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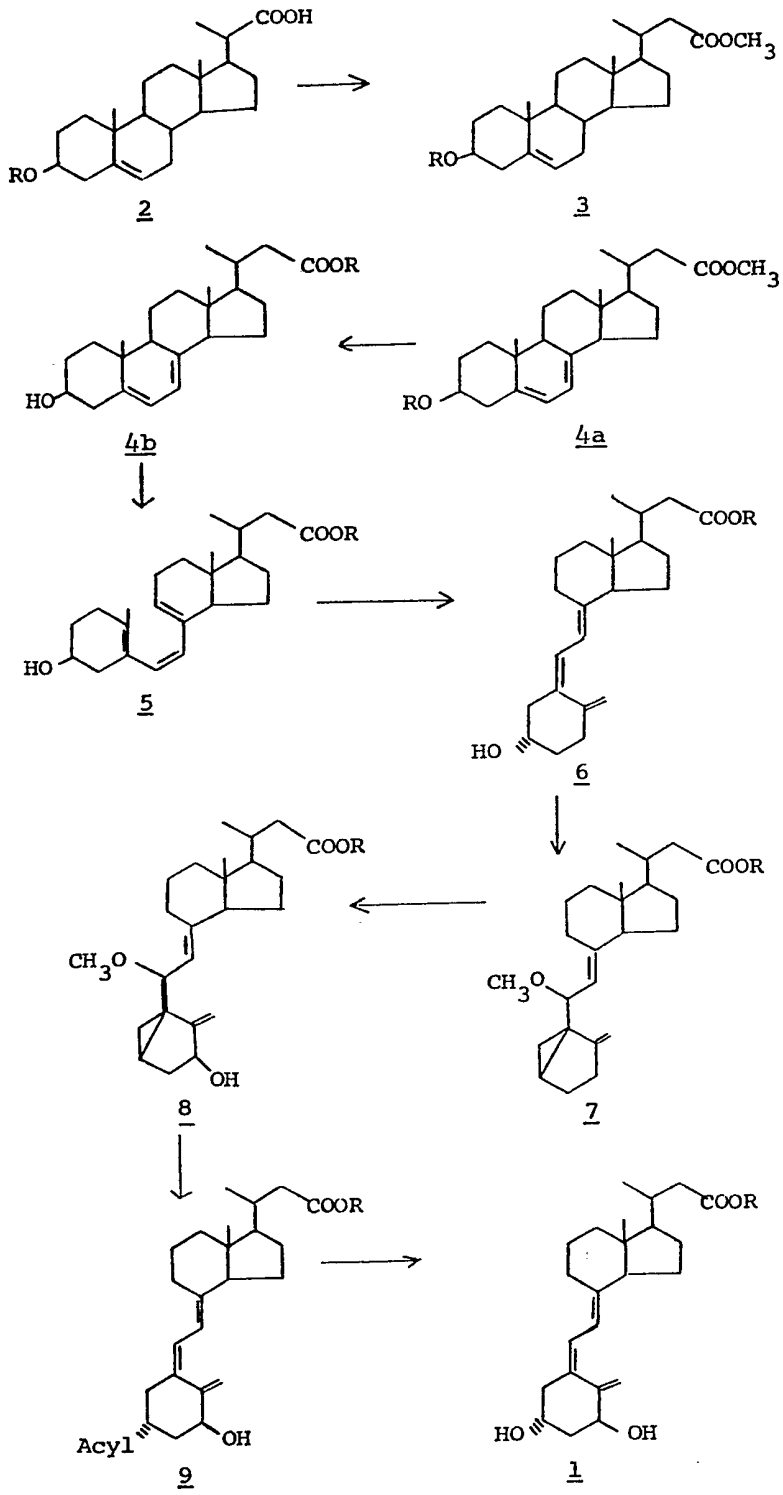
(54) **Synthesis of Calcitroic Acid and 5,6-trans-calcitroic Acid**

(57) Calcitroic acid (1; R=H) and its 5,6-*trans*-isomer (not shown) are synthesised via novel intermediates as follows (R=H or protecting group):



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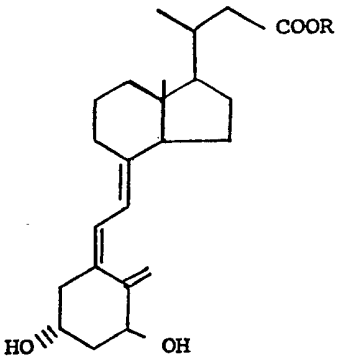
SPECIFICATION

Processes for Preparing Calcitroic Acid and Esters Thereof

This invention relates to biologically active vitamin D compounds, in particular esters of calcitroic acid and to methods for their preparation.

