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        **524268**

(71) Applicant
    **Wisconsin Alumni Research Foundation**
    **(USA—Wisconsin),**
    **614 North Walnut Street, Madison, Wisconsin 53705,**
    **United States of America**

(72) Inventors
    **Hector F DeLuca**
    **Yoko Tanaka**
    **Nobuo Ikekawa**
    **Yoshiro Kobayashi**

(74) Agent and/or Address for Service
    **J A Kemp & Co,**
    **14 South Square, Gray's Inn, London WC1R 5EU**

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(52) Domestic classification
    **C2V 8 A1**
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    **GB A 2026494**

(58) Field of search
    **C2V**

(54) **Vitamin D$_3$ derivatives**

(57) New derivatives of vitamin D$_3$ of formula
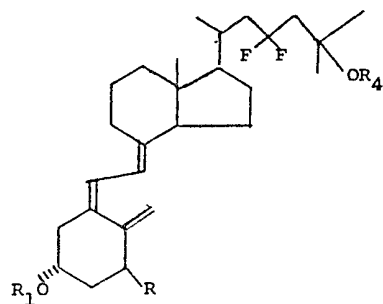


wherein R is H, OH, O-aliphatic acyl or O-benzoyl and R$_1$ and R$_4$ are H, aliphatic acyl or benzoyl, are characterized by vitamin D-like activity as evidence by their ability to increase intestinal calcium transport and serum calcium. They are further characterized by resistance to hydroxylation at C–23.
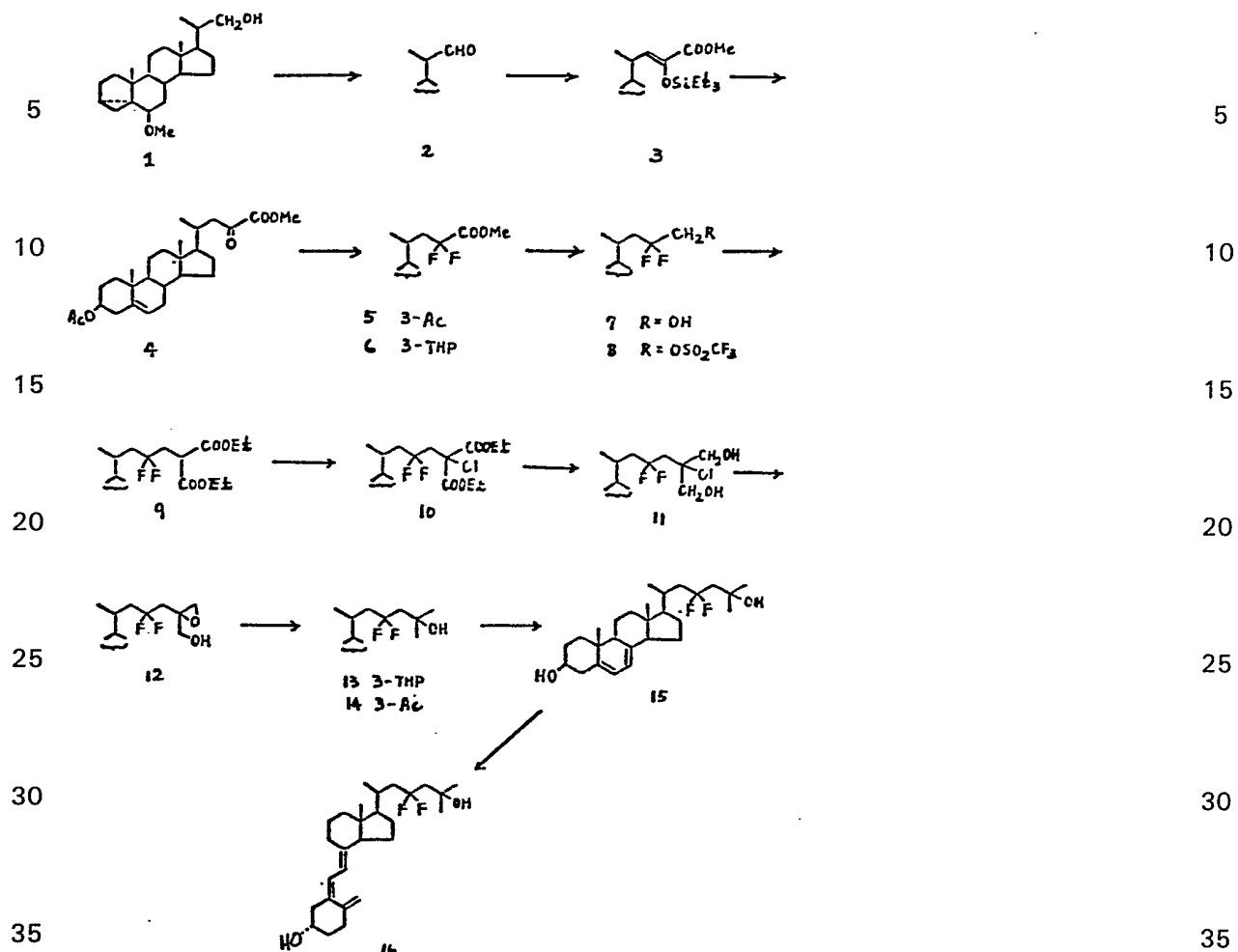    23,23 difluoro-steroids of formulæ

 and 
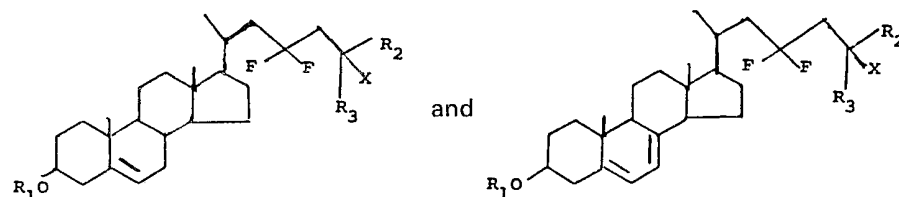
wherein R$_1$ is acryl or tetrahydropyranyl
    R$_2$ and R$_3$ are H, COO alkyl or CH$_2$OH and
    X is halogen, hydroxy or O-acyl are also disclosed.

