The Chemistry of 'Vitamin' D: The Hormonal Calciferols

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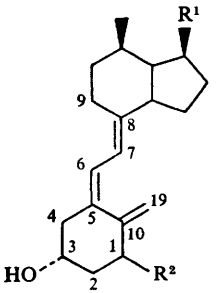
1 Introduction

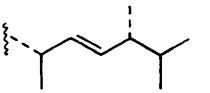
The relationship between the disease rickets and 'vitamin D' has been known for over 50 years, but it is only very recently that a better understanding of this relationship has begun to emerge. The discovery of the cause and cure of rickets has been described as one of the great triumphs of biochemical medicine,[1] and as a result its occurrence has been drastically reduced. Nevertheless, reports of recent findings[2] of relatively high incidence of rickets in Britain suggest that much public health education still needs to be done in many countries.

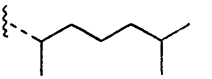
Mellanby[3] first produced the disease experimentally in 1919. It quickly became thought of as a nutritional disease since it could be cured by the administration of cod-liver oil. McCollum established that 'vitamin D' was the antirachitic factor in the cod-liver oil. However, the observations that rickets could also be cured by exposure to adequate sunlight led to much confusion.

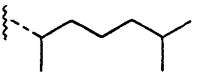
Steenbock and Hess independently discovered that antirachitic activity could be produced in some foods which were subjected to u.v. radiation. These findings eventually led to the isolation and structural elucidation of the active factors. Askew and Windaus in 1932 first identified vitamin D_2, or ergocalciferol (1a).

Ergocalciferol is the 'unnatural' form of 'vitamin D' since it is derived from ergosterol (2a) a plant sterol which is commercially readily available from yeast, and is not produced in animals. The 'natural' form, vitamin D_3, or cholecalciferol

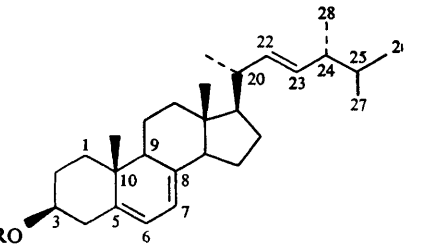


(1) a; R^1 =  , R^2 = H

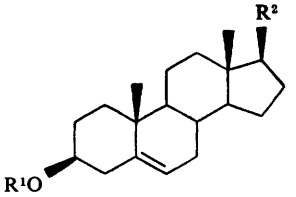
b; R^1 =  , R^2 = H

c; R^1 =  , R^2 = OH

[1] W. F. Loomis, *Sci. American*, 1970, **223**, 77.
[2] M. A. Preece, S. Tomlinson, C. A. Ribot, J. Pietrek, H. T. Korn, D. M. Davies, J. A. Ford, M. G. Dunnigan, and J. L. H. O'Riordan, *Quart. J. Medicine*, 1975, **44**, 575.
[3] For references and an excellent review of the early work on 'vitamin D' see L. F. Fieser and M. Fieser, 'Steroids', Reinhold Publishing Corporation, New York, 1959, pp. 91—168.



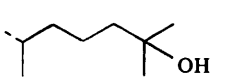
(2) a; ergosterol, R = H
b; R = Ac



(3) a; cholesterol, R^1 = H, R^2 =

b; R^1 = Ac, R^2 =

c; R^1 = H, R^2 =

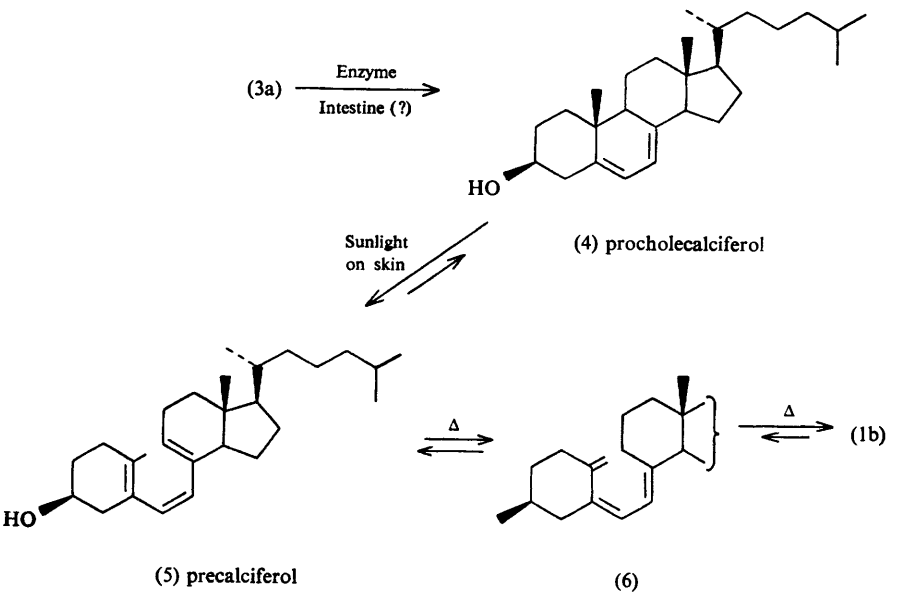


(1b) which is derived from cholesterol (3a), and which is the active factor in cod-liver oil, was not identified until 1936 by Windaus. In humans it is produced in the skin from 7-dehydrocholesterol or 'procholecalciferol' (4) by the action of u.v. radiation from sunlight, followed by the isomerizations outlined in Scheme 1.

The antirachitic activities of these two calciferols in humans is comparable, although in chickens, and in rats the activity varies. The chemistry and metabolism of the more intensively studied cholecalciferols, however, will be the major subject of this review. The photo- and thermo-chemistry of these compounds has been the subject of some very extensive and elegant studies and these have been recently reviewed elsewhere.[4]

Rickets can conveniently be described biochemically as a disease in which the calcification process cannot keep pace with the synthesis of the organic matrix of bone.[5] In this condition, it is found that blood plasma is undersaturated with respect to calcium and phosphate ions. The physiological action of the cholecalciferols is to elevate the concentrations of these ions to supersaturation levels, which is the normal situation. This is achieved by two basic effects;

[4] E. Havinga, *Experimentia*, 1973, **29**, 1181.
[5] 'Fat Soluble Vitamins', ed. H. F. DeLuca and J. W. Suttie, University of Wisconsin Press, 1969, p. 3.