Calcitroic acid is an *in vivo* metabolite of 1α,25-dihydroxyvitamin D_3 (1α,25-$(OH)_2D_3$). This latter compound is the most potent known metabolite in the vitamin D series for the regulation of calcium and phosphate homeostasis (see DeLuca and Schnoes, *Ann. Rev. Biochem. 45,* 631, 1976). Recently it was discovered that rats rapidly metabolize 1α,25-$(OH)_2D_3$ to a compound having an acid function in the steroid side chain. This novel metabolite was isolated as the corresponding methyl ester and identified as methyl 1α,3β-dihydroxy-24-nor-9,10-secochola-5,7,10(19)-trien-23-oate depicted by structure *1* (R=CH_3) for which the trivial name methyl calcitroate has been proposed: (Esvelt et al, Biochemistry 18, 3977, 1979). The *in vivo* metabolite is therefore the corresponding free acid, namely calcitroic acid as shown by structure 1(R=H) below. (An alternative trivial name for this compound is 1α-hydroxycalcitroic acid).



1: R=H calcitroic acid.
R=CH_3 methyl calcitroate.

A process for the chemical synthesis of calcitroic acid and of esters thereof (where R is hydrogen, alkyl or benzyl) has now been developed. The general process is outlined in Process Schematic 1.

Arabic numerals identifying specific intermediates and products refer to this Process Schematic.

In this specification the term "alkyl" denotes a lower alkyl radical of from 1 to, say, 5 carbons which may be straight chain or branched (e.g. methyl, ethyl, butyl, isopropyl, isobutyl) and the term "acyl" denotes an aliphatic acyl group of from 1 to, say, 5 carbons, such as formyl, acetyl, propionyl, butyryl, or an aromatic acyl group such as benzoyl or substituted benzoyl, e.g. toluoyl, nitrobenzolyl or halobenzoyl.

The process uses commercially available acid *2* (where R is a hydroxy-protecting group, e.g. acyl, tetrahydropyranyl, methoxymethyl, alkylsilyl) as starting material which, by an Arndt-Eistert homologation sequency using the general method of Ryer and Gebert (*J. Am. Chem. Soc. 74,* 43, 1959) gives the side chain desired in the final product. Use of methanol in the silver oxide-catalyzed Wolff rearrangement gives the methyl ester *3* (e.g. R=acyl) in about 60% yield after recrystallization. Ester *3* is converted to diene *4a*, by allylic bromination and dehydrobromination according to well-known procedures (Hunziker and Mullner, *Helv. Chim. Acta 41,* 70, 1958; Napoli et al *Arch. Biochem. Biophys. 197,* 119, 1979). Removal of the hydroxy-protecting group (e.g. by mild acid or base hydrolysis, depending on the protecting group present, and according to well-known procedures) yields the hydroxy ester *4b* (R=CH_3). More vigorous basic hydrolysis (e.g. 10% NaOH/dil. methanol, 60—100°C, 1—3 hr) also cleaves the ester function to give the acid (*4b*, R=H) and from this acid other alkyl esters are readily prepared, if desired. For example, esterification of the acid or the acid halide with any desired low molecular weight alcohol according to procedures well known in the art, yields other alkyl or aryl esters suitable for subsequent interconversions (e.g. *4b* where R is lower alkyl such as methyl, ethyl, propyl, butyl, or a benzyl group). Irradiation of ester *4b* (R=alkyl) with ultraviolet light yields the previtamin ester *5* (R=alkyl), which is isomerized by heating (60—80°C) in an alcohol or benzene solvent to the vitamin ester *6* (R=alkyl). Alternatively, ester *6* (R=alkyl) can, of course, also be obtained by subjecting the 3-O-protected derivatives of the 5,7-diene intermediate of general structure *4b* to the irradiation/thermal isomerization sequence described above, to give the 3-O-protected derivative of ester *6*. Subsequent removal of the protecting group yields hydroxy ester *6*. The free acid (compound *6*, R=H) can readily be obtained from the ester *6* (R=alkyl) by vigorous base hydrolysis (e.g. 10% NaOH, e.g. alcohol, 1—3 hr, 60—90°C) and from the acid the corresponding 3-O-protected derivatives are readily prepared, if desired, by standard procedures. The 3-O-acylates are preferred derivatives.