SPECIFICATION

## 23,23-Difluoro-25-hydroxy- and 1α,25-dihydroxy-vitamin D₃

5    This invention relates to derivatives of vitamin $D_3$.                                            5
     Vitamin $D_3$ is a well-known agent for the control of calcium and phosphorus homeostatis. In
the normal animal or human this compound is known to stimulate intestinal calcium transport
and bone-calcium mobilization and is effective in preventing rickets.
     It is also now well-known that to be effective, vitamin $D_3$ must be converted *in vivo* to its
10  hydroxylated forms. For example, the vitamin is first hydroxylated in the liver to form 25-          10
hydroxy-vitamin $D_3$ and is further hydroxylated in the kidney to produce 1α,25-hydroxy-vitamin
$D_3$ of 24,25-dihydroxy-vitamin $D_3$. The 1-hydroxylated form of the vitamin is generally
considered to be the physiologically active or hormonal form of the vitamin and to be
responsible for what are termed the vitamin D-like activities, such as increasing intestinal
15  absorption of calcium and phosphate, mobilizing bone mineral, and causing reabsorption of       15
calium in the kidneys.
     Since the discovery of biologically active metabolites of vitamin D there has been much
interest in the preparation of structural analogs thereof because such compounds may respresent
useful therapeutic agents for the treatment of diseases resulting from calcium metabolism
20  disorders. It is generally accepted that 1α,25-dihydroxycholecalciferol is the circulating hormonal  20
form of vitamin D.
     New derivatives of vitamin D have now been found which are at least as potent as 25-
hydroxyvitamin $D_3$ as measured by their ability to stimulate calcium transport in the intestine or
its ability to mobilize calcium from bone. These derivatives which form the subject of the present
25  invention are 23,23-difluoro-25-hydroxycholecalciferol (23,23-difluoro-25-hydroxy vitamin $D_3$ or  25
23,23-$F_2$-25(OH)$D_3$) and 23,23-difluoro-1α,25-dihydroxycholecalciferol (23,23-difluoro-1,25-
dihydroxy vitamin $D_3$ or 23,23-$F_2$-1,25(OH)$_2$$D_3$), and acylates thereof.
     A major pathway for inactivation of vitamin D is believed to be 23S-hydroxylation of 25-
hydroxy vitamin $D_3$ (see Biochemistry 20, 3875–3879, 1981) and its subsequent conversion to
30  25R-hydroxy-26,23S-lactone (see Proc. Nat'l. Acad. Sci. USA 78, 4805–4808, 1981).              30
Accordingly it would appear that the derivatives of the present invention, because of the fluorine
substituents at C-23, would not be readily hydroxylated at that carbon and that, therefore, they
would be characterized by prolonged vitamin D-like activity—an obvious advantage in many
therapeutic applications.
35    23,23-Difluoro-25-hydroxyvitamin D can be obtained in accordance with the process herein-    35
after described and shown in the accompanying schematic wherein like numbers refer to like
compounds.
     The steroid-1-ether (1) is oxidized to the C-22 aldehyde by pyridium chlorochromate or other
suitable alcohol oxidizing reagent. This C-22 aldehyde is converted to a silyl ether carboxy ester
40  (3) by a Wittig type condensation. Hydrolysis in acetic acid and TsOH afford the corresponding  40
C-23α -keto methyl ester and converts the 1 ether to the 3 acetoxy function (4). The ketone is
fluorinated with DAST (diethyl amino sulphur trifluoride) to provide the C-23 difluorocarboxyme-
thyl-3 acetate. Hydrolysis of the acetoxy group and treatment with 2,3-dihydropyran and TsOH
gave the 3-THP protected difluoro 24 ester. Reduction with lithium aluminium hydride produced
45  the C-24 alcohol (7). Treatment of this alcohol with a mixture of trifluoromethanesulphonic     45
anhydride and pyridine provides the trifluoromethanesulphonyl ester which undergoes a malonic
ester condensation to yield the C-26,27 diethyl ester (9). N-chlorosuccinimide is used to convert
(9) to the C-25 chloro derivative (10) which, upon reduction with lithium aluminium hydride,
affords the C-26,27 chlorodialcohol (11). Treatment of that chlorodiol with sodium hydride in
50  dimethoxyethane yields the 25,26 epoxy 26-alcohol (12). Treatment with methanesulphonyl     50
chloride and triethylamine provided the mesylate which upon reduction with lithium aluminium
hydride yielded 23,23-difluoro, 25-hydroxyl-3-1-tetrahydropyranyl cholesterol (13) which was
converted to the acetate (14). This compound was then converted to the 5,7-diene by the usual
allylic bromination followed by dehydrobromination in collidine. This diene was then photolyzed
55  to provide the corresponding previtamin which during the temperature of work up results in the   55
23,23-difluoro-25-hydroxyvitamin $D_3$ (16).

The structural reaction schemes (compounds 1 through 16) appear in the upper portion of the page.

23,23-Difluoro-1α,25-dihydroxy-vitamin $D_3$ can be readily prepared from 23,23-difluoro-25-hydroxy-vitamin $D_3$ by in vitro enzymatic hydroxylation of the latter compound at carbon 1, for
40   example by incubation with a homogenate prepared from kidney tissue of vitamin D-deficient       40
chickens, as follows:
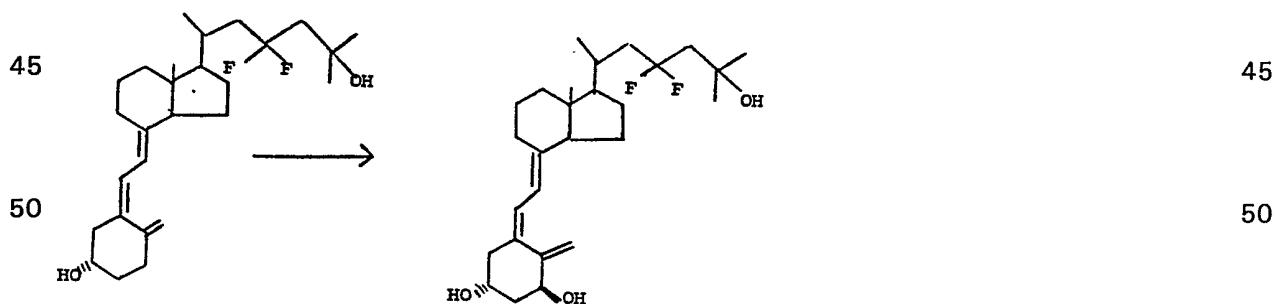


