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(21) International Application Number: PCT/US81/00961 (22) International Filing Date: 16 July 1981 (16.07.81) (31) Priority Application Numbers: 174,944 228,486 (32) Priority Dates: 4 August 1980 (04.08.80) 26 January 1981 (26.01.81) (33) Priority Country: US (71) Applicant: WISCONSIN ALUMNI RESEARCH FOUNDATION [US/US]; 614 North Walnut Street, Madison, WI 53705 (US). (72) Inventors: DeLUCA, Hector, F.; 5130 Minocqua Cres- cent, Madison, WI 53705 (US). SCHNOES, Heinrich, K.; 1806 Summit Avenue, Madison, WI 53705 (US). PAAREN, Herbert, E.; 6360 Pheasant Lane, Verona, WI 53593 (US). WICHMANN, Joseph, K.; 2750 Union Street, Madison, WI 53704 (US). FIVIZZANI, Mary, A.; 2924 Harvey Street, Madison, WI 53705 (US).		(74) Agent: BREMER, Howard, W.; 614 North Walnut Street, Madison, WI 53705 (US). (81) Designated States: AU, CH, DE, DK, GB, JP. Published <i>With international search report</i>
(54) Title: PROCESS FOR PREPARING VITAMIN D-LACTONES (57) Abstract Methods for preparing the various isomeric forms of 25-hydroxyvitamin D ₃ -26, 23-lactone utilizing cyanohydrin forma- tion with readily prepared steroid materials followed by actinic radiation of the products and recovery of the desired 25-hydroxy- vitamin D ₃ -26, 23-lactone. The invention also provides new intermediate compounds in their various isomeric forms derived during the process for preparing the said vitamin D ₃ lactones. 25-Hydroxyvitamin D ₃ -26, 23-lactone is recognized as having vi- tamin D-like activity and is believed to play an important role in the regulation of calcium and phosphate levels in the animal and human organism. The present invention provides a convenient method for preparing the various isomeric forms of this com- pound.		

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- 1 -

DescriptionProcess for Preparing Vitamin D-LactonesTechnical Field

5 This application is a continuation-in-part of application Serial No. 174,944, filed August 4, 1980.

This invention relates to the preparation of biologically active vitamin D derivatives.

10 More specifically this invention relates to a process for the preparation of 25-hydroxyvitamin D₃-26,23-lactone and to novel compounds which are synthetic intermediates in this process.

It is now well-established that the biological function of vitamin D in the animal or human is dependent on metabolism of the vitamin to hydroxylated forms. A number of metabolites have been identified, e.g. 25-hydroxyvitamin D₃, 24,25-dihydroxy-
15 vitamin D₃, 1,25-dihydroxyvitamin D₃, and it is now generally accepted that one or more of these metabolites are the compounds responsible for the biological action associated with vitamin D, namely the control of calcium and phosphorus homeostasis in the
20 animal or human.

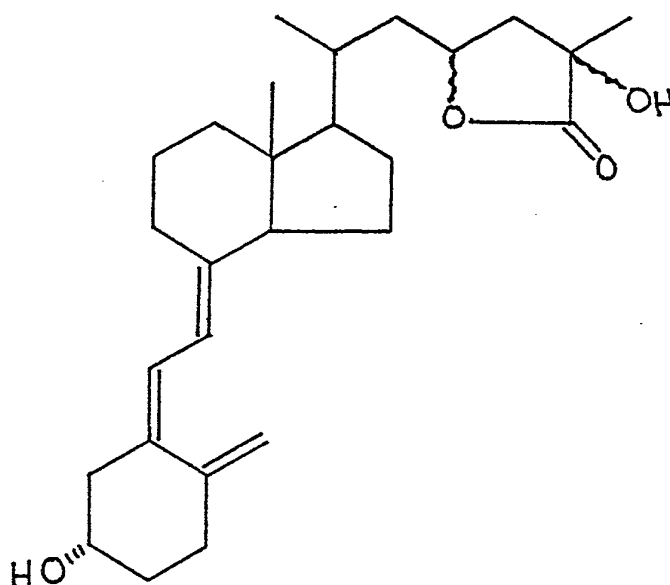
Background Art

Because of their biological potency, vitamin D metabolites are of great therapeutic interest, and their preparation, efficacy and use is amply documented in the patent and other literature, e.g. U.S. Letters Patents Nos. 3,880,894 (1,25-dihydroxyergo-
25 calciferol); 3,879,548 (method of treating milk fever in dairy cattle with 1 α -hydroxycholecalciferol); 3,715,374 (24,25-dihydroxycholecalciferol); 3,697,559 (1,25-dihydroxycholecalciferol). In addition, various structural analogs of these compounds are of
30 scientific and commercial interest as substitutes for the natural metabolites in various clinical application, e.g. U.S. Letters Patent Nos. 4,201,881 (24,24-difluoro-1 α ,25-dihydroxycholecalciferol); 4,196,133 (24,24-difluoro-25-hydroxycholecalciferol); 3,786,062 (22-dehydro-25-hydroxycholecalciferol).



- 2 -

More recently a novel vitamin D₃ metabolite, characterized by an unusual lactone unit in the steroid side chain was discovered (Wichmann et al. Biochemistry 18, 4775-4780, 1979). The compound, 25-hydroxyvitamin D₃-26,23-lactone, may be represented by the structure shown below,



The 25-hydroxy-vitamin D₃-lactone, exhibits vitamin D-like activity and is believed to play an important role in the control of calcium and phosphate levels in the animal organism.

Disclosure of Invention

10 The present invention provides a process for preparing 25-hydroxyvitamin D₃-26,23-lactone from readily available steroid starting materials as is more fully described in the following specification and as claimed in the appended claims.

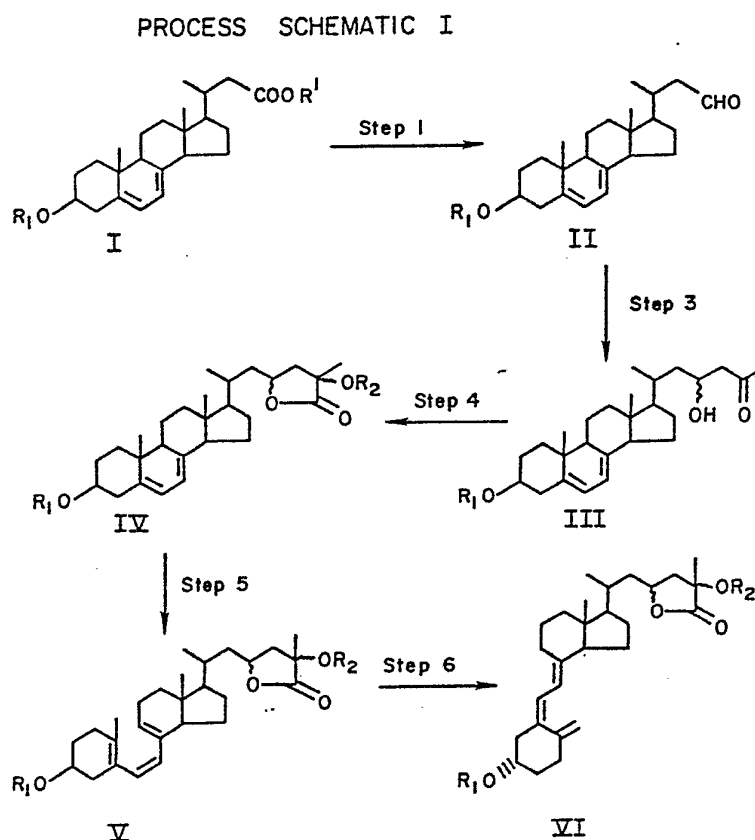
15 In this Description and in the claims the word "acyl" means an aliphatic acyl group of from 1 to about 5 carbon atoms, such as acetyl, propionyl, butyryl, pentoyl and their isomeric forms, or an aromatic acyl group, such as benzoyl, or substituted benzoyl, e.g. methyl benzoyl, nitrobenzoyl or halobenzoyl. The term "alkyl" denotes a hydrocarbon radical of

- 3 -

from 1 to about 5 carbon atoms such as methyl, ethyl, propyl, etc., or their corresponding isomeric forms.

