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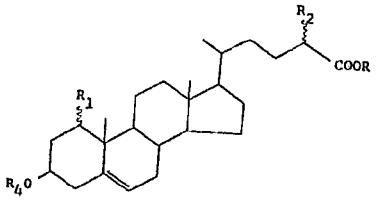
(54) **Vitamin D Derivatives**

(57) This invention relates to vitamin D intermediates of formulae



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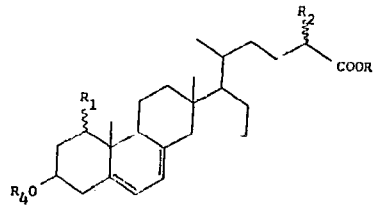

where each of R_1 and R_2 is hydrogen, hydroxy or O-acyl, with the proviso that R_1 and R_2 cannot both be hydrogen, R is alkyl and R_4 is hydrogen or acyl. They can be used, in particular, for preparing 26,27-isotopically labeled vitamin D_3 compounds of high specific activity.

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SPECIFICATION

Vitamin D Derivatives

This invention relates to vitamin D derivatives.

Vitamin D_3 is a well-known agent for the control of calcium and phosphorus homeostasis. In the normal animal or human this compound is known to stimulate intestinal absorption of calcium and phosphate, mobilizing bone mineral, and retaining calcium in the kidneys, 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 hydroxylated forms. For example, the vitamin is first hydroxylated in the liver to form 25-hydroxy vitamin D_3 and is further hydroxylated in the kidney to produce 1α,25-dihydroxy vitamin D_3 or 24,25-dihydroxy vitamin D_3. The 1α-hydroxylated form of the vitamin is generally considered to be the physiologically active or hormonal form of the vitamin.

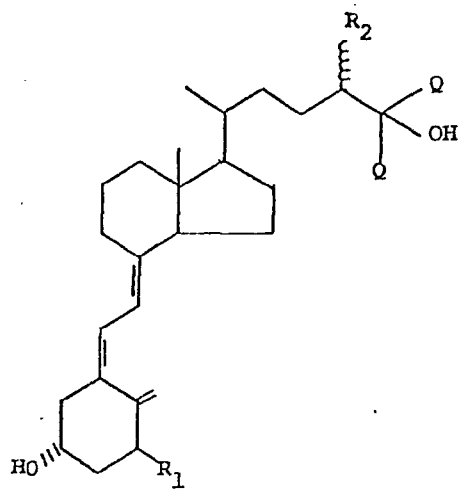
Essential to the establishment of the above facts was the availability of a variety of radiolabeled vitamin D derivatives (see, for example, Suda et al, *Anal. Biochem.* 43, 139 (1971), Neville and Deluca, *Biochemistry, 5,* 2201 (1966), Jones et al, *Biochemistry 14,* 1250 (1975), Bell and Scott, *J. Label. Compds. 9,* 339 (1973), De Luca et al, *Arch, Biochem, Biophys. 124,* 122 (1968), and Yamada et al, *Anal Biochem.* 85, 34 (1978)). However, such radiolabeled compounds were either of low specific activity (0.2 to 10.6 Ci/mmol) and/or required cumbersome syntheses.

In our Application No 80 16584 we describe and claim isotopically labeled vitamin D compounds (radiotracers) characterized by very high specific activity, specifically 26,27-radiolabeled derivatives of 25-hydroxycholecalciferol (25-OH-D_3) and 1α,25-dihydroxycholecalciferol (1α,25-$(OH)_2D_3$) and the 24-hydroxy analogs of these compounds, and an efficient and versatile method for synthesizing such compounds.

These radiolabeled vitamin D derivatives of this invention find ready application in the determination of vitamin D metabolite levels in the blood and tissues of man and animals and can therefore be of inestimable value in determining the presence or absence of disease states, such as osteomalacia, osteodystrophy and hyperparathyroidism. For example, such compounds are useful in known assays for 25-OH-D_3 (Bayard et al, *Europ. J. Clin. Invest* 2, 195 (1972), Belsey et al, *J. Clin. Endocrinol. Metab.* 33, 554 (1971), Bouilon et al, *Clin. Chem. 22,* 364 (1976), Edelstein et al, *Clin. Sci. Mol. Med. 46,* 231 (1974), Eisman et al, *Anal. Biochem. 80,* 298, (1977), Garcia-Pascual et al, *Clin. Chim. Acta 68,* 99 (1976), Haddad and Chyu, *J. Clin. Endocrinol, Metab. 33,* 992 (1971), Haddad et al, *J. Clin. Endocrinol. Metab. 43,* 86 (1976), Jones, *Clin. Chem. 24,* 287 (1978), and Preece et al, *Clin. Chem. Acta 54,* 235 (1974)), or for 1α,25-$(OH)_2D_3$ (Brumbaugh et al, *Science 183,* 1089 (1974), Eisman et al, *Arch. Biochem. Biophys. 176,* 235 (1976) and Clemens et al. *Clin. Science Mol. Med. 54,* 329 (1978)) or for multiple assays for both 25-OH-D_3 and 1,25-$(OH_2D_3$ as well as other metabolites (Caldas et al, *J. Lab. Clin. Med. 91,* 840 (1978), Hughes et al, *J. Clin. Invest. 58,* 61 (1970)), or in the continued investigations of vitamin D function, binding-protein studies, target-tissue receptor isolation and characterization, autoradiographic studies, and further investigations into vitamin D metabolism.

Vitamin D compounds labeled with stable heavy isotopes of carbon or hydrogen (^{13}C or ^{2}H) are also useful for metabolite analysis in blood and tissues especially by mass spectrometric methods as shown by Bjorkhem and Holmberg, *Clin. Chim. Acta 68,* 215 (1976)).

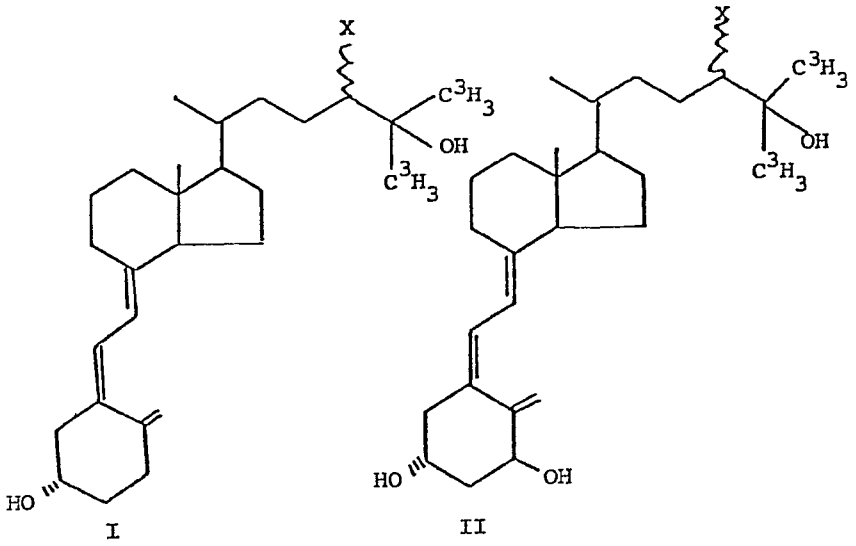
These compounds have the general structure:



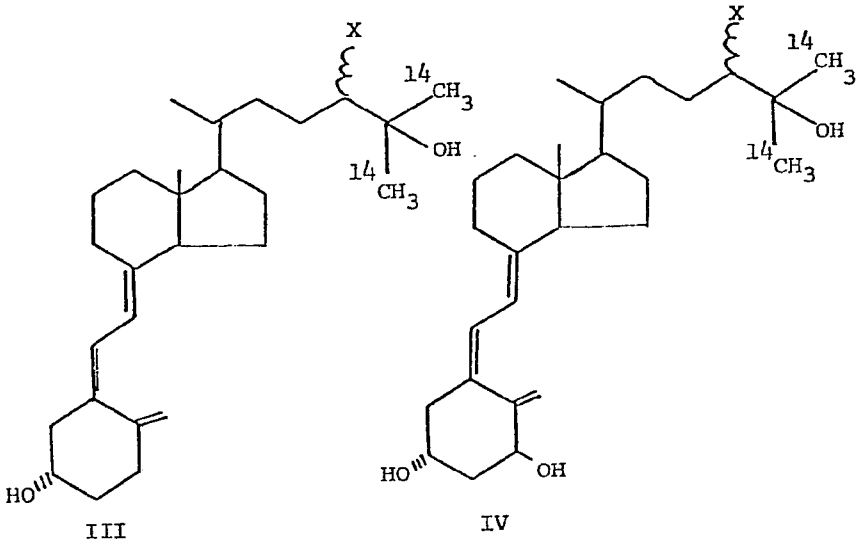
(A)

where R_1 and R_2 each is hydrogen, hydroxy, O-alkyl or O-acyl and R_2 may additionally be alkyl and each Q is a methyl group such that at least 4 of the 6 hydrogen atoms and/or at least one of the two carbon atoms, in said methyl groups, are heavy isotopes.

Preferred compounds are radiolabeled vitamin D compounds having the following general structures:



I II

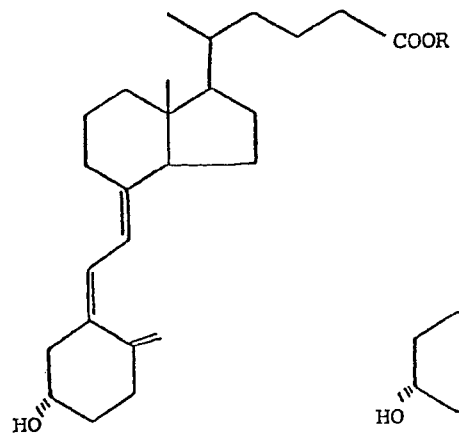


III IV

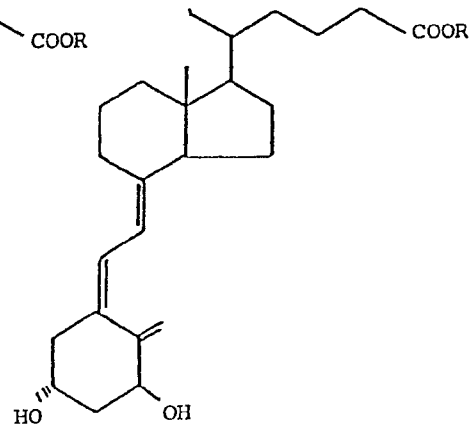
where X is hydrogen, hydroxy, alkyl, O-alkyl or O-acyl, and where the substituent X may have *R* or *S* stereo-chemical configuration.

Throughout this specification the term "alkyl" refers to a lower alkyl group, generally having from 1 to 4 carbon atoms, such as methyl, ethyl, propyl, isopropyl, butyl, sec. butyl, isobutyl, tert. butyl, and the term "acyl" implies either a lower aliphatic acyl group, having from, say, 1 to 4 carbon atoms, such as formyl, acetyl, propionyl, butyryl, or an aromatic acyl group such as benzoyl or nitrobenzoyl.

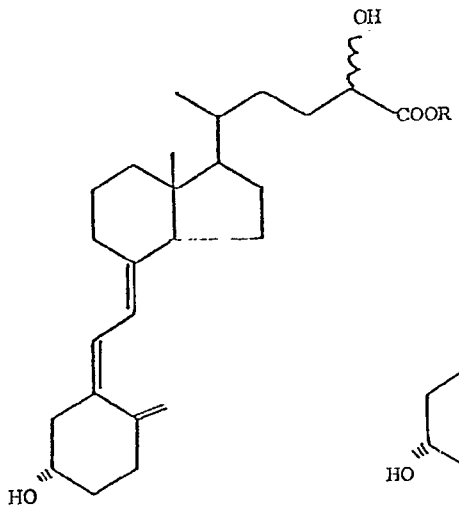
These isotopically labeled compounds are readily prepared by a process which involves treatment of an ester of 24-X-26,27-dinor-vitamin D-25-carboxylic acid (X having the designation given above, or an ester of 1α-hydroxy-24-X-26,27-dinor-vitamin D-25-carboxylic acid, with a Grignard reagent such as C^3H_3MgBr or $^{14}CH_3MgBr$, or other alkyl metal such as C^3H_3Li or $^{14}CH_3Li$, or the analogous deuterated or ^{13}C-labeled Grignard or methyl lithium reagents. Examples of such esters are V, VI, VII, and VIII below, where R represents a hydrocarbon, generally an alkyl, group, in particular a lower alkyl group, preferably having from, say, 1 to 4 carbon atoms.



