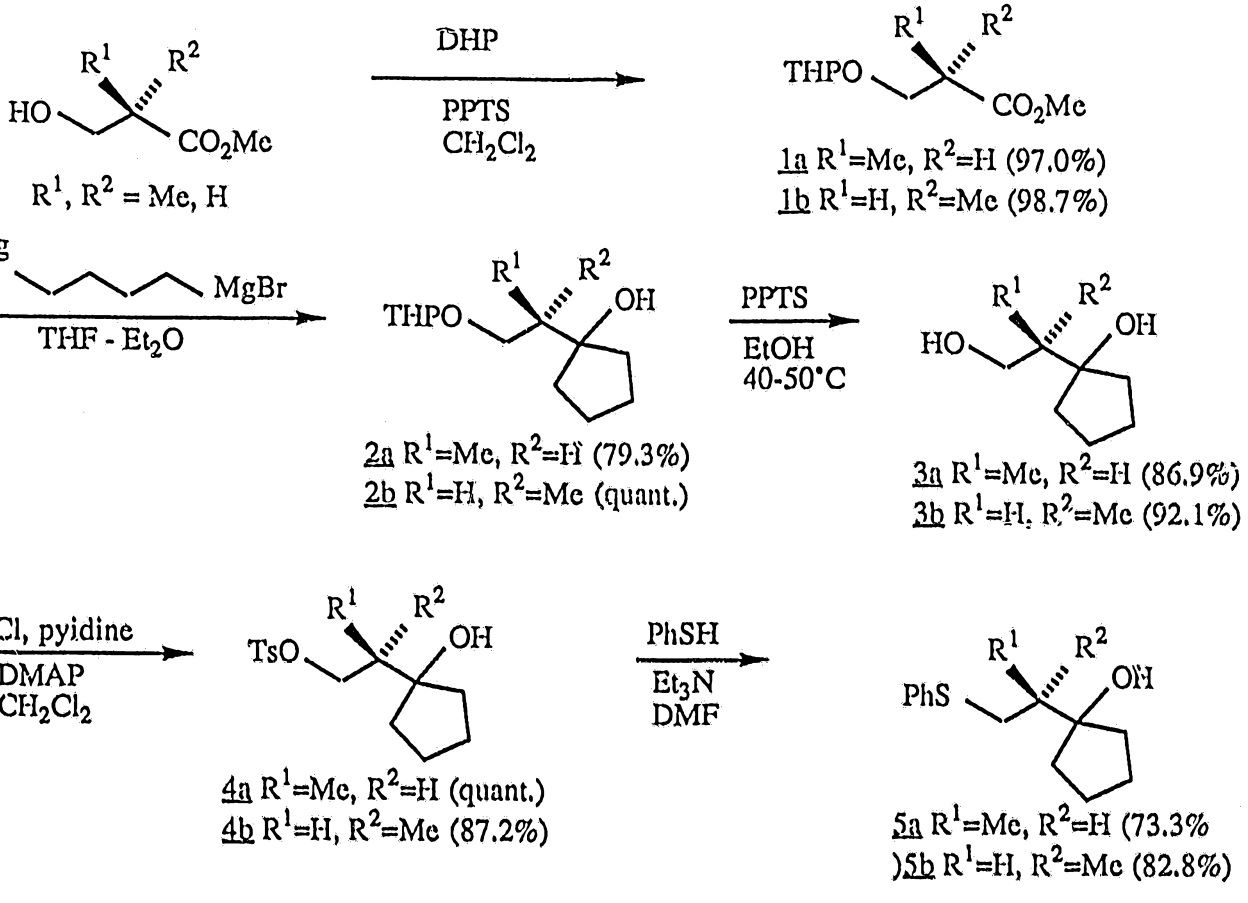
For treatement purposes, the novel compounds of this invention may be formulated for pharmaceutical applications as a solution in innocuous solvents, or as an emulsion, suspension or dispersion in suitable solvents or carriers, or as pills, tablets or capsules, together with solid carriers, according to conventional methods known in the art. Any such formulations may also contain other pharmaceutically-acceptable and non-toxic excipients such as stabilizers, anti-oxidants, binders, coloring agents or emulsifying or taste-modifying agents.