Best Mode for Carrying Out the Invention

As depicted in Process Schematic I, the present process
 5 utilizes the 5,7-diene ester (I) as the starting material, where R_1 is hydrogen or a hydroxy protecting group such as acyl, alkylsilyl, or tetrahydropyranyl and where R' is hydrogen or an alkyl group of from 1-5 carbons. This 5,7-diene ester is readily
 10 obtained from the known 24-nor-5-cholenic acid or its esters employing well known processes for introducing the 7,8-double bond.

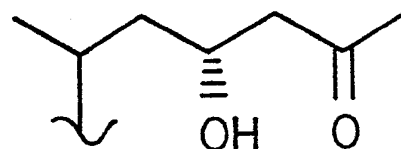
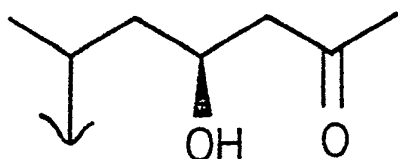


In accordance with the process shown in Process Schematic I the 23-acid or ester function in I is subjected to a hydride reduction to form the corresponding aldehyde represented by
 15 structure II. This reduction is conducted in a suitable solvent (e.g. ether, THF, benzene, etc.) at moderate or low temperatures

- 4 -

with sterically bulky aluminum hydrides, such as di-t-butyl aluminum hydrides as preferred reagents since, with such reagents, the reaction may be stopped at the aldehyde stage. If the hydroxy protecting group R_1 in compound I is an acyl group, such a group would, of course, be removed in the
5 hydride reduction step to yield aldehyde II, where $R_1 = H$. Reprotection is not necessary, but if desired, can be accomplished readily by well-known procedures (e.g. acylation, alkylsilylation, tetrahydropyranylation) to yield aldehyde II, where $R_1 =$ acyl,
10 alkylsilyl or THP. Alternatively, and especially in those cases where the protecting group R_1 in compound I is a group stable to hydride reagents (e.g. tetrahydropyranyl (THP) or alkylsilyl group) the conversion of I to aldehyde II can be conducted as a two-step process, involving first the reduction of the acid or
15 ester function to alcohol and then reoxidation of the 23-alcohol to the aldehyde. Such processes are well known in the art.

The resulting aldehyde II (where R_1 is hydrogen or a hydroxy-protecting group) is then subjected to an aldol condensation reaction (step 2 in Process Schematic I) with acetone, or
20 the synthetic equivalent of the acetone reagent, e.g. the derived imine such as acetonecyclohexylimine, in the presence of a catalyst and suitable solvent (e.g. acetone, ether, etc). With acetone as the reagent, a basic catalyst is preferred, such as a solution of potassium hydroxide or similar base. Organic bases
25 may also be used, e.g. $NaOCH_3$, or potassium-*t*-butoxide, lithium isopropylamide, etc. The product of this aldol condensation reaction is the hydroxy ketone, represented as structure II| in Process Schematic I, where R_1 is hydrogen or a hydroxy protecting group as previously defined. This hydroxy-ketone product is a
30 mixture of two stereoisomers, differing in C-23-hydroxy-configuration in accordance with the following structural formulas.



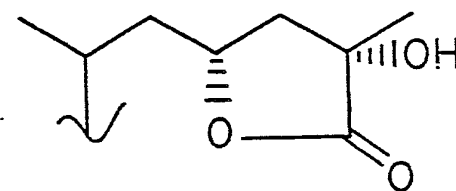
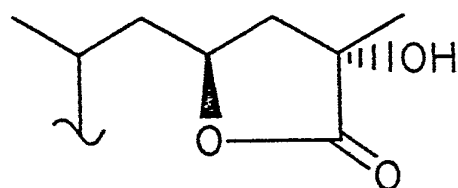
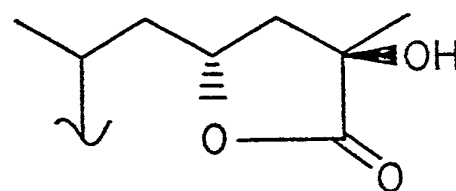
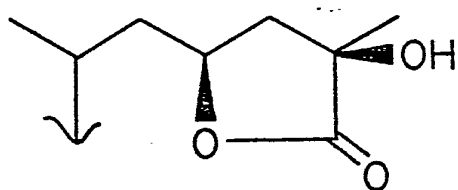
This mixture can be separated by any of the well known isomer separation methods, e.g. crystallization, or, preferably chromatography on either thin layer plates or efficient columns, e.g. by high-performance liquid chromatography (HPLC) and the two
5 C-23-hydroxy epimers can then be subjected separately to the subsequent steps of the process.

Alternatively, the hydroxy-ketone product (III) can be subjected directly to the next step of the process (step 3 in Process Schematic I) which involves, lactone formation in the
10 side chain. The transformation is accomplished by treating hydroxy-ketone III with cyanide (e.g. KCN) in the presence of base (e.g. hydroxide) and a suitable solvent, e.g. alcohol or alcohol/water mixtures, to obtain the intermediate cyanohydrin at carbon 25, which is not isolated but directly converted under
15 these conditions to the hydroxy-acid and then lactonized in acid (e.g. HCl) to form the desired lactone product represented by the structure IV in Process Schematic I. C-3-Hydroxy-protecting groups that may be present in the hydroxy-ketone III subjected

- 6 -

to lactone formation will usually be removed during this lactonization process involving both base and acid treatment and the lactone product obtained can normally be represented by structure IV, where $R_1 = H$, $R_2 = H$. Reprotection of the hydroxy group is not necessary, but can, if desired, be accomplished by any of the known methods of acylation, alkylsilylation, etc. to obtain lactone derivatives of structure IV where R_1 or R_2 or both are hydroxy-protecting groups, such as acyl, alkylsilyl, etc.

Cyanohydrin formation can also be accomplished by a cyanohydrin exchange reaction involving, for example, treatment of hydroxy ketone III with acetone cyanohydrin in the presence of a suitable catalyst, e.g. KOH, NaOCH_3 , potassium t-butoxide. Since formation of the cyanohydrin results in generation of another asymmetric center at C-25, lactone IV, as prepared from the hydroxy-ketone III (representing two C-23-hydroxy stereo-isomers, *supra*), now consists of a mixture of four stereo-isomeric products differing in steric configuration at C-23 and C-25 as represented by the following structural formulas.



- 7 -

The stereoisomeric mixture of lactone IV may be separated at this stage, preferable by high-performance liquid chromatography (HPLC), to obtain the four stereo isomeric forms, designated here for convenience as IVA, IVB, IVC and IVD, in the order of their elution from HPLC, in pure form. Each of the lactone stereoisomers is then subjected to photolysis under ultraviolet irradiation (step 4 in Process Schematic I) in a suitable solvent (e.g. ether, alcohol, benzene, or mixtures of ether and other solvents, e.g. benzene) to obtain the corresponding previtamin D lactone derivatives represented by general structure V (where $R_1 = R_2 = H$, or a hydroxy-protecting group).

Subsequent isomerization of each of the previtamin lactone isomers depicted by general structure V to the corresponding vitamin lactone, represented by general structure VI (where $R_1 = R_2 = H$ or where any or both of R_1 and R_2 may represent a hydroxy-protecting group) (step 5 in Process Schematic I) is accomplished by heating the previtamin intermediates to moderate temperature (e.g. 50-80°C) in a suitable solvent, e.g. on low molecular weight alcohol, or benzene, or toluene. This two-step irradiation/thermal isomerization sequence yields from lactone isomer IVA the corresponding vitamin lactone VIA, from isomer IVB, the vitamin lactone VIB, from isomer IVC the corresponding vitamin lactone VIC, and from isomer IVD the vitamin lactone VID, all in pure form. If vitamin lactones IV, contains hydroxy-protecting groups at C-3 and/or C-25, such groups may be removed by acid or base hydrolysis (depending on the protecting group present, as is well known in the art) to obtain the corresponding free hydroxy-lactone product VI where $R_1 = R_2 = H$.

Direct comparison with natural 25-hydroxyvitamin D₃-26,23-lactone shows that synthetic isomer VIC (where $R_1 = R_2 = H$) is identical with the natural product.