55   The acylates i.e. where one or more of the hydroxy groups in the 1 (if present), 3 an 25       55
positions is O-aliphatic acyl of 1 to 4 carbon atoms, such as O-acetyl, O-propionyl or O-butyryl,
or O-benzoyl, can readily be obtained from the free vitamins by treatment with the appropriate
acid chloride or anhydride, typically in the presence of pyridine, from ambient temperature to
reflux. For example, treatment of the free vitamin (1 mg) with acetic anhydride (0.1 ml) in
60   pyridine (0.1 ml) at ambient temperature for 1.5 hours yields the corresponding 1,3-diacetoxy       60
derivative. The corresponding 1,3,25-triacetoxy derivative can be readily obtained by utilizing
the same reagents at elevated temperatures, e.g. 75° to 90°C. Similarly, the corresponding
benzoate compound can be prepared by reaction of the free vitamin with benzoyl chloride in
pyridine at room temperature for 3 hours.
65   The present invention also provides the compounds of the formula:                               65
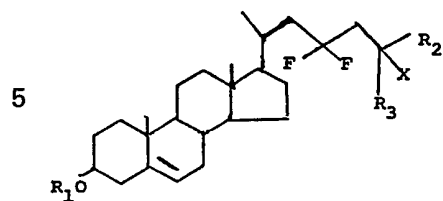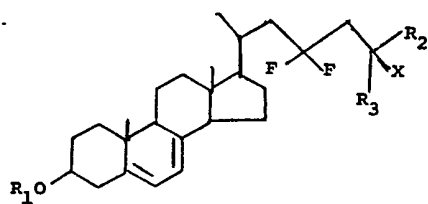
5



10

wherein R₁ represents acyl or tetrahydro-pyranyl
    X represents halogen or hydroxy and
    R₂ and R₃ independently represent $COOP_4$, $CH_2OH$ or hydrogen where R₄ is lower alkyl and of
the formula:
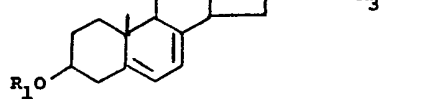
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20

25 wherein R₁ represents hydrogen or acyl,
    X represents hydroxyl or O-acyl and R₂ and R₃ are as defined above.
    The various physico-chemical characteristics of the compounds in the following synthesis were
determined as follows:
    Melting points were determined on a hot stage microscope and were uncorrected. UV spectra
30 were obtained in ethanol solution with a Shimadzu UV–200 double beam spectrophotometer. IR
spectra were taken with a JEOL IRA-1 diffraction grating infrared spectrophotometer. ¹H-NMR
spectra were recorded on a Varian EM-360L spectrometer in $CDCl_3$ unless otherwise stated, with
tetramethylsilane as an internal reference. ¹⁹F-NMR spectra were recorded on a Varian EM-360L
spectrometer in $CDCl_3$ solution, with benzotrifluoride as an internal reference (a plus means high
35 field). Mass spectra were obtained with a HITACHI double focusing mass spectrometer RMU-7L.
Column chromatography was effected with silica gal (Merck, 70–23 mesh). Preparative thin
layer chromatography was carried out on precoated plates of silica gel (Merck, silica gel 60
$F_{254}$). The "usual work-up" refers to dilution with water, extraction with an organic solvent,
washing to neutrality, drying over magnesium sulfate, filtration, and removal of the solvent
40 under reduced pressure. The following abbreviations were used: THF-tetrahydrofuran; ether-
diethyl ether; HPA-hexamethylphosphoramide; TsOH-p-toluensulfonic acid; THP-tetrahydropyra-
nyl; s-singlet; d-doublet; t-triplet; q-quartet; m-multiplet; bs-broaden singlet.

*Synthesis*
45    *6β-Methoxy-3α,5-cyclo-23,24-dinor-5α-cholan-22-al (2)*
    6β-Methoxy-3α,5-cyclo-23,24-dinor-5α-cholan-22ol (1) (2.0 g, 15.8 mmol), which was pre-
pared according to Helvetica Chimica Acta, *57*, FASC 3, pp. 764–771 (1974), was added to a
suspension of pyridinium chlorochemoate (3.8 g) and sodium acetate (1.4 g) in dichloromethane
(40 ml); this mixture was stirred at room temperature for 2.5 hr. Then, to this solution ether
50 (100 ml) was added and the resultant precipitates were filtered off and washed with ether (100
ml). The combined filtrate was successively washed with 5% $NaHCO_3$ and brine, and dried over
magnesium sulfate. After removal of the solvent *in vacuo*, the residue was applied to a column
of silica gel (300 g). Elution with n-hexane-ether (10:1) provided the aldehyde (2) (1.44 g,
73%), amorphous. H-NMR δ: 0.76 (3H, s, 18-H₃), 1.30 (3H, d, J = 6Hz, 21-H₃), 1.17 (3H, s,
55 19-H₃), 2.76 (1H, m, 6-H), 3.33 (3H, s, –O$CH_3$), 9.51 (1H, d, J = 3.5Hz, –C$HO$). MS m/z:
344 (M⁺), 329, 312.
    *6β-Methoxy-23-triethylsilyloxy-3ᵃ,5-cyclo-5ᵃ-cholan-22-en-24-oic Acid Methyl Ester (3)*
    To a solution of diisopropylamin (1.05 ml, 7.5 mmol) in THF (10 ml) n-butyllithium (6 mmol)
was added at − 78°C under argon atmosphere and this solution was stirred for 5 min. To this
60 solution methyl α-triethylsilyloxy-α-dimethylphosphonoacetate (1.56 g, 5 mmol) in THF (10 ml)
was added and this mixture was stirred at room temperature for 15 min. Then, to the resulting
solution the aldehyde (2) (491 mg, 1.43 mmol) in THF (10 ml) was added and this mixture was
stirred at room temperature for 4 hr. The usual work-up (ether for extraction) gave a crude
product, which was applied to a column of silica gel (150 g). Elution with n-hexane-ether (15:1)
65 provided the unsaturated ester (3) (615 mg, 81%), colorless oil. ¹H-NMR δ: 3.30 (3H, s,

$-OCH_3$), 3.73 (3H, s, $-CO_2CH_3$), 5.26 (1H, d, J = 10Hz, 22-H). MS m/z: 530 (M$^+$), 501, 469.

### 3β-Acetoxy-23-oxochol-5-en-24-oic Acid Methyl Ester (4)

A solution of the unsaturated ester (3) (1.53 g, 2.9 mmol) in acetic acid (7 ml) was heated at 80–90°C for 6 hr. The usual work-up (ether for extraction) gave a crude product. This and a catalytic amount of TsOH in dioxane (10 ml) and water (10 ml) were heated at 85–95°C for 7 hr. The usual work-up (ether for extraction) gave a crude product, which was applied to a column of silica gel (300 g). Elution with n-hexane-ether (15:1) provided the α-keto ester (4) (768 mg, 76%), mp 146–147°C (n-hexane).

IR$\gamma_{max}^{KBr}$cm$^{-1}$: 1720, 1240.