Scheme 1

namely, by increasing the absorption of these ions by the intestine,[6] and by increasing their mobilization from the bone.[7]

In order to accomplish these functions, it is now established that cholecalciferol (or ergocalciferol) must first undergo several metabolic steps which activate and regulate the activitities of these compounds. It was a well-known fact that there is a time lag of 8—10 h before the effect of administered cholecalciferol could be observed, and that this suggested the existence of a more active metabolite. However, it was only in 1968 that DeLuca and co-workers,[8] using suitably labelled radioactive cholecalciferol together with chromatographic and spectroscopic techniques, identified a major polar metabolite. This was shown to be calciferol which is hydroxylated at the C-25 position (7), and is referred to as 25-HCC (25-hydroxycholecalciferol) (see Scheme 2). This compound is more effective than its precursor in inducing the intestinal absorption and bone mobilization of calcium. The site of its synthesis in the body was shown to be the liver[9] and it is the major circulating form of cholecalciferol in the blood plasma. For a time it was erroneously thought to be the active form of the 'vitamin' at the tissue level, despite the fact that there is a delay of 3—4 h after its administration before its biological activity can be observed.

[6] R. Nicolaysen, *Acta Physiol. Scand.*, 1951, **22**, 260.
[7] A. Carlsson, *Acta Physiol. Scand.*, 1952, **26**, 212.
[8] J. W. Blunt, H. F. DeLuca, and H. K. Schnoes, *Biochemistry*, 1968, **7**, 3317.
[9] M. Horsting and H. F. DeLuca, *Biochem. Biophys. Res. Comm.*, 1969, **36**, 251.

Fraser and Kodicek[10] and Norman *et al.*[11] reported that the kidney, surprisingly enough, was the site of synthesis of a second, more polar, and more active metabolite which is derived from 25-HCC. It was eventually identified in 1971 as 1(*S*),25-dihydroxycholecalciferol (8), and is referred to as 1,25-DHCC. At present, it is considered to be the active form of cholecalciferol. It is capable of stimulating intestinal calcium and phosphate transport, and bone-mineral mobilization in anephric animals. As we shall see subsequently, the behaviour of this compound closely corresponds to that of other steroid hormones, and the label 'vitamin D' is really a misnomer.

2 Of Vitamins and Hormones

Since rickets could be cured by the administration of cod-liver oil, or by the ingestion of some foods that had been irradiated with u.v. radiation or had been supplemented with ergocalciferol, it is easy to understand why the disease was considered to be diet-dependent. At the time, discoveries were being made of other trace compounds which were found to be essential for good health. These compounds are acquired almost exclusively from dietary sources, and were termed 'vitamins'. The antirachitic factors were therefore named 'vitamin D', and are still erroneously considered by many to be classic vitamins. Vitamins are either precursor of coenzymes, or are coenzymes themselves, and the fact that coenzymes, like enzymes function as catalysts explains why vitamins are needed only in trace amounts.

The hormones on the other hand have functional characteristics which are relatively easier to define: (i) they are produced in various endocrine glands *e.g.* the pituitary, testis, *etc.*, (ii) they interact at 'target' tissues which contain hormone receptors that are specialized proteins capable of binding to the hormone with very high specificity and affinity; (iii) the hormone–receptor complex causes the formation of intracellular messenger molecules which stimulate or depress some characteristic biochemical activity of the target cell.

DeLuca[12a] and Norman *et al.*[12b] in the U.S., and Kodicek[12c] in the U.K., have clearly shown that 1,25-DHCC has all of these hormonal characteristics: (i) it is produced in the kidney; (ii) it, in conjunction with another hormone, parathyroid hormone (PTH), a protein containing 84 amino-acid units, is transported *via* the blood plasma and interacts at target tissues. These include the intestine, bone, muscle, and probably the kidney[12a] itself; (iii) the overall characteristic physiological activity of these target cells is to regulate normal plasma concentration of calcium and phosphate ions. The homeostasis of these ions are essential to numerous cellular functions, including the prevention of rickets.

Another characteristic of hormones is that they are regulated by a 'feedback'

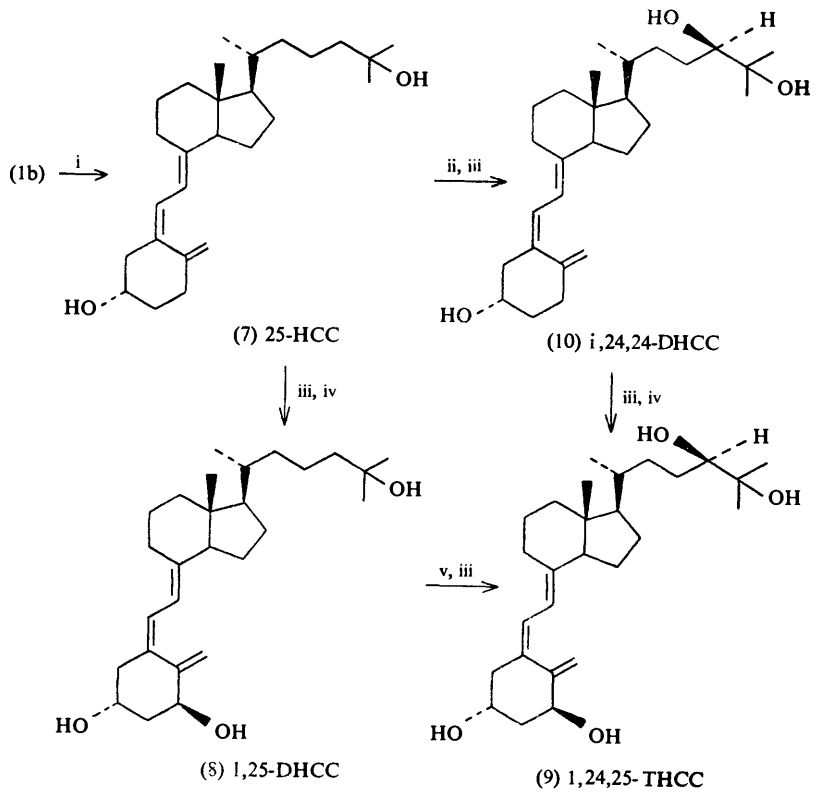
[10] D. R. Fraser and E. Kodicek, *Nature*, 1970, **228**, 764.
[11] A. W. Norman, R. J. Midgett, J. F. Myrtle, and H. G. Nowicki, *Biochem. Biophys. Res. Comm.*, 1971, **42**, 1082.
[12] For recent reviews of the physiological studies of this and related compounds, see (*a*) H. F. DeLuca, *J. Lab. Clin. Med.*, 1976, **87**, 7; (*b*) A. W. Norman, D. A. Procsal, W. K. Okamura, and R. M. Wing, *J. Steroid Biochem.*, 1975, **6**, 461; (*c*) E. Kodicek, *Lancet*, 1974, 325.

mechanism. How is 1,25-DHCC itself regulated? It is clear that 25-HCC is the major circulating form of cholecalciferol in the plasma. Its concentration is dependent on both the dietary, and more importantly the endogenous source of cholecalciferol which is produced in the skin by the action of u.v. light on (4a). Its production does not appear to be regulated by the calcium and phosphate levels in the plasma, but appears to be controlled by the levels of 25-HCC present in the liver itself. After 25-HCC is transported to the kidney, its fate of oxidation by the 1-hydroxylase system is determined by several factors. Thus, when plasma levels of calcium and phosphate are low, and in the presence of PTH, the production of 1,25-DHCC is stimulated. Once it appears, a regulation mechanism is initiated by its presence wherein two metabolites[13] 1(*S*),24(*R*),25-trihydroxycholecalciferol [1,24,25-THCC, (9)], and 24(*R*),25-dihydroxycholecalciferol [24,25-DHCC, (10)] are produced. These compounds are in general, less active than their precursors, but at present the exact functions of these metabolites have not been unambiguously established.