Alternatively the steroid lactone intermediate IV, as a mixture of its four stereoisomeric forms, may be subjected to photolysis by actinic irradiation as described above, to yield the corresponding mixture of the previtamin D lactone stereoisomers of general structure V. This mixture in turn is subjected to



- 8 -

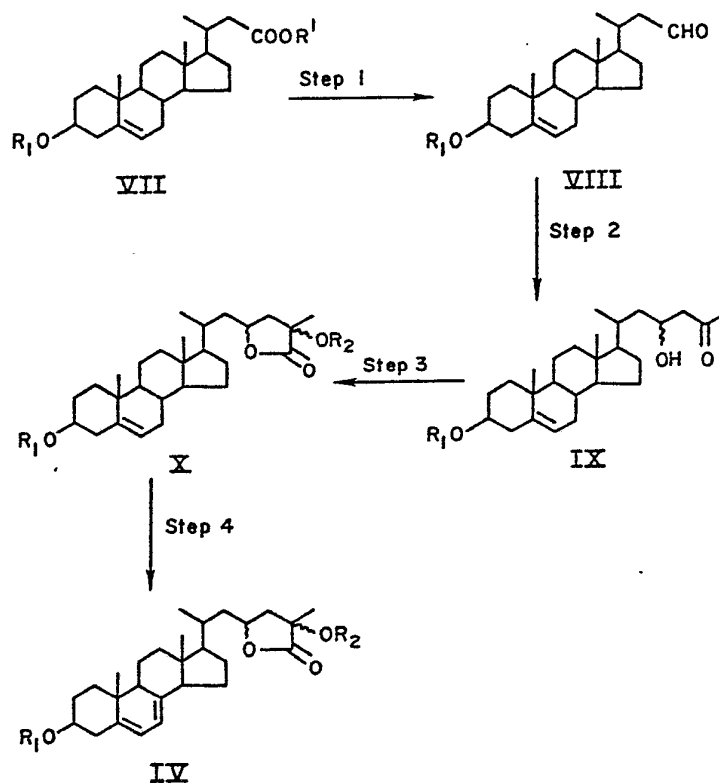
thermal isomerization as described above, to yield 25-hydroxyvitamin D₃-26,23-lactone, represented by general structure VI as a mixture of the four possible lactone stereo isomers. These stereo isomers can now be separated, preferably by HPLC, to yield in
5 order of elution from the column, lactone stereo isomers, VIA, VIB, VIC, and CID, in pure form with isomer VIC corresponding to the natural product.

If desired, the synthetic process can also be conducted, by effecting an initial separation of the two C-23-hydroxy
10 stereo-isomers of hydroxy-ketone III resulting from the aldol condensation step as described above by crystallization, or preferably, chromatography, to yield the two C-23-stereo-isomers in pure form (designated IIIA and IIIB for convenience here) and then subjecting each isomer separately to the subsequent
15 steps of the described process (i.e. lactone formation, photolysis and thermal isomerization, steps 3, 4 and 5) to yield the desired vitamin lactone product of structure VI. In this manner, hydroxy-ketone isomer IIIA, subjected to the lactonization reaction (step 3) yields a mixture of lactone stereo-isomers IVB
20 and IVD, which can be separated, preferably by HPLC, and separately converted by photolysis and thermal isomerization (steps 4 and 5) as described above to vitamin lactone products VIB and VID, respectively. Similarly, after the lactonization of the hydroxy-ketone isomer IIIB (step 3), a mixture of lactone
25 isomers IVA and IVC is obtained, which, after separation, are converted by photolysis and isomerization (steps 4 and 5), to vitamin lactones VIA and VIC, respectively, where VIC, as mentioned above, corresponds to the natural product.

If desired, the steroid-lactone intermediate IV, shown in
30 Process Schematic 1, can be obtained by an alternative but chemically analogous sequence, as depicted in Process Schematic 2.

- 9 -

PROCESS SCHEMATIC 2



- This process utilizes the known 24-nor-5-cholen-23-oic acid or its esters represented by structure VII (where R_1 and R' designate substituents as defined for compound I in Process Schematic I), as starting material. Ester VII is reduced in step
- 5 I to aldehyde VIII (where R_1 is hydrogen or a hydroxy protecting group) in a process entirely analogous to that discussed previously (i.e. step I of Process Schematic I) and aldehyde VIII is subjected to an aldol condensation with acetone or an acetone equivalent to yield hydroxy-ketone IX, as a mixture of the two possible
- 10 hydroxy stereo-isomers at carbon 23. Treatment of hydroxy-ketone IX (where R_1 is hydrogen or a hydroxy protecting group) with cyanide and subsequent hydrolysis using conditions analogous to those described above for step 3 of Process Schematic I yields the lactone intermediate X (where $R_1 = R_2 = H$) as a
- 15 mixture of four possible (C-23 and C-25) stereo-isomeric forms. This product can then be converted to the corresponding 5,7-diene lactone IV described above, by well established methods, e.g.

- 10 -

protection of the C-3-hydroxy group by acylation using standard conditions, followed by allylic bromination and dehydrobromination and removal of the C-3-acyl group by mild base hydrolysis. The four stereo-isomers of this product (compound IV) can now
5 be separated, to yield in order of elution on the HPLC isomers IVA, IVB, IVC and IVD, as previously described. It is, of course, also possible to separate (preferably by HPLC) the two C-23-hydroxy stereo-isomers of hydroxy-ketone IX and to subject each epimer separately to the subsequent steps of the
10 process (steps 3 and 4 of Process Schematic 2) in a manner entirely analogous to that described previously to obtain the same four stereo-isomers of lactone IV. Similarly, separation of the four lactone stereo-isomers can be effected at the stage of compound X (Process Schematic 2) and each of the lactone
15 isomers XA, XB, XC and XD can then be separately converted to the corresponding 5,7-diene IVA, IVB, IVC and IVD, respectively, by the standard allylic bromination/dehydrobromination process.

Although in therapeutic applications of 25-hydroxyvitamin D₃ 26,23-lactone, the free hydroxy-lactone, represented by
20 structure VI, where $R_1 = R_2 = H$, is normally the preferred form for administration, it is to be noted that a variety of derivatives of lactone VI, that may be desirable for certain applications can be readily prepared. Thus, acylation at moderate or low temperatures using acyl anhydride reagents and base
25 catalysts provides the C-3-O-acyl derivatives of VI ($R_1 = \text{acyl}$, $R_2 = H$), whereas acylation at elevated temperatures (50-70°C) yields 3,25-di-O-acyl products (VI, $R_1 = R_2 = \text{acyl}$). For example, treatment of VI ($R_1 = R_2 = H$) with acetic anhydride and pyridine at 20°C for 1-2 hours gives the corresponding
30 3-acetate derivative, whereas the 3,25-diacetate is readily formed when using the same reagents at 60°C. Similarly, alkylsilyl derivatives or tetrahydropyranyl derivatives of VI can be prepared by established procedures, and because of the differential reactivity of the C-3 and C-25-hydroxy groups, the
35 3-mono, or 3,25-di-protected derivatives are readily obtained.

- 11 -

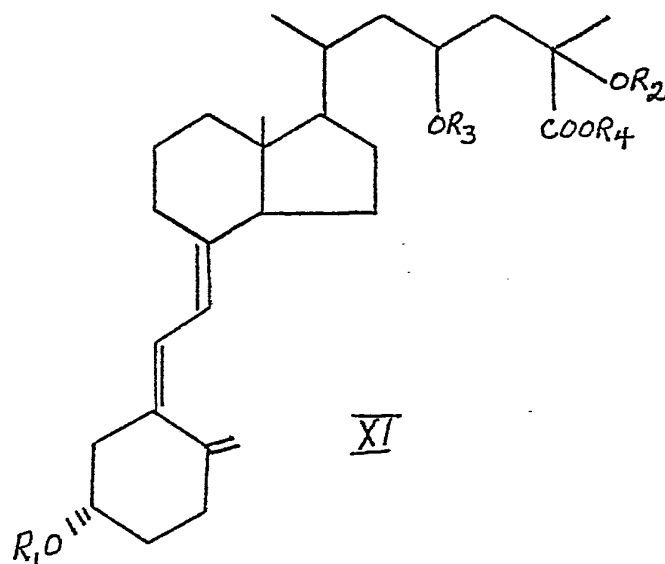
The 3-mono-acylated product, can be further derivatized at the C-25-hydroxy group, e.g. by acylation with a different acyl group or by alkylsilylation (with trimethylchlorosilane or similar reagents, or t-butyl-dimethylchlorosilane, etc) or tetrahydro-
5 pyranylation according to procedures well known in the art.

