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The Synthesis of Active Metabolites and Analogues of Vitamin D_3

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The literature data on the synthesis of the active metabolites and analogues of vitamin D_3 (cholecalciferol), which play an important role in regulating the homeostatis of calcium in the organism, are reviewed.
The bibliography includes 150 references.

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I. INTRODUCTION

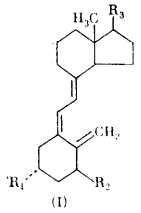
A rigorously defined concentration of calcium in the blood is essential for the normal functioning of the living organism. The factors responsible for maintaining a constant level of calcium are the parathyroid hormone, which mobilises calcium cations from the bones when the calcium concentration falls, as well as calcitonin, which lowers the level of calcium, when its concentration increases, by the calcification of the bone tissue. Both of these hormones maintain a constant level of calcium in the blood by drawing on the internal reserves of the organism[1]. A possible regulator of the homeostasis of calcium entering the organism is vitamin D_3 (cholecalciferol) (Ia), which is a natural and the most active vitamin of the D group.

Vitamins of the D group control the entry of exogenic calcium into the organism by absorbing it from the food in the intestine; in addition, they participate in the mobilisation of calcium from the bones and promote the calcification of the bone tissue and an increase of the concentration of phosphate in the blood. These functions are impaired in rickets, a common disease of a growing organism, and vitamins D are virtually the only radical anti-rickets preparations.

It was believed for a long time that vitamin D_3 acts in the organism without undergoing any changes. The difficulty of studying the mechanism of the action and metabolism of vitamin D_3 is due to the fact that the amounts of the vitamin to which animals respond are vanishingly small and extremely sensitive methods are required for their detection. It was not until the end of the 1960's, after the synthesis of radioactive preparations of vitamin D_3 with a high specific activity, the development of new effective thin-layer and column chromatographic methods, and the application of high-resolution mass spectrometric and NMR methods, that it was possible to investigate the transformations of vitamin D_3 in the organism and to elucidate the mechanism of the action of its metabolites.

In 1966 American investigators[2,3] detected the first metabolite of vitamin D_3, which was isolated and identified as 25-hydroxycholecalciferol (25-OHD_3) (Ib).[4,5] DeLuca and coworkers[6] established that 25-hydroxylation of vitamin D_3 takes place in the liver and that this results in the formation of a metabolite 1.4 times more active than vitamin D_3 in the treatment of rickets and acting three times as fast[7,8].

The synthesis of radioactive 25-OHD_3 made it possible to follow the further transformations of this metabolite in the organism. In 1971 three groups of investigators, headed by Kodicek[9], DeLuca[10,11], and Norman[12], isolated almost simultaneously a new metabolite—1α, 25-dihydroxycholecalciferol [1α, 25-$(OH)_2D_3$] (Ic). It is biosynthesised in the kidneys[13] and is not formed in nephrectomised animals[14]:



(I)

(a) D_3: R_1 = OH; R_2 = H; R_3 =

(b) 25-OHD_3: R_1 = OH; R_2 = H; R_3 =

(c) 1α,25-$(OH)_2D_3$: R_1 = OH; R_2 = OH; R_3 =

(d) 1α-OHD_3: R_1 = OH; R_2 = OH; R_3 =

(e) 3-deoxy-1α-OHD_3: R_1 = H; R_2 = OH; R_3 =

(f) 24*R*, 25-$(OH)_2D_3$: R_1 = OH; R_2 = H; R_3 =

(g) 25, 26-$(OH)_2D_3$: R_1 = OH; R_2 = H; R_3 =

(h) 1α, 24*R*, 25-$(OH)_3D_3$: R_1 = OH; R_2 = OH; R_3 =

The metabolite 1α, 25-$(OH)_2D_3$ is the most active hormonal form of vitamin D_3, being 10–15 times more active than the latter[1,15]. It acts very rapdily on all known systems, which are acted upon by vitamin D_3, stimulating the absorption of calcium in the intestine, the mobilisation of calcium from the bone, and the calcification of the bone tissue; it also increases the concentration of phosphate in blood serum. A very important property of 1α, 25-$(OH)_2D_3$ is that it induces all these processes also in the case of kidney diseases, when neither vitamin D_3 nor its other metabolites have any activity. 1α, 25-$(OH)_2D_3$ has therefore been used successfully

in the treatment of various bone diseases associated with kidney affections, such as osteomalacia, osteoporosis, etc. However, the complexity of the synthesis of 1α, 25-$(OH)_2D_3$ makes it so far relatively unavailable for wide-scale application. The search for a synthetic analogue of 1α, 25-$(OH)_2D_3$ led DeLuca to the synthesis in 1973 [16] of 1α-hydroxycholecalciferol (1α-OHD_3) (Id) which was shown by extensive studies of its biological activity[16-20] to be capable of replacing 1α, 25-$(OH)_2D_3$ in therapeutic practice[21,22].

3-Deoxy-1α-hydroxycholecalciferol (3-deoxy-1α-OHD_3) (Ie), synthesised in 1974 in the laboratories of Norman[23] and DeLuca[24,25], also proved to be an analogue of 1α, 25-$(OH)_2D_3$ with a high biological activity. It stimulates the transport of calcium in the intestine almost as actively as 1α, 25-$(OH)_2D_3$,[23,24,26] but its effect on the bone system is less pronounced. This specific property makes this compound extremely important in the treatment of bone diseases in those cases where it is necessary to stimulate the transport of calcium in the intestine without causing its mobilisation from the bone.

Several other metabolites, in addition to 25-OHD_3, and 1α, 25-$(OH)_2D_3$, were isolated in the study of the metabolism of vitamin D_3. In 1970 DeLuca and coworkers[27,28] isolated 24, 25-dihydroxycholecalciferol [24, 25-$(OH)_2D_3$] (If), which is formed when animals are on a normal or a hypercalcemic diet[29]. The synthesis of two stereoisomers and the study of the biological activity of 24*R*- and 24*S*-25-$(OH)_2D_3$ showed that the 24*R*-isomer is formed in the organism and is almost as active as 25-OHD_3.[30,31]

25,26-Dihydroxycholecalciferol [25,26-$(OH)_2D_3$] (Ig) [32] and 1α, 24,25-trihydroxycholecalciferol [1α, 24,25-$(OH)_3D_3$] (Ih) [33] were isolated from the blood plasma of animals administered radioactive vitamin D_3. These metabolites are active in the transport of calcium in the intestine, but are relatively ineffective in the treatment of rickets and in the mobilisation of calcium from bone[33-37]. However, one cannot rule out the possibility that, on being formed in the organism, these metabolites play an important physiological role †.

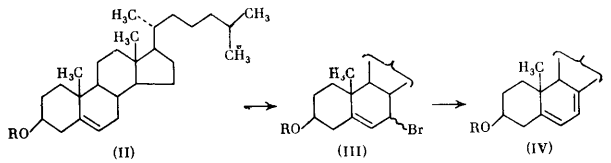
The high activity of vitamin D_3 metabolites not only in the treatment of rickets but also in other severe diseases associated with the breakdown of the homeostasis of calcium in the organism led chemists in many countries to join in the search for a solution to the problem of the chemical synthesis of these compounds. During the last few years, different versions of complex chemical syntheses of active metabolites of vitamin D_3 and some of its active analogues were therefore proposed.

The biochemical synthesis of the metabolites in the organism takes place with the aid of the corresponding enzyme system in the liver or in the kidneys and proceeds in such a way that direct transition from the molecule of vitamin D_3 to that of its hydroxy-derivative is acheived. Chemical syntheses of the active metabolites and analogues of vitamin D_3 involve complex multistage transformations of the entire structure of the steroid molecule of the initial substance and are therefore in most instances mainly of theoretical interest. Practical applications of these important biologically active compounds have unfortunately not been found so far.

† The relation between the structure and biological activity of vitamins D, their metabolites, and synthetic analogues was described in greater detail in a monograph by the author of the present review[38].