Introduction of the 1α-hydroxy function can be achieved by the method of Paaren et al (*Proc. Nat. Acad. Sci.* USA *75,* 2080, 1978). Ester *6* (R=alkyl) is converted to the cyclovitamin ester *7*, by a two-

step process involving tosylation of *6* to the 3-O-tosyl derivative followed by bicarbonate buffer methanolysis of the tosylate (Paaren et al, supra; Sheves und Mazur, *J. Am. Chem. Soc. 97,* 6249, 1975). Subsequent allylic oxidation of *7* (R=alkyl) with selenium dioxide and t-butyl hydroperoxide in a halocarbon solvent yields the desired 1α-hydroxy cyclovitamin ester *8* (R=alkyl). Vigorous base hydrolysis of either *7* or *8* (where R=alkyl) using conditions as described for ester *6* provides the corresponding free acids. The 1-hydroxy group of ester *8* (R=alkyl) can be protected (e.g. by acyl, tetrahydropyranyl, methoxy-methyl, alkylsilyl groups) using standard derivatization conditions, the 1-O-acylates (e.g. acetyl, formyl, benzoyl) being preferred derivatives. The 1-O-acylates can be subjected to acid catalyzed solvolysis (e.g. using formic, acetic or paratoluene sulfonic acid) as described by Paaren et al (supra.) to yield either 1,4-di-O-acyl-, or 1-O-acyl-3-hydroxy-vitamin D esters, depending upon the solvolysis conditions chosen, as a mixture of the 5,6-*cis* and 5,6-*trans*-isomers. For example, solvolysis in carboxylic acids (e.g. formic or acetic acid) leads to 1,3-di-O-acylates where the C-3-acyl group corresponds to the acyl moiety of the acid used, whereas solvolysis with sulfonic acids in aqueous media yields 1-O-acyl-3-hydroxy-products, as fully described by Paaren et al. After separation of the 5,6-*cis* and -*trans*- mixture, the 5,6-*cis* product is hydrolyzed in mild alkali to yield the desired calcitroic acid ester (compound *1*, R=alkyl).

A preferred procedure consists of the direct solvolysis of unprotected hydroxy ester *8* with a low molecular weight carboxylic acid (acetic acid being a preferred acid) to give 1α-hydroxy vitamin D ester 3-O-acyl (*9*, R=alkyl) and the corresponding 5,6-*trans*-isomer (1α-hydroxy-5,6-*trans* vitamin D ester 3-O-acyl) in a mixture ratio of ca. 3:1. These isomers are readily separated by chromatography (e.g. high pressure liquid chromatography, thin layer chromatography). Mild basic hydrolysis of *9* (R=alkyl) then yields the desired calcitroic acid ester *1* (R=alkyl). Vigorous alkaline hydrolysis of the ester (e.g. 10% NaOH, dilute alcohol, 60—100°C, 1—3 hr) provides the corresponding acid, calcitroic acid, compound *1* (R=H), in pure form.

Acylated derivatives of these calcitroic acid esters (e.g. compounds of structure 1, R=alkyl) with 1-O-acyl or 1,3-di-O-acyl groups can be obtained by the use of alternative solvolysis conditions as described above or by acylation of intermediate *9* to the corresponding 1,3-di-O-acyl derivative (where the acyl groups may be the same or different) using well known acylation procedures. Similarly, the 1,3-di-O-acyl derivatives of calcitroic acid (*1*, R=H) can readily be obtained by direct acylation of this acid. If calcitroic acid or its esters are desired with other 1,3-O-protecting groups, such groups may be conveniently selected from tetrahydropyranyl, methoxy-methyl or alkylsilyl.