Also in 3,25-di-protected derivatives of compound VI, the C-3-protecting group can be selectively removed by base or acid hydrolysis (depending on the protecting group present) to generate the 3-hydroxy-25-protected derivative (compound VI
10 where $R_1 = H$, $R_2 =$ hydroxy protecting group) and the 3-hydroxy group in such compounds may then be selectively derivatized with a group different from that present at C-25.

Alternatively and conveniently, free hydroxy groups in the steroid precursors to vitamin lactone VI can be protected
15 and these hydroxy-protected derivatives can then be converted by the process steps detailed above to vitamin lactone VI, where R_1 or R_2 , or both, represent hydroxy-protecting groups. Thus one or both of the hydroxy groups of 5,7-diene lactone IV ($R_1 = R_2 = H$ in Process Schematic 1) or of Δ^5 -lactone X
20 ($R_1 = R_2 = H$, in Process Schematic 2) can be derivatized by acylation, alkylsilylation, tetrahydropyranylation, etc.) exactly analogously to the procedures discussed earlier, to yield the corresponding mono- or di-hydroxy-protected derivatives (where the hydroxy protecting groups R_1 and R_2 can be the same or
25 different). These derivatives can then be carried through the final process steps (e.g. steps 4 and 5 in Process Schematic 1 which are not affected by the nature of the hydroxy-protecting group present) to yield hydroxy-protected previtamin D lactone of structure V (R_1 or $R_2 =$ hydrogen or hydroxy protecting
30 group) and then the desired mono- or di-hydroxy-protected vitamin D lactone of structure IV. It should be obvious also that these hydroxy-derivatization procedures can be applied to both mixtures of stereo-isomers (e.g. the mixtures of lactone isomers of compounds IV, X, VI or X) as well as the individual
35 separated isomers (e.g. IV A, B, C, D, or VI A, B, C, D, etc.), although it is generally preferred to derivatize the individual stereo-isomers separately.



Other pharmaceutically useful derivatives of the lactone compounds described above can also be prepared. These are the corresponding hydroxy-carboxylic acids, resulting from lactone opening, i. e. compounds of the general structure XI



- 5 wherein each of R_1 , R_2 and R_3 is hydrogen and where R_4 is hydrogen or a negative charge (i. e. carboxylate anion).

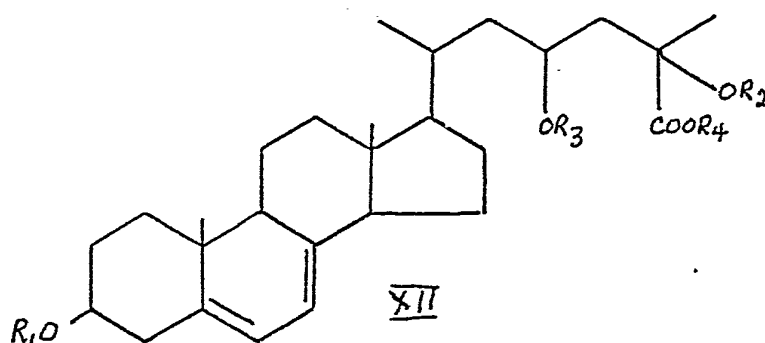
Such compounds are of particular interest because they are water-soluble derivatives of the described lactones. Because of their close structural relationship to the lactones they would be expected to possess intrinsic biological activity, or to express biological activity by virtue of their recyclization of the lactones in vivo (i. e. formation of compounds of type VI) since an equilibrium between lactone and hydroxy-carboxylic acid (or hydroxy carboxylate) must necessarily exist under in vivo conditions.

- 15 The hydroxy-acids of general structure XI are readily produced from lactones of general structure VI by hydrolysis of the lactone ring in base. Thus, treatment of the lactones VI in 0.01 to 0.1 M base (e. g. KOH or NaOH in H_2O or H_2O /dioxane

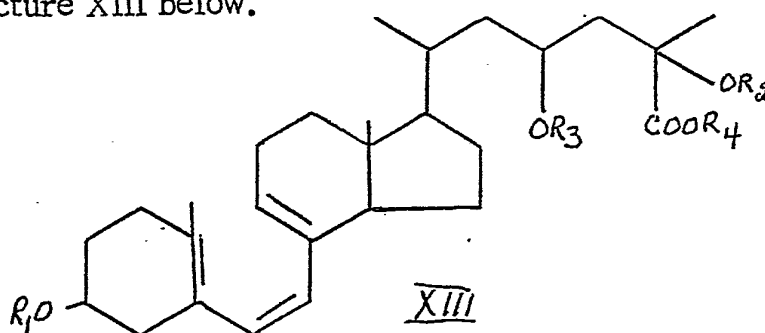
mixtures of H₂O/MeOH mixtures) at 25-50° C yields the corresponding ring-opened hydroxy-carboxylates, which, after careful acidification to pH 5-7, provide the hydroxy-carboxylic acids of structure IX (where R₁, R₂, R₃ and R₄ are hydrogen). The corresponding hydroxy-esters of structure XI, where R₁, R₂ and R₃ represent hydrogen and R₄ is an alkyl group can be produced in analogous fashion, by cleavage of the lactone in alcoholic base. For example, treatment of the lactones with sodium ethoxide in ethanol yields the ethyl ester of structure XI, wherein R₁, R₂ and R₃ are hydrogen and R₄ is ethyl. Other esters, e.g. the methyl propyl or butyl esters, can be prepared by analogous procedures utilizing the appropriate equivalent alcoholic base.

Additional derivatives of the hydroxy-esters such as may be desired for pharmaceutical preparations or other uses can be conveniently prepared by known methods. For example, using the derivatization procedures (acylation, alkylsilylation, etc. or combination of these procedures) discussed hereinbefore derivatives carrying acyl, alkylsilyl or tetrahydropyranyl groups (or any combination of these groups) on any or all of the C-3, 23 or 25 hydroxy groups (i.e. compounds of general structure XI wherein each of R₁, R₂ and R₃ is selected from hydrogen, acyl, alkylsilyl and tetrahydropyranyl, and where R₄ is alkyl) can be readily prepared.

It should be noted also that hydroxy-acids or hydroxy-esters of general structure XI, or their O-protected (acylated, alkylsilylated) derivatives can also be prepared from the corresponding steroid intermediates. For example, the base hydrolysis of the lactone ring of the 5,7-diene intermediate IV (Process Schematic I), utilizing a procedure analogous to that described above for lactone opening in the case of vitamin-lactone VI, yields the hydroxy-acid of the general structure



wherein R_1 , R_2 , R_3 and R_4 are hydrogen. Ultraviolet irradiation of this substance, as described for the analogous step in Process Schematic I, yields the corresponding pre-vitamin D-hydroxy-acid having structure XIII below.

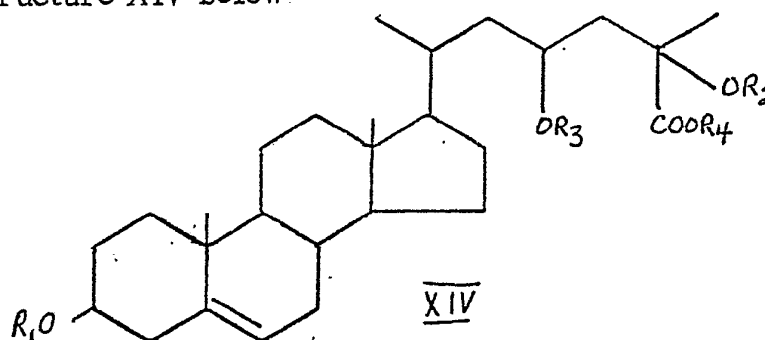


- 5 This pre-vitamin compound can be isomerized by heating in an inert solvent as described previously to give the vitamin hydroxy acid XI (R_1 , R_2 , R_3 and R_4 =H). Similarly alcoholysis of the 5,7-diene-lactone IV (e. g. NaOMe in MeOH; NaOEt in EtOH) yields the corresponding ester of structure XII (where R_1 , R_2 and R_3 =H and R_4 =alkyl) from which hydroxy-ester hydroxy-protected derivatives (e. g. O-acyl, O-alkylsilyl, O-tetrahydropyranyl represented by structure XII, wherein each of R_1 , R_2 and R_3 is selected from hydrogen, acyl, alkylsilyl, and tetrahydropyranyl and where R_4 =alkyl) can be readily obtained by the derivatization procedures previously discussed. Ultraviolet irradiation of the hydroxy ester or its O-protected derivatives yields the previtamin D compounds of general structure XIII where
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each of R_1 , R_2 and R_3 is selected from hydrogen, acyl, alkylsilyl and tetrahydropyranyl and where R_4 is alkyl. Subsequent thermal isomerization of these previtamin-intermediates yields the hydroxy esters or their corresponding O-protected derivatives of general structure VI, where each of R_1 , R_2 and R_3 is selected from hydrogen, acyl, alkylsilyl and tetrahydropyranyl and where R_4 is alkyl.