II. THE SYNTHESIS OF CHOLECALCIFEROL

The synthesis of vitamin D_3, achieved as early as 1936 by Windaus and coworkers[39,40], includes two main stages: the formation of the provitamin and its photochemical isomerisation. A distinctive feature of provitamins D is the presence of two conjugated double bonds at the ring $C_{(5)}-C_{(6)}-C_{(7)}-C_{(8)}$ atoms of the steroid molecule. 7-Dehydrochloesterol, which is a provitamin of cholecalciferol, is cholesterol (IIa) dehydrogenated at $C_{(7)}-C_{(8)}$. The introduction of an additional double bond into the cholesterol molecule is achieved by different methods: by the oxidisation of a cholesterol ester to the 7-oxo-derivative and then by reduction, benzoylation, and elimination of benzoic acid from 7-hydroxycholesterol 3,7-dibenzoate[41-42]; the formation of the tosylhydrazone from the 7-oxo-derivative by the Bamford–Stevens reaction has been described. It is subsequently converted into the provitamin on interaction with LiH.[43] The method involving the allylic bromination of the cholesterol ester (IIb) with subsequent dehydrobromination of the 7-bromo-derivative (III) and the hydrolysis of the 7-dehydrocholesterol ester (IVb) to 7-dehydrocholesterol (IVa) is most widely used[44]:

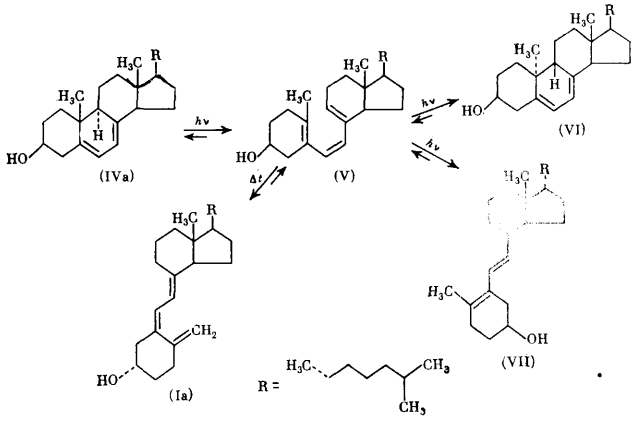


The allylic bromination of cholesterol esters (usually the benzoates) is carried out with 1,3-dibromo-5,5-dimethylhydantoin in a benzene-petroleum ether mixture[45] or in CCl_4 in the presence of radical initiators[46,47]. The yield of compound (III) reaches 92–94%.[47] Depending on the dehydrobrominating agents and the reaction conditions, the dehydrobromination of (III) can lead to the formation, apart from the required $\Delta^{5,7}$-diene (IVb), also of other cholestadienes (the $\Delta^{4,6}$-, $\Delta^{5,8(9)}$-, $\Delta^{6,8(14)}$-, and $\Delta^{8,14}$- derivatives) and cholestatrienes (the $\Delta^{2,4,6}$- and $\Delta^{4,6,8(14)}$- derivatives). The maximum yield of the $\Delta^{5,7}$-diene (up to 60%) was obtained on dehydrobromination in boiling xylene with various organophosphorus compounds (trialkyl phosphites and dioxaphospholans)[45,48-50] as well as $NaHCO_3$ in the presence of catalytic amounts of α-picoline[46,51]. The main side product under these conditions is the $\Delta^{4,6}$-diene.

Provitamins derived from cholecalciferol are usually synthesised in accordance with the above scheme, but in certain cases it is impossible to introduce the double bond at $C_{(7)}-C_{(8)}$ by the methods described or the yield of provitamins is very low[23,52,53]. Certain new methods for the introduction of conjugated double bonds into the B ring, which will be considered directly in connection with the synthesis of hydroxylated cholecalciferols, have therefore been proposed[54-56].

The photochemical isomerisation of provitamins D is accompanied by the dissociation of the $C_{(9)}-C_{(10)}$ bond and the opening of the B ring with the formation of the direct precursor of vitamins D—previtamin D (V). When the previtamin is irradiated with ultraviolet light, two photoisomers are formed—lumisterol (VI) and tachysterol (VII), while thermal isomerisation leads to the formation of vitamins D (I). The mechanism of these reversible transformations, which is nowadays generally accepted, was established by the studies of Velluz and coworkers[57] and Havinga and coworkers[58-60] over a period of many years.

The photochemical isomerisation of provitamins D is usually carried out in solution in ethanol, ether, benzene, and other solvents up to not more than 50% conversion, in order to reduce the accumulation of side products—tachysterol and lumisterol. The yield of the previtamin is a maximum when unfiltered ultraviolet light at $\lambda = 295$ nm [61] or erythemal lamps E-3 phosphor (transmission maximum at 302–305 nm) [62] are used:



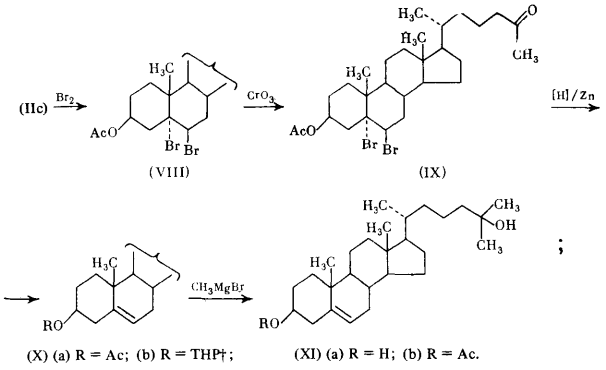
III. THE SYNTHESIS OF 25-HYDROXYCHOLECALCIFEROL

The chemical synthesis of 25-hydroxycholecalciferol was achieved by Blunt and DeLuca[63,64] to confirm the structure of the active vitamin D_3 metabolite isolated from blood plasma, and Haltes and van Vliet[65] obtained for the first time crystalline 25-OHD_3 in the form of the monohydrate.

The main difficulty in the synthesis of 25-OHD_3 is the formation of the key compound—25-hydroxycholesterol. Numerous studies on the synthesis of this compound, differing mainly in the starting material, have therefore been published in recent years. 25-Hydroxycholesterol is converted into 25-OHD_3 as described in Section II.

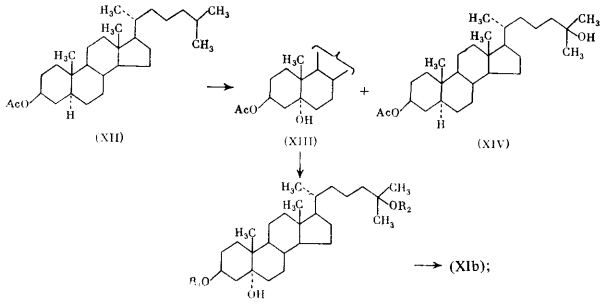
1. Synthesis from Cholesterol and Cholestanol

25-Hydroxycholesterol is formed in a small amount in the autoxidation or photolysis of cholesterol together with other hydroxylated derivatives[66,67]. DeLuca and coworkers[63,64] and Halkes and van Vliet[65] synthesised 25-hydroxycholesterol from cholesterol by the method described as early as 1950.[68–70] The bromination of cholesteryl acetate (IIc), the oxidation of the dibromide (VIII) with CrO_3 in acetic acid to the 27-nor-25-oxo-derivative (IX), and treatment of the latter with zinc dust in acetic acid lead to 25-oxo-27-norcholest-5-en-3β-yl acetate (Xa). The reaction with methylmagnesium bromide yields the 3β-acetate of 25-hydroxycholesterol (XIb). However, the yields of (Xa) and (XIb) are very insignificant.



(X) (a) R = Ac; (b) R = THP†; (XI) (a) R = H; (b) R = Ac.

On irradiating cholestan-3β-yl acetate (XII) ultraviolet light at $\lambda = 300$ nm in acetic acid + hydrogen peroxide solution, Rotman and Mazur[71] obtained 37.5% yield of the 25-hydroxy-derivative (XIV) and a 30% yield of the 5α-hydroxy-derivative (XIII). On further irradiation of compound (XIII), the 3β-acetate of cholestane-3β, 5α, 25-triol (XVa) is formed, giving rise to the 3β-acetate (XIb) after acetylation and treatment of the diacetate (XVb) with a mixture of acetic acid and toluene-*p*-sulphonic acid and hydrolysis:



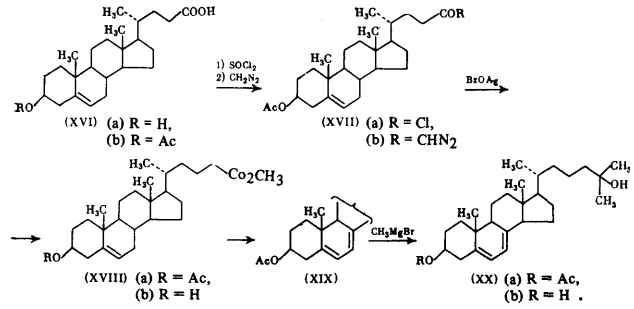
(XV) (a) R_1 = Ac, R_2 = OH; (b) $R_1 = R_2$ = Ac.

2. Synthesis from 3β-Hydroxychol-5-enoic Acid

3β-Hydroxychol-5-enoic acid (XVIa), which is a relatively readily available substance formed on oxidation of the cholesterol side chain, has been used[72,73] to synthesise the provitamin 25-OHD_3. The acid (XVIa) was converted via the 3β-acetate (XVIB) and the acid chloride (XVIIa) into the diazoketone (XVIIb) and then into the 25-homoester (XVIIIa) on interaction with silver benzoate. After these transformations in the side chain, the 5,7-diene (XIX) was obtained by the usual method involving allylic bromination and dehydrobromination. The synthesis of the provitamin–cholesta-5,7-diene-3β, 25-diol (XXb)—is completed by the reaction

† THP = tetrahydropyranyl ether.

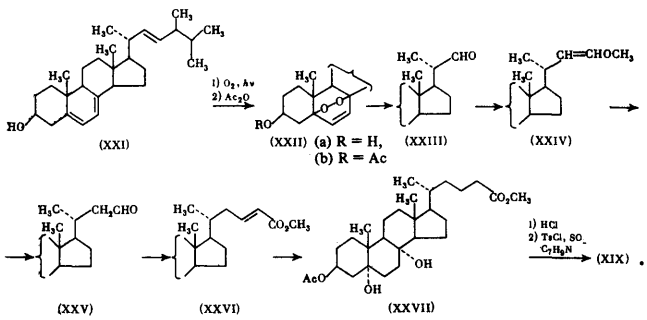
with methylmagnesium bromide and hydrolysis:



(XVI) (a) R = H, (b) R = Ac; (XVII) (a) R = Cl, (b) R = CHN_2; (XVIII) (a) R = Ac, (b) R = H; (XIX); (XX) (a) R = Ac, (b) R = H.