The 5,6-*trans* intermediates obtained after solvolysis of the cyclovitamin intermediate (e.g. compound *9* with 5,6-*trans* double bond configuration) can be converted by hydrolysis of the acyl group to 5,6-*trans* calcitroic acid esters and further hydrolysis of the ester yields, 5,6-*trans* calcitroic acid. These hydrolysis steps can be conducted exactly as described for the 5,6-*cis* compounds. Any of these 5,6-*trans* intermediates or products are of course convertible to the corresponding natural 5,6-*cis* compound, by the well-known photochemical isomerization process of Inhoffen et al, Chem. Ber. *90,* 2544 (1957).

For the following specific Examples NMR were taken in $CDCl_3$ with a Bruker WH-270 FT spectrometer. Mass spectra were obtained at 110—112°C above ambient at 70 eV with an AEI MS-9 spectrometer coupled to a DS-60 data system. Ultraviolet (UV) absorption spectra were recorded in methanol with a Beckman Model 24 recording spectrophotometer. HPLC was performed on a Waters Associates Model ALC/GPC 204 using Zorbax-SIL (Dupont) 6.4 mm×25 cm or 4.8 mm×25 cm columns monitoring at 313 nm for preparative samples or 254 nm for analytical samples. Liquid scintillation counting of radioactivity was determined with a Packard model 3255 using a scintillation solution consisting of 0.4% 2,5-diphenyl oxazole and 0.03% dimethyl-1,4-bis(2(5-phenyloxazolyl)) benzene in toluene. All reactions are preferably conducted under an inert atmosphere.

Example 1

Methyl 3β-Acetoxy-24-nor-5-cholen-23-oate (3, R=Acetyl)

A solution of 5 g (12.3 nm) of *2* (R=acetyl) in 10 ml of freshly distilled thionyl chloride was stirred at 25°C for 90 min. Excess thionyl chloride was removed by distillation following 5 additions of 20 ml benzene. The brown residue was suspended in 50 ml benzene and slowly added to a 130 ml ether solution containing approximately 2 g of diazomethane (2-fold excess) at 0°C. The reaction mixture was left at room temperature for 18 h resulting in the formation of pale yellow crystals. Solvents were evaporated and the crude diazoketone, dissolved in 50 ml benzene and 110 ml methanol, was heated to 60°C and a suspension of 6 mmoles silver oxide in 50 ml methanol was added slowly. After refluxing at 70°C for 20 h the solvents were evaporated and the residue (taken up in ether) was filtered through Celite. The ether solution was adjusted to 100 ml and washed with 0.1 NHCl, dilute $NaHCO_3$, water, and dried over sodium sulfate. The methyl ester product, *3* (R=acetyl), was recrystallized from 10% acetone in methanol and 100% ethanol to give 3.2 g (60%) of white needles. mp 126.0—127.2°C; mass spectrum *m/e* (rel. int.) 356 (100 M^+ — HOAc), 341 (29), 325 (2.4), 282 (4.5), 255 (24); nmr ($CDCl_3$) δ .66 (s, 3 H, 18-CH_3), .93 (d, 3 H, 21-CH_3), .97 (s, 3 H, 19-CH_3), 2.01 (s, 3 H, —OAc), 2.27 (d, 2 H, 22-CH_2), 3.60 (s, 3 H, OACMe), 4.5 (m, 1 H, 3α-H), 5.33 (m, 1 H, 6-H).

Example 2
Methyl 3β-Hydroxy-24-norchola-5,7-dien-23-oate (4b, R=CH_3)