$^1$H-NMR δ: 0.73 (3H, s, 18-H$_3$), 0.93 (3H, d, J = 6Hz, 21-H$_3$), 1.03 (3H, s, 19-H$_3$), 2.03 (3H, s, acetyl), 3.88 (3H, s, $-CO_2CH_3$), 4.63 (1H, m, 3-H), 5.41 (1H, m, 6-H). MS m/z: 384 (M$^+$ $-CH_3COOH$), 369. Anal. Calcd for C$_{27}$H$_{40}$O$_5$: C, 72.92; H, 9.08. Found: C, 72.63; H, 913.

### 3β-Acetoxy-23,23-difluorochol-5-en-24-oic Acid Methyl Ester (5)

A mixture of α-ketoester (4) (400 mg, 0.9 mmol) and diethylaminosulfurtrifluoride (1.5 ml, 9.5 mmol) in dichloromethane (15 ml) was stirred at room temperature for 16 hr. The usual work-up (ether for extraction) gave a crude product, which was applied to a column of silica gel (100 g). Elution with n-hexane-ether (10:1) provided the difluoroester (5) (312 mg, 74%), mp 132–132.5°C (n-hexane).

IR$\gamma_{max}^{KBr}$cm$^{-1}$: 1770, 1730, 1255.

$^1$H-NMR δ: 0.70 (3H, s, 18-H$_3$), 1.0. (3H, s, 19-H$_3$), 1.10 (3H, d, J = 6Hz, 21-H$_3$), 2.03 (3H, s, acetyl), 3.87 (3H, s, $-CO_2CH_3$), 4.60 (1H, m, 3-H), 5.38 (1H, m, 6-H). $^{19}$F-NMR: + 40.3. MS m/z: 406 (M$^+$ $-CH_3COOH$). Anal. Calcd for CH$_{27}$H$_{40}$O$_4$F$_2$: C, 69.50; H, 8.64; F, 8.14. Found: C, 69.75; H, 8.75; F, 8.26.

### 23,23-Difluoro-3β-tetrahydropyranyloxychol-5-en-24-oic Acid Methyl Ester (6)

The difluoroester (5) (880 mg, 1.9 mmol) was treated with 2% KOH-MeOH (39 ml) at room temperature for 2 hr. The usual work-up (ether for exttaction) gave a crude acid. This in ether (10 ml) was treated with an ethereal solution of diazomethane until the gas evolution was ceased. This solution was concentrated under reduced pressure to leave the residue. This in dioxane (10 ml) was treated with 2,3-dihydropyran (516 µl) and TsOH (10 mg) at room temperature for 3 hr. The usual work-up (ether for extraction) gave a crude product, which was applied to a column of silica gel (200 g). Elution with n-hexane-ether (15:1) provided the THP-ester (6) (907 mg, 95%), amorphous. $^1$H-NMR δ: 0.70 (3H, s, 18-H$_3$), 1.03 (3H, s, 19-H$_3$), 1.10 (3H, d, J = 6Hz, 21-H$_3$), 3.53 (2H, m, THP), 3.86 (3H, s, $-CO_2CH_3$), 3.93 (1H, m, 3-H), 4.73 (1H, m, THP), 5.36 (1H, m, 6-H). F-NMR δ: + 40.0. MS m/z: 424 (M$^+$-DHP), 406, 391.

### 23,23-Difluorochol-5-ene-3β,24-diol 3-THP Ether (7)

To a suspension of lithium aluminium hydride (63 mg, 1.65 mmol) in ether (10 ml) the difluoroester (6) (1.40 g, 2.76 mmol) in ether (10 ml) was added and the mixture was stirred at 0°C for 10 min and then stirred at room temperature for 10 min. The usual work-up (ether for extraction) gave a crude product, which was applied to a column of silica gel (100 g). Elution with n-hexane-ether (5:1) gave the alcohol (7) (1.13 g, 86%), viscous oil. $^1$H-NMR δ: 0.73 (3H, s, 18-H$_3$), 1.03 (3H, s, 19-H$_3$), 1.13 (3H, d, J = 6Hz, 21-H$_3$), 3.33–4.10 (5H, m, 24-H$_2$, 3-H, and THP), 4.76 (1H, m, THP), 5.38 (1H, m, 6-H). $^{19}$F-NMR δ: + 43.3. MS m/z: 396 (M + -DHP), 378.

### 23,23-Difluoro-24-trifluoromethanesulfonyloxychol-5-en-3β-ol 3-THP Ether (8)

The mixture of pyridine (124 µl) and trifluoromethanesulfonic anhydride (206 µl) in dichloromethane (5 ml) was stirred at − 20°C under argon atmosphere for 5 min. To this solution the alcohol (7) (400 mg, 1.02 mmol) in dichloromethane (10 ml) was added and the mixture was stirred at room temperature for 40 min. The usual work-up (dichloromethane for extraction) gave the triflate (8) (612 mg), which was used in the next step without further purification. $^1$H-NMR δ: 0.73 (3H, s, 18-H$_3$), 1.00 (3H, s, 19-H$_3$), 1.15 (3H, d, J = 6Hz, 21-H$_3$), 3.56 (2H, m, THP), 3.85 (1H, m, 3-H), 4.50 (2H, t, J = 12Hz, 24-H$_2$), 4.70 (1H, m, THP), 5.37 (1H, m, 6-H). $^{19}$F-NMR δ: + 12.2 (3F), + 41.3 (2F).

### 23,23-Difluoro-3β-tetrahydropyranyloxycholest-5-ene-26,27-dioic Acid Diethyl Ester (9)

A mixture of potassium tert-butoxide (1.1 g, 9.6 mmol) and diethyl malonate (3.8 g, 24 mmol) in THF (25 ml) and HMPA (8 ml) was stirred at room temperature under argcn atomsphere for 1 hr. To this solution the triflate (8) (1.47 g, 2.4 mmol) in THF (20 ml) was added and the mixture was stirred at room temperature for 26 hr. The usual work-up (ether for extraction) gave a crude product, which was applied to a column of silica gel (100 g). Elution with n-hexane-

ether (5:1) provided the diester (9) (1.20 g, 81%), mp 79–80°C (ethanol).

IR$\gamma^{KBr}_{max}$cm$^{-1}$: 1750, 1740.

$^1$H-NMR $\delta$: 0.73 (3H, s, 18-H$_3$), 1.00 (3H, s, 19-H$_3$), 1.10 (3H, d, J = 6Hz, 21-H$_3$), 1.27 (6H, t, J = 7Hz, –CO$_2$CH$_2$CH$_3$), 3.46 (2H, m, THP), 3.62 (1H, t, J = 6Hz, 25-H), 3.80 (1H,m, 3-H), 4.14 (4H,q,J = JHz, –COCH$_2$CH$_3$), 4.64 (1H,m,THP), 5.30 (1H, m, 6-H). MS m/z: 538 (M$^+$-DHP), 520, 505. Anal. Calcd for C$_{36}$H$_{56}$O$_6$F$_2$: C, 69.4; H, 9.06; F, 6.10. Found: C, 69.19; H, 9.11; F, 5.85.