When calcium and phosphate levels are elevated to normality, and when PTH levels are correspondingly low, 24-hydroxylase activity is initiated, and 1,25-DHCC is hydroxylated to produce (9). At the same time, the oxidation of 25-HCC by the 24-hydroxylase also occurs to produce (10), which is ultimately oxidized to (9) when the 1-hydroxylase activity resumes again. Only once the formation of the 24-hydroxylase system occurs, does a 'feedback' regulation system by calcium and phosphate become initiated. The formation of (9) and (10) appear to be the initial signals for inactivation and excretion of the cholecalciferols.[12a] However, it is important to note that it has not yet been established whether or not 1,25-DHCC is metabolized further to a more active hormonal form. Recently,[12a] DeLuca has found that $^{14}CO_2$ is produced within 4 h of administration to cholecalciferol-deficient hypocalcaemic rats of 1,25-DHCC labelled with ^{14}C at C-26 and C-27. This appears rapidly enough to be potentially of some significance towards intestinal calcium transport but this is by no means certain yet. The site of this oxidative side-chain degradation is not known, nor has the metabolite which is produced been isolated or identified. Nevertheless, this metabolic pathway is of quantitative significance since it accounts for at least one-third of the labelled precursor, 32 h after its administration. Scheme 2 summarizes these findings.

The isolation of these metabolites from blood plasma is a very laborious procedure and affords only small quantities. Synthetic sources of these compounds and their analogues are thus required in order to facilitate the elucidation of their exact biological functions. In addition, specifically radioactively labelled precursors are also required which will enable the metabolic reactions and products

[13] M. F. Holick, H. K. Schnoes, H. F. DeLuca, R. W. Gray, I. T. Boyle, and T. Suda, *Biochemistry*, 1972, **11**, 4251; M. F. Holick, A. Kleiner-Bossaller, H. K. Schnoes, P. M, Kasten, I. T. Boyle, and H. F. DeLuca, *J. Biol. Chem.*, 1973, **248**, 6691; Y. Tanaka, H. F. DeLuca, N. Ikekawa, M. Morisaki, and N. Koizumi, *Arch. Biochem. Biophys.* 1975, **170**. 620.



Reagents: i, 25-hydroxylase [liver]; ii, normal Ca^{2+}, PO_4^{3-} concentration levels; iii, 24-hydroxylase [kidney]; iii, parathyroid hormone, low Ca^{2+}, PO_4^{3-} levels; iv, 1-hydroxylase [kidney]; v, low parathyroid hormone, normal Ca^{2+}, PO_4^{3-} levels.

Scheme 2

to be monitored. The more important synthetic approaches to these compounds will be reviewed here.

3 Total Chemical Synthesis

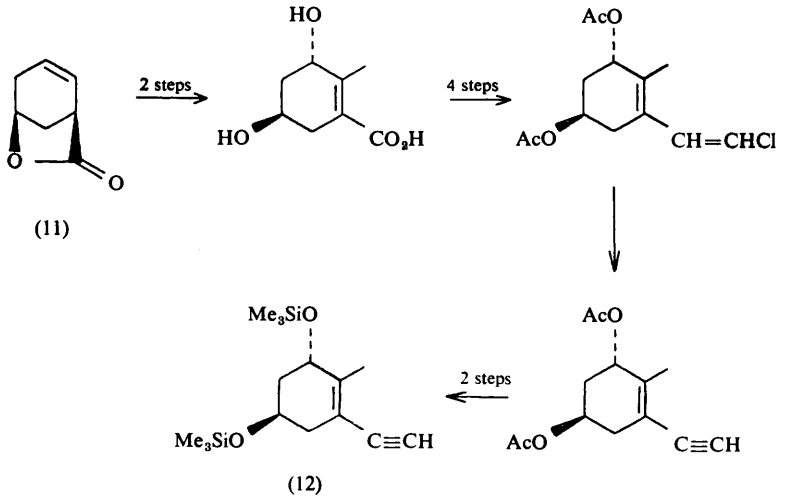
Two groups of workers have dominated the developments in the total synthesis of the cholecalciferols. Inhoffen's group at Braunschweig reported the first total synthesis of cholecalciferol in 1960,[14] and Lythgoe's group in Leeds reported the total synthesis of precalciferol (5) in 1970.[15] In general, although much valuable information about these and related compounds has been discovered during these studies, their syntheses can be much more efficiently achieved from

[14] H. H. Inhoffen, *Angew. Chem.*, 1960, **72**, 875.
[15] T. M. Dawson, J. Dixon, P. S. Littlewood, B. Lythgoe, and A. K. Saksena, *Chem. Comm.*, 1970, 993; *J. Chem. Soc. (C)*, 1971, 2960.

partial synthetic sequences using cholesterol, for example, as starting material.

The discovery of (8) with its two additional functional groups however, initially promised to close this efficiency gap. The first partial synthesis of this compound required a lengthy reaction sequence and resulted in a product whose physical constants were not reported.[16] Furthermore, DeLuca[17] and Barton *et al.*[18] reported that a synthetic analogue of (8), the mono-hydroxylated 1(*S*)-hydroxycholecalciferol [1-HCC, (1c)] had biological activity comparable with that of (8) itself. Hence (1c) became in itself another important target for total synthesis.