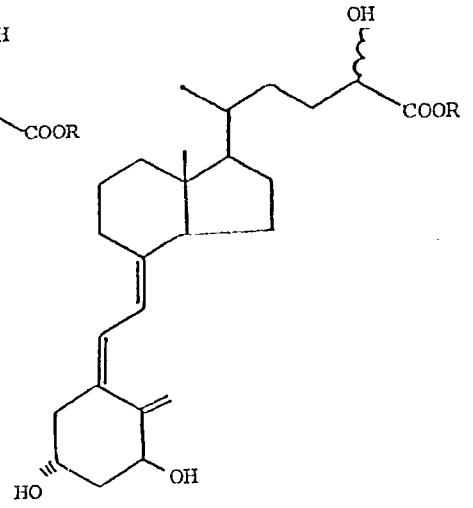
V



VI



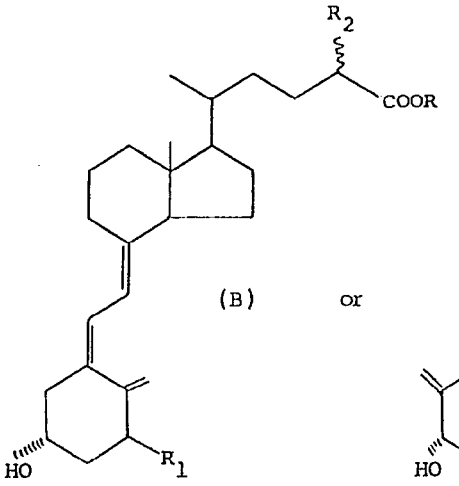
VII



VIII

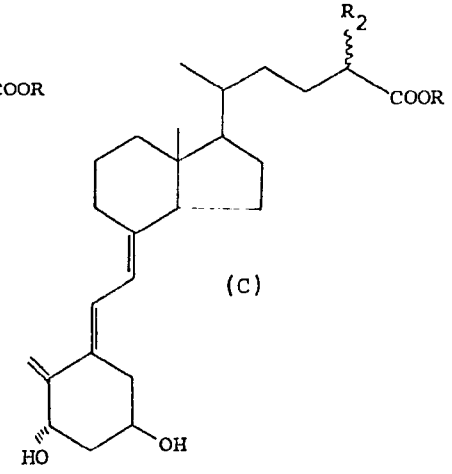
The production of isotopically labeled compounds of structures I—IV thus comprises two phases: the synthesis of appropriate unlabeled starting materials followed by the introduction of the desired isotopic label at the 26 and 27 positions.

The present invention provides the esters of the formula:



(B)

or



(C)

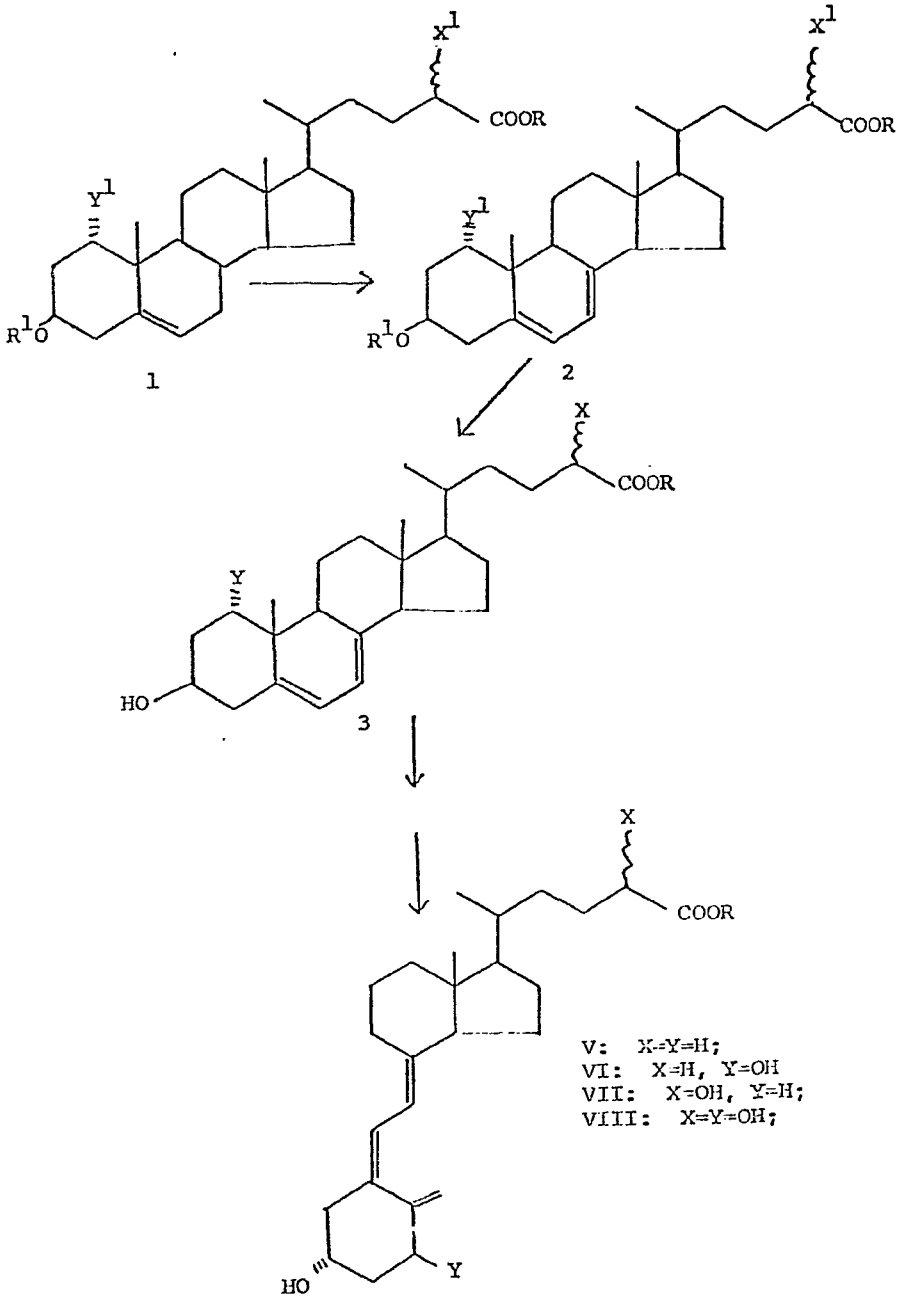
where R is lower alkyl or a protected derivative thereof and each of R_1 and R_2 is hydrogen or hydroxy with the proviso that R_1 and R_2 are not both hydrogen.

These compounds are typically prepared from starting materials such as the esters of homocholenic acid or hydroxy-substituted homocholenic acid esters according to the general scheme shown in process schematic I.

The production of isotopically labeled compounds of structures I—IV thus comprises two phases: the synthesis of appropriate unlabeled starting materials followed by the introduction of the desired isotopic label at the 26 and 27 positions.

Compounds V—VIII are typically prepared from starting materials such as the esters of homocholenic acid or hydroxy-substituted homocholenic acid esters according to the general scheme shown in process schematic I.