To a solution of *3* (R=acetyl) (500 mg, 1.2 mmoles) in 22 ml benzene and 17 ml hexane was added 500 mg $NaHCO_3$ and 1.5 eg. 1,3-dibromo-5,5-dimethyl-hydantoin. The reaction mixture was refluxed at 75°C for 20 minutes then rapidly cooled and filtered. The residue obtained upon solvent evaporation was dissolved in 17 ml xylene and 4 ml s-collidine and refluxed for 90 minutes. Ether was added and the organic phase was thoroughly washed with 1 N HCl, dilute $NaHCO_3$, water, saturated NaCl, and then dried over Na_2SO_4. The residue (containing 5,7- and 4,6-diene products) was heated in dry dioxane with 80 mg p-toluene sulfonic acid at 70°C for 35 minutes. The mixture was diluted with ether and washed with water, dilute bicarbonate, water and saturated NaCl. The dried residue was chromatographed on a silica gel column (2×15 cm) eluted with 15% EtOAc in hexane. The product (*4a*) (R=acetyl) eluting between 51 and 102 ml obtained in 29% yield from *3* was stirred in 10 ml ether and 10 ml 5% (w/v) KOH in 95% methanol for 30 minutes at room temperature. The reaction mixture was diluted with ether and the organic phase washed as above. The product was purified by tlc (40% EtOAc in hexane, developed twice, Rf 0.33) to give 96 mg of *4b* (R=CH_3) (21% from 3). UV λ_{max} 262, 271, 282, 292, nm. High resolution mass spectrum, calc'd. for $C_{24}H_{36}O_3$: 372.2664; found: 372.2652. nmr δ 0.66 (s, 3 H, 18-CH_3), .94 (s, 3 H, 19-CH_3), 1.01 (d, 3 H, 21-CH_3), 3.67 (s, 3 H, $COOCH_3$), 2.76 (m, 1 H, 3α-H), 5.39 (d, 1 H, 7-H), 5.56 (d, 1 H, 6-H).

Example 3
Methyl 3β-Hydroxy-24-nor-9,10-seco-chola-5,7,10(19)trien-23-oate (6) (R=CH_3)

Ether solutions of approximately 20 mg of *4b* (R=CH_3) were irradiated on ice and under nitrogen for 10 minutes with a mercury arc lamp (Hanovia 9A-1) fitted with a Corex filter. The residues obtained after solvent evaporation were chromatographed on HPLC (6.4 mm×25 cm Zorbax-SIL, 4 ml/min 1500 psi) eluted with 1.5% 2-propanol in hexane. Pure previtamin, *5*, (R=CH_3) was collected at 45 ml (UV: λ_{max} 260 nm λ_{min} 231 nm). The combined previtamin containing fractions were heated in 10 ml ethanol at 80°C for 150 minutes to yield 10.8 mg of *6* (R=CH_3) (11% yield from *4*). UV, λ_{max} 264 nm, λ_{min} 228 nm. High resolution mass spectrum calc'd. for $C_{24}H_{36}O_3$: 372.2664; found: 372.2661; *m/e* (rel. int.) 372 (44), 354 (3), 341 (6), 313 (4), 298 (1), 271 (4), 253 (7), 136 (97), 118 (100). nmr δ .58 (s, 3 H, 18-CH_3), .99 (d, 3 H, 21-CH_3), 3.67 (s, 3 H, $COOCH_3$) 3.95 (m, 1 H, 3α-H), 4.81 (s, 1 H, 19(*Z*)-H), 5.05 (s, 1 H, 19(*E*)-H), 6.03 (d, 1 H, 7-H), 6.23 (d, 1 H, 6-H).

Example 4
Methyl 1α,3β-dihydroxy-24-nor-9,10-seco-5,7,10(19)-cholatrien-23-oate (Calcitroic Acid Methyl Ester, R=CH_3)