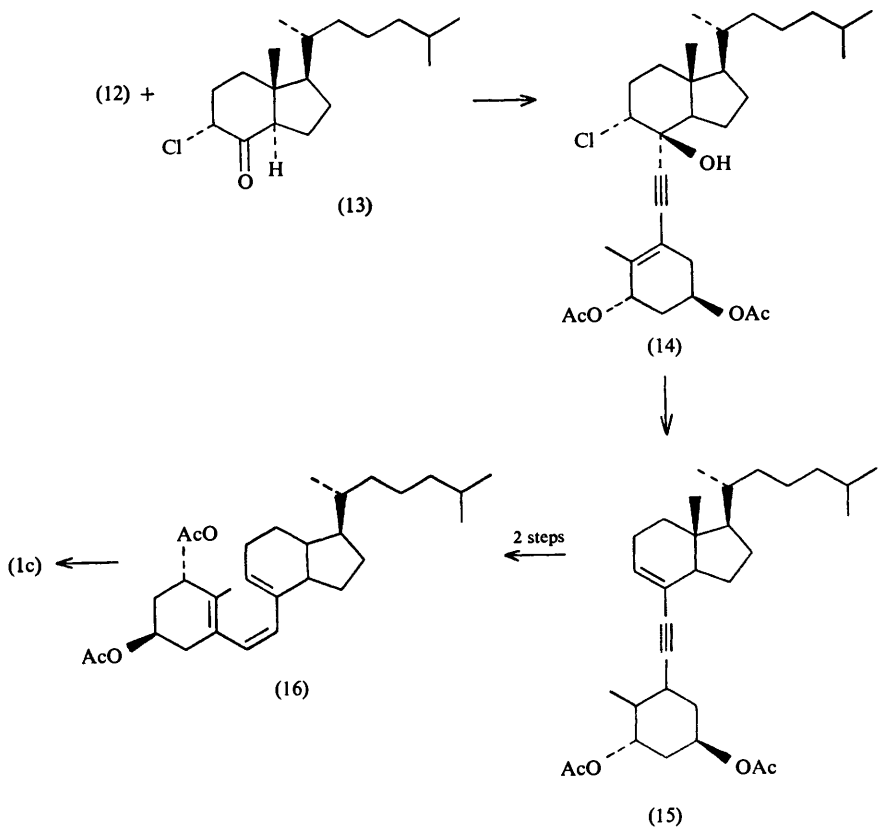
Lythgoe reported the total synthesis[19] of (1c) by a modification of his procedure for the total synthesis of cholecalciferol. Thus, the optically active lactone (11) was transformed into (12) in 17% overall yield by a sequence of nine steps (Scheme 3). The 9α-chloro-*des*-AB-cholestan-8-one (13) which is also obtained



Scheme 3

by a total synthesis[15] was alkylated with the lithium derivative of (12) to give, after hydrolysis and acetylation, (14). Treatment with bis(ethylenediamine)-chromium(II) in DMF, gave the dienyne (15) which was partially hydrogenated with Lindlar's catalyst to give (16). This precalciferol was transformed into (1c) by heating in benzene, followed by saponification. The product was crystalline and well characterized and was obtained in 22% yield from (13) (Scheme 4).

[16] E. J. Semmler, M. F. Holick, H. K. Schnoes, and H. F. DeLuca, *Tetrahedron Letters*, 1972, 4147.
[17] M. F. Holick, E. J. Semmler, H. K. Schnoes, and H. F. DeLuca, *Science*, 1973, **180**, 190.
[18] D. H. R. Barton, R. H. Hesse, M. M. Pechet, and E. Rizzardo, (*a*) *J. Amer. Chem. Soc.*, 1973, **95**, 2748; (*b*) *J.C.S. Chem. Comm.*, 1974, 203.
[19] R. G. Harrison, B. Lythgoe, and P. W. Wright, *Tetrahedron Letters*, 1973, 3649.



Scheme 4

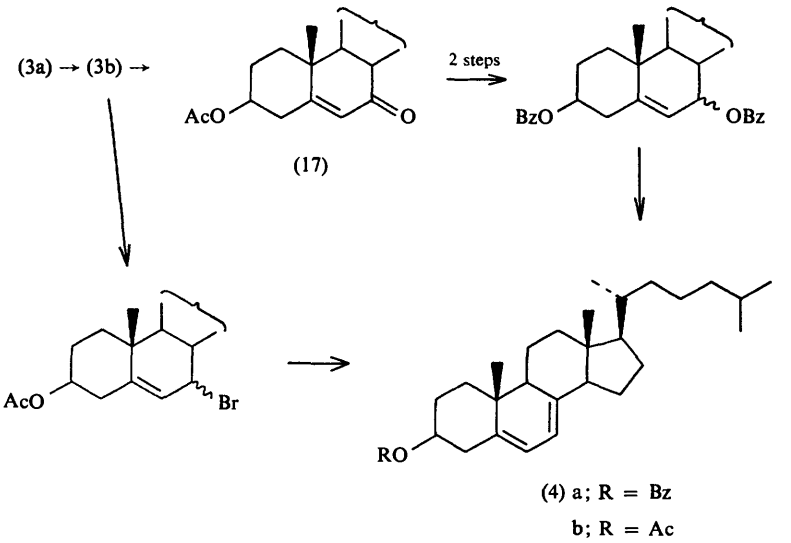
4 Partial Chemical Synthesis

By far the greatest amount of synthetic effort towards the various calciferols reported to date has been concerned with the transformation of readily available naturally occurring sterols.

A. Procholecalciferol.—Most of the naturally occurring sterols with the exception of ergosterol, that have been used in partial synthesis contain a Δ^5-olefin. The calciferols are essentially seco-steroids formed when the C-9—C-10 bond of the diene-containing ring B is photochemically cleaved. Thus, generation of a $\Delta^{5,7}$-diene system in ring B to form 'procalciferols' such as (4) from cholesterol, represented the first synthetic challenge.

This transformation was first studied by Windaus. In his approach,[3] cholesterol was first protected as the acetate (3b), then oxidized with chromium trioxide

to give the 7-keto-derivative (17). Meerwein–Pondorf reduction, followed by benzoylation gave a dibenzoate which could be readily pyrolysed to give (4a). A more efficient synthesis was devised by Hunziker and Müllner,[20a] using a mild allylic bromination–dehydrobromination sequence. Thus, (3b) is brominated with *NN'*-dibromodimethylhydantoin to the 7-bromo-derivative which is then dehydrobrominated with trimethylphosphite to give (4b) (Scheme 5). This method



Scheme 5

has been the one of choice in numerous syntheses of the procalciferols and their hydroxylated derivatives. Recently, a more efficient procedure has been described[20b] with the formation of (4a) reported to be in 75% yield compared with 51% by the older method.

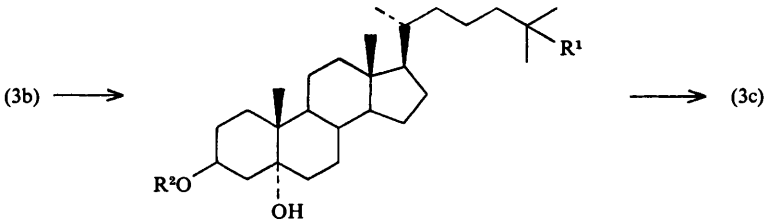
Furthermore, Williams[21] has reported on the advantages of using fluorenone as a photosensitizer to increase the yield of (1b) from the procholecalciferol (5).

B. 25-Hydroxycholesterol.—Since any cholesterol derivative could now in principle be easily converted into the appropriate precalciferol, the next synthetic target was (3c), the synthetic precursor to 25-HCC.

(i) *From Cholesterol.* Autoxidation of crystalline cholesterol on storage has been found[3] to produce (3c), although in low yield. Rotman and Mazur[22] have reported an alternative procedure to produce (3c) directly by a photochemical method.