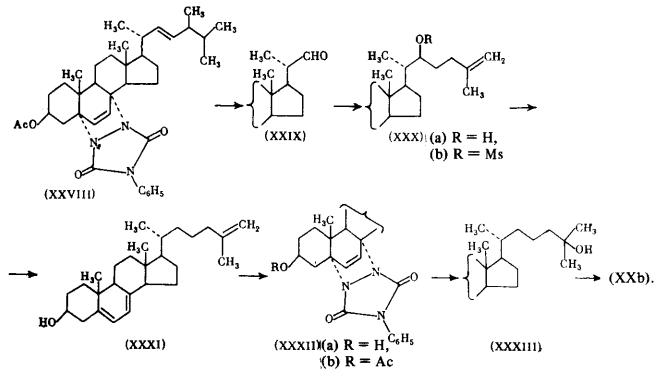
3. Synthesis from Ergosterol

The 5,7-diene (XIX) has been obtained[74-77] by the transformation of the ergosterol side chain. The photosensitised addition of oxygen to ergosterol (XXI) leads to the peroxide (XXIIa), while the cleavage of the double bond in the side chain by the ozonolysis of the acetate (XXIb) yields the aldehyde (XXIII). The growth of the side chain was achieved by condensation with triphenylmethoxymethylenephosphorane to the methyl ether (XXIV), which gives rise to the aldehyde (XXV) after hydrolysis with formic acid. On treatment with triphenylmethoxycarbonylmethylenephosphorane, the aldehyde is converted into a mixture of the *trans*- and *cis*-isomers of the methyl esters of 3β-acetoxy-5,8-epidihydroxyhomochola-6,23-diene-25-ioc acid (XXVI). The methyl ester of the *trans*-acid was hydrogenated in the presence of palladium and the dehydration leading to the formation of compound (XIX) was carried out without isolating the triol acetate (XXVII). The conversion of the ester (XIX) into 25-OHD_3 was described above[72,73]:



(XXI); (XXII) (a) R = H, (b) R = Ac; (XXIII); (XXIV); (XXV); (XXVI); (XXVII); (XIX).

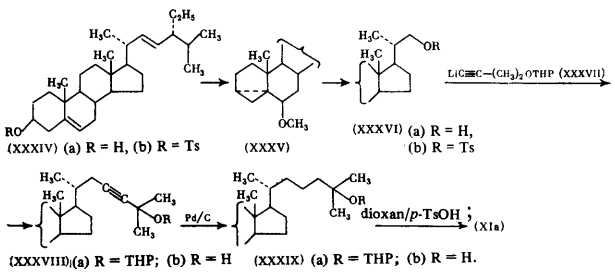
A more convenient pathway from ergosterol to the 25-hydroxy-provitamin (XXb) has been proposed by Eyley and Williams[56,78]. The adduct of the ergosteryl acetate and 4-phenyl-1,2,4-triazoline-3,5-dione (XXVIII) was obtained by the method of Barton et al.[79] and was converted into the aldehyde (XXIX) by ozonolysis. The reaction of compound (XXIX) with the Grignard reagent obtained from 4-chloro-2-methylbut-1-ene in tetrahydrofuran (THF) at 30 °C gave the alcohol (XXXa), which gave rise to the trienol (XXXI) after conversion into the methanesulphonate (XXXb) and reduction with $LiAlH_4$. The 5,7-double bonds were again protected via the adduct (XXXIIa) and the acetylation of the latter, the oxidation of the diene (XXXIIb) with $Hg(OAc)_2$ to the 25-hydroxy-derivative (XXXIII), and reduction with $LiAlH_4$ gave compound (XXb):



(XXVIII); (XXIX); (XXX) (a) R = H, (b) R = Ms; (XXXI); (XXXII) (a) R = H, (b) R = Ac; (XXXIII); (XXb).

4. Synthesis from Stigmasterol

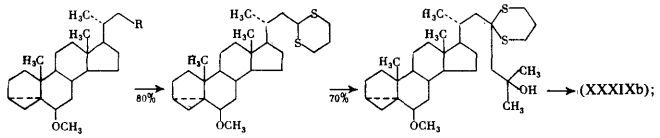
The synthesis of compound (XIa) from stigmasterol (XXXIVa), which is a fairly readily available plant sterol obtained from soya beans, is also based on the cleavage of the double bond in the side chain. Partridge and coworkers[80,81] achieved this cleavage in the following way. The 5,6-double bonds of stigmasterol were initially protected by the formation of the *i*-methyl ester (XXXV), which is achieved by treating the toluene-*p*-sulphonate (XXXIVb) with methanol and pyridine. The *i*-methyl ester (XXXV) was ozonised at -78 °C and then reduced with sodium bis-(2-methyoxyethoxy)aluminium hydride to the alcohol (XXXVIa), which was converted into the crystalline toluene-*p*-sulphonate (XXXVIb). Alkylation of the latter with lithium acetylide (XXXVII) (obtained from n-butyl-lithium and 3-methyl-1-but-3-ynyl tetrahydropyranyl ether) gave a 90% yield of compound (XXXVIIIa). The acetylenic bond in (XXXVIIIa) was hydrogenated over PtO_2 or Pd/C and compound (XXXIXa) was isolated in quantitative yield. The authors then propose several versions of the synthesis of compound (XIa). One of these involves the conversion of compound (XXXIXa) into the alcohol (XXXIXb), which, on treatment with aqueous dioxan in the presence of catalytic amounts of toluene-*p*-sulphonic acid, gave compound (XIa). The overall yield of compound (XIa) was 30% with respect to stigmasterol.



(XXXIV) (a) R = H, (b) R = Ts; (XXXV); (XXXVI) (a) R = H, (b) R = Ts; (XXXVIII) (a) R = THP; (b) R = H; (XXXIX) (a) R = THP; (b) R = H; (XIa).

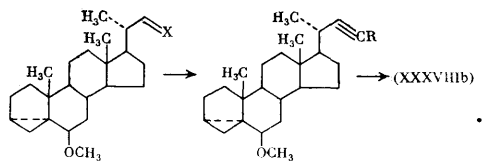
By the reaction of the bromide (XLa) or the iodide (XLb), obtained by Partridge et al.[80] from the alcohol (XXXVIa), with 2-lithiodithian, Salmond and Maisto[82] obtained the dithioacetal (XLI), which gives rise to the alcohol (XLII) after treatment with n-butyl-lithium and subsequent condensation with 1,2-epoxy-2-methylpropanol. When the alcohol (XLII) is refluxed with $LiAlH_4$ + $TiCl_4$ in THF, compound (XXXIXb)

is obtained:



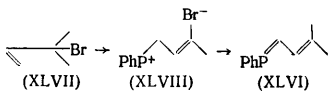
(XL) (a) R = Br; (b) R = I. (XLI) (XLII)

Salmond et al.[83] also used the aldehyde (XLIIIa) [obtained by the ozonolysis of compound (XXXV)], which, on reaction with dibromomethylenetriphenylphosphorane, gives a high yield of 20*R*-dibromovinylpregnane (XLIIIb). Treatment of the dibromo-olefin with n-butyl-lithium at -68 °C leads to the lithio-derivative (XLIV), from which 20*R*-ethynylpregnane (XLV) was obtained by hydrolysis. Its interaction with 1,2-epoxy-2-propanol in the presence of hexamethylphosphoramide results in the formation of the alcohol (XXXVIIIb) in 70% yield. The hydrogenation of the acetylenic bond over PtO_2 results in the formation of the alcohol (XXXIXb):



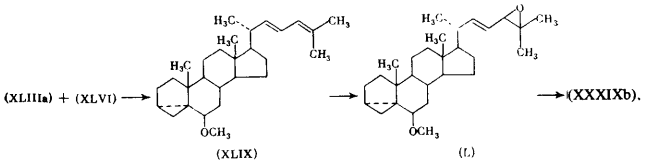
(XLIII) (a) X = O, (XLIV) R = Li
(b) X = CBr_2 (XLV) R = H

Salmond and Sobala[84] recently proposed a still simpler procedure for the formation of compound (XXXIXb) from stigmasterol with an overall yield in excess of 50%. Although the total number of stages was 7, as in the work of Partridge et al.[80], it was necessary to isolate and crystallise the reaction products only three times, which greatly simplifies the synthesis. The first three stages, namely the conversion of stigmasterol into the toluene-*p*-sulphonate (XXXIVb) and the *i*-methyl ester (XXXV) and ozonisation to the aldehyde (XLIIIa), were described above. In order to introduce the side chain, the diene (XLVI) was synthesised from the bromide (XLVII) via the salt (XLVIII):



(XLVII) (XLVIII) (XLVI)

The condensation of the aldehyde (XLIIIa) with (XLVI) yields the diene (XLIX):

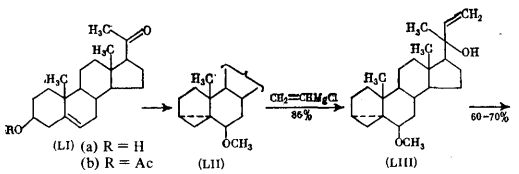


(XLIX) (L)

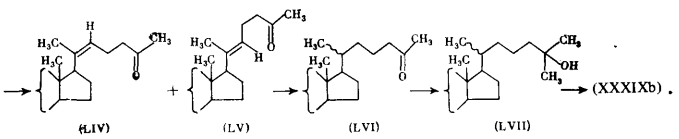
Next the Δ^{24}-double bond was selectively epoxidised with peracetic acid and the epoxide (L) was obtained; it was reduced over Pd/C to compound (XXXIXb) in 56% yield with respect to (XXXIVb). When the *i*-ester (XXXIXb) is refluxed in acetic acid, compound (XIb) is formed in 94% yield.