A solution of *6* (R=CH_3) (10.2 mg, 27 μmoles) in pyridine (0.2 ml) was treated with 30 mg p-toluene sulfonyl chloride at 4° for 22 hr. After addition of dilute bicarbonate (2 ml) the product was extracted with $CHCl_3$ ether (10 ml); the combined organic phases were washed with 1 N HCl, dilute bicarbonate, water, and saturated NaCl and dried over $MgSO_4$. The 3-tosyl derivative was then solvolyzed in 0.3 ml benzene, 2 ml methanol, and 50 mg $NaHCO_3$, heated to 56°C for 18 hr. The resulting cyclovitamin product (*7*) (R=CH_3) was extracted into ether, washed with water and saturated NaCl, dried, and purified on silica gel tlc (30% EtOAc/hexane, Rf. 54). This product in 0.7 ml CH_2Cl_2 was then added to an ice-cooled solution containing 0.5 eg. SeO_2 and 2 eq. t-BuOOH in 0.5 ml CH_2Cl_2. The reaction, followed by tlc, was allowed to proceed at room temperature for a total of 40 minutes and was stopped with the addition of $NaHCO_3$ and ether. The organic phase was washed with dilute bicarbonate, water, and saturated NaCl, and dried over $MgSO_4$. Evaporation of solvent gave 1α-hydroxy derivative *8*, (R=CH_3) which was dissolved in 0.5 ml glacial acetic acid and heated at 55° for 15 mins. Products (*9*, R=CH_3) and the corresponding 5,6-*trans* isomer in *ca* 3:1 ratio) were extracted with ether, and the ether phase was washed as before. Compound *9* (R=CH_3) was purified by tlc (50% EtOAc in hexane rf. 0.32) followed by HPLC, (6.4×250 mM column, 2.5% of 2-propanol in hexane, at 2 ml/min and 900 psi). Product *9* (R=CH_3) eluting at 63 ml, was recycled through the column and obtained in pure form in 7.1% yield from *6* (UV λ_{max} 264, λ_{min} 228 nm). Mild hydrolysis of *9* (75 μl 0.1 M KOH/MeOH and 200 μl ether, 15°, 60 min) provided 1 (R=CH_3); UV λ_{max} 264 nm, λ_{min} 228 nm; high resolution mass spectrum: calc'd for $C_{24}H_{36}O_4$ 388.2614; found 388.2645; *m/e* (rel. int.) 388 (18), 370 (61), 357 (3), 352 (24), 314 (1), 287 (1), 269 (4), 251 (7), 152 (31), 134 (100); nmr δ 0.58 (s, 3 H, 18 CH_3), 0.99 (d, 3 H, 21 CH_3), 3.67 (s, 3 H, $COOCH_3$), 4.23 (m, 1 H, 3α-H), 4.43 (m, 1 H, 1β-H), 5.00 (s, 1 H, 19(*Z*)-H), 5.33 (s, 1 H, 19(*E*)-H), 6.02 (d, 1 H, 7-H), 6.38 (d, 1 H, 6-H).

Example 5
Calcitroic Acid (1, R=H)

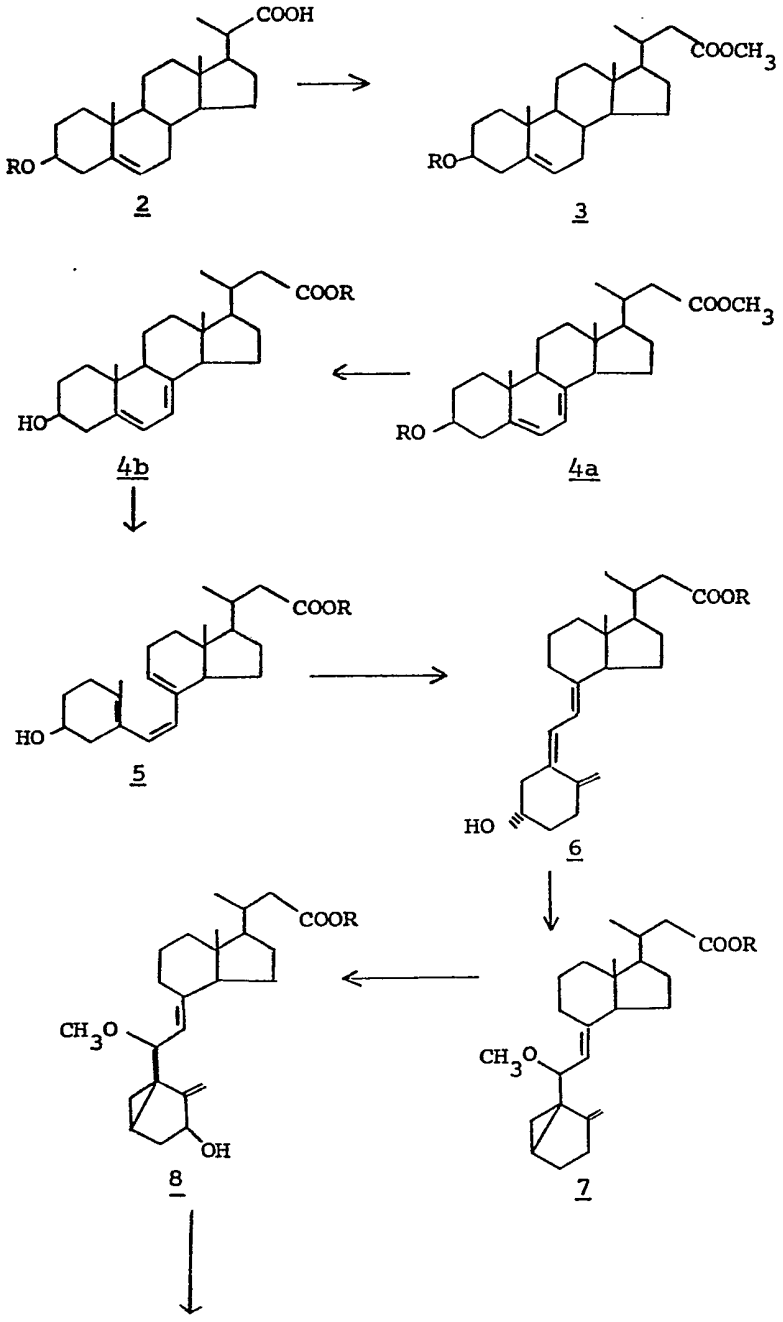
Hydrolysis of 1 (R=CH_3) in 10% KOH/90% methanol at 60°C for 30 minutes followed by neutralization and filtration in 100% ethanol gives quantitative yields (by tlc and UV) of the natural product *1* (R=H). UV λ_{max} 264, λ_{min} 228 nm.