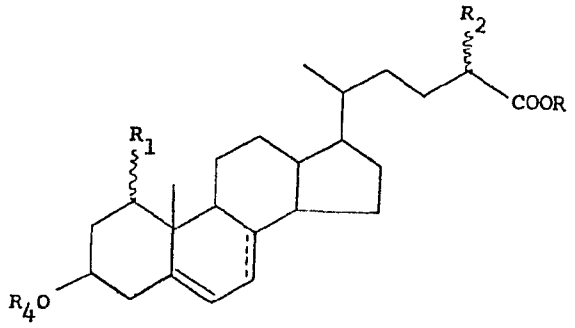
Process Schematic 1



In these formulae R^1 is an alcohol protecting group such as an acyl group (e.g. acetyl or benzoyl), X and Y each independently is hydrogen or hydroxyl, and X^1 and Y^1 each independently is hydrogen or O-acyl. Preparation of compounds V—VIII involves the conversion of the acyl-protected homocholenic acid ester (1) (itself generated by acylation of free hydroxy groups, by well-known acylation procedures, e.g. treatment with acetic anhydride/pyridine, or benzoyl chloride/pyridine) to the corresponding 5,7-diene (2) using, for example, the dehydrogenation procedure of Hunzicker and Müllner (Helv. Chim. Acta *61*, 70 (1958)). Subsequent removal of the acyl protecting groups by hydrolysis in mild alkali

yields the hydroxy ester *3*. This diene is converted to the desired vitamin D product by the well-known procedure of irradiating it with ultraviolet light, to yield the previtamin D intermediate which is then thermally isomerized to the vitamin D ester (V to VIII). These conversions are well-known in the art (see, for example, U.S. Patent Nos. 3,907,843, 3,741,996 and 3,772,361). Alternatively, the acyl-protected 5,7-diene intermediate of structure *2*, can, of course, be irradiated directly to yield the acylated previtamin D ester intermediate, which is then thermally isomerized (e.g. heating to 80°—90°C in the presence of mild base (e.g. 10% KOH/methanol)) to effect both conversion to the vitamin 5,6-*cis*-triene structure and removal of the acyl groups to produce the products of structure V—VIII.

The present invention provides the compounds of formula 1, 2 and 3 i.e. compounds of the formula



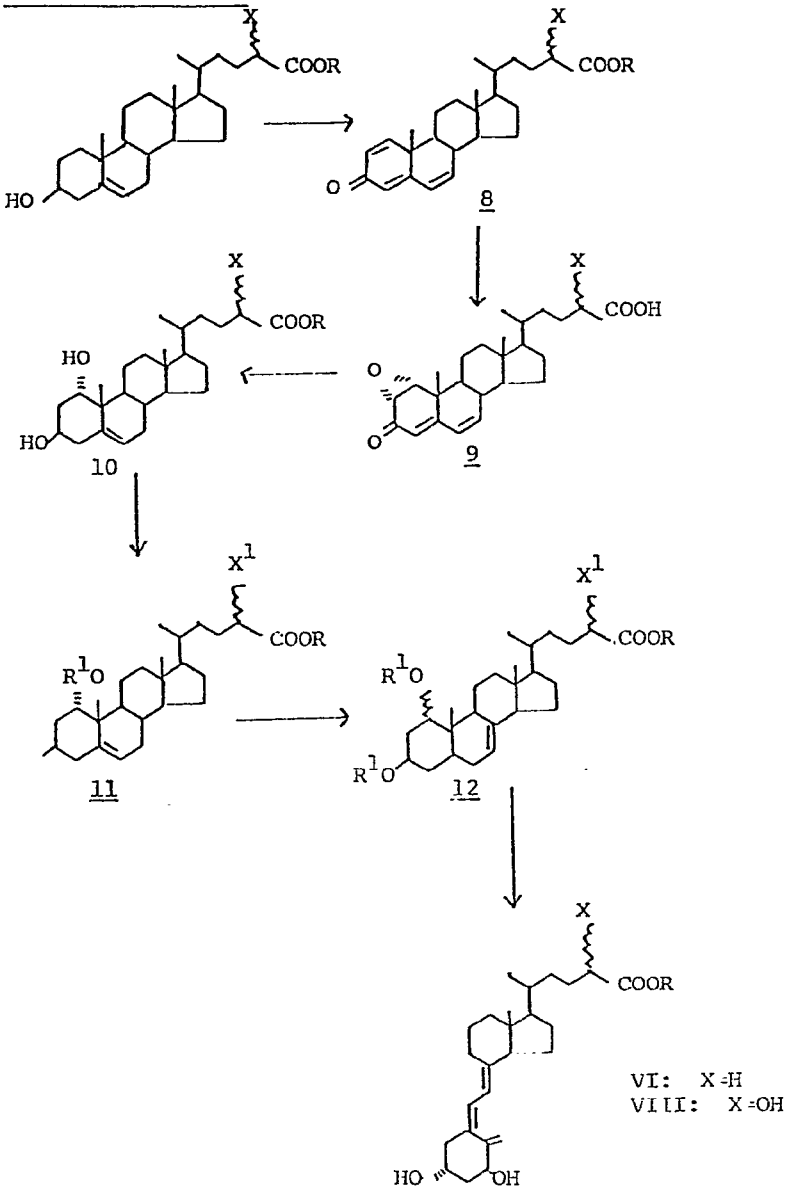
where ⁼⁼⁼ represents a single or double bond, each of R_1 and R_2 is hydrogen, hydroxy or O-acyl with the proviso that R_1 and R_2 cannot both be hydrogen, R is lower alkyl and R_4 is hydrogen or acyl.

The preparation of the vitamin D ester, V, can be accomplished by direct application of the scheme above to homocholenic acid esters or 3-O-acyl derivatives thereof (e.g. structure *1* above, where R is methyl, R^1 is benzoyl, X^1 is hydrogen) which are known compounds (see, for example, Campbell et al, Steroids *13*, 567—577 (1969)).

The vitamin D esters of structures VI—VIII can be similarly prepared.

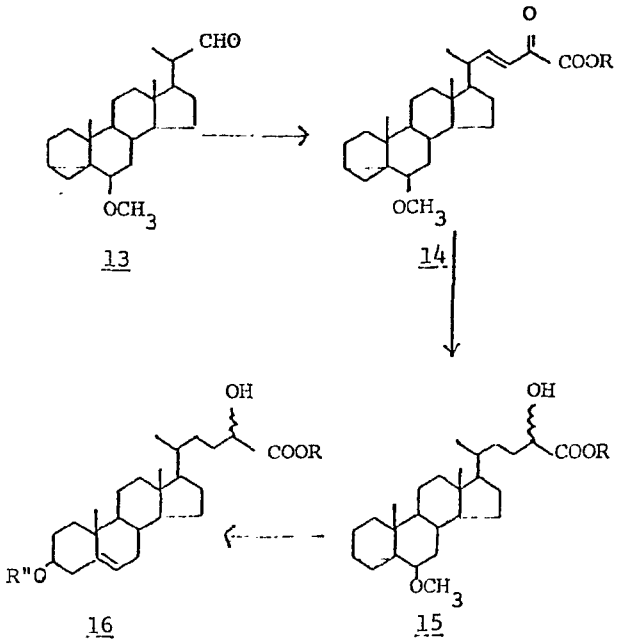
An alternative preparation for 1α-hydroxy compounds VI or VIII from homocholenic acid esters is outlined in process schematic 3. This process involves the introduction of a 1α-hydroxy function via an intermediate 1α,2α-epoxy-4,6-dien-3-one steroid (e.g. product *9*), following the general procedures of Barton et al (J Am Chem Soc *95*, 2748 (1973)). Conversion of the resulting 1α-hydroxy-5-ene compound (compounds *10* and *11*) to the 5,7-diene (e.g. *12*) and subsequent irradiation and thermal isomerization follow the conventional practices already discussed in connection with process schematic 1, to yield the desired 1α-hydroxy compounds VI or VIII.