If desired, the lactone opening reaction, using procedures entirely analogous to those described above can also be applied to the lactone steroid intermediate X (Process Schematic 2) to yield the corresponding hydroxy acids or hydroxy esters represented by general structure XIV below.



where R_1 , R_2 and R_3 are hydrogen and R_4 is hydrogen or alkyl. Such analogs, or their O-protected derivatives, can be readily converted to the corresponding vitamin hydroxy-acids or esters of structure XI, via dehydrogenation to compounds of general structure XII, subsequent photochemical conversion to intermediates of type XIII and thermal isomerization to the final products of structure XI using established and well known procedures.

- 16 -

Example 1

To 269 mg of methyl 3 β -hydroxy-24-norchola-5,7-dien-23-oate 3-acetate (compound I, where R₁ = acetyl and R' = methyl) in 12 ml toluene at -78°C is added 0.72 ml of a 25% solution of lithium diisobutyl aluminum hydride in toluene. After 30 min, the solution is warmed to 4°C and 4 ml of saturated NH₄Cl is added and the mixture is stirred for 5 min. Water (25 ml) is then added and the reaction mixture is extracted with 100 ml Et₂O. The ether phase is separated and washed with 25 ml each of 1 N HCl, saturated NaHCO₃, saturated NaCl. The crude reaction mixture is applied to a 1 x 30 cm silica gel column and eluted with 6% EtOAc in hexane which elutes compound I and then with 16% and 22% EtOAc/hexane until the desired aldehyde product, compound II, is recovered (127.5 mg). The 23-aldehyde (compound II with R₁ = H) exhibits the following spectral properties: U.V. absorption, λ_{max} = 282, 272, 292 (shoulder); mass spectrum: m/e 342.2545 (calcd. 342.2558), 100%, M⁺; m/e 309, 65%, M⁺-H₂O-CH₃; m/e 283, 40%, M⁺-2CH₃-CHO; m/e 143, 50%, C₁₁H₁₁⁺; NMR: δ 9.77, s, 1H, C-23; 5.56, m, 1H, C-6; 5.40, m, 1H, C-7; 3.66, m, 1H, C-3; 1.05, d, J = 6.2, 3H, C-21; 0.95, s, 3H, C-19; 0.67, s, 3H, C-18.

Example 2

A mixture of 1.5 ml of acetone and 30 μ l of 1.0 M KOH in methanol is prepared, and after 15 min at 0°C is added to 122 mg compound II (R₁ = H) dissolved in 0.5 ml acetone. The reaction mixture is stirred at 0°C for 1.5 hr then 50 ml H₂O is added and the mixture extracted 3X with 75 ml CH₂Cl₂. The crude reaction mixture is subjected to HPLC on a 0.79 x 30 cm silica gel column (μ Porasil, a product of Waters Associates, Medford, Mass.) eluted with 2.25% isopropyl alcohol in CH₂Cl₂ at a flow rate of 3 ml/min. This procedure elutes 37.5 mg of starting material (compound II) at 12 ml followed by the desired hydroxy-ketone C-23-stereo isomers (compound III, R₁ = H) namely isomer IIIA (39.5 mg) eluting at 42 ml and isomer IIIB (37.3 mg) eluting at 46.5 ml. Spectral data for isomer IIIA:

- 17 -

U.V. absorption spectrum, λ_{\max} = 282, 272, 292 (shoulder),
mass spectrum, m/e 400.2983 (calcd. 400.2978) 100%, M^+ ; 382,
22%, $M^+ - H_2O$; 367, 30%, $M^+ - H_2O - CH_3$; 342, 60%, $M^+ - C_3H_6O$; 309,
28%, $M^+ - C_3H_6O - CH_3 - H_2O$; 271, 26%, M^+ -side chain; 253, 12%,
5 M^+ -side chain- H_2O ; NMR: δ 5.56, m, 1H, C-6; 5.39, m, 1H,
C-7; 4.17, m, 1H, C-23; 3.63, m, 1H, C-3; 2.17, s, 3H, C-26;
0.99, d, J = 6.2, 3H, C-21; 0.94, s, 3H, C-19; 0.65, s, 3H,
C-18. For isomer IIIB: U.V. absorption spectrum, λ_{\max} = 282,
272, 292 (shoulder); mass spectrum: m/e 400.2983 (calcd.
10 400.2978) 100%, M^+ ; 382, 19%, $M^+ - H_2O$; 367, 25%, $M^+ - H_2O - CH_3$;
342, 93%, $M^+ - C_3H_6O$; 309, 38%, $M^+ - C_3H_6O - CH_3 - H_2O$; 271, 28%,
 M^+ -side chain; 253, 15%, M^+ -side chain- H_2O ; NMR: δ , 5.56, m,
1H, C-6; 5.40, m, 1H, C-7; 4.14, m, 1H, C-23; 3.64, m, 1H,
C-3; 2.19, s, 3H, C-26; 1.00, d, J = 5.5, 3H, C-21; 0.94, s,
15 3H, C-19; 0.63, s, 3H, C-18.

Example 3

To a solution of 34 mg of hydroxy-ketone isomer IIIA (R_1
= H) in 0.8 ml of EtOH at 50°C is added 0.1 ml of a slurry of
cyanide (prepared by grinding 340 mg NaCN and 540 mg $CaCl_2 \cdot 2H_2O$
20 in a mortar to homogeneity, and slurrying 45 mg of the resulting
mixture in 1 ml of water), and after 5 and 10 min, additional 0.1
ml aliquots of the same slurry are added. After 1 hr, 0.15 ml
of cyanide slurry is added together with 0.5 ml EtOH, and this
addition is repeated at 2.5 hr. After 4.5 hr, 50 ml H_2O is
25 added and pH adjusted to ca. 1.5 with 1 N HCl. The reaction
mixture is extracted with 4 x 50 ml CH_2Cl_2 . The crude products,
in 5 ml EtOH, are then treated with 1 ml 1 N HCl added at 45°C
for 1 hr. Water (25 ml) is then added and product is extracted
with three 50 ml portions of CH_2Cl_2 .

30 The crude product is subjected to HPLC on 2 Zorbax SIL
semi-preparative columns (0.62 x 25 cm) in series eluted with
5% isopropanol in hexane at a flow rate of 3 ml/min to give
lactone IV (R_1 = H, R_2 = H) as the expected two C-25-stereoisomers,
designated IVB (4.3 mg) eluting at 90 ml, and IVD (4.0 mg)
35 eluting at 129 ml. Spectral data for IVB (R_1 = R_2 = H),

- 18 -

U.V. absorption spectrum, $\lambda_{\max} = 282, 272, 292$ (shoulder);
 mass spectrum: m/e 428.2935 (calcd. 428.2927), 100%, M^+ ; 410,
 14%, $M^+ - H_2O$; 395, 53%, $M^+ - H_2O - CH_3$; 369, 22%, $M^+ - C_3H_7O$; 271,
 58%, M^+ -side chain; 253, 28%, M^+ -side chain- H_2O ; 143, 63%,
 5 $C_{11}H_{11}^+$; NMR: δ 5.57, m, 1H, C-6; 5.40, m, 1H, C-7; 4.75, m,
 1H, C-23; 3.64, m, 1H, C-3; 1.52, s, 3H, C-27; 1.04, d, J =
 5.9, 3H, C-21; 0.94, s, 3H, C-19; 0.64, s, 3H, C-18.