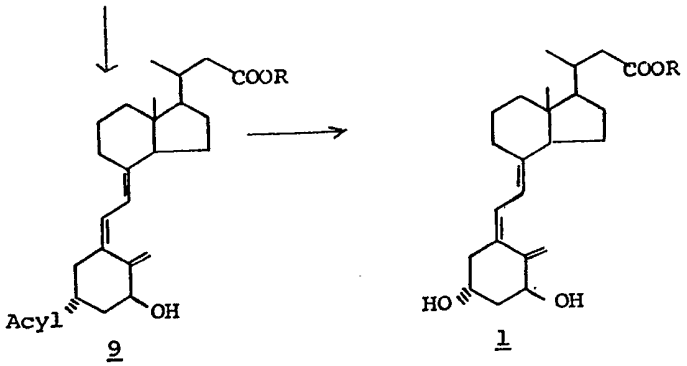
Example 6

Comparison with Biologically Generated 1 (R=CH_3)

The low resolution mass spectrum and the UV spectrum for synthetic *1* (R=CH_3) were identical with the spectra obtained for the methylated metabolite isolated from 1,25-$(OH)_2D_3$-treated rats (Esvelt et al, supra). (Direct comparison of nmr spectra was not possible because the low quantitites of available natural product precluded nmr measurements). To confirm chromatographic identity, (3α-^{3}H)-calcitroic acid was obtained from the livers of rats dosed with 1α,25-dihydroxy(3α-^{3}H)D_3, and converted to its methyl ester (*1*) (R=CH_3) as described by Esvelt et al (supra). This material (6500 dpm) was combined with 2 μg of synthetic *1* (R=CH_3) and the mixture was chromatographed on HPLC using the 4.6 mm column eluted with 8% 2-propanol in hexane, and the absorbance was monitored at 254 nm. Fractions were collected, evaporated, and counted. Radioactivity co-eluted exactly with the UV-absorbing peak due to synthetic *1* (R=CH_3) (elution volume, 40 ml). Spectral and chromatographic properties establish the identity between synthetic *1* (R=CH_3) and the methylated natural product.

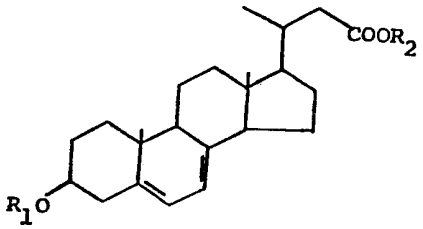
Process Schematic 1





Claims

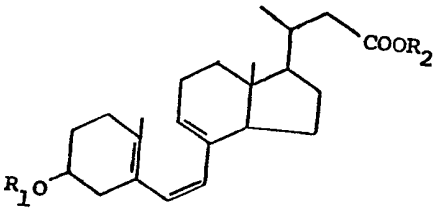
1. A compound having the formula



where R_1 is hydrogen, or a hydroxy-protecting group and R_2 is hydrogen, alkyl or benzyl.