Process Schematic 3



For the preparation of 24-hydroxyvitamin D esters of general structures VII and VIII by the processes outlined in schematics 1, 2 or 3 above, a 24-hydroxylated homocholenic acid ester is required as the ultimate precursor. Such compounds can be prepared by several methods. One convenient route to 24-hydroxy homocholenic acid esters is illustrated in process schematic 4.

Process Schematic 4

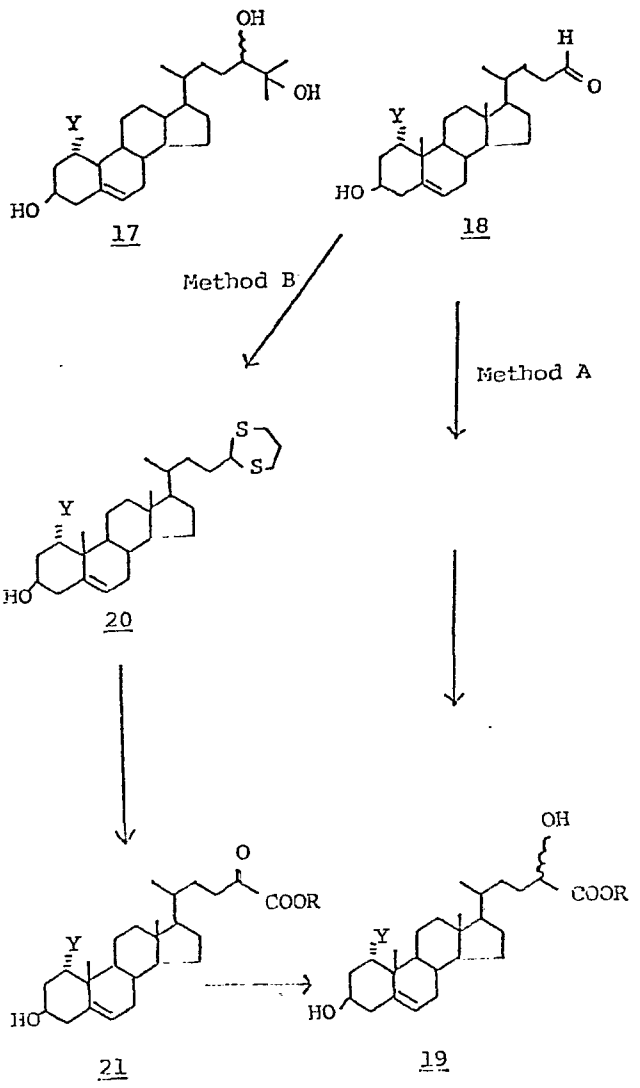


The 22-aldehyde *13*, readily available from stigmasterol by the procedure of Partridge et al (Helv Chim Acta *57*, 764 (1974)), is condensed (aldol reaction) with pyruvic acid to yield, after esterification, the unsaturated keto ester *14*, using, for example, the general procedures of Eyley and Williams (J Chem Soc Perkin Trans I p 727 and 731 (1976)). Treatment of ester *14* with $NaBH_4$ in pyridine, effects reduction of both the double bond and the keto group and yields the hydroxy ester *15* (see Eyley and Williams, supra). Solvolysis of compound *15*, using well-established conditions (see for example Partridge et al, supra) yields the 24-hydroxy-homocholenic acid ester *16* where R^{11} is hydrogen or an acyl group (e.g. acetyl) depending on the solvolysis conditions chosen.

Compound *16* can be readily converted to the 24-hydroxyvitamin D ester VII, by the process shown in Process Schematic 1. Compound *16* (with R^{11}=H) can also be converted to 1α,24-dihydroxyester VIII, by the process shown in Process Schematic 3.

C-24-Hydroxylated homocholenic ester analogs can also be conveniently prepared from 24,25-dihydroxycholesterol or from 1α,24,25-trihydroxycholesterol both of which are known compounds (Lam et al, Biochemistry *12*, 4851 (1973); Seki et al, Chem Pharm Bull (Japan) *21* 2783—2785 (1973); Ikekawa et al, Chem Pharm Bull (Japan) *23*, 695—697 (1975)). The conversion of these cholesterol derivatives to 24-hydroxyhomocholenic acid esters or to 1α,24-dihydroxyhomocholenic acid esters is shown in Process Schematic 5.

Process Schematic 5



Treatment of a methanol solution of the 24,25-dihydroxycholesterol starting material (*17* where Y= hydrogen or hydroxy) with an excess of saturated methanol solution of sodium metaperiodate at room temperature for, say, 2 hours yields the expected cleavage product, the 24-aldehyde (18). This intermediate (after protection of the hydroxy functions as the silyl ethers) can be directly alkylated with 2-lithio-2-methylmercapto-1, 3-dithiane (Method A, Process Schematic 5) and the resulting 24-hydroxy-25-orthotrithio ester adduct can be directly converted to the 24-hydroxy-homocholenic acid ester 19 by oxidative alcoholysis of the orthothio ester. (Seebach, Angew. Chem. *79*, 469—470 (1969); Seebach, Synthesis p. 17—36 (1969); Ellison et al, J. Org. Chem *37*, 2757 (1972)).

Alternatively (Method B, Process Schematic 5), the 24-aldehyde intermediate *18* can be converted to the 24-thioacetal (*20*), by treatment with 1,3-propanedithiol in chloroform solution containing BF_3-etherate as catalyst. Reaction of compound (*20*) (after temporary blocking of the hydroxy groups as ether functions, (e.g. trimethylsilyl ethers) with n-butyl lithium in tetrahydofuran solution at low temperature (e.g. —20° to —70°C) under nitrogen or argon atmosphere will generate the 24-lithio derivatives which can be directly carboethyoxylated by addition of an excess of alkyl chloroformate (e.g. ethyl chloroformate) according to the general procedures of Corey and Seebach (Angewandte Chemie, *77*, 1135—1136 (1965)) to give the 24-thioketal 25-carboxylic acid ester intermediate. Treatment of this ester with $HgO/HgCl_2$ in aqueous acetone results in hydrolysis of the thio-ketal with formation of the α-keto ester (*21*) which can be directly reduced with $NaBH_4$ in methanol to the desired 24-hydroxy-25-homocholenic ester of structure *19* (where Y is hydrogen or hydroxy).

Ester *19* (with Y=hydrogen) after suitable acylation of the hydroxy groups (e.g. acetylation or benzoylation) can then be converted to 24-hydroxyvitamin D ester VII by the method of Process

Schematic 1, whereas ester *19* (with Y=hydroxy) yields the corresponding 1α,25-dihydroxyvitamin D_3 ester VIII by the same process.