*25-Chloro-23,23-difluoro-3β-tetrahydropyranyloxycholest-5-ene-26,27-dioic Acid Diethyl Ester (10)*

The diester (9) (700 mg, 1.125 mmol) was treated with sodium hydride (39 mg, 1.625 mmol) in dimethoxyethane (20 ml) at room temperature under argon atmosphere for 1 hr. Then, to this solution N-chlorosuccinimide (180 mg, 1.35 mmol) was added and the mixture was stirred at room temperature for 1 hr. The usual work-up (ether for extraction) gave a crude product, which was applied to a column of silica gel (20 g). Elution with n-hexane-ether (10:1) provided the chlorodiester (10) (730 mg, 99%), glass. $^1$H-NMR: 0.72 (3H, s, 18-H$_3$), 1.02 (3H, s, 19-H$_3$), 1.10 (3H, d, J = 6Hz, 21-H$_3$), 1.30 (6H, t, J = 7.5Hz, –CO$_2$CH$_2$CH$_3$), 2.95 (2H, t, J = 15Hz, 24-H$_2$), 3.52 (2H, m, THP), 3.88 (1H, m, 3-H), 4.32 (4H, q, J = 7.5Jz, –CO$_2$CO$_2$CH$_3$), 4.72 (1H, m, THP), 5.38 (1H, m, 6-H). MS m/z: 554, 520.

*25-Chloro-23,23-difluorocholest-5-ene-3β,26,27-triol 3-THP Ether (11)*

To a solution of the chlorodiester (10) (730 mg, 1.1 mmol) in ether (15 ml) lithium aluminium hydride (48 mg) was added and the mixture was stirred at 0°C for 1 hr. and then stirred at room temperature for 2 hr. The usual work-up (ether for extraction) gave a crude product, which was applied to a column of silica gel (50 g). Elution with dichloromethane provided the chlorodiol (11) (250 mg, 39%) mp 152–153°C (n-hexane-ether). $^1$H-NMR $\delta$(CDCl$_3$-acetone d$_6$-DMSO d$_6$): 0.77 (3H, s, 18-H$_3$), 1.00 (3H, s, 19–H$_3$), 1.10 (3H, d, J = 6Hz, 21-H$_3$), 3.50-4.50 (7H, m, 3-H, 26-H$_2$, 27-H$_2$, and THP), 4.77 (3H, m 26-OH, 27-OH, and THP), 5.38 (1H, m, 6-H); $\delta$(CDCl$_3$-acetone d$_6$-DMSOd$_6$-D$_2$O): 3.60 (2H, m, THP), 3.77 (4H, s, 26-H$_2$ and 27-H$_2$), 4.77 (1H, m, THP). MS m/z: 434, 416, 404. Anal. Calcd for C$_{32}$H$_{51}$O$_4$ClF$_2$: C, 67.05; H, 8.97; Cl, 6.19; F, 6.63. Found: C, 67.08; H, 8.89; Cl, 5.99; F, 6.53.

*25ε)-25,26-Epoxy-23,23-difluorocholest-5-ene-3β, 27-diol 3-THP Ether (12)*

The chlorodiol (11) (183 mg, 0.32 mmol) was treated with sodium hydride (18 mg, 0.75 mmol) in dimethoxyethane (18 ml) at room temperature for 6 days. The usual work-up (ether for extraction) gave a crude product, which was applied to a column of silica gel (100 g). Elution with dichoromethane provided the epoxyalcohol (12) (56 mg, 32%), glass. $^1$H-NMr $\delta$: 2.92 (2H, m, 26-H$_2$), 3.67–4.16 (3H, m, 3-H and 27-H$_2$). MS m/z: 434 (M$^+$-THP OH), 416, 404, and the recovery of chlorodiol 11 (92 mg, 50%).

*23,23-Difluorocholest-5-ene-3β,25-diol 3-THP Ether (13)*

The epoxyalcohol (12) (55 mg, 0.103 mmol) was treated with methanesulfonyl chloride (20 μl) and triethylamine (30 μl) in dichloromethane (10 ml) at room temperature for 13 hr. The usual work-up (ether for extraction) gave the crude mesylate (69 mg). This mesylate was treated with lithium aluminum hydride (5 mg) in ether (10 ml) at 0°C for 1.5 hr. The usual work-up (ether for extraction) gave a crude product, which was applied to a column of silica gel (20 g). Elution with n-hexane-ether (5:2) provided the 25-ol (13) (43.3 mg, 80%), mp 148–149°C (n-hexane-cyclohexane). $^1$H-NMR $\delta$: 0.72 (3H, s, 18-H$_3$), 1.01 (3H; s,19-H$_3$, 1.10 (3H,d,J = 6Hz, 21-H), 1.35 (6H,s,26-H$_3$ and 27-H$_3$), 3.53 (2H, m, THP), 3.87 (1H, m, 3-H), 4.71 (1H, m, THP), 5.37 (1H, m, 6-H). MS m/z:420 (M$^+$-TEPOH), 405. High resolution MS Calcd for C$_{27}$H$_{42}$F$_2$O (M$^+$-THPOH): 420, 3214. Found: 420, 3208.

*23,23-Difluorocholest-5-ene-3β,25-diol 3-Acetate (14)*

The THP-ether (13) (26 mg, 0.0498 mmol) in methanol (4 ml) and THP (4 ml) was treated with a catalytic amount of TsOH at room temperature for 1 hr. The usual work-up (ethyl acetate for extraction) gave the crude diol (21.4 mg). This diol was treated with acetic anhydride (1 ml) and pyridine (1 ml) at room temperature for 14 hr. The usual work-up (ethyl acetate for extraction) gave a crude product, which was applied to a column of silica gel (5 g). Elution with benzene-ethyl acetate (10:1) provided the acetate (14) (23.0 mg, 96%); mp 168–170°C (methanol). $^1$H-NMR $\delta$: 0.82 (3H, s, 18-H$_3$), 1.02 (3H, s, 19-H$_3$), 1.07 (3H, d, J = 6Hz, 21-H$_3$), 1.35 (6H, s, 26-H$_3$ and 27-H$_3$), 2.03 (3H, s, acetyl), 4.55 (1H, m, 3-H), 5.36 (1H, m, 6-H). High resolution MS Calcd for C$_{27}$H$_{42}$F$_2$O (M$^+$-CH$_3$COOH): 420, 3202. Found: 420, 3206.