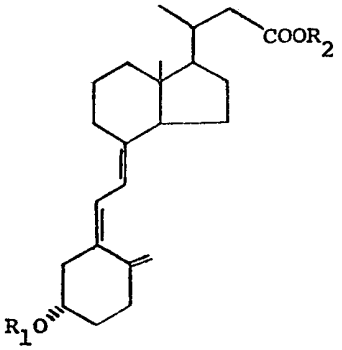
2. A compound of claim 1 where R_1 is hydrogen or acyl and R_2 is alkyl.

3. A compound having the formula



where R_1 is hydrogen, or a hydroxy-protecting group and R_2 is alkyl or benzyl.

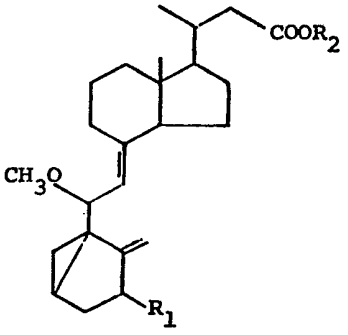
4. A compound having the formula



where R_1 is hydrogen or a hydroxy-protecting group and R_2 is hydrogen, alkyl or benzyl.

5. A compound of claim 4 where R_1 is hydrogen or acyl and R_2 is hydrogen or alkyl.

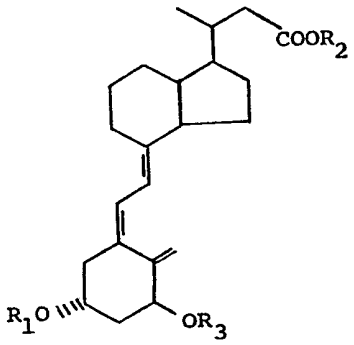
6. A compound having the formula



where R_1 is hydrogen, hydroxy, or a protected hydroxyl group and R_2 is hydrogen, alkyl or benzyl.

7. A compound of claim 6 where R_1 is hydrogen or hydroxy.

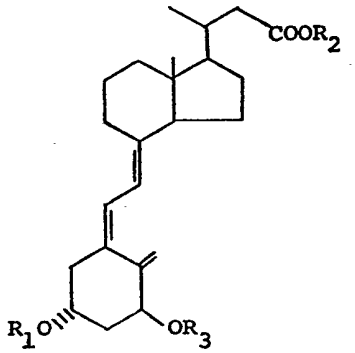
8. A compound having the formula



where each of R_1 and R_3, which may be the same or different, is a hydroxy-protecting group and R_2 is hydrogen, alkyl or benzyl.

9. A compound of claim 8 where each of R_1 and R_3 is acyl.

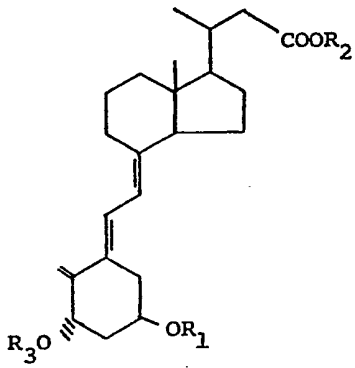
10. A compound having the formula



where R_1 and R_3 is hydrogen or acyl, such that when R_1 is hydrogen, R_3 is acyl and when R_3 is hydrogen, R_1 is acyl, and R_2 is hydrogen, alkyl or benzyl.

11. A compound of claim 10 where the acyl group is acetyl.

12. A compound having the formula



where each of R_1 and R_3, which may be the same or different, is hydrogen, or a hydroxy-protecting group and R_2 is hydrogen, alkyl or benzyl.

13. A compound of claim 12 where R_1 and R_3 are independently hydrogen or acyl.

14. A compound according to any one of claims 1, 3, 4, 8 or 12 where the hydroxy-protecting group is acyl, methoxymethyl, tetrahydropyranyl or alkylsilyl.

15. A process for preparing a compound of the formula given in claim 8, wherein R_1 and R_3 are both hydrogen and R_2 is as defined in claim 8 which comprises subjecting a compound as claimed in claim 6 to solvolysis and hydrolysing the resulting 5,6-cis product in mild alkali.

16. A compound of the formula given in claim 8, wherein R_1 and R_3 are both hydrogen and R_2 is as defined in claim 8 whenever prepared by a process as claimed in claim 15.

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