Although the present invention has been discussed in relation to vitamin D esters of structures V—VIII, in which the substituent at carbon 24 is hydrogen or hydroxy, for the preparation of 26,27-labeled vitamin D compounds, it is obvious that vitamin D ester analogs, bearing C-24 substituents such as alkyl, O-alkyl or O-acyl, are equally suitable substrates which, by the isotope-labeling process of this invention yield the corresponding 26,27-labeled-vitamin D compounds of structures I—IV in which X is alkyl, O-alkyl and hydroxy respectively.

Acylated derivatives, or other O-protected derivatives (e.g. O-silyl ethers) of vitamin D esters of structure V to VIII are, of course, suitable alternative starting materials. Such O-protected derivatives are available as intermediates of the preparation of esters V—VIII or are readily prepared from esters V—VIII, by acylation or silylation.

It should be noted also that certain intermediates of the syntheses of vitamin D esters V to VIII are also suitable substrates for the introduction of isotopic labels, and such labeled intermediates can subsequently be converted to the labeled vitamin D substances, I—IV.

The 5,7-diene intermediates of structures *2* or *3* of Process Schematic 1, can be converted to 26,27-labeled-5,7-diene-steroids which after the usual irradiation/thermal isomerisation sequence yields any of the compounds I—IV as desired. Because with such intermediates label introduction occurs at a stage further removed from the final product, such modifications of the general process are, in general, not so preferred. They do, however, represent an attractive alternative in certain circumstances. Thus the introduction of radio-label into 5,7-diene intermediates (e.g. compounds *3* or *12*) would be useful if the 26,27-labeled previtamin D compounds (or 26,27-labeled tachysterol compounds) are desired since the latter are the immediate products of irradiation of the 5,7-dienes.

In the following Examples which further illustrate the present invention ultraviolet absorbance (UV) spectra were taken in ethanol with a Beckman Model 24 recording spectrophotometer (Beckman Instruments, Fullerton, Cal); nuclear magnetic resonance (NMR) spectra were obtained in $CDCl_3$ with a Bruker WH-270 spectrometer (Bruker Instruments Inc, Wheaton, ILL); mass spectra were obtained at 100°C above ambient with an AEI (Associated Electrical Industries) MS-9 coupled to a DS-50 data system; high-pressure liquid chromatography (HPLC) was done with a Waters Associates Model ALC/GPC-204 liquid chromatograph (Waters Associates, Milford, Mass); irradiations were done in a quartz reaction vessel with a 125 watt Hanovia 8A36 lamp fitted with a Corex filter; radioactivity was measured with a Packard Model 3255 liquid scintillation counter (Packard Instrument Company, Inc., Downers Grove, Ill.). Sephadex LH-20 is a hydroxypropyl ether derivative of a polydextran marketed by Pharmacia Chemicals, Piscataway, New Jersey; Lipidex 5000 is a 50% saturated hydroxyalkoxypropylation product of Sephadex LH-20 with an average alkoxy group chain length of 15 carbons available from Packard Instruments, Inc., Downers Grove, Illinois; the semi-preparative HPLC column was 0.6x25 cm and was packed with microparticulate silica gel (5 micron particles), preparative-layer chromatography was done on 20x20 cm silica gel plates with a bed thickness of 0.75 or 0.25 cm.

The structural designations in the following Examples, by Roman or Arabic numerals, refer to the structures so identified in the preceding specification and the process schematics.

Example 1

Methyl 3β-hydroxy-25-homo-5,7-choladien-25-oate (*3*, where X=Y=H and R=methyl). Methyl 3β-hydroxy-25-homo-5-cholen-25-oate 3-benzoate, *1* (X^1=Y^1=H, R=methyl, R^1=benzoyl) (0.5 g, 0.99 mmol), sodium bicarbonate (0.55 g, 6.5 mmol), and 1.3-dibromo-5,5-dimethylhydantoin (0.16 g, 0.56 mmol) in hexane 10 ml) are heated under nitrogen for 20 min at 80°C. The reaction mixture is then cooled and filtered. The residue obtained after evaporating the solvent from the filtrate is dissolved in a solution of 2,4,6-trimethylpyridine (1 ml) in xylene (10 ml) and is heated at reflux under nitrogen for 1.5 hr. The reaction mixture is cooled, diluted with benzene, and washed successively with 1 N HCl, dilute $NaHCO_3$, and water. The solvent is removed, and the residue is dissolved in a solution of *p*-toluenesulfonic acid (0.06 g) in dioxane (12 ml), and heated at 70°C for 40 min. After cooling the reaction mixture, water and ether are added. The phases are separated, and the organic phase is washed with dilute sodium bicarbonate, water, and brine. The solvent is removed and the residue (compound *2*, where X^1=Y^1=H, R=methyl, and R^1 is benzoyl) is dissolved in a mixture of ether (3.0 ml) and 0.4 M methanolic potassium hydroxide (5 ml). After 2.5 hr at room temperature, water and ether are added, and the organic phase is separated and washed repeatedly with water. The solvent is evaporated and the residue is chromatographed on a silica gel column (1x15 cm) eluted with 25% ethyl acetate/hexane to give the product methyl 3β-hydroxy-25-homo-5,7-choladien-25-oate (*3*, X=Y=H, R=Me) (0.08 g, 0.2 mmol): UV 294, 282, 272, 264 (shoulder) nm: NMR 0.67 (s, 18-CH_3), 1.01 (d, J=6.5 Hz, 21-CH_3), 1.04 (s, 19-CH_3), 3.72 (s, —CO_2CH_3), 5.43, 5.64 (2 m, 6H, 7H).

Example 2

a) Methyl 25-homo-1,4,6-cholatrien-3-on-25-oate (*8*, R=methyl X=hydrogen). A mixture of homocholenic acid methyl ester and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (3.5 molar excess) in

dry dioxane is heated under reflux for 20 to 24 hr. The reaction mixture is cooled and filtered. The residue obtained after evaporation of the solvent is filtered through a neutral alumina column eluted with methylene chloride. The material obtained is purified by chromatography over silica gel eluted with acetone/hexane, and then used as such for the next step.

b) 1α,2α-oxido-25-homochola-4,6-dien-3-on-25-oic acid (*9*, X=H). Trienone *8* obtained as in part a in ether/methanol is hydrolyzed with aqueous potassium hydroxide to convert ester to acid. The cooled mixture is diluted with H_2O and extracted with ether to remove organic soluble material. The aqueous phase is then acidified with 1 N hydrochloric acid, and is thoroughly extracted with several portions of ether. The combined organic phases are washed with water and brine and dried over magnesium sulfate. A portion of the residue obtained after evaporation of the solvent equivalent to 3.6 mmol of steroid is dissolved in methanol so that the final concentration of steroid is from 0.05 to 0.1 M. To this solution is added 10% sodium hydroxide (0.45 ml) and 30% H_2O_2 (2.5 ml), and the resulting mixture is allowed to stand at room temperature for 16 hr. The reaction mixture is acidified with methanolic hydrochloric acid; the solvent is concentrated; and the precipitate representing the desired 1α,2α-epoxy derivative *9*(X=H) is collected and used for the next step.