5. Synthesis from Pregnenolone

Narwid and coworkers[85,86] used a commercial product—pregnenolone (LIa)—and its acetate (LIb) to synthesise 25-hydroxycholesterol. As in the case of stigmasterol, the 5,6-double bonds in compound (LIa) were first protected by conversion into the *i*-steroid (LII), which gives rise to the allyl alcohol (LIII) after reaction with vinylmagnesium chloride. Next the side chain was lengthened to 7 carbon atoms by treating the alcohol (LIII) with an excess of diketen, giving a 1 : 2 mixture of the *cis*- and *trans*-oxo-isomers (LIV) and (LV), which was reduced over PtO_2 to a mixture of saturated ketones (LVI). After treating the latter with CH_3MgI, a mixture of alcohols (LVII) was obtained. The natural 20*R*-isomer (XXXIXb) was separated by crystallisation and obtained in 26% yield, from the alcohol (LIII). The overall yield of 25-hydroxycholesterol with respect to pregnenolone was 17%:

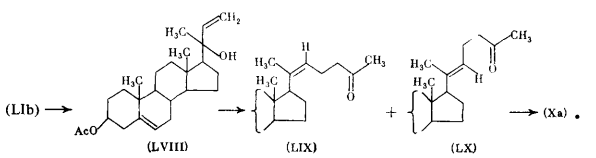


(LI) (a) R = H
(b) R = Ac (LII) (LIII)



(LIV) (LV) (LVI) (LVII)

Compound (XIa) can be synthesised from the acetate of pregnenolone (LIb) without the preliminary protection of the 5,6-double bond. For this purpose, the alcohol (LVIII) was obtained from the acetate (LIb) and vinylmagnesium chloride[85]. The side chain was lengthened by interaction with diketen. A mixture of the *cis*- and *trans*-keto-olefins (LIX) and (LX) was obtained with an overall yield of 72%. Catalytic hydrogenation of this mixture over PtO_2 leads to the 20*R*-ketone (Xa). (Xa) was converted into (XIa) in 88% yield by the Grignard reaction with subsequent hydrolysis of the ester. The overall yield of compound (XI) with respect to compound (LIb) was 25–28%:



(LVIII) (LIX) (LX)

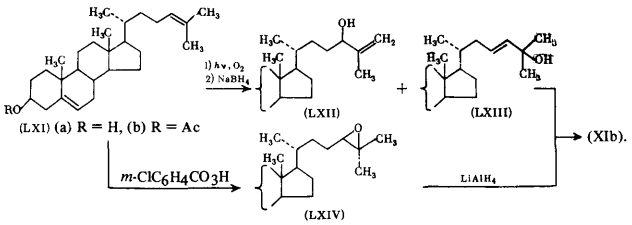
6. Synthesis from 3β-Hydroxyandrost-5-en-17-one

The stereospecific synthesis of 25-hydroxycholecalciferol from 3β-hydroxyandrost-5-en-17-one has been described[87]. Although the overall yield is 42%, the authors carried out more than 10 transformations in order to introduce the cholesterol side chain with the 25-hydroxy-group.

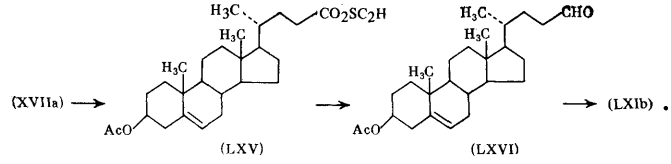
7. Synthesis from Desmosterol

A series of investigations by Japanese workers on the synthesis of active forms of vitamin D was published in 1973[88-92]. Desmosterol (LXIa), the structure of which is very convenient for the introduction of a hydroxy-group at $C_{(25)}$ by various methods, was chosen as the starting material.

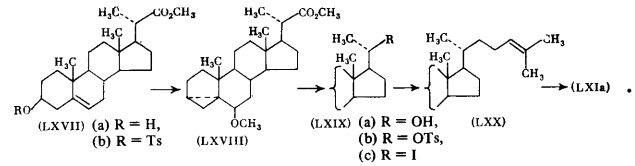
When a solution of desmosteryl acetate (LXIb) [88] was irradiated with sunlight in isopropyl alcohol in the presence of a sensitiser and the unpurified product was reduced with $NaBH_4$, two substances were obtained—the 3β-acetates of cholesta-5, 25-diene-3β,24-diol (LXII) (25%) and cholesta-5,23-diene-3β, 25-diol (LXIII) (35%). The catalytic hydrogenation of the latter over Pd/C gives compound (XIb) in 60% yield. Another way of obtaining compound (XIb) from (LXIb) involves the selective epoxidation of the double bond at $C_{(24)}$–$C_{(25)}$ in *m*-chloroperbenzoic acid with formation of the 24,25-monoepoxide (LXIV), which is then reduced with $LiAlH_4$ to compound (XIb) with an overall yield of 50%. Finally, the third and most convenient method of synthesis of compound (XI) involves the treatment of compound (LXIb) with $Hg(OAc)_2$ or HgBr followed by reduction with $NaBH_4$. After chromatographic purification on silica gel, compound (XIb) is obtained in 85% yield. This is apparently the simplest and most effective method of synthesising 25-hydroxycholesterol among all those described.



Since desmosterol proved to be a very convenient starting material for the synthesis not only of 25-OHD_3 but also of other active vitamin D_3 metabolites, numerous studies have now been carried out on its preparation[94–99]. The Wittig synthesis of desmosterol from the acetate of 3β-hydroxycholest-5-enoic acid (XVIb) has been described[100]. The acid was converted into the chloride (XVIIa), the condensation of which with ethanethiol gave ethyl thiocholenoate (LXV), which was converted into the aldehyde (LXVI) by treatment with Raney nickel, and finally the reaction with the Wittig reagent gave compound (LXIb):

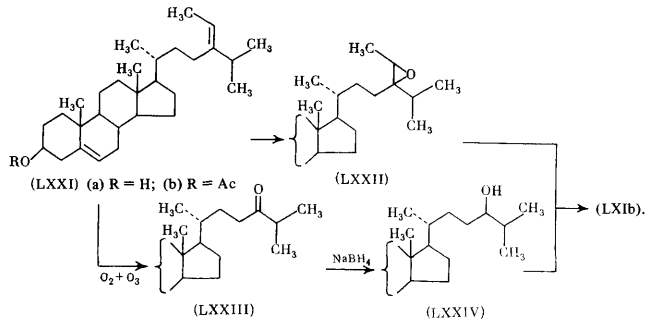


3β-Hydroxybisnorchol-5-enoic and 3β-hydroxynorchol-5-enoic acids can also serve as the starting materials for the synthesis of compound (LXIa).[94] The methyl ester (LXVIIa) was converted into the toluene-*p*-sulphonate (LXVIIb) and then into the *i*-ester (LXVIII), which was reduced with $LiAH_4$ and converted into the 22-toluene-*p*-sulphonate (LXIXb) without isolating the alcohol (LXIXa). The reaction of the toluene-*p*-sulphonate with NaI gave the corresponding iodide (LXIXc):



Next the iodide was converted by treatment with π-(dimethylallyl)nickel bromide into the desmosterol *i*-ester (LXX), whose treatment with $HClO_4$ gave desmosterol (LXIa) in an overall yield of 20%. Bergmann and Dusza[96] obtained demosterol from 25-oxocholesterol.