*23,23-Difluorocholesta-5,7-diene-3,25-diol (15)*

To a solution of the acetate (14) (19 mg, 0.0396 mmol) in carbontetrachloride (2 ml), N-bromosuccinimide (10 mg, 0.0571 mmol) was added and this mixture was refluxed under argon atmosphere for 20 min. After cooling to 0°C, the resulting precipitate was filtered off. The filtrate was concentrated below 40°C to leave the residue. This residue in xylene (2 ml) was

added dropwise to a refluxing solution of S-collidine (0.5) and xylene (1.5 ml) and refluxing was continued for 20 min. The usual work-up (ethyl acetate for extraction) gave the crude diene. This diene in acetone (10 ml) was treated with a catalytic amount of TsOH at room temperature under argon atmosphere in the dark for 14 hr. The usual work-up (ethyl acetate for extraction)
5  gave the crude 5,7-diene acetate. This acetate in THF (5 ml) was treated with 5% KOH-MeOH (1.0 ml) at room temperature under argon atmosphere in the dark for 30 min. The usual work-up (ethyl acetate for extraction) gave a crude product, which was submitted to preparation TLC (benzene-ethyl acetate 2:1, developed twice). The band of Rf value 0.47 was scraped off and eluted with ethyl acetate. Removal of the solvent provided the 5,7-diene (15) (3.75 mg,
10  21.7%). UV $\lambda_{max}$ mm: 294, 282, 272.

   *23,23-Difluoro-25-hydroxyvitamin $D_3$ (16)*
   A solution of the 5,7-diene (15) (3.75 mg, 8.60 $\mu$mol) in benzene (90 ml) and ethanol (40 ml) was irradiated with a medium pressure mercury lamp through a Vycor filter with ice cooling under argon atmosphere for 2.5 min. Removal of the solvent under reduced pressure gave a
15  crude product, which was submitted to preparative TLC (benzene-ethyl acetate 2:1, developed twice). The band of Rf value 0.59 was scraped off and eluted with ethyl acetate. Removal of the solvent provided the vitamin $D_3$ derivative (16) (0.96 mg, 25.6%). This was further purified by high performance liquid chromatography on a Zorbax SIL normal phase column (4.6 mm$\Phi$ × 15 cm) at a flow rate of 2 ml/min with hexane-dichloromethane (1:2) as an eluent. The retention
20  time of (16) was 7.4 min. UV$\lambda_{max}$nm: 265, $\lambda_{min}$ nm: 228. $^1$H-NMR $\delta$: 0.58 (3H, s, 18-$H_3$), 1.07 (3H, d, J = 6.1Hz, 21-$H_3$) 1.34 (6H, s, 26-$H_3$ and 27-$H_3$), 3.95 (1H, m, 3-H), 4.81 (1H, bs, 19-H), 5.04 (1H, bs, 19-H), 6.03 (1H, d, J = 10.7Hz, 7-H), 6.23 (1H, d, J = 10.7Hz, 6-H). MS m/z: 436 (M$^+$), 418, 403, 398, 380, 378, 300, 271, 265, 145, 118. High resolution MS calcd for $C_{27}H_{42}F_2O_2$: 436, 3150. Found: 436, 3155.
25     It will be apparent that other reactants may be utilized to provide equivalent substituents at various places in the compounds. For example, in compound 4 the acetoxy shown in the 3-position in the molecule could readily be some other acyloxy group where the acyl group contains from about 1 to 4 carbon atoms or tetrahydropyranyl. Also the ethyl ester shown in the 26 and 27 positions in compounds 9 and 10 can readily be another alkyl ester whre the alkyl
30  group is a lower alkyl group containing from about 1 to about 4 carbon atoms. Likewise other halogen atoms can be introduced for the chlorine in compounds 10 and 11.

   *23,23-Difluoro-1$\alpha$,25-dihydroxyvitamin $D_3$*
   One day-old leghorn chickens were fed a vitamin D-deficient diet containing 1% calcium for two weeks (Omdahl et al, Biochemistry, 10, 2935–2940 (1971)). They were then killed, their
35  kidneys were removed, and a 20% (W/V) homogenate was prepared in ice-cold 0.19M sucrose solution containing 15 mM. Tris-acetate (trihydroxymethylaminoethane acetate) (pH 7.4 at room temperature) and 1.9 mM magnesium aetate (Tanaka, Y. et al, Arch. Biochem. Biophys, 171, 521–526 (1975)). The incubation involved the addition of 9$\mu$g of 23,23-difluoro-25-hydroxyvitamin $D_3$ dissolved in 100 $\mu$l of 95% ethanol to a 125 ml Erlenmeyer flask which contained 1g
40  of kidney tissue, 0.19 M sucrose, 1.5 mM Trisacetate, 1.9 mM magnesium acetate and 25 mM succinate in a final volume of 7.5 ml. After shaking the mixture at 37°C for 2 hrs., the reaction was stopped with 15 ml of MeOG and 7 5 ml of $CH_2Cl_2$. After another 7.5 ml of $CH_2Cl_2$ was added to the organic phase, the resulting mixture was separated and evaporated under vacuum. The residue containing the desired 23,23-difluoro-1,25-dihydroxyvitamin $D_3$ was then subjected
45  to chromatographic purification by high pressure liquid chromatography using a model ALC/GPC 204 high pressure liquid chromatograph (Waters Associates, Medford, Mass.) equipped with an ultraviolet detector operating at 254 nm. The residue, dissolved in 100 $\mu$l of 10% 2-propanol in hexane, was injected onto a silica gel column (Zorbax-SIL, 0.46 × 25 cm, Dupont, Inc.) operating under a pressure of 1000 psi which produced a flow rate of 2 ml/min.
50  Using a solvent system containing 10% 2-propanol in hexane, the sample was purified twice through this column and then collected. Putative 23,23-difluoro-1,25-dihydroxyvitamin $D_3$ was further purified on a reverse-phase column (Lichrosorb RP-15, 0.46 × 25 cm, E. Merck, Darmstadt, Federal Republic of Germany) using the same high pressure liquid chromatograph operating at a pressure of 2000 psi. The product was eluted with a solvent mixture of
55  $H_2O$/MeOH (1/4) and collected. The residue was rechromatographed on the Zorbax SIL column using conditions exactly as described above.
   The identity of the product as 23,23-difluoro-1,25-dihydroxy vitamin $D_3$ was confirmed by its spectroscopic properties. The compound showed the typical vitamin D-like ultraviolet absorption with a maximum at 264 nm. The mass spectrum of the product contained a molecular ion at
60  m/e 452 as required for a 23,23-difluoro-1,25-dihydroxyvitamin $D_3$. Fragments at m/e 434 and 416 represent elimination of one and two molecules of $H_2O$. Loss of the entire side chain results in the fragment of m/e 287 which, by elimination of one and two molecules of $H_2O$, gives rise to peaks at m/e 269 and 251. In addition, the spectrum shows prominent peaks at m/e 152 and m/e 134 (elimination of one molecule of $H_2O$) which represent ring A fragments
65  and are diagnostic for 1$\alpha$,3-dihydroxy-vitamin $D_3$ compounds.