The compounds may be administered orally, parenterally or transdermally. The compounds are advantageously administered by injection or by intravenous infusion of suitable sterile solutions, or in the form of liquid or solid doses via the alimentary canal, or in the form of creams, ointments, patches, or similar vehicles suitable for transdermal applications. Doses of from 0.5 µg to 50 µg per day of the compounds are appropriate for treatment purposes, such doses being

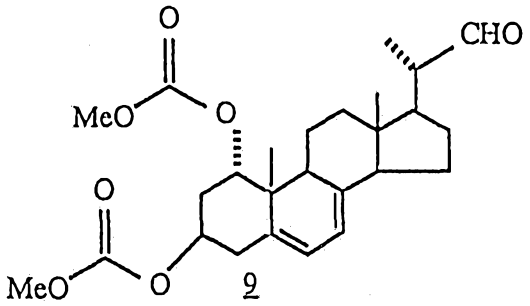
adjusted according to the disease to be treated, its severity and the response of the subject as is well understood in the art. Since the new compounds exhibit specificity of action, each may be suitably administered alone, in situations where only calcium transport stimulation is desired, or together with graded doses of another active vitamin D compound -- e.g. 1α-hydroxyvitamin D_2 or D_3, or 1α,25-dihydroxyvitamin D_3 -- in situations where some degree of bone mineral mobilization (together with calcium transport stimulation) is found to be advantageous.

The claims form part of the disclosure of this specification.

SCHEME II

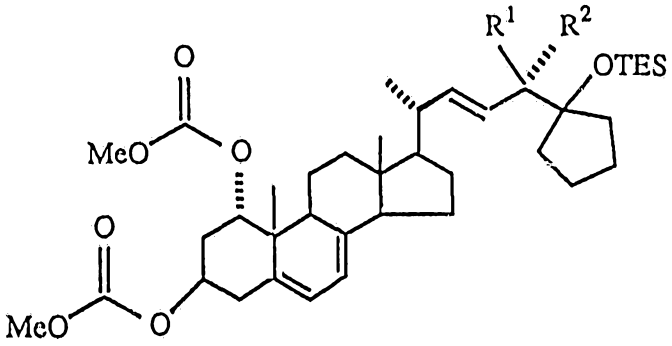

DHP
PPTS
CH_2Cl_2
R^1, R^2 = Me, H
1a R^1=Me, R^2=H (97.0%)
1b R^1=H, R^2=Me (98.7%)
BrMg
MgBr
THF - Et_2O
2a R^1=Me, R^2=H (79.3%)
2b R^1=H, R^2=Me (quant.)
PPTS
EtOH
40-50°C
3a R^1=Me, R^2=H (86.9%)
3b R^1=H, R^2=Me (92.1%)
TsCl, pyidine
DMAP
CH_2Cl_2
4a R^1=Me, R^2=H (quant.)
4b R^1=H, R^2=Me (87.2%)
PhSH
Et_3N
DMF
5a R^1=Me, R^2=H (73.3%
)5b R^1=H, R^2=Me (82.8%)
mCPBA
$NaHCO_3$
CH_2Cl_2 - H_2O
6a R^1=Me, R^2=H (quant.)
6b R^1=H, R^2=Me (92.5%)
Et_3SiCl
DMF
imidazole
7a R^1=Me, R^2=H (quant.)
7b R^1=H, R^2=Me (quant.)

SCHEME III



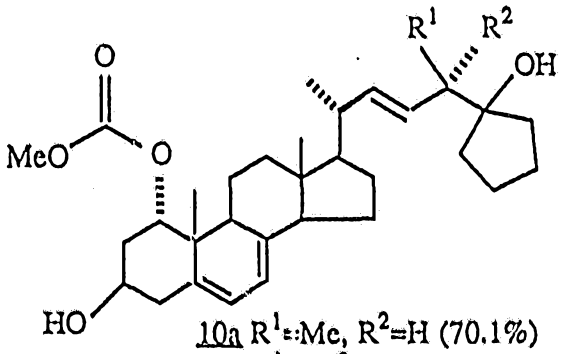
9

1) 7a or 7b, $LiNEt_2$, THF
2) Ac_2O, DMAP, CH_2Cl_2
3) Na-Hg, $NaHCO_3$, MeOH, THF