[20] (*a*) F. Hunziker and F. X. Müllner, *Helv. Chim. Acta*, 1958, **41**, 70; (*b*) J. J. Kaminski and N. Boder, *Tetrahedron*, 1976, **32**, 1097.
[21] S. C. Eyley and D. H. Williams, *J.C.S. Chem. Comm.*, 1975, 858.
[22] A. Rotman and Y. Mazur, *J.C.S. Chem. Comm.*, 1974, 15; G.P. 2 415 676.

In this procedure it was necessary first to protect the double bond of (3b) by transforming it to the 5α-hydroxy derivative (18a). This was then treated with an excess of peracetic acid in a quartz tube and irradiated with 300 nm light. The diol–acetate (18b) was formed in 38% overall yield, based on reacted starting material. Acylation, followed by dehydration and hydrolysis gave (3c) (Scheme 6).



(18) a; R^1 = Ac, R^2 = H

b; R^1 = Ac, R^2 = OH

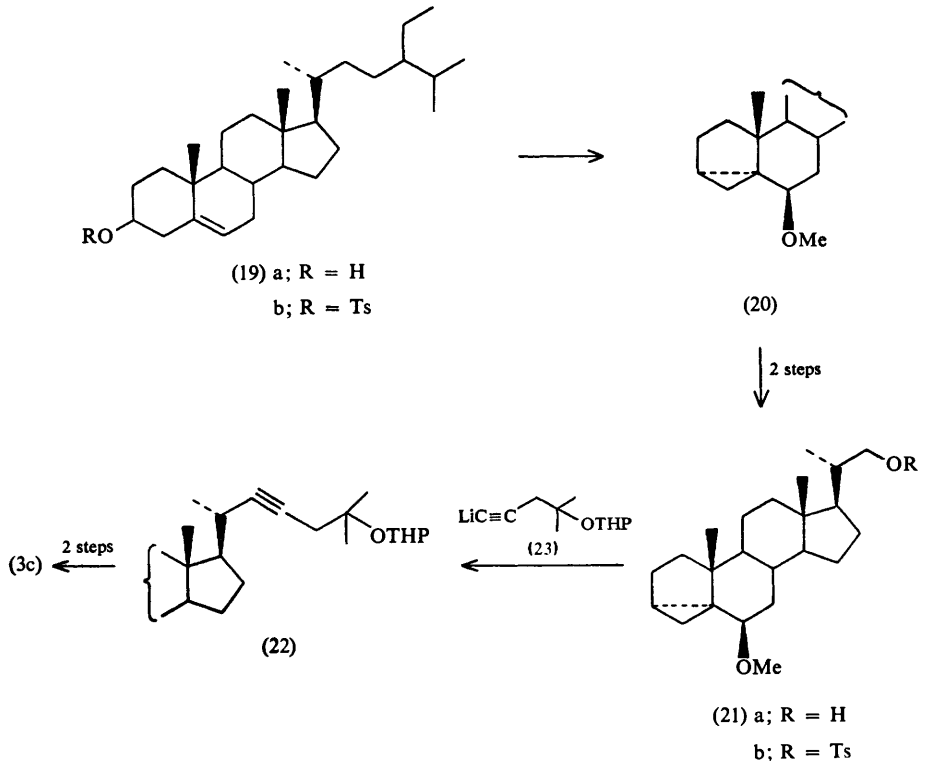
Scheme 6

The synthesis by Blunt and De Luca[23] of (3c) employed 26-norcholestene-25-one, a low-yield product obtained from the oxidation of cholesterol.

(ii) *From Stigmasterol.* The Hoffman–LaRoche group have developed an efficient synthesis of (3c) starting from the readily available stigmasterol[24] (19a), a sterol which is isolated commercially from soya beans. The Δ^5-olefin was protected by formation of the isostigmasteryl methyl ether (20). Thus, stigmasteryl tosylate (19b) was solvolysed with methanol and pyridine to give (20). Ozonolysis, followed by reduction, gave the alcohol (21a), which was converted into the tosylate (21b). The tosylate was converted into (22) in 90% yield, upon treatment with a one-fold excess of (23). However, (23) was first produced in dioxan solution to precipitate any lithium chloride present which would compete in the nucleophilic displacement reaction. Hydrogenation of the acetylenic bond, followed by retro-iso-rearrangement of the cyclopropyl group in aqueous acidic dioxan gave (3c). The overall yield for the seven-step sequence was 30% (Scheme 7).

(iii) *From Pregnenolone.* In another attractive synthesis[25] by the Hoffmann–LaRoche group, the acetate of pregnenolone (24), a readily available compound which is commercially obtained from diosgenin, was the starting material. Treatment of (24) vinylmagnesium chloride at −78 °C gave the alcohol (25). Chain extension was achieved by reaction with diketen to give a 2:1 mixture of the crystalline *cis*- and *trans*-keto-olefins (26) and (27) respectively. Catalytic

[23] J. N. Blunt and H. F. DeLuca, *Biochemistry*, 1969, **8**, 671.
[24] J. J. Partridge, S. Faber, and M. R. Uskoković, *Helv. Chim. Acta*, 1974, **57**, 764.
[25] T. A. Narwid, K. E. Cooney, and M. R. Uskoković, *Helv. Chim. Acta*, 1974, **57**, 771.

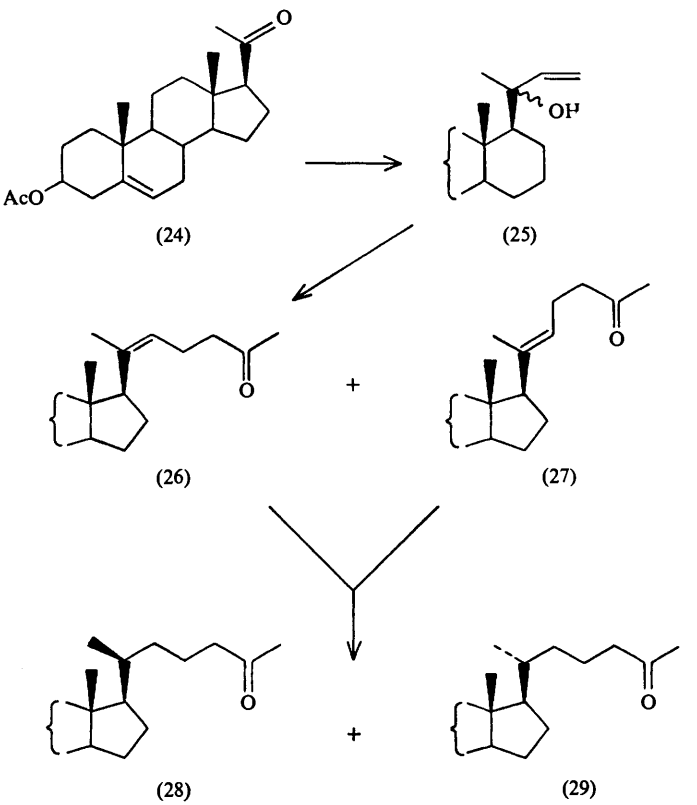


Scheme 7

hydrogenation of the mixture gave a 50% yield of the desired 20(*R*)-ketone (28) which was separated from its 20(*S*) isomer (29) by crystallization. Reaction of (28) with methylmagnesium chloride gave (3c). An overall yield of 25-28% of (3c) for the seven-step sequence was obtained (Scheme 8).