Spectral data for IVD: U.V. absorption spectrum, λ_{\max}
 = 282, 272, 292 (shoulder); mass spectrum: m/e 428.2927
 10 (calcd. 428.2927), 100%, M^+ ; 410, 15%, $M^+ - H_2O$; 395, 65%,
 $M^+ - H_2O - CH_3$; 369, 30%, $M^+ - C_3H_7O$; 271, 44%, M^+ -side chain;
 253, 25%, M^+ -side chain- H_2O ; 143, 90%, $C_{11}H_{11}^+$; NMR: δ 5.56,
 m, 1H, C-6; 5.40, m, 1H, C-7; 4.47, m, 1H, C-23; 3.63, m, 1H,
 C-3; 1.50, s, 3H, C-27; 1.03, d, J = 6.6, 3H, C-21; 0.94, s,
 15 3H, C-19; 0.65, s, 3H, C-18.

Hydroxy-ketone stereoisomer IIIB ($R_1 = H$) subjected to
 the identical lactonization sequence also yields (from 29 mg of
 IIIB) two lactone stereoisomers, namely IVA ($R_1 = R_2 = H$) (2.9
 mg) eluting at 80 ml in the HPLC system described above and
 20 lactone isomer IVC ($R_1 = R_2 = H$) (2.4 mg) eluting at 107 ml.
 Spectral data for IVA: U.V. absorption spectrum, $\lambda_{\max} = 282,$
 272, 292 (shoulder); mass spectrum: m/e 428.2931 (calcd.
 428.2927) 100%, M^+ ; 410, 18%, $M^+ - H_2O$; 395, 62%, $M^+ - H_2O - CH_3$;
 369, 33%, $M^+ - C_3H_7O$; 271, 26%, M^+ -side chain; 253, 19%, M^+ -side
 25 chain- H_2O ; 143, 70%, $C_{11}H_{11}^+$. NMR: δ 5.57, m, 1H, C-6; 5.40,
 m, 1H, C-7; 4.72, m, 1H, C-23; 3.65, m, 1H, C-3; 1.51, s, 3H,
 C-27; 1.05, d, J = 6.1, 3H, C-21; 0.95, s, 3H, C-19; 0.63, s,
 3H, C-18.

Spectral data for IVC: U.V. absorption spectrum, $\lambda_{\max} =$
 30 282, 272, 292 (shoulder); mass spectrum: m/e 428.2917 (calcd.
 428.2927) 100%, M^+ ; 410, 16%, $M^+ - H_2O$; 395, 68%, $M^+ - H_2O - CH_3$;
 369, 37%, $M^+ - C_3H_7O$; 271, 16%, M^+ -side chain; 253, 16%, M^+ -side
 chain- H_2O ; 143, 70%, $C_{11}H_{11}^+$. NMR: δ , 5.57, m, 1H, C-6;
 5.40, m, 1H, C-7; 4.44, m, 1H, C-23; 3.64, m, 1H, C-3; 1.49,
 35 s, 3H, C-27; 1.04, d, J = 6.7, 3H, C-21; 0.94, s, 3H, C-19;
 0.63, s, 3H, C-18.

- 19 -

Example 4

One mg each of the lactone isomers IVA, IVB, IVC and IVD as obtained in Example 3, are photolysed separately in 150 ml of 20% benzene in diethylether using a quartz immersion well and Hanovia 608A36 lamp with corex filter. After 15 min irradiation each reaction mixture is subjected to HPLC on a Zorbax-SIL semi-preparative column 0.62 x 25 cm using 2.25% 2-propanol in methylene chloride as solvent. From lactone isomer IVA there is obtained the desired previtamin lactone VA ($R_1 = R_2 = H$) eluting at 34.5 ml; from isomer IVB there is obtained previtamin lactone VB eluting at 34 ml; from isomer IVC, the previtamin lactone VC, eluting at 33 ml is obtained, and from lactone isomer IVD, the corresponding previtamin lactone VD, eluting at 33 ml is obtained.

Example 5

Each of the previtamin lactones VA, VB, VC and VD are immediately isomerized to the desired vitamin lactone of structure VI in 1 ml EtOH at 70°C for 2 hr. Each reaction mixture is then subjected to HPLC on a Zorbax SIL semi-preparative column (0.62 x 25 cm) using 6% 2-propanol in hexane as eluant. Vitamin lactone VIA ($R_1 = R_2 = H$) (500 μ g) is collected at 29.5 ml; vitamin lactone isomer VIB (500 μ g) is collected at 31.5 ml; vitamin lactone isomer VIC (500 μ g) is collected at 39.75 ml, and 500 μ g of isomer VID is collected at 45.0 ml.

Spectral Data: VIA; mass spectrum, m/e 428.2923 (calcd. 428.2927) 24%, M^+ ; 410, 3%, $M^+ - H_2O$; 395, 11%, $M^+ - H_2O - CH_3$; 271, 2%, M^+ -side chain; 253, 9%, M^+ -side chain- H_2O ; 136, 100%, A ring + $C_6 + C_7^+$; 118, 82%, A ring + $C_6 + C_7^+ - H_2O$; NMR: δ 6.28, d, J = 11.8, 1H, C-6; 6.03, d, J = 11.0, 1H, C-7; 5.05, m(sharp), 1H, C-19(E); 4.82, m (sharp), 1H, C-19(Z); 4.72, m, 1H, C-23; 3.96, m, 1H, C-3; 1.51, s, 3H, C-27; 1.03, d, J = 5.5, 3H, C-21; 0.56, s, 3H, C-18; Fourier transform infrared spectrum (FT-IR); 1780 cm^{-1} (lactone C = O); UV: $\lambda_{max} = 265$ nm, $\lambda_{min} = 228$ nm.

- 20 -

- VIB; mass spectrum, m/e 428.2927 (calcd. 428.2927), 27%, M^+ ; 410, 2%, $M^+ - H_2O$; 395, 11%, $M^+ - H_2O - CH_3$; 271, 2%, M^+ -side chain; 253, 7%, M^+ -side chain- H_2O ; 136, 100%, A ring + $C_6 + C_7^+$; 118, 92%, A ring + $C_6 + C_7^+ - H_2O$; NMR: δ 6.28, d, $J = 11.7$, 1H, C-6; 6.03, d, $J = 11.1$, 1H, C-7; 5.05, m (sharp), 1H, C-19(E); 4.82, m (sharp), 1H, C-19(Z); 4.75, m, 1H, C-23; 3.96, m, 1H, C-3; 1.52, s, 3H, C-27; 1.03, d, $J = 5.6$, 3H, C-21; 0.56, s, 3H, C-18; FT-IR: 1781 cm^{-1} (lactone C = O); UV: $\lambda_{\text{max}} = 265\text{ nm}$, $\lambda_{\text{min}} = 228\text{ nm}$.
- 10 VIC; mass spectrum, m/e 428.2919 (calcd. 428.2927), 26%, M^+ ; 410, 2%, $M^+ - H_2O$; 395, 9%, $M^+ - H_2O - CH_3$; 271, 1%, M^+ -side chain; 253, 8%, M^+ -side chain- H_2O ; 136, 100%, A ring + $C_6 + C_7^+$; 118, 83%, A ring + $C_6 + C_7^+ - H_2O$; NMR: δ 6.28, d, $J = 11.8$, 1H, C-6; 6.03, d, $J = 10.7$, 1H, C-7; 5.05, m (sharp), 1H, C-19(E); 4.82, m (sharp), 1H, C-19(Z); 4.44, m, 1H, C-23; 3.96, m, 1H, C-3; 1.49, s, 3H, C-27; 1.03, d, $J = 5.2$, 3H, C-21; 0.56, s, 3H, C-18; FT-IR: 1784 cm^{-1} (lactone C = O); UV: $\lambda_{\text{max}} = 265\text{ nm}$, $\lambda_{\text{min}} = 228\text{ nm}$.
- 15
- VID: mass spectrum, m/e 428.2927 (calcd. 428.2927), 26%, M^+ ; 410, 1%, $M^+ - H_2O$; 395, 11%, $M^+ - H_2O - CH_3$; 271, 2%, M^+ -side chain; 253, 7%, M^+ -side chain- H_2O ; 136, 100%, A ring + $C_6 + C_7^+$; 118, 86%, A ring + $C_6 + C_7^+ - H_2O$; NMR: δ 6.28, d, $J = 11.8$, 1H, C-6; 6.03, d, $J = 11.0$, 1H, C-7; 5.05, m (sharp), 1H, C-19(E); 4.82, m (sharp), C-19(Z); 4.47 m, 1H, C-23; 3.96, m, 1H, C-3; 1.50, s, 3H, C-27; 1.03, d, $J = 5.5$, 3H, C-21; 0.56, s, 3H, C-18; FT-IR: 1784 cm^{-1} (lactone C = O); UV: $\lambda_{\text{max}} = 265\text{ nm}$, $\lambda_{\text{min}} = 285\text{ nm}$.
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- 25

- 21 -

Example 6

Methyl 24-nor-5-cholen-23-oate (0.7 g) is reacted with 2 ml dihydropyran and 0.2 ml phosphorus oxychloride in 15 ml CH_2Cl_2 at room temperature for 40 min; 50 ml Et_2O is added and the mixture extracted with 2 x 25 ml saturated NaHCO_3 and 1 x 25 ml saturated NaCl . The Et_2O phase is evaporated to yield the crude tetrahydropyranyl derivative (compound VII of Process Schematic 2, where R_1 = tetrahydropyranyl (THP) and R' = Me).