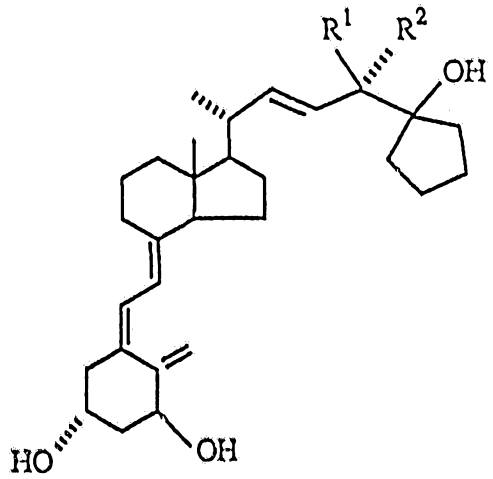
8a R^1=Me, R^2=H (65.2%)
8b R^1=H, R^2=Me (67.4%)

1) TBAF, THF
2) K_2CO_3, MeOH



10a R^1=Me, R^2=H (70.1%)
10b R^1=, R^2=Me (68.7%)

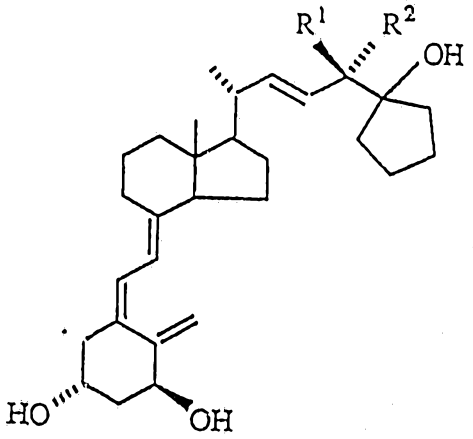
1) hv, Et_2O-benzene
2) Δ
3) $LiOH \cdot H_2O$, MeOH



11a R^1=Me, R^2=H (30.0%) (47.2% based on recovery)
11b R^1=H, R^2=Me (26.5%) (55.4% based on recovery)

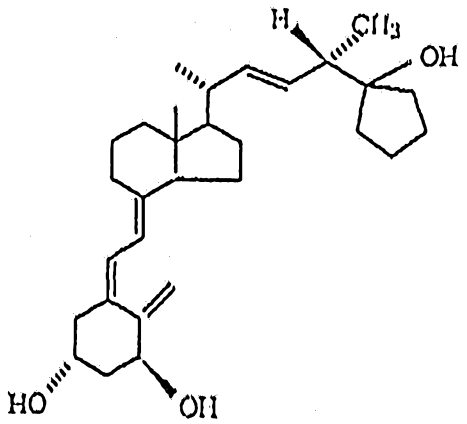
The claims defining the invention are as follows:

1. A compound having the formula



where R^1 and R^2 may be hydrogen or methyl with the proviso that when R^1 is hydrogen R^2 cannot be hydrogen and when R^1 is methyl R^2 cannot be methyl.

2. 26,27-dimethylene-1α,25-dihydroxyvitamin D_2 having the structure:



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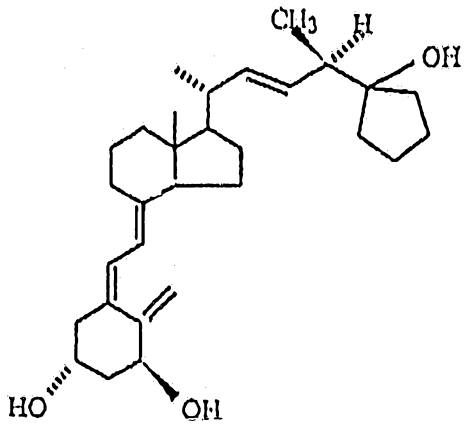
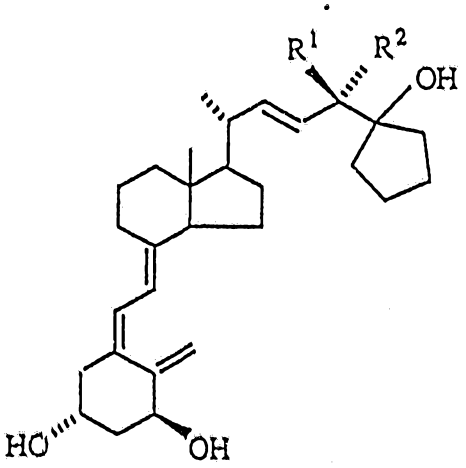
3. 26,27-dimethylene-24-epi-1α,25-dihydroxyvitamin D_2 having the structure:



4. A method of treating metabolic bone disease where it is desired to maintain or increase bone mass comprising administering to a patient with said disease a compound having the formula



where R^1 and R^2 may be hydrogen or methyl with the proviso that when R^1 is hydrogen R^2 cannot be hydrogen and when R^1 is methyl R^2 cannot be methyl.

5. The method of claim 4 where the disease is osteoporosis.

6. The method of claim 4 where the disease is osteomalacia.

7. The method of claim 4 where the disease is renal osteodystrophy.

8. The method of claim 4 wherein the compound is administered orally.

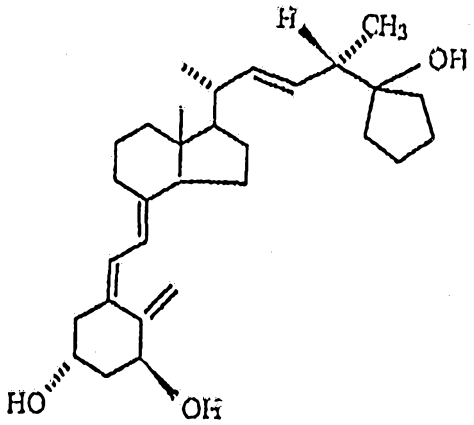
9. The method of claim 4 wherein the compound is administered parenterally.

10. The method of claim 4 wherein the compound is administered transdermally.

11. The method of claim 4 wherein the compound is administered in a dosage of from 0.5μg to 50μg per day.

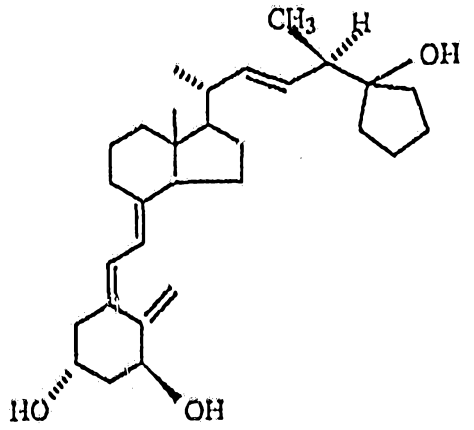
12. A pharmaceutical composition containing at least one compound as claimed in claim 1 together with a pharmaceutically acceptable excipient.

13. The pharmaceutical composition of claim 12 containing 26,27-dimethylene-1α,25-dihydroxyvitamin D_2 having the structure:



in an amount from 0.5μg to 50μg.

14. The pharmaceutical composition of claim 12 containing 26,27-dimethylene-24-epi-1α,25-dihydroxyvitamin D_2 having the structure:



in an amount from 0.5μg to 50μg.

15. Compounds according to any one of claims 1 to 3 substantially as hereinbefore described with reference to any one or more of the non-comparative examples.

16. A method of treating metabolic bone disease according to any one of claims 4 to 11 substantially as hereinbefore described.

17. A pharmaceutical composition according to any one of claims 12 to 14 substantially as hereinbefore described.

DATED: 23 November 1994

CARTER SMITH & BEADLE

Patent Attorneys for the Applicant:

WISCONSIN ALUMNI RESEARCH FOUNDATION

Abstract of the Disclosure

Vitamin D_2 analogs in which a cyclopentane ring is introduced onto the 25-carbon of the side chain of 1α,25-dihydroxyvitamin D_2 and its 24-epimer. The compounds are characterized by a marked intestinal calcium transport activity while exhibiting much lower activity than 1α,25-dihydroxyvitamin D_3 in their ability to mobilize calcium from bone. Because of their preferential calcemic activity, these compounds would be useful for the treatment of diseases where bone formation is desired, such as osteoporosis.