(iv) *From Androstenolone.* A stereospecific construction of the hydroxylated side-chain was achieved[26] by starting from the acetate of androstenolone (30) which is also readily available from diosgenin. The ester (31) was formed by a Reformatsky reaction with ethyl bromoacetate, followed by dehydration, selective hydrogenation of the Δ^{17-20}-olefin, and exchanging the protecting groups. Alkylation of (31) with the dioxolan of 1-bromopentan-4-one, using di-isopropyllithium amide and hexamethylphosphorotriamide at low temperatures, gave the stereoselectively formed 20(*R*)-product (32). This was easily converted into (3c) by a sequence of six routine steps. The overall yield of (3c) from (30) was reported to be 42% (Scheme 9). The Hoffman–LaRoche syntheses, however, are more direct and thus more convenient.

[26] J. Wicha and K. Bal, *J.C.S. Chem. Comm.*, 1975, 968.

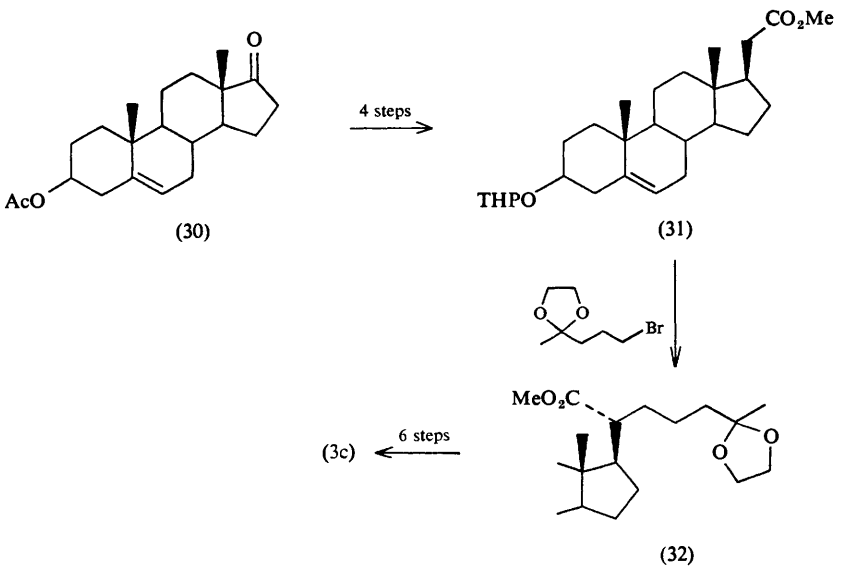


Scheme 8

(v) *From Ergosterol.* The methods described above all require as a final step the bromination–dehydrobromination sequence described earlier to produce the procalciferol. Although high yields have been obtained with simple cholesterol esters, yields of the corresponding side-chain hydroxylated cholesterol derivatives have not been greater than 25%.

Ergosterol (2a) therefore, appeared[27] to be the ideal choice as starting material for (3c) since it already possesses the $\Delta^{5,7}$-diene system, and the side-chain olefin provides a means for the modification of the side-chain. Barton *et al.*[28] had shown that the Diels–Alder adduct (33), of ergosteryl acetate (2b), with 4-phenyl-1,2,4-triazoline-3,5-dione [PTAD (34)] could undergo selective ozonolysis of the side-chain olefin to give the hexanoraldehyde (35). They also showed that the protecting group could be easily removed. Thus, it was envisaged

[27] P. E. Georghiou and G. Just, *J.C.S. Perkin I*, 1973, 888.
[28] D. H. R. Barton, T. Shioiri, and D. A. Widdowson, *J. Chem. Soc.* (*C*), 1971, 1968.



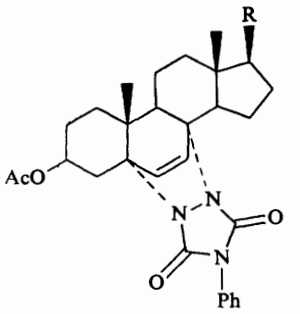
Scheme 9

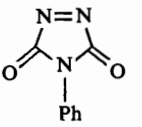
that a Wittig reaction with the phosphorane of (36), followed by selective catalytic hydrogenation of the side-chain olefin and removal of the protecting groups would lead to 25-hydroxyprocholecalciferol (37) directly. However, it was found that selective hydrogenation of the side-chain olefin of the adduct (33), or of (38) could not be effected. Instead, the ring B olefin was reduced much more quickly.[27] Thus, attempts at nucleophilic displacements of the C-22 iodide (39) obtained from (35) were investigated, using the lithium and copper 'ate' salts of (36). These attempts however, were unsuccessful[27] and similar results were later obtained by Eyley and Williams.[29]

Nevertheless, (37) has now been synthesized[29b] in 43% yield from (35). Thus a reaction of (35) with the Grignard reagent derived from (40) gave the alcohol (41a). The alcohol was converted into the mesylate (41b) which was selectively reduced with sodium borohydride in DMSO to (41c). Oxidation of the terminal olefin with mercury(II) acetate, followed by removal of the protecting groups gave (37).

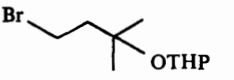
C. 1α-Hydroxycholesterol, and 1α,25-Dihydroxycholesterol.—The importance of (8) led to much synthetic effort being directed towards finding efficient methods for the hydroxylation of cholesterol, and of (3c) in the 1α-position. These compounds 1α-hydroxycholesterol (42), and 1α,25-dihydroxycholesterol (43) would of course be the precursors of (1c) and (8), respectively.

[29] (*a*) S. C. Eyley and D. H. Williams, *J.C.S. Perkin I*, 1976, 727; (*b*) *ibid.*, p. 731.



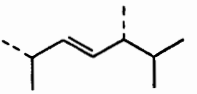


(34)

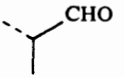


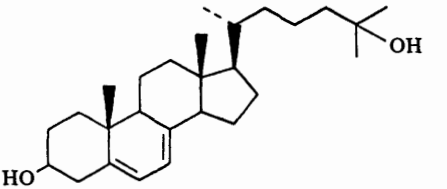
(36)

(33) R =



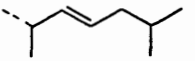
(35) R =

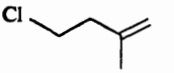




(37)

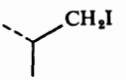
(38) R =



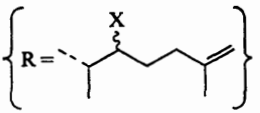


(40)

(39) R =



(41a)
(41b) R =
(41c)



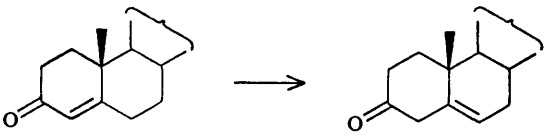
X = OH
X = OMs
X = H

The initial approaches to (42) and (43) all involved the classical approach of conjugate addition to a Δ^1-3-keto-system and subsequent transformation into the cholecalciferols. Pelc and Kodicek's synthesis of (42) in 1970 required a 14-step sequence, starting from cholesterol.[30] DeLuca's group reported[16] a synthesis of (43) which involved a 17-step sequence starting from a nor-cholanic acid.