Japanese workers[97–99] developed several methods for the preparation of demosterol from fucosterol (LXXIa), the principal sterol in brown algae[101,102]. Fucosteryl acetate (LXXIb) was oxidised with *m*-chloroperbenzoic acid to the 24,28-epoxide (LXXII) and, after treatment of the latter with BF_3. .($SnCl_4$, $AlCl_3$), desmosteryl acetate was obtained in 30% yield. When the 24,28-epoxide was treated with silica gel, a zeolite, and other Lewis acids, the yield of compound (LXIb) was 40%.[99] Fucosteryl acetate was converted into compound (LXIb) via the ketone obtained by the ozonolysis of compound (LXXIb) with a $O_3 + O_2$ mixture in CH_2Cl_2 at -78 °C after its reduction to the alcohol (LXXIV) and dehydration of the latter latter by P_2O_5 in benzene:



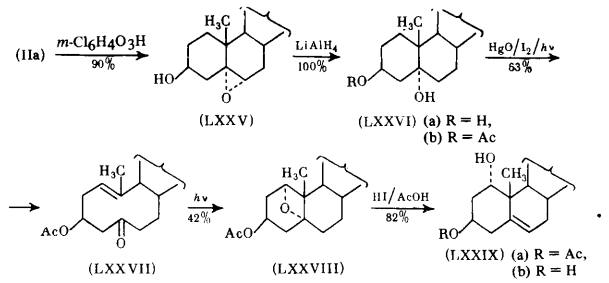
Other methods for the introduction of a hydroxy-group at $C_{(25)}$ are also known[103–105], but unfortunately they all involve many stages and give fairly low yields.

IV. THE SYNTHESIS OF 1α-HYDROXYCHOLECALCIFEROL

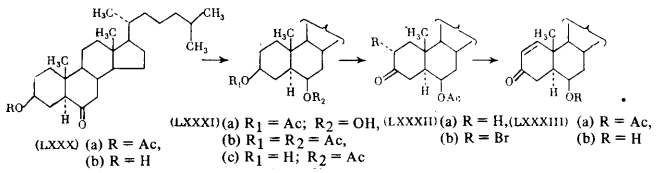
The high biological activity of 1α-hydroxycholecalciferol, comparable to that of 1α, 25-$(OH)_2D_3$,[16–22] and its simpler synthesis, involving the transformation of only the A ring, make this compound the most promising synthetic analogue of 1α, 25-$(OH)_2D_3$—the active hormonal form of vitamin D_3. During the last few years, numerous reports of the synthesis of 1α-OHD_3 have therefore been published.

As in the case of 25-OHD_3, the most complex feature of the synthesis of 1α-OHD_3 is the formation of the 1α-hydroxy-derivative of cholesterol, which is converted into 1α-OHD_3 by the usual methods. 1α-Hydroxycholesterol has been obtained[106] in a study of the photochemical cyclisation of 5,10-seco-steroids together with other products in a fairly low overall yield. At that time the authors' aim was not the development of a 1α-hydroxylation method, which is so necessary for the synthesis of 1α, 25-$(OH)_2D_3$ and 1α-OHD_3. In 1977 Mihailović et al.[107] improved the method of synthesis of 1α-hydroxycholesterol via the seco-derivative and proposed a fairly simple method with an overall yield of approximately 20%. Cholesterol (IIa) was oxidised with *m*-chloroperbenzoic acid to give a 90% yield of the 5α, 6α-epoxide (LXXV), which was reduced quantitatively with $LiAlH_4$ to the 3β, 5α-diol (LXXVIa) and the latter was converted into the 3β-acetate (LXXVIb). Treatment of the acetate with HgO, I_2, and ultraviolet light gave a 63% yield of the 3β-acetate of 5,10-seco-cholest-1(10)-en-5-one (LXXVI), whose photochemical cyclisation gives compound (LXXVIII)(42%). Treatment of compound (LXXVIII) with HI led to an 82% yield of the 3β-acetate of 1α-hydroxy-

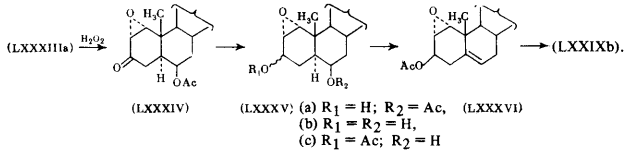
cholesterol (LXXIXa):



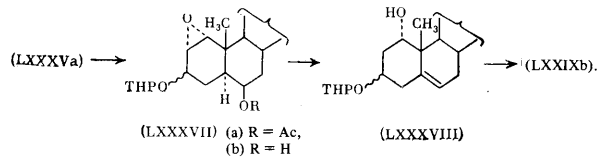
The first study on the specific synthesis of 1α-hydroxycholesterol (LXXIXb) was carried out in 1970 by Pelc and Kodicek[108], who detected, in the study of the metabolism of [1-^{3}H] vitamin D_3, a metabolite with a deficiency of radioactive hydrogen and more polar than 25-OHD_3, probably as a result of the incorporation of an additional oxygen atom at $C_{(1)}$;[109,110] an attempt was therefore made to synthesise the 1α-hydroxy-analogue of vitamin D_3. These workers began with 6-oxo-5α-cholestan-3β-yl acetate (LXXXa), which can be readily obtained from cholesteryl acetate by nitration and subsequent reduction of the nitro-compound and hydrolysis. The 6-oxo-derivative (LXXXa) was reduced with $NaBH_4$ to the 6β-hydroxy-derivative (LXXXIa) in a high yield and the latter was then acylated to the 3β,6β-diacetate (LXXXIb), which was converted by mild aklakine hydrolysis into the 6β-acetate (LXXXIc):



The oxidation of the 3β-hydroxy-group to the carbonyl group with sodium dichromate in acetic acid and then bromination of the ketone (LXXXIIa) with bromine and treatment of the 2α-bromo-derivative (LXXXIIb) with $CaCO_3$ in dimethylformamide (DMF) gave the 6β-acetate (LXXXIIIa). Hydrolysis of the 6β-acetate gave 6β-hydroxy-5α-cholest-1-en-3-one (LXXXIIIb). Pelc and Kodicek were unable to complete the synthesis of 1α-hydroxycholesterol[111], but the ketone (LXXXIIIb) which they synthesised was used by other investigators as the starting material for the preparation of compound (LXXIXb). By oxidising compound (LXXXIIIa) with 35% H_2O_2, Morisaki and coworkers[89,112] obtained in 1973 the 1α, 2α-epoxide (LXXXIV). The reduction of the keto-group with $NaBH_4$ led to a 4 : 1 mixture of the epimeric 3β- and 3α-alcohols (LXXXVa) and, after hydrolysis, a mixture of the epimeric diols (LXXXVb). Chromatographic separation and acetylation with half an equivalent of acetic anhydride led to the isolation of the 3β-acetate (LXXXVc) in an overall yield of 24% with respect to the initial compound (LXXXIIIa). The dehydration of the 3β-acetate (LXXXVc) with $POCl_3$ gives a 75% yield of the Δ^5-olefin (LXXXVI), whose reduction with $LiAlH_4$ leads to the formation of compound (LXXIXb):

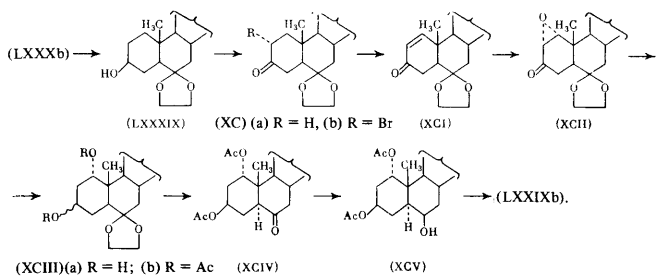


Furst et al.[113] carried out an analogous synthesis of compound (LXXIXb), but the starting material was the ketone (LXXXIIIb), the provitamin and 1α-OHD_3 being obtained in a crystalline form. The conversion of compound (LXXXVa) into (LXXIXb), without the isolation of intermediates and with an overall yield of 30% has been described[89]: compound (LXXXVa) was converted into the tetrahydropyranyl ether (LXXXVIIa), which was hydrolysed to compound (LXXXVIIb). Dehydration with $POCl_3$ and subsequent reduction with $LiAlH_4$ gave a mixture of the 3β- and 3α-tetrahydropyranyl ethers (LXXXVIII), whose hydrolysis led to compound (LXXIXb):



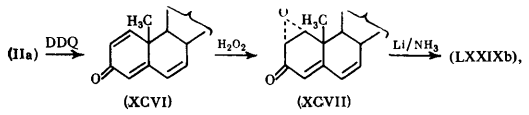
Yet another method of synthesis of 1α-OHD_3 was proposed by Morisaki et al.[114], but the overall yield of the final product was not more than 1%.