FIG.1

HL-60 DIFFERENTIATION ACTIVITY

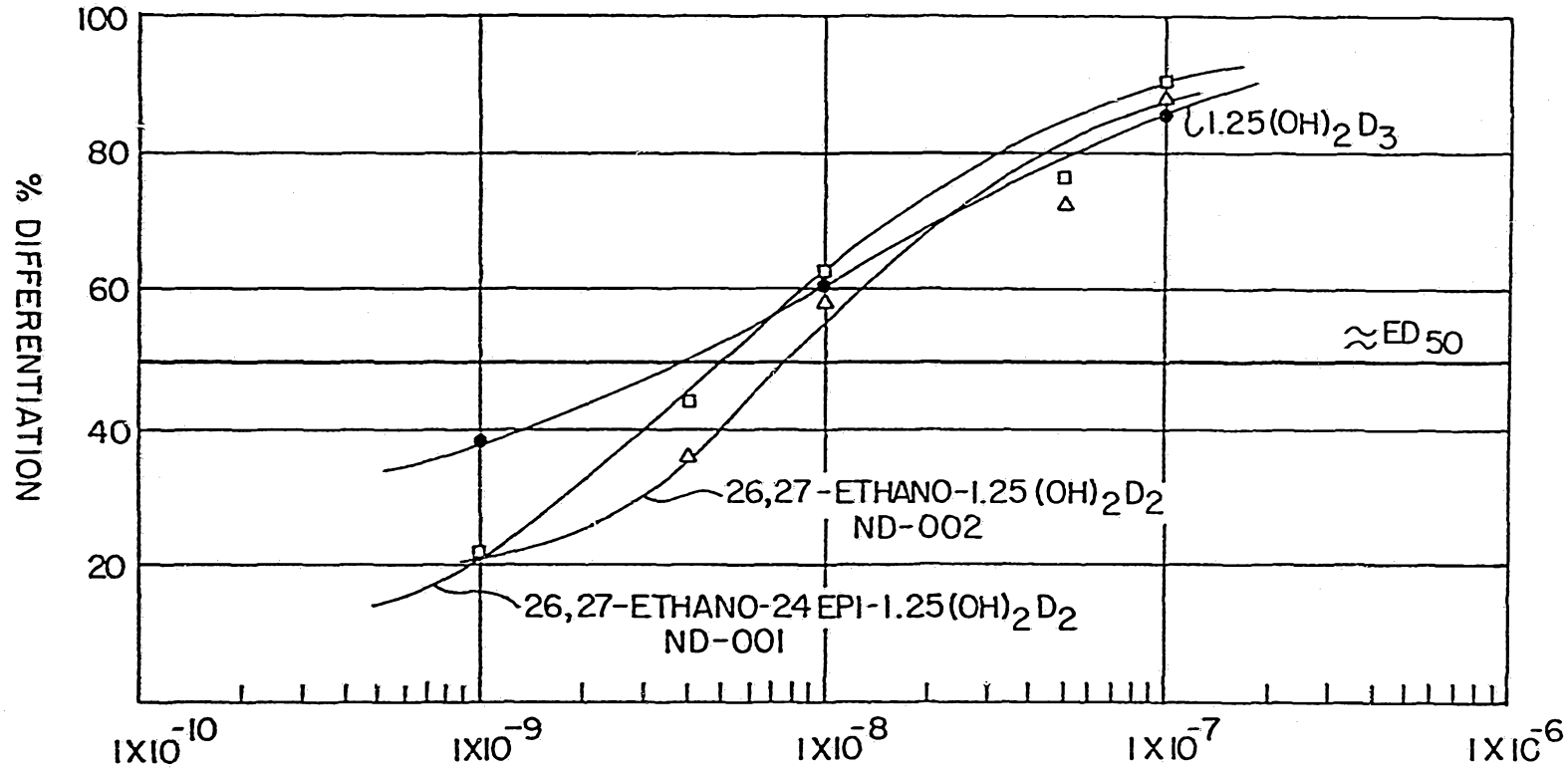


FIG.2

COMPETITIVE BINDING