23,23-Difluoro-25-hydroxy- and 1,25-dihydroxy-vitamin $D_3$ can be obtained in crystalline form if desired by recrystallization from appropriate hydrocarbon solvents, or combinations of such solvents with alcoholic solvents, e.g. a combination of hexane and methanol, as is well-known.

5 The desired compounds can be obtained in crystalline form if desired by recrystallization from appropriate hydrocarbon solvents, or combinations of such solvents with alcoholic solvents, e.g. a combination of hexane and methanol, as is well-known in the organic chemical art.

*Biological Activity*

The biological activity of the new analogs is evidenced by appropriate in vivo assays in the
10 rat.

*23,23-Difluoro-25-hydroxyvitamin $D_3$*

Male weanling rats (Holtzman Company, Madison, Wis.) were fed a low calcium vitamin D-deficient diet (0.02% calcium, 0.3% phosphorus—J. Nutr. 100, 1045–1052 (1970)) for 3
15 weeks. They are then divided into three groups of 6 rats each. Rats in the control group were given 0.05 ml of 95% ethanol by intrajugular injection. Rats in the second group were administered, in same manner, a dose of 650 omole of 25-hydroxyvitamin $D_3$ (25-OHD$_3$) dissolved in 0.05 ml ethanol, while rats in the third group were injected with a dose of 650 omole of 23,23-difluoro-25-hydroxyvitamin $D_3$ (23,23-$F_2$-25-OHD$_3$) dissolved in 0.05 ml ethanol
20 for comparative purposes. Twenty four hours after dosing, the effect of the test compounds on intestinal calcium transport and on bone calcium mobilization measured as by the serum calcium concentration were determined by the assay methods of Martin and DeLuca (Am. J. Physiol. 216, 1351–1359 (1969)) and of Tanaka et al (Biochemistry, 14, 3293–3296 (1975)) respectively. Results are shown in Table 1.

*Table 1*

| Compound given | Intestinal Calcium transport (Ca serosal/Ca mucosal) | Serum calcium (mg/100 ml) |
|---|---|---|
| Vehicle (ethanol | $2.8 \pm 0.4^{a)*}$ | $2.8 \pm 0.1^{d)}$ |
| 25-OHD$_2$ | $5.5 \pm 0.7^{b)}$ | $3.5 \pm 0.05^{e)}$ |
| 23,23-$F_2$-25-OHD$_3$ | $5.0 \pm 1.4^{c)}$ | $3.4 \pm 0.2^{f)}$ |
| Significance of Difference: | b) & c) from a) $p<0.005$ b) from c) N.S. | e) & f) from d) $p<0.001$ e) from f) N.S. |

*Standard deviation of the mean

The foregoing data indicate that 23,23-$F_2$-25-OHD$_3$ is active in both intestine and bone and that the compound exhibits vitamin D-like activity at least as great as that exhibited by 25-
45 hydroxyvitamin $D_3$, strongly suggesting its use as a substitute for that vitamin D derivative or for vitamin D.

*23,23-Difluoro-1α,25-dihydroxyvitamin $D_3$*

Male weanling rats (Holtzman Company, Madison, Wis.) were fed the low calcium vitzamin D-
50 deficient diet for two weeks. They were divided into three groups of 6–7 rats each. Rats in the control group were given 0.05 ml of 95% ethanol by intrajugular injection. Rats in the other two groups were each administered, in the same manner, a dose, respectively, of 100 *p*moles of 1,25-dihydroxyvitamin $D_3$ (1,25-(OH)$_2$D$_3$) in 0.05 ml of ethanol or 23,23-difluoro-1α,25-dihydroxyvitamin $D_3$ (23,23-$F_2$-1,25-(OH$_2$)D$_3$ in 0.05 ml ethanol. 96 Hours after dosing the
55 effect of the compounds on intestinal calcium transport was determined by the method of Martin and DeLuca. Results are shown in the Table below.

*Table 1*

| Compound Given | Intestinal Calcium Transport (Ca serosol/Ca mucosal) (Avg. ± SEM) |
| --- | --- |
| None (vehicle only) | 2.6 ± 0.1[a] |
| 1,25-$(OH)_2D_3$ | 4.6 ± 0.4[b] |
| 23,23-$F_2$-1,25-$(OH)_2D_3$ | 4.5 ± 0.3[c] |

Significance of difference: b) & c) from a) $p < 0.001$

The foregoing data indicate that 23,25-$F_2$-1,25-$(OH)_2D_3$ is as active in promoting intestinal calcium transport as 1,25-$(OH)_2D_3$, strongly suggesting its use as a substitute for the hormonal form of the vitamin where pharmacologically increased intestinal calcium transport is indicated.

The compounds of this invention may typically be readily administered as sterile parenteral solutions by injection or intravenously or by alimentary canal in the form of oral dosages, or by suppository. Doses of from about 0.1 μg to about 10 μg per day of 23,23-$F_2$-1α,25-$(OH_2)$-$D_3$ and from 1 μg to about 25 μg per day of 23,23-$F_2$-25-OH-$D_3$ are generally effective in obtaining the physiological calcium balance responses described (which are characteristic of vitamin D-like activity) with maintenance doses of about 0.25 μg of 23,23-$F_2$-1α,25-$(OH)_2$-$D_3$ and about 5 μg of 23,23-$F_2$-25-OH-$D_3$ being suitable.

Dosage forms can be prepared by combining the compound with a non-toxic pharmaceutically acceptable carrier as is well-known in the art. Such carriers may be either solid or liquid such as corn starch, lactose, sucrose, peanut oil, olive oil, sesame oil and water. If a solid carrier is used the dosage forms include tablets, capsules, powders, troches or lozenges. If a liquid carrier is used, soft gelatin capsules, or syrup or liquid suspensions, emulsions or solutions may be the dosage form. The dosage forms may also contain adjuvants, such as preserving, stabilizing, wetting or emulsifying agents or solution promoters. They may also contain other therapeutically valuable substances.