c) *Methyl 1α,3β-dihydroxy-25-homo-5-cholen-25-oate 1,3-diacetate* (*11*, R=Me, R^2=acetyl, X=hydrogen). Epoxydienone *9* (X=H) (0.25 g) dissolved in dry tetrahydrofuran at −33°C is added in one portion to a solution of sodium (20-fold excess) in liquid ammonia for a final steroid concentration of 0.015 M. After 10 min ammonium chloride (2.5 g) is added in small portions during 1 hr. The ammonia is allowed to evaporate and 1 N hydrochloric acid and ether are cautiously added to the reaction mixture. The phases are separated and the aqueous phase is repeateldy extracted with ether. The combined organic phases are washed with water and brine and dried over magnesium sulfate. The solution is concentrated and treated with excess diazomethane in ether. The solvent is evaporated and the residue is purified by silica gel chromatography eluted with acetone/hexane to give the 1α-hydroxy-intermediate *10* (X=H, R=methyl).

This 1α-hydroxylated compound is heated (60°) with acetic anhydride/pyridine (1:1) until acetylation is complete (ca. 3 hr, conveniently checked by TLC). The mixture is poured onto ice. Ether is added and potassium carbonate is added until effervescence ceases. The phases are separated and the organic phase is washed with 1 N hydrochloric acid, dilute sodium bicarbonate, water, and brine and dried over sodium sulfate. Evaporation of the solvent provides ester *11* (R=methyl, R^1=acetyl, X^1=H).

d) Methyl 1α,3β-dihydroxy-25-homo-5,7-choladien-25-oate 1,3-diacetate (*12*, R=methyl, R^1=acetyl, X^1=hydrogen). Compound *11* (X=H) (1.2 mmol), sodium bicarbonate (500 mg), and 1,3-dibromo-5,5-dimethylhydantoin (0.84 mmol) are heated at 75° under nitrogen for 20 min. The reaction mixture is cooled, filtered, and the solvent is removed. The residue is dissolved in xylene (15 ml) and collidine (3.5 ml) and heated at reflux under nitrogen for 1.5 hr. Benzene is added and the organic phase is washed with 1 N hydrochloric acid, dilute sodium bicarbonate, brine, and dried over sodium sulfate. The residue obtained after evaporation of the solvent is dissolved in dioxane (14 ml) to which *p*-toluenesulfonic acid (65 mg) is added, and is heated at 70°C under nitrogen for 35 min. Ether is added, and the organic phase is washed with dilute sodium bicarbonate water, saturated sodium chloride, and dried over sodium sulfate. The residue obtained after evaporation of the solvent is purified by silica gel thin layer chromatography eluted twice with 10% acetone/hexane, to yield product *12* (R=Me, R^1=acetyl, X^1=H).

Example 3

a) Aldol condensation to keto-ester (*14* (R=methyl). To 20 ml of di-isopropyl amine in 14 ml of dry THF, cooled to 0°C, are added 8.9 ml of n-butyl lithium (1.6 M in hexane); 0.5 ml of pyruvic acid in THF (1.0 ml) is then added dropwise and the solution is stirred at 0°C for 1 hr. The mixture is cooled to −78°C and 2.0 g of the 22-aldehyde (*13*) dissolved in 10 ml dry THF is added. After 1 hr the solution is allowed to warm to 0°C and then stirred for a further 3 hr. The reaction is quenched with 1 ml glacial acetic acid, diluted with ether and H_2O and the layers separated. The organic phase is washed with 1 N HCl, water and saturated NaCl (aq). The organic layer is dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue is taken up in ether and treated with etheral diazomethane to convert the acid to its methyl ester. The resulting oil is chromatographed over silica gel (100—200 mesh) with 5% ethyl acetate in hexane as eluant to give keto ester *14* (R=methyl).

b) Reduction of compound 14 to α-hydroxy ester 15 (R=methyl). To a solution of 1 g of the enone (*14*) in 30 ml of dry pyridine, 178 mg of $NaBH_4$ is added and the mixture stirred at room temperature for about 48 hr. The mixture is poured into H_2O and extracted with ether. The combined extracts are washed with 1 N HCl, 1 N $NaHCO_3$, saturated NaCl (aq) and dried over anhydrous Na_2SO_4. The organic layer is concentrated *in vacuo* to an oil, chromatographed over silica gel (100—200 mesh) using 5% ethyl acetate in hexane as eluant and 800 mg of α-hydroxy-ester *15* is obtained.

c) Solvolysis of 15 to 24-hydroxy homocholenic ester 16 (R=methyl, R^1=acetyl). A solution of 648 mg of the compound *15* in 30 ml of glacial acetic acid is warmed to 70°C for 16 hr. The solution is cooled and neutralized with 10% NaOH (aq) (iced). The solution is then extracted with ether. The combined extracts is washed with 1 N NCL 1N $NaHCO_3$, saturated NaCl (aq), dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The crude solid is chromatographed over silica gel (100—200 mesh) with 5% ethyl acetate/hexane as eluant, to give methyl 3β,24-dihydroxy-25-homo-5-cholen-25-oate 3-acetate (*16*, where R=methyl, R^1=acetyl) in *ca.* 80% yield.

Example 4

a) 3β-hydroxychol-5-en-24-al (*18*, Y=H). To a methanol (10 ml) solution of 1.5 g of 24,25-dihydroxycholesterol (*17*, Y=H) (prepared as described by Lam et al, Biochemistry *12*, 4851 (1973)) is added a saturated methanol solution of sodium metaperiodate. After 2 hours at room temperature, the reaction mixture is diluted with water and extracted with chloroform. The organic extracts are washed with water and brine, dried over potassium carbonate and evaporated to yield *ca.* 1 g of the desired 24-aldehyde (*18*, Y=H).