The most elegant procedure for 1-hydroxylation reported to date, is that of Barton and Hesse.[18a] This group exploited the well-known deconjugation reaction of Δ^4-3-keto-steroids [equation (1)].

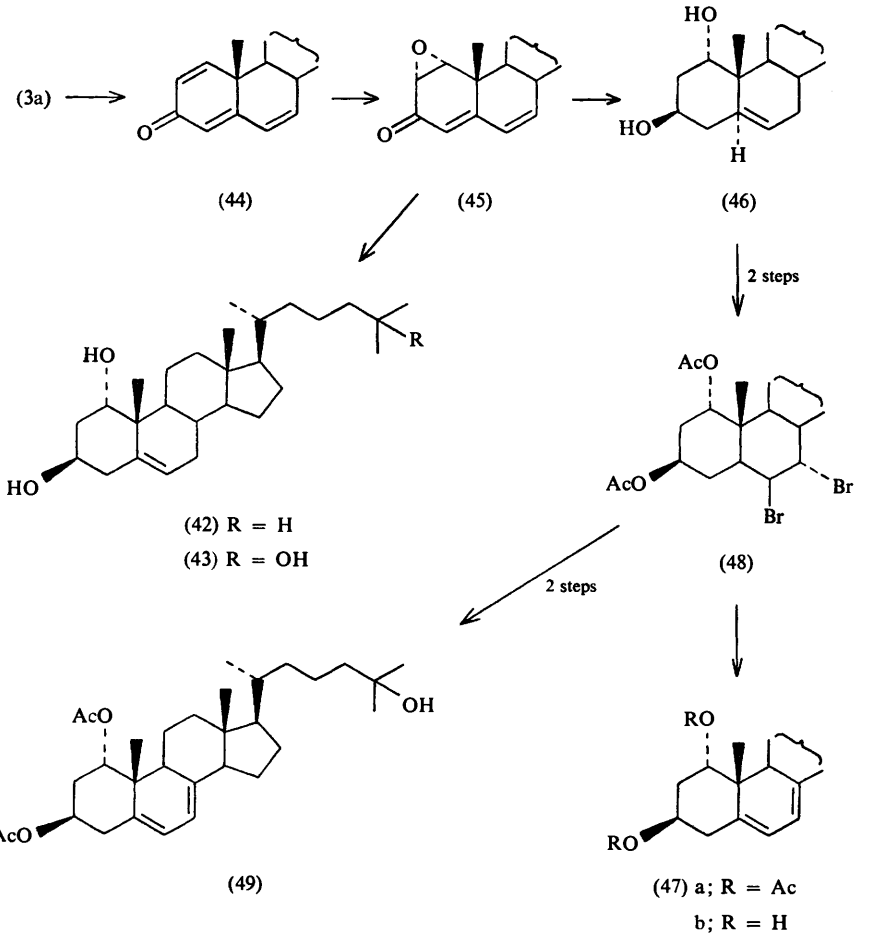
Thus, in their approach, cholesterol was dehydrogenated with dichlorodicyanobenzoquinone to the trienone (44). Base-catalysed epoxidation gave the

[30] B. Pelc and E. Kodicek, *J. Chem. Soc. (C)*, 1970, 1624.



(1)

α-epoxide (45) which was treated with 'large excesses' of lithium metal and ammonium chloride in liquid ammonia–THF solution. The product obtained was (42) and was obtained in 27% overall yield from cholesterol. The transformation most likely proceeds *via* a series of enolization–protonation steps. The same sequence of reactions[18b] was used by this group using (3c) as starting material in their synthesis of well-characterized and crystalline 1,25-DHCC.



Scheme 10

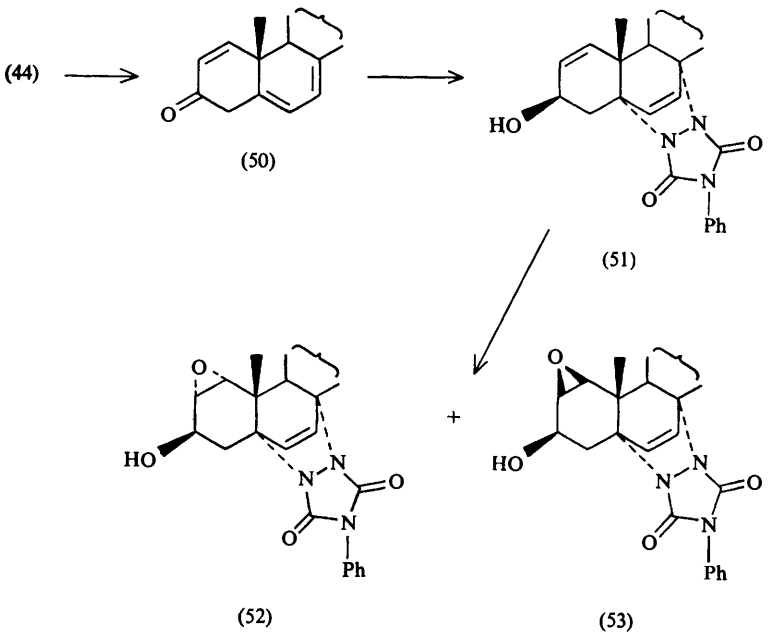
Mazur[31a] has described in detail a procedure in which the lithium–ammonia reduction of (45) produces the Δ^6-olefin (46) in 45% yield, together with (42) in 20% yield. The transformation of (46) into the $\Delta^{5,7}$-diene (47), was effected by acetylation and bromination of the Δ^6-olefin to give (48) (Scheme 10). This dibromide was then dehydrobrominated to give a quantitative yield of a 1.3 : 1 mixture of (47a) and a $\Delta^{4,6}$-diene which could be easily separated. A further development from Mazur's laboratories is an application of his method of 'dry ozonation'. In this report,[31b] the dibromide (48) is adsorbed onto silica gel, it is saturated with ozone at −78 °C, and then the mixture allowed to warm to room temperature. Elution of the product and chromatographic separation afford the unreacted (48) and its 25-hydroxylated derivative, in 11% conversion and 51% yield. This is then transformed by dehydrobromination into (49), after first protecting the 25-hydroxyl group as the trifluoroacetate. This direct introduction of the hydroxyl group into the side-chain of cholesterol implies that the procalciferol (49) can now be obtained directly from cholesterol by a seven-step synthesis, in an overall yield of 1.7—2.0%, as presently described. It seems likely that this yield could be improved in the future.