The method for the introduction of the 1α-hydroxy-group used by DeLuca and coworkers[16,115,116] in the synthesis of 1α-OHD_3 and 1α, 25-$(OH)_2D_3$ involves a fairly large number of transformations in the A and B rings and consists in the conversion of cholesterol into the 6-oxo-derivative (LXXXb), its treatment with diketen, and the formation of 6-ethylenedioxy-5α-cholestan-3β-ol (LXXXIX). The 3β-hydroxy-group is then oxidised to the carbonyl group [compound (XCa)] by CrO_3 in pyridine and the ketone is brominated with bromine to give the 2α-bromo-derivative (XCb). The dehydrobromination of the latter with *sym*-collidine gives the Δ^1-diene (XCI). The conversion of the diene (XCI) to the epoxide (XCII) and the reduction of the latter with $LiAlH_4$ lead to a mixture of the 3α- and 3β-epimers of the 1α, 3-diol (XCIIIa) and, after acetylation, to a mixture of the 1α, 3-diacetates (XCIIIb). Mild acid hydrolysis yields the ketone (XCIV), whose reduction with $NaBH_4$ gives rise to the 1α, 3-diacetate of the triol (XCV); the dehydration of the latter with $POCl_3$ and hydrolysis lead to 1α-hydroxycholesterol:

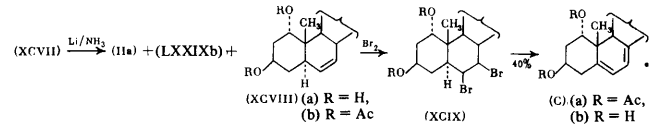


A fairly simple method of synthesis of 1α-OHD_3 has been proposed by Barton and coworkers[17,117]. By dehydrating cholesterol (IIa) with dichlorodicyanobenzoquinone (DDQ),[118] the authors obtained the trienone (XCVI),[119] which was converted by treatment with alkaline hydrogen peroxide[120] into 1α, 2α-epoxycholesta-4,6-diene-3-one (XCVII) with an overall yield of 45%. Treatment of the epoxide (XCVII) with a large excess of metallic lithium in a 1 : 1 NH_3–THF mixture or with sodium in ammonia[121] leads to 1α-hydroxycholesterol in 60% yield. The overall yield with respect to cholesterol is 27%.

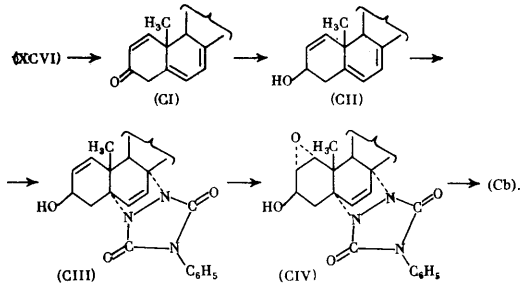
The conversion of 1α-hydroxycholesterol into the provitamin 1α-OHD_3,



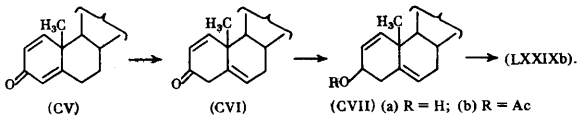
by the usual methods (bromination–dehydrobromination) involves low yields of the $\Delta^{5,7}$-diene. Methods have therefore been developed for the direct synthesis of the provitamin 1α-OHD_3. Mazur and coworkers[54] modified somewhat the method of Barton et al.,[17] in the reduction of the epoxydienone (XCVII) with lithium in liquid ammonia and obtained a mixture of three products: cholesterol (IIa) (15%), 1α-hydroxycholesterol (LXXIXb) (20%), and 5α-cholest-6-ene-1α, 3β-diol (XCVIIIa). The latter was acetylated to the diacetate (XCVIIIb), and brominated with bromine to the diaxial dibromide (XCIX), which was dehydrobrominated to the provitamin (Ca):



The provitamin (Cb) was obtained by Kaneko and coworkers[55,122,123], who used the familiar deconjugation reaction to isomerise the 1,4,6-trienone (XCVI), obtained by the method of Barton et al.[17], to the 1,5,7-trienone (CI) with potassium t-butoxide in dimethyl sulphoxide (DMSO) and then reduced it to the 3β-hydroxy-derivative (CII) with $Ca(BH_4)_2$. After protecting the 5,7-double bonds by forming the cyclic 1,4-adduct (CIII) with 4-phenyl-1,2,4-triazoline-3,5-dione[79], the epoxide (CIV) was obtained by oxidation with *m*-chloroperbenzoic acid. Its reduction with $LiAlH_4$ leads to compound (Cb):



Kaneko et al.[124] used the isomerisation reaction to convert cholesta-1,4-dien-3-one (CV) into the 1,5-dienone (CVI), which was then reduced with $NaBH_4$ or (better) with $Ca(BH_4)_2$ to the 3β-hydroxy-derivative (CVIIa). Hydroboration with B_2H_2 and oxidation with alkaline H_2O_2 give a low yield (10–15%) of compound (LXXIXb). A somewhat higher yield of this compound was obtained by treating the 3β-acetate (CVIIb) with HgO in the presence of trifluoroacetic acid and then reducing with $NaBH_4$:[125,126]

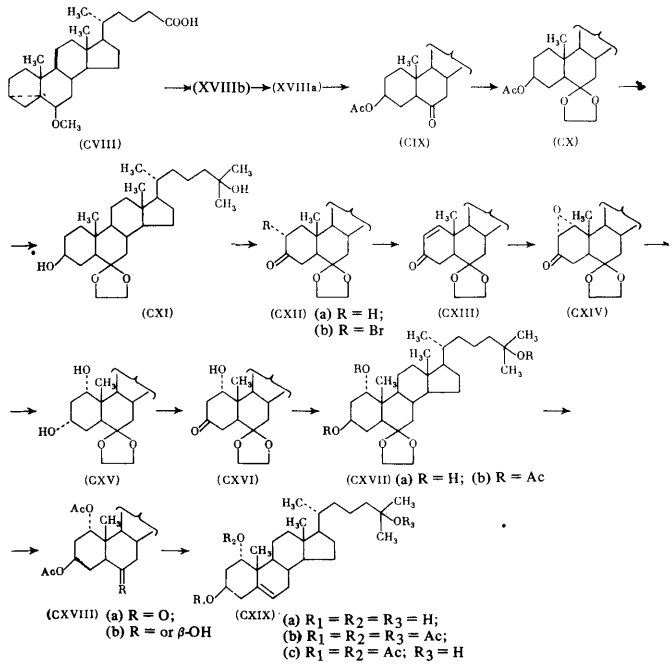


Harrison et al.[127,128] achieved the total synthesis of 1α-OHD_3 in 1973.

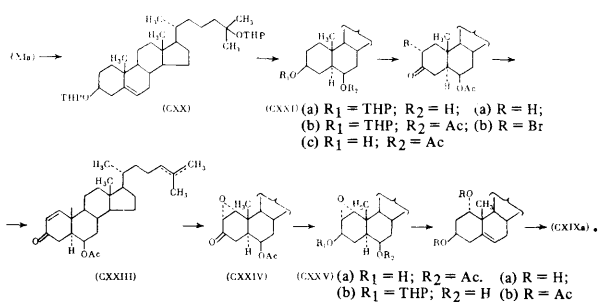
V. THE SYNTHESIS OF 1α,25-DIHYDROXYCHOLECALCIFEROL

The synthesis of 1α, 25-$(OH)_2D_3$, the active hormonal form of vitamin D_3, which involves the introduction of two hydroxy-groups into the molecule of vitamin D_3, still remains fairly complex. All the methods for the introduction of the 1α- and 25-hydroxy-groups described above can be used in the synthesis of 1α, 25-$(OH)_2D_3$. However, the hydroxy-groups are not always introduced consecutively; this usually involves simultaneous transformations in the A and B rings and in the side chain, which leads to the synthesis of new and hitherto unknown intermediates.

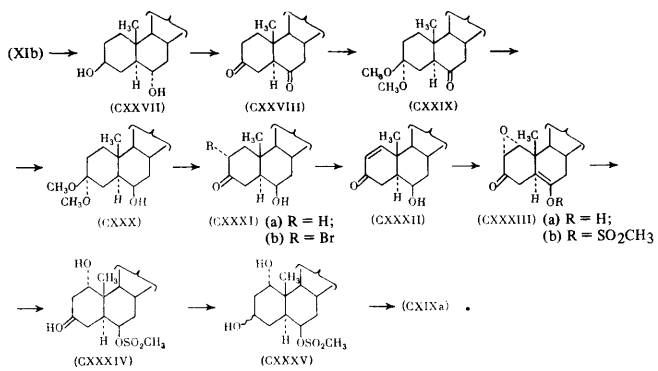
The synthesis of 1α, 25-$(OH)_2D_3$ by DeLuca and coworkers[115], who were the first to obtain this metabolite in 1971, was based on the conversion of the methyl ester of isohomocholanoic acid (CVIII) into a derivative of cholenoic acid (XVIIIb) by esterification with diazomethane and subsequent acid hydrolysis. After nitration, reduction, and acid hydrolysis, the acetate (XVIIIa) was converted into the ketone (CIX) and after the protection of the keto-group, the 25-hydroxy-derivative (CXI) was obtained via the ketal (CX) by the Grignard reaction. The oxidation of the 25-hydroxy-derivative with CrO_3 gives the 3-oxo-derivative (CXIIa), which is converted via the 2α-bromo-derivative (CXIIb) into the Δ^1-compound (CXIII). Its treatment with H_2O_2 leads to the 1α, 2α-epoxide (CXIV), whose reduction with $LiAlH_4$ yields the 1α, 3α-diol (CXV). In order to obtain the required 1α, 3β-diol, compound (CXV) was subjected to selective oxidation with *N*-bromosuccinimide, which gave the 3-oxo-derivative (CXVI), whose reduction with $NaBH_4$ led to the 1α, 3β-isomer (CXVIIa), which was then converted into the triacetate (CXVIIb) and then by mild acid hydrolysis into the 6-oxo-derivative (CXVIIIa). The reduction by $NaBH_4$ to compound (CXVIIIb) and dehydration of the 6-hydroxy-derivative with $POCl_3$ led to the triacetate of 1α, 25-dihydroxycholesterol (CXIXb), which was converted into 1α, 25-$(OH)_2D_3$ in the usual way:



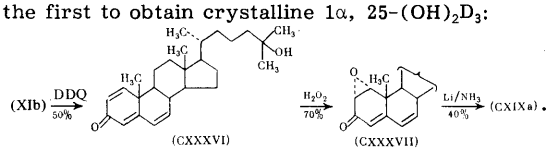
The syntheses of 1α, 25-dihydroxycholesterol described by Japanese workers are similar multistage processes[90,120,130]. One of them consists in the successive treatment of the dihydropyranyl ether (CXX), synthesised from 25-hydroxycholesterol (XIa), with B_2H_6 and H_2O_2 to form the 6-hydroxy-derivative (CXXIa) and then the acetate (CXXIb), whose hydrolysis led to the 3β, 25-diol (CXXIc). Its oxidation with CrO_3 gave the 6β-acetate of 6β, 25-dihydroxy-5α-cholestan-3-one (CXIIa) in an overall yield of 52% with respect to compound (XIa). The 1α-hydroxy group was introduced into compound (CXXIIa) by bromination to the 2α-bromo-derivative (CXXIIb) followed by dehydrobromination to give a mixture of the $\Delta^{1,24(25)}$- and $\Delta^{1,25(26)}$-dienes (CXXIII). The conversion into the 1α-hydroxy-derivative was achieved via the epoxykentone (CXXIV), the alcohol (CXXVa), and the tetrahydropyranyl ether (CXXVb). The dehydration of the latter and alkaline hydrolysis gave the 1α, 3β-diol (CXXVIa). The 25-hydroxy-group was introduced by treating the acetate (CXXVIb) with $Hg(OAc)_2$ and reducing with $NaBH_4$. The hydrolysis of the 1α, 3β-diacetate (CXIXc) gave 1α, 25-dihydroxycholesterol (CXIXa):



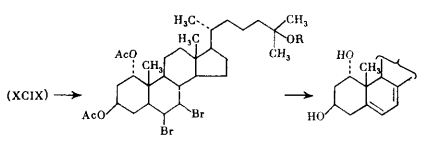
The synthesis of 1α, 25-dihydroxycholesterol from 25-hydroxycholesterol, involving transformations in the A and B rings only to give an 18% yield with respect to 25-hydroxycholesteryl acetate (XIb), has been achieved[131-133]. The hydroboration of (XIb) with diborane followed by treatment with alkaline H_2O_2 gives a mixture of alcohols, among which the main product is the triol (CXXVII). The unpurified mixture of alcohols was oxidised with the Jones reagent ($CrO_3 + H_2SO_4 + H_2O$) to the diketone (CXXVIII) with an overall yield of 74%. In order to convert the 6-oxo-group into the β-hydroxy-group, required for the subsequent introduction of the Δ^5-double bond, the 3-oxo-group in compound (CXXVIII) was converted into the dimethoxy-group in compound (CXXIX) and the latter was then reduced with $NaBH_4$ to the 6β-hydroxy-derivative (CXXX), whose hydrolysis gave the hydroxyketone (CXXXIa). The bromination of the latter to 2α-bromoketone (CXXXIb) followed by dehydrobromination led to the introduction of the Δ^1-double bond. Treatment of compound (CXXXII) with alkaline H_2O_2 gave the epoxyketone (CXXXIIIa). Next the 6β-hydroxy-group was protected by conversion into the methanesulphonate (CXXXIIIb) and the epoxy- compound was cleaved with aluminium amalgam to give the 1α-hydroxyketone (CXXXIV). Its reduction with $NaBH_4$ to a mixture of epimeric 3-hydroxy-derivatives (CXXXV), treatment of the mixture with $LiCO_3$ in DMF, and chromatographic separation of the epimers made it possible to isolate the pure compound (CXIXa):



Using the methods described above for the introduction of the 1α- and 25-hydroxy-groups, Japanese workers[91] achieved the synthesis of 1α, 25-$(OH)_2D_3$ from fucosterol. Fucosterol (LXXI) was converted into desmosterol (LXIb) via the ketone (LXXIII) in 45% yield. After treatment with $Hg(OAc)_2$ and then $NaBH_4$, the 3β-acetate of 25-hydroxycholesterol (XIb) was obtained from desmosterol in 85% yield; the overall yield of compound (XIb) with respect to (LXXI) was 38%. Next the synthesis was continued by the method of Barton and coworkers[17,134]. The dehydrogenation of compound (XIb) with the aid of DDQ leads to 25-hydroxy-1,4,6-trien-3-one (CXXXVI). The synthesis of 1α, 25-dihydroxycholesterol is completed by the conversion of the trienone into the triol (CXIXa) via the epoxide (CXXXVII). The overall yield with respect to fucosterol is not more than 5%. Barton et al.[134] were the first to obtain crystalline 1α, 25-$(OH)_2D_3$:



A relatively simple method of synthesising 1α, 25-$(OH)_2D_3$ has been developed by Mazur and coworkers[105]. The dibromide (XCIX) described above was adsorbed on silica gel and saturated with ozone at -78 °C and the mixture was then slowly heated to room temperature. Chromatographic separation of the mixture gave 51% of compound (CXXXVIIIa) for an 11% conversion of compound (XCIX). The hydroxy-group at $C_{(25)}$ was protected by the formation of the trifuloroacetate (CXXXVIIIb). Compound (CXXXVIIIb) was dehydrobrominated to the provitamin (CXXXIX), as described above[54]. The synthesis of 1α, 25-$(OH)_2D_3$ from cholesterol includes only seven stages:

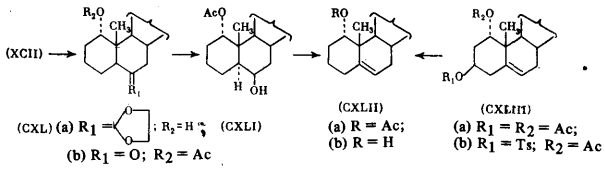


VI. THE SYNTHESIS OF 3-DEOXY-1α-HYDROXYCHOLECALCIFEROL

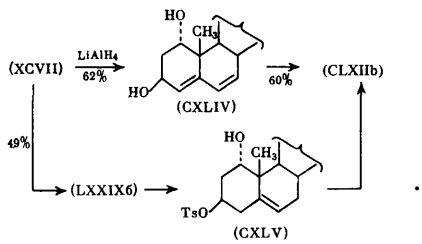
The presence of three hydroxy-groups, at $C_{(1)}$, $C_{(3)}$, and $C_{(25)}$, in the molecule of 1α, 25-$(OH)_2D_3$—the most active metabolite of vitamin D_3—stimulated attempts to elucidate the functional role of each of them. The synthesis of 1α-OHD_3 and the study of its biological activity showed that the absence of the hydroxy-group at $C_{(25)}$ hardly lowers the biological activity. The synthesis of 3-deoxy-1α-OHD_3 (Id) and its

high biological activity made it possible to demonstrate for the first time the exceptionally small role of the 3β-hydroxy-group in the manifestation of biological activity.

3-Deoxy-1α-OHD_3 was synthesised in 1974 by Norman and coworkers[22,135] and by DeLuca and coworkers[24,25]. DeLuca and coworkers synthesised this compound from the epoxy-ketone (XCII) obtained in the synthesis of 1α-OHD_3 [16,25]:



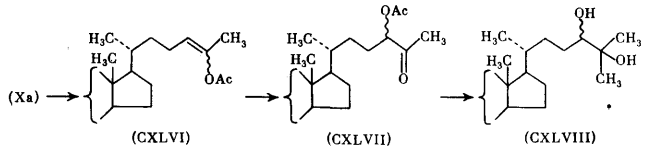
Treatment of the epoxyketone with hydrazine hydrate followed by catalytic hydrogenation led to the formation of the 1α-derivative (CXLa). Hydrolysis of the ketal under mild conditions with subsequent acetylation gave rise to the 6-oxo-derivative (CXLb), which was converted into the 6β-hydroxy-derivative (CXLI) after reduction with $NaBH_4$, while dehydration with $POCl_3$ gave cholest-5-en-1α-yl acetate (CXLIIIa). A second method of synthesis of compound (CXLII), based on the diacetate of cholest-5-ene-1α, 3β-diol (CXLIIIa), is possible. Partial hydrolysis of the acetate group at $C_{(3)}$, the formation of the toluene-*p*-sulphonate (CXLIII), and reduction with $LiAlH_4$ lead to compound (CXLIIb). Norman and coworkers[23,135] obtained compound (CXLIIb) from cholesterol via the epoxyketone (XCVII) on reduction of compound (XCVII) with $LiAlH_4$ to the diol (CXLIV) and then with Li/NH_3 to compound (CXLIIb). If compound (XCVII) is reduced initially by the method of Barton et al.[17] to 1α-hydroxycholesterol (LXXIXb) with Li/NH_3, then subsequent treatment of its 3β-toluene-*p*-sulphonate (CXLV) with $LiAlH_4$ leads to the formation of compound (CXLIIb):



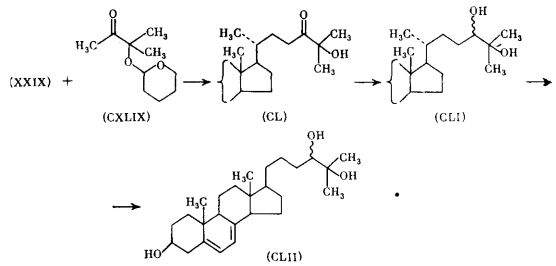
Successive bromination of compound (CXLIIa), dehydrobromination, and photochemical and thermal isomerisation lead to 3-deoxy-1α-OHD_3 with very low yields (8–10%) at each stage.