To a slurry of 0.3 g LAH in 15 ml Et_2O is added crude VII dissolved in 15 ml Et_2O at -78°C . At 30 min after final addition, the reaction is warmed to 0°C and 10% NaOH in H_2O is slowly added with stirring until all flocculant material is white. The mixture is extracted with 100 ml Et_2O vs 3 x 50 ml H_2O , dried with MgSO_4 and concentrated to yield crude 23-alcohol.

To a solution of 12 molar excess pyridine in 30 ml CH_2Cl_2 on ice is added a 6 molar excess of CrO_3 . The mixture is stirred for 30 min at which time crude 23-alcohol obtained as above in 20 ml CH_2Cl_2 is added. At 15 min the reaction is extracted 3 x 25 ml 50% NaHCO_3 , dried with MgSO_4 and applied to a 2 x 36 cm silica column eluted with 5% EtOAc /hexane to recover 0.63 g of 23-aldehyde, represented by structure VIII of Process Schematic 2 where R_1 = THP (78.6% yield from VII). Mass spectrum: m/e 428, 0.5%, M^+ ; 326, 80%, $\text{M}^+ - \text{HOTHP}$; 298, 22%, $\text{M}^+ - \text{HOTHP} - \text{CO}$; 85, 100%, $\text{C}_5\text{H}_9\text{O}^+$.

Example 7

n-Butyl lithium (0.672 mmol) is slowly added to a solution of 0.672 mmol of diisopropyl amine in 5 ml Et_2O at -78°C ; 20 min after addition, 0.672 mmol of acetonecyclohexylimine is added and after 15 min, 250 mg of aldehyde VIII (R_1 = THP) is slowly added in 10 ml Et_2O . After 30 min, the reaction is warmed to 0°C and 10 ml H_2O is added and the mixture is stirred for 10 min. An additional 30 ml H_2O is then added and the mixture is extracted 3 times with 30 ml of diethyl ether.

The ether phase is dried with MgSO_4 and applied to four

- 22 -

20 x 20 cm x 750 μ m silica TLC plates eluted with 25% EtOAc/hexane to give 60 mg of hydroxyketone IX (R_1 = THP) (21% yield from VIII) as the two possible C-23-hydroxy stereoisomers. Mass spectrum: m/e, 486, 0.5%, M^+ ; 384, 35%, M^+ -HOTHP; 366, 21%, M^+ -HOTHP- H_2O ; 326, 68%, M^+ -HOTHP- C_3H_6O ; 85, 100%, $C_5H_9O^+$.

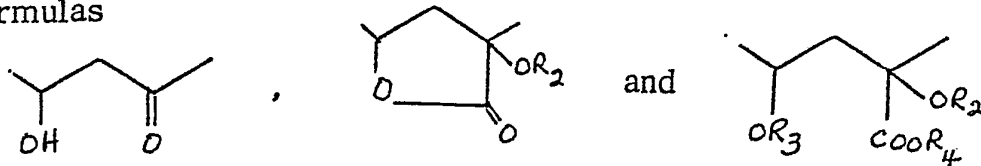
Example 8

To a solution of 37 mg IX (R_1 = THP) in 1 ml EtOH is added 0.250 ml of acetonecyanohydrin. The mixture is reacted at room temperature for 12 hr at which time 0.32 g of KOH is added in 4 ml 1:1 H_2O :EtOH. The temperature is raised to 50°C for 1 hr and enough 6 N HCl is then slowly added to bring the pH to ca. 1.0. The resulting mixture is stirred for 30 min at room temperature, then 30 ml H_2O is added and extracted 3 times with 30 ml of CH_2Cl_2 . The CH_2Cl_2 phase is evaporated to yield after preliminary purification 18.6 mg of lactone X (R_1 = H), representing a mixture of the four possible C-23 and C-25 stereoisomers (yield 57% from 5); mass spectrum: m/e 430, 88%, M^+ ; 412, 100%, M^+ - H_2O ; 397, 47%, M^+ - H_2O - CH_3 ; 345, 30%; 319, 45%; 213, 93%. The four lactone stereoisomers can be separated by HPLC chromatography on silica gel (semi-preparative Zorbax-Sil column 0.62 x 25 cm) using 4.5 % 2-propanol in hexane as eluting solvent.

Lactone X can be converted to 7-dehydrolactone IV by established procedures. Thus acetylation of X as obtained above with pyridine acetic anhydride yields the corresponding acetate which is subjected to allylic bromination (following well-known conditions dibromodimethylhydantoin) and then dehydrobrominated with trimethyl phosphite or collidine to yield the 5,7-diene lactone IV (R = acetyl) as a mixture of C-23 and C-25 epimers. These four epimers are separated as described in Example 3 to yield each stereoisomer, i.e. IVA, IVB, IVC and IVD in pure form.

- 23 -

As is evident from the foregoing specification, the structural formulas



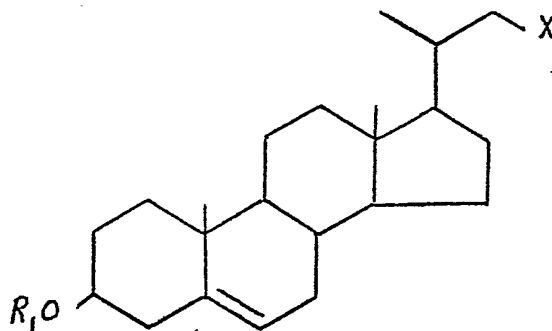
wherever they appear therein or in the appended claims are indicative of all their isomeric forms.

Having thus described the invention what is claimed is:

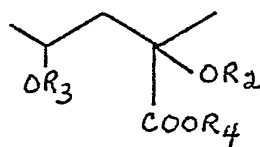
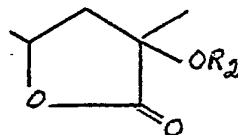
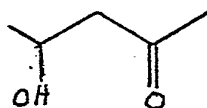
- 24 -

Claims

1. A process for preparing 25-hydroxyvitamin D₃-26,23-lactone, which comprises subjecting 3 β ,25-dihydroxy-26-norcholesta-5,7-diene-25-one to cyanohydrin formation, hydrolysis, and subsequent treatment with acid, to obtain 3 β ,25-hydroxy-cholesta-5,7-diene-26,23-lactone, subjecting said lactone to actinic irradiation to obtain 25-hydroxy-previtamin D₃-26,23-lactone, and isomerizing said product by heating and recovering the desired 25-hydroxyvitamin D₃-26,23-lactone product.
2. Compounds having the formula



where X is selected from



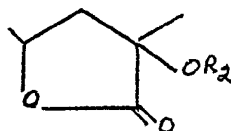
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R_1 , R_2 and R_3 are selected from the group consisting of hydrogen, acyl, alkylsilyl and tetrahydropyranyl and

R_4 is hydrogen or alkyl

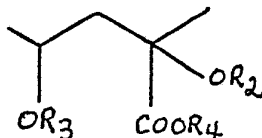
in all their isomeric forms.

3. Compounds according to Claim 2 wherein X is



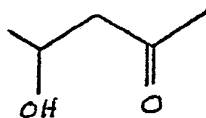
- 5 and each of R_1 and R_2 , which may be the same or different, is selected from the group consisting of hydrogen, acyl, alkylsilyl and tetrahydropyranyl in all their isomeric forms.