The 24-aldehyde is converted to its 3-O-silyl ether derivative in the usual fashion: reaction of a THF/pyridine (1:1) solution of the aldehyde with hexamethyldisilazane (1.5 ml) and trimethylchlorosilane (1 ml) at room temperature for 0.5 hr. gives the 3-O-trimethylsilyl product, which dissolved in THF is rigorously dried with K_2CO_3 and then used as a solution in THF for the next step.

b) Ethyl 3β,24-dihydroxy-25-homochol-5-en-25-oate (*19*, Y=H, R=Et). To a tetrahydrofuran solution of the lithium salt of 2-methylmercapto-1,3-dithiane (prepared as described by Seebach, Angew. Chem. *79*, 469—470 (1967); Ellison et al, J. Org. Chem. *37*, 2757 (1972)) maintained at —78° under an atmosphere of argon is added dropwise a THF-solution of the 24-aldehyde 3-O-silyl ether (prepared as in a) above) (ca. 0.8 mole of aldehyde per mole of lithium orthothio-ester reagent). After addition of the aldehyde the mixture is allowed to warm to room temperature and then worked up by addition of water and extraction of the product into $CHCl_3$. The chloroform solution is washed with dilute KOH solution and then water and dried over Na_2SO_4. The 24-hydroxy-25-orthothioester intermediate obtained in this manner is then added to a mixture of $HgCl_2$ (3.5 equiv.) and HgO (2.0 equiv.) in 95% ethanol to affect ethanolysis of the orthothioester to the 25-carboxylic ester (Ellison el al supra). The temperature is raised to 50° and the mixture is stirred overnight under argon. Precipitated solids are filtered and washed with CH_2Cl_2 and the filtrate is diluted with water extracted with CH_2Cl_2, washed with ammonium chloride, sodium bicarbonate, followed by water and brine, drying of the solution (Na_2SO_4) and evaporation of solvent gives the desired hydrolysis product ethyl 3β-24-dihydroxy-25-homochol-5-en-25-oate.

Example 5

a) Preparation of 24-thioacetal (*20*, Y=H). To a chloroform solution of the 24-aldehyde *18* (Y=H) (1 g) prepared as described in Example 7 a) is added an excess of 1,3-dithiopropane (2 ml) and the mixture is stirred at room temperature. Then after ca. 30 min, the mixture is cooled in an ice bath and 1 ml of BF_3 etherate is added, and the resulting solution is allowed to stand overnight in the cold. The cold solution is then washed with 5% aqueous KOH solution, followed by washing with H_2O and drying over K_2CO_3. Evaporation of the solvent then yields the desired 24,24-dithioacetal derivative *20*, (Y=H). This material is converted to its 3-O-trimethylsilyl ethyl derivative as described in Example 7 a).

b) Ethyl 3β-hydroxy-24-oxo-25-homochol-5-en-25-oate (*21*, Y=H, R=ethyl). A solution (0.1 M) of the 3-O-silylated-thioacetal derivative obtained as described above in dry tetrahydrofuran maintained at —25° under an atmosphere of argon is treated with 1.5 equivalents of n-butyllithium in hexane (1.5 M solution) and the mixture is stirred for 3.5 hr to yield the desired 24-lithio derivative. The temperature is lowered to —70° and a large excess of ethyl chloroformate (ClCOOEt) is added slowly as a solution in dry THF and the reaction is stirred for 7 hr. It is then allowed to warm to room temperature, water is added, and the mixture is extracted with $CHCl_3$. The $CHCl_3$ extracts are washed with dilute KOH and H_2O and then dried and evaporated to yield the 25-carboethoxy-24,24-thioketal derivative. The thioketal is directly hydrolyzed by adding an acetonitrile of solution of the above product (1 mmol) to a solution of excess N-chloro-succinimide (5 mmol) and $AgNO_3$ (4 mmol) in acetonitrile/H_2O (5:1). After 1 hr at 50°, saturated NaCl solution is added, the precipitate is removed by filtration and the filtrate after dilution with CH_2Cl_2 is washed with sodium bisulfite, and sodium bicarbonate solutions followed by water and brine. After drying and evaporation of solvent, the desired 24-keto-25-ethyl ester product (*21*, Y=H, R=Et) is obtained.

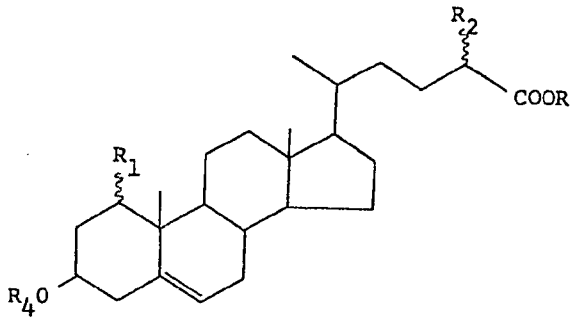
c) Ethyl 3β,24-dihydroxy-25-homochol-5-en-25-oate (*19*, Y=H, R=Et). A methanol solution (10 ml) of the 24-keto product (100 mg) obained as in b) above is treated with excess $NaBH_4$ at room temperature for 45 min. Addition of dilute aqueous acetic acid, and extraction with methylene chloride yields after chromatography on silica gel column developed with ethylacetate/hexane, 85 mg, the desired 24-hydroxy-25-ester product (*19*, Y=H, R=Et).

Example 6

A methanol solution of 1α, 24,25-trihydroxycholesterol is subjected to periodate cleavage exactly as in Example 7a) to obtain the 1α-hydroxy-24-aldehyde intermediate (*18*, Y=OH). This aldehyde is converted to its 1,3-ditrimethylsilyl ether using the conditions given in Example 7a). The silyl ether derivative is then treated with 2-lithio-2-methylmercapto-1,3-dithiane exactly as described in Example 7b) and the resulting 24-hydroxy-25-ortho-thioester is subjected to ethanolysis using the conditions given in Example 7b), to obtain ethyl 1α, 3β,24-trihydroxy-25-homochol-5-en-25-oate (*19*, R=ethyl, Y=OH). Acetylation of this triol ester with excess.

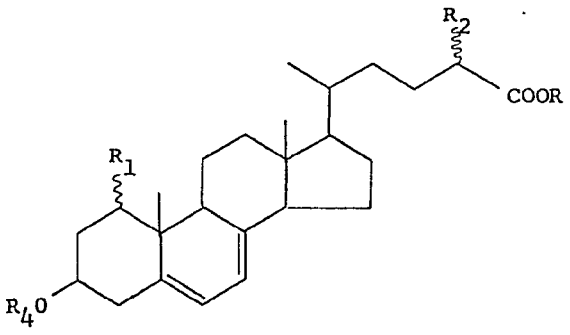
Claims

1. A compound of the structure



where each of R_1 and R_2 is hydrogen, hydroxy, or O-acyl with the proviso that R_1 and R_2 cannot both be hydrogen, R is lower alkyl and R_4 is hydrogen or acyl.

2. A compound of the structure



where R, R_1, R_2 and R_4 are as defined in claim 1.

3. A compound according to claim 1 or 2 wherein R_1 is hydroxy, each of R_2 and R_4 is hydrogen, and R_3 is methyl or ethyl or an acylate thereof.

4. A compound according to claim 1 or 2 wherein both R_1 and R_4 are hydrogen, R_2 is hydroxy, and R_3 is methyl or ethyl or an acylate thereof.

5. A compound according to claim 1 or 2 wherein both R_1 and R_2 are hydroxy, R_3 is methyl or ethyl and R_4 is hydrogen or an acylate thereof.

6. A compound according to claim 1 or 2 specifically identified herein.

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