VII. THE SYNTHESIS OF 24*R*,25-DIHYDROXYCHOLECALCIFEROL

Synthetic 24,25-$(OH)_2D_3$ was obtained by DeLuca and coworkers[136,137] in 1973 from 25-oxo-27-norcholest-5-en-3β-yl acetate (Xa). Refluxing of compound (Xa) with acetic anhydride in the presence of toluene-*p*-sulphonic acid leads to a mixture of the *cis*- and *trans*-acetates of the Δ^{24}-enols (CXLVI). The 24-acetate of the 25-oxo-derivative (CXLVII) was obtained from this mixture via the intermediate bromo- and iodo-derivatives using the method of Moffett and Weisblat[138]. The reaction with a Grignard reagent leads to 24ξ, 25-dihydroxycholesterol (CXLVIII). The triacetate of the latter was converted into the provitamin and then into 24,25-$(OH)_2D_3$ by standard methods:



Eyley and Williams[56] obtained the provitamin 24,25-$(OH)_2D_3$ directly from ergosterol. The aldehyde (XXIX), obtained by the ozonolysis of the adduct of ergosterol with 4-phenyl-1,2,4-triazoline-3,5-dione, undergoes the aldol condensation with the enolate of 3-methyl-3-tetrahydropyranyloxybutan-2-one [which is formed when the ketone (CXLIX) is treated with lithium di-isopropylamide] to give the 24-oxo-derivative (CL):

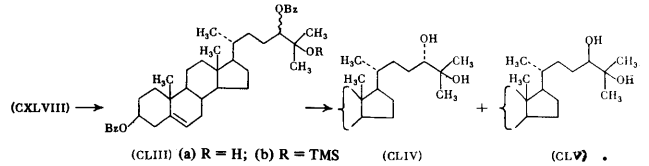


The reduction of this ketone with $NaBH_4$ leads to the adduct of the 3β, 24ξ, 25-triol (CLI). Treatment of the adduct with $LiAlH_4$ yields the provitamin (CLII), the overall yield of which is 38% with respect to the aldehyde (XXIX).

Japanese workers developed a simple method for the synthesis of 24,25-dihydroxycholesterol from desmosterol. Desmosteryl acetate (LXIb) was oxidised with *m*-chloroperbenzoic acid to the 24,25-epoxide (LXIV), whose treatment with H_2SO_4 gave compound (CXLVIII) [92]:

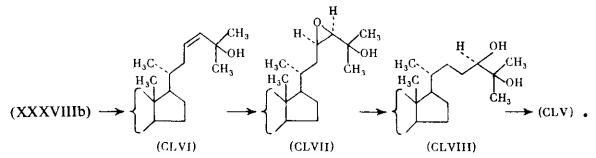
(LXIb) → (LXIV) → (CXLVIII).

An even simpler method of synthesis of compound (CXLVIII) consists in the oxidation of compound (LXIb) with OsO_4 in anhydrous ether[130], the yield being almost quantitative. Kodicek and coworkers[140,141] established that the oxidation of compound (LXIb) with osmic acid in benzene gives rise to a mixture of epimers with respect to $C_{(24)}$ (*R* and *S*). Next the authors obtained a mixture of epimers of 24(*R*,*S*), 25-$(OH)_2D_3$, one of which was the active metabolite isolated by Suda et al.[27] from a living organism. In 1975 Japanese workers[142,143] separated a mixture of 24(*R*,*S*), 25-dihydroxycholesterols into epimers. For this purpose, the mixture (CXLVIII) was converted into the 3β, 24ξ-dibenzoate (CLIIIa), which gave rise to the 25-trimethylsilyl ether (CLIIIb). The latter was resolved on a silica gel column and after hydrolysis two epimers were obtained: 24*S*, 25- and 24*R*, 25-dihydroxycholesterols (CLIV) and (CLV):



Their conversion into 24*S*, 25-$(OH)_2D_3$ and 24*R*, 25-$(OH)_2D_3$ and the study of their biological activity showed that 24*R*, 25-$(OH)_2D_3$ is the natural and active product.

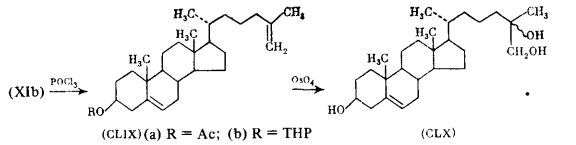
The sterospecific synthesis of 24*R*, 25-dihydroxycholesterol was achieved by Partridge and coworkers[144,145]. The hydrogenation of the unsaturated alcohol (XXXVIIIb) over the Lindlar catalyst gave a 90% yield of the *Z*-allyl alcohol (CLVI). Its oxidation with a peracid or better with t-butyl peroxide gives the 23*R*, 24*R*-epoxyalcohol (CLVII), whose reduction with $LiAlH_4$ results in the formation of the 24*R*, 25-diol (CLVIII). After treating the latter with acid dioxan, 24*R*, 25-dihydroxycholesterol (CLV) was obtained:



VIII. THE SYNTHESIS OF 25,26-DIHYDROXYCHOLECALCIFEROL

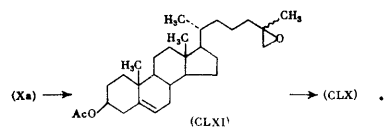
25,26-Dihydroxycholecalciferol was isolated and identified by DeLuca and coworkers in 1970.[32] The same investigators[146] patented a biochemical method of preparing this metabolite from the blood plasma of animals to which labelled vitamin D_3 had been administered. The configuration of the hydroxy-group at $C_{(25)}$ has not so far been established, but biological tests on a synthetic preparation showed that only one of the diastereoisomers with respect to $C_{(25)}$ is biologically active.

25,26-Dihydroxycholesterol was synthesised[92,139] from the acetate of 25-hydroxycholesterol (XIb), whose degradation with $POCl_3$ gave the $\Delta^{5,25}$-diene (CLIXa); the oxidation of the latter with OsO_4 gives a high yield of 25(*R*,*S*), 26-diol (CLX):



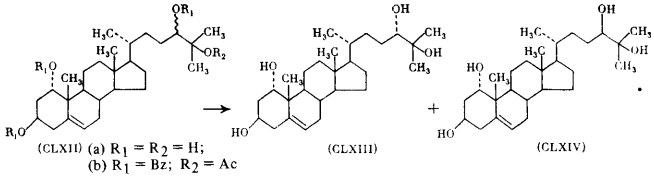
The chemical synthesis of $25,26\text{-}(OH)_2D_3$ was carried out by Kodicek and coworkers[147,148] in 1973. The tetrahydropyranyl ether of 3β-hydroxy-27-norcholest-5-en-25-one (Xb) was converted by the Wittig reaction into the tetrahydropyranyl ether of the $\Delta^{5,25}$-diene (CLIXb). After hydrolysis (with HCl/MeOH) and acetylation, the acetate (CLIXa) was oxidised to compound (CLX) with OsO_4, as described above. The provitamin and $25,26\text{-}(OH)_2D_3$ were obtained by the usual methods.

Lam et al.[34] developed another method for the conversion of compound (Xa) into $25,26\text{-}(OH)_2D_3$. Treatment of compound (Xa) with a mixture of sodium hydride and trimethylsulphonium iodide in DMF yielded the 25,26-epoxide (CLXI), which was converted by treatment with alkali into the crystalline 3β, 25,26-triol (CLX):



IX. THE SYNTHESIS OF 1α, 24*R*, 25-TRIHYDROXYCHOLECALCIFEROL

Ikakawa et al.[149] synthesised the two epimers 24*R*- and 24*S*-1α, $24,25\text{-}(OH)_2D_3$ in order to elucidate the configuration of the natural metabolite. 1α, 24,25-Trihydroxycholesterol was synthesised by the methods described above: 24,25-dihydroxycholesterol was obtained by the method of Seki et al.[92], after which the 1α-hydroxy-grlup was introduced by the method of Barton et al.[17] and a mixture of 1α, 24(*R*,*S*)25-trihydroxycholesterol (CLXIIa) epimers was obtained. They were then esterified and the mixture of tribenzoate acetates (CLXIIb) was separated chromatographically into two epimers. It was established by NMR that the more polar product (CLXIII) is the 24*R*-epimer, while the less polar product (CLXIV) is the 24*R*-epimer:



The conversion of these cholesterol derivatives into 1α, 24*R*, $25\text{-}(OH)_3D_3$ and 1α, 24*R*, $25\text{-}(OH)_3D_3$ [150] and the study of their biological properties showed that the natural metabolite is 1α, 24*R*, $25\text{-}(OH)_3D_3$.

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