4. Compounds according to Claim 2 where X is



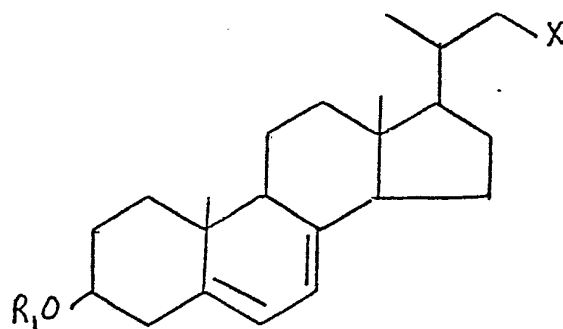
R_1 , R_2 and R_3 are hydrogen, and R_4 is hydrogen or alkyl.

- 10 5. The compounds of Claim 2 wherein X is



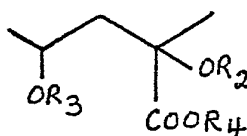
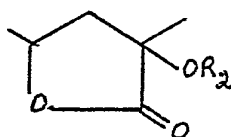
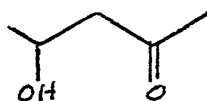
and R_1 is selected from hydrogen, acyl, alkylsilyl and tetrahydropyranyl.

6. Compounds having the formula



- 26 -

where X is selected from

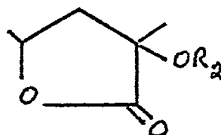


R_1 , R_2 and R_3 are selected from the group consisting of hydrogen, acyl, alkylsilyl and tetrahydropyranyl and

R_4 is hydrogen or alkyl

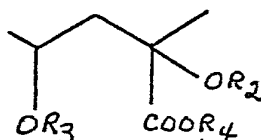
5 in all their isomeric forms.

7. Compounds according to Claim 6 wherein X is



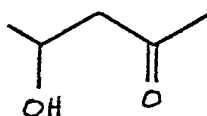
and each of R_1 and R_2 , which may be the same or different, is selected from the group consisting of hydrogen, acyl, alkylsilyl and tetrahydropyranyl in all their isomeric forms.

10 8. Compounds according to Claim 6 wherein X is



and R_1 , R_2 , R_3 and R_4 are hydrogen.

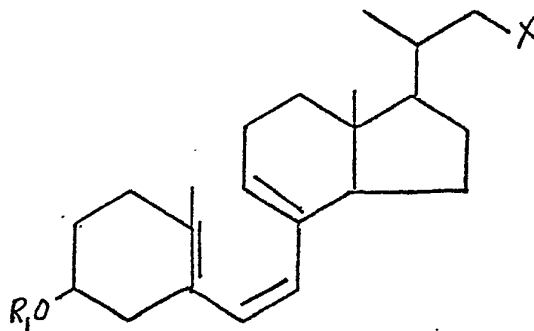
9. The compounds of Claim 6 wherein X is



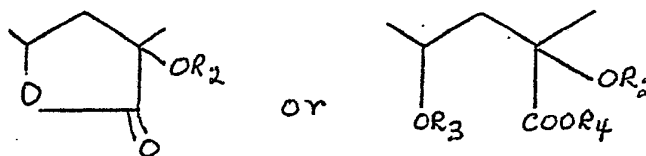
- 27 -

and R_1 is selected from hydrogen, acyl, alkylsilyl and tetrahydropyranyl.

10. Compounds having the formula



where X is selected from

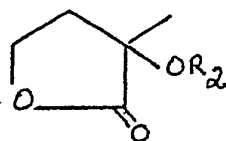


- 5 R_1 , R_2 and R_3 are selected from the group consisting of hydrogen, acyl, alkylsilyl and tetrahydropyranyl and

R_4 is hydrogen or alkyl

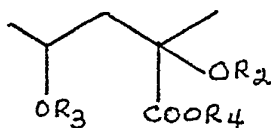
in all their isomeric forms.

11. Compounds according to Claim 10 wherein X is



- 10 and each of R_1 and R_2 , which may be the same or different, is selected from the group consisting of hydrogen, acyl, alkylsilyl and tetrahydropyranyl in all their isomeric forms.

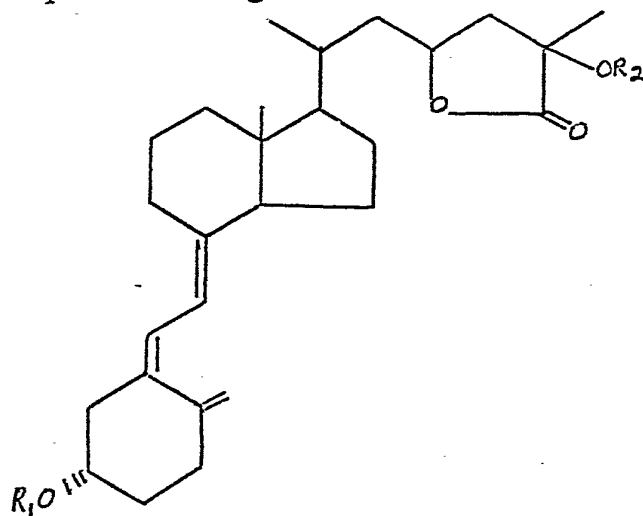
12. Compounds according to Claim 10 wherein X is



R_1 , R_2 and R_3 are hydrogen and R_4 is hydrogen or alkyl.

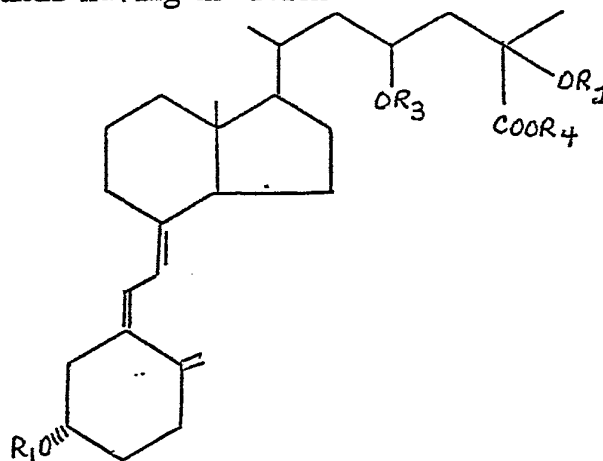
- 28 -

13. The compounds having the formula



wherein each of R_1 and R_2 , which may be the same or different, is selected from the group consisting of hydrogen, acyl, alkylsilyl and tetrahydropyranyl, except that, when the stereochemical configuration at carbon-23 and 25 is as it occurs in 25-hydroxyvitamin D_3 -26,23-lactone derived from natural sources, R_1 and R_2 may not both be hydrogen.

14. Compounds having the formula



where R_1 , R_2 and R_3 are selected from the group consisting of hydrogen, acyl, alkylsilyl and tetrahydropyranyl and R_4 is hydrogen or alkyl.

15. The compounds of Claim 14 wherein R_1 , R_2 , R_3 and R_4 are hydrogen.
16. The compounds of Claim 14 wherein R_1 , R_2 and R_3 are hydrogen and R_4 is alkyl.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US/00961

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ³		
According to International Patent Classification (IPC) or to both National Classification and IPC		
Int. Cl. ³	C07J 9/00	A61K 31/59
U.S. Cl.	260/397.2	424/236
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁴		
Classification System	Classification Symbols	
U.S.	260/397.2; 424/236	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁵		
III. DOCUMENTS CONSIDERED TO BE RELEVANT ¹⁴		
Category ⁶	Citation of Document, ¹⁶ with indication, where appropriate, of the relevant passages ¹⁷	Relevant to Claim No. ¹⁸
A	USA, 4,026,882 Published May 31, 1977 by Baggiolini et al.	
A	USA, 4,199,577 Published April 22, 1980 by Takeshita et al.	
A, P	USA, 4,268,453 Published May 19, 1981 by Barner et al.	
<p>* Special categories of cited documents: ¹⁵</p> <p>"A" document defining the general state of the art</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document cited for special reason other than those referred to in the other categories</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but on or after the priority date claimed</p> <p>"T" later document published on or after the international filing date or priority date and not in conflict with the application, but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance</p>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search ²		Date of Mailing of this International Search Report ²
30 September 1981		23 OCT 1981
International Searching Authority ¹		Signature of Authorized Officer ¹⁹
ISA/US		