It should be understood that although dosage ranges are given, the particular dose to be administered to a host will depend upon the specific disease state being treated, the end results being sought, as well as other factors known to those skilled in the therapeutic use of such medicinal agents.
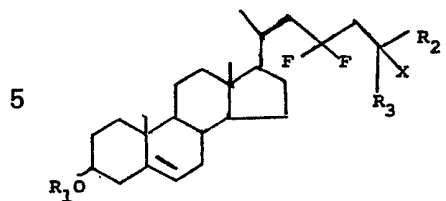
CLAIMS

1. A compound of the formula:



wherein R represents hydrogen, hydroxyl or O-aliphatic acyl or 1 to 4 carbon atoms or O-benzoyl and $R_1$ and $R_4$ individually represent hydrogen, aliphatic acyl of 1 to 4 carbon atoms or benzoyl.

2. A compound according to claim 1 wherein $R_1$ and $R_4$ represent hydrogen and R represents hydroxyl.

3. A compound according to claim 1 wherein R, $R_1$ and $R_4$ represent hydrogen.

4. A compound according to claim 2 or 3 in crystalline form.

5. 3,23-difluoro-cholesta-5,7-diene.

6. A compound having the formula:

5



10

wherein R represents acyl or tetrahydro-pyranyl
    X represents halogen or hydroxy and
    $R_2$ and $R_3$ independently represent $COOR_4$, $CH_2OH$ or hydrogen where $R_4$ is lower alkyl.
    7.   A compound according to claim 6 wherein $R_1$ is acetyl and $R_2$ and $R_3$ are $COOC_2H_5$.
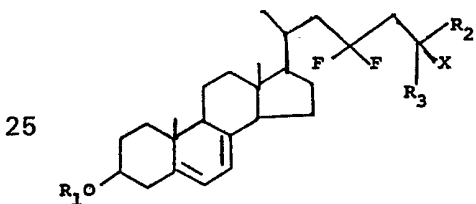15     8.   A compound according to claim 6 wherein $R_1$ is acetyl, X is chlorine and $R_2$ and $R_3$ are     15
$COOC_2H_5$.
    9.   A compound according to claim 6 wherein $R_1$ is acetyl, X is hydroxy and $R_2$ and $R_3$ are
hydrogen.
    10.   A compound having the formula:

20



25

30 wherein $R_1$ represents hydrogen, acyl or tetrahydropyranyl,                                      30
    X represents hydroxyl or O-acyl and $R_2$ and $R_3$ are as defined in claim 6.
    11.   A compound according to claim 10 wherein $R_1$ represents hydrogen and X represents
hydroxyl.
    12.   A process for preparing a compound as claimed in claim 1 wherein R is other than
35 hydrogen which comprises enzymatically hydroxylating a compound as claimed in claim 1     35
wherein R represents hydrogen.
    13.   A process for preparing a compound as claimed in claim 1 wherein R represents
hydrogen which comprises
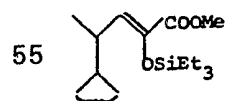    (i) converting the compound of the formula:

40



45
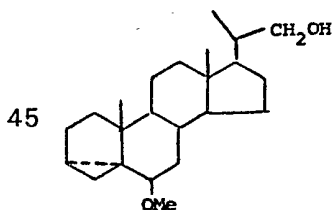
50
to the corresponding silyl ether carboxy ester of the formula:



55

by Wittig type condensation.
60    (ii) converting this ester to the compound of the formula:                                      60

5



10

where R represents an aliphatic acyl group of 1 to 4 carbon atoms or tetrahydropyranyl and converting this compound to the ester of the formula:

15



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wherein $R_4$ represents lower alkyl by malonic acid condensation, and
   (iii) converting the resulting ester to the corresponding epoxy alcohol of the formula:

25



30 and converting this epoxy alcohol to the corresponding mesylate and reacting that with lithium 30
aluminium hydride to provide the alcohol of the formula:

35



35
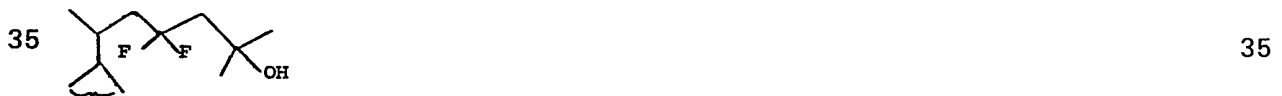
   14.  A compound as claimed in claim 1 whenever prepared by a process as claimed in claim
40 12 or 13.                                                                                              40
   15.  A pharmaceutical composition which comprises at least one compound as claimed in
any one of claims 1 to 4 and 14 and a pharmaceutically acceptable diluent or carrier.

   CLAIMS
45  $R_2$ and $R_3$ independently represent $COOR_4$, $CH_2OH$ or methyl where $R_4$ is lower alkyl.      45
   7.  A compound according to claim 6 wherein $R_1$ is acetyl and $R_2$ and $R_3$ are $COOC_2H_5$.
   8.  A compound according to claim 6 wherein $R_1$ is acetyl, X is chlorine and $R_2$ and $R_3$ are
$COOC_2H_5$.
   9.  A compound according to claim 6 wherein $R_1$ is acetyl, X is hydroxy and $R_2$ and $R_3$ are
50 methyl.                                                                                                50
   10.  A compound having the formula:

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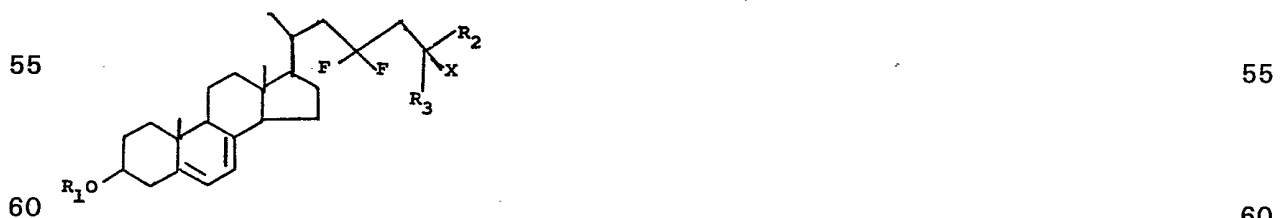
wherein $R_1$ represents hydrogen, acyl or tetrahydropyranyl,
      X represents hydroxy or O-acyl and $R_2$ and $R_3$ are as defined in claim 6.
   11.  A compound according to claim 10 wherein $R_1$ represents hydrogen and X represents
65 hydroxyl.                                                                                             65

12.  A process for preparing a compound as claimed in claim 1 wherein R is other than hydrogen which comprises enzymatically hydroxylating a compound as claimed in claim 1 wherein R represents hydrogen.

13.  A process for preparing a compound as claimed in claim 1 wherein R represents
5 hydrogen which comprises                                                                                    5
     (i) converting the compound of the formula:

10                                                                                                            10

15                                                                                                            15