Kaneko's group has developed an alternative Δ^4-3-keto deconjugation method.[32] (Scheme 11) Thus, (44) is deconjugated by treatment with t-butanol in DMSO, followed by addition into ice–water to give (50). The ketone was reduced with calcium borohydride and the product purified as the PTAD-adduct (51) which also served to protect the $\Delta^{5,7}$-diene system. Thus, epoxidation gave a mixture of the α- and β-epoxides (52) and (53). These were separated and each was treated with lithium aluminium hydride, which reduced the epoxide and also regenerated the $\Delta^{5,7}$-diene. Epoxide (52) gave the diol (47b) in approximately 2% overall yield from cholesterol.

D. 1α,24(*R*),25-Trihydroxycholesterol and 24(*R*),25-Dihydroxycholesterol.— The first syntheses of C-24 epimeric 24,25-dihydroxycholesterol (54a), a synthetic precursor for (10) were accomplished by DeLuca,[33a] Kodicek,[33b] and their respective co-workers, both groups using 26-norcholesten-25-one as starting compound. Their syntheses produced 1 : 1 mixtures of the C-24 epimers. Ikekawa and co-workers[34] resolved the epimeric pair of C-24 alcohols by conversion into their tribenzoates and separation on silica gel chromatography. They also determined the absolute stereochemistry of these epimers.

DeLuca, and the Hoffman–LaRoche group later independently assigned the 24(*R*) absolute configuration to the naturally produced metabolite. The latter

[31] (*a*) D. Freeman, A. Archer, and Y. Mazur, *Tetrahedron Letters*, 1975, 261; (*b*) Z. Cohen, E. Keinan, Y. Mazur, and A. Ulman, *J. Org. Chem.*, 1976, **41**, 2651.
[32] C. Kaneko, A. Sugimoto, Y. Eguchi, S. Yamada, M. Ishikawa, S. Sasaki, and T. Suda, *Tetrahedron*, 1974, **30**, 2701.
[33] (*a*) H.-Yat Lam, H. K. Schnoes, H. F. DeLuca, and T. C. Chen, *Biochemistry*, 1973, **12**, 4851; (*b*) J. Redel, N. Bazely, Y. Calando, F. Delbarre, P. A. Bell, E. Kodicek, *J. Steroid Biochem.*, 1975, **6**, 117.
[34] M. Seki, N. Koizumi, M. Morisaki, and N. Ikekawa, *Tetrahedron Letters*, 1975, 15.

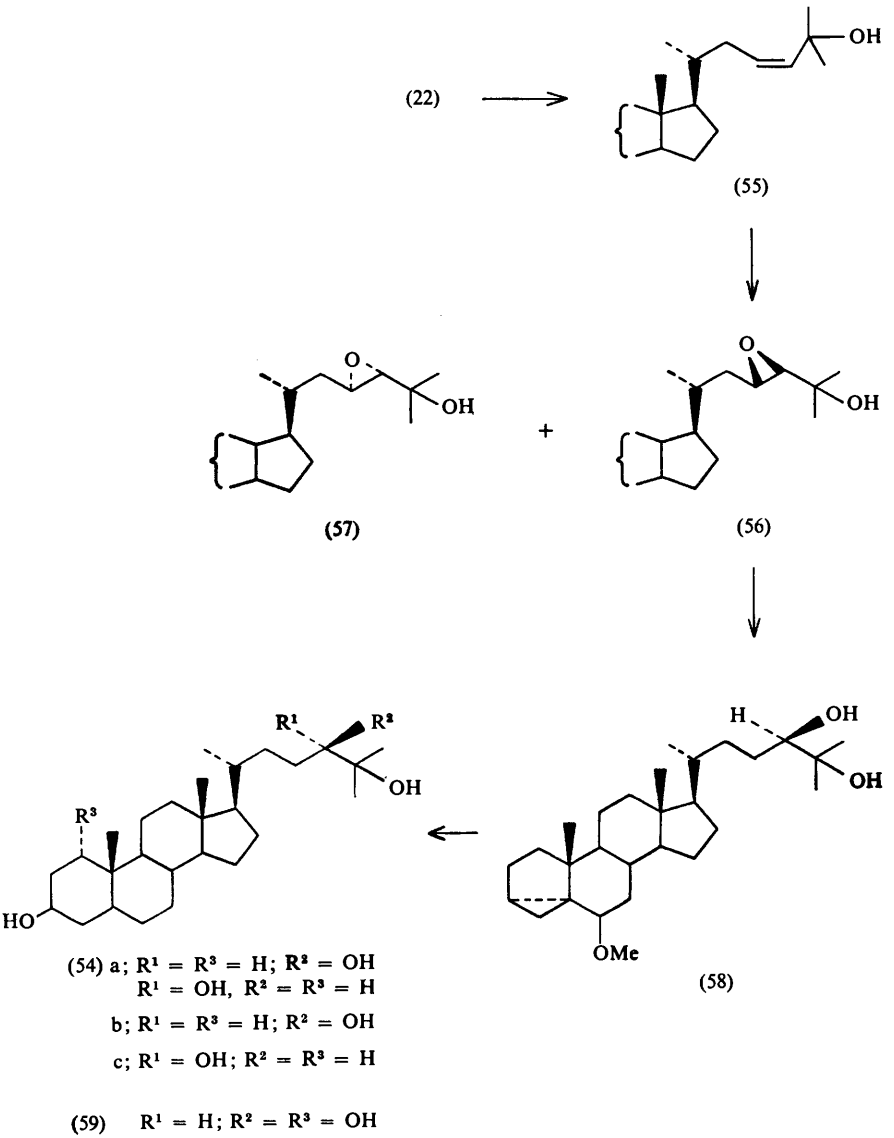


Scheme 11

group has recently published[35a] a stereoselective synthesis of the 24(*R*),25-dihydroxycholesterol (54b) (Scheme 12). Their starting compound was the acetylenic alcohol (22) which was used in their earlier synthesis of (3c). Catalytic partial hydrogenation of (22) gave the *Z*-allylic alcohol (55). This was stereoselectively epoxidized by Sharpless's method using t-butylhydroperoxide and a catalytic amount of vanadyl acetoacetate in toluene, at −78 °C, to produce an 85 : 15 mixture of (56) and (57) (Scheme 12). These epoxyalcohols were separated on silica gel, and (56) was reduced with lithium aluminium hydride to (58). Treatment of (58) with aqueous acidic dioxan gave the desired compound (54b) in an unspecified overall yield. The 24(*S*),25-dihydroxycholesterol (54c) was also stereoselectively synthesized by a modification of this route.

The absolute configurations of these compounds were determined by a useful modification of the induced split-circular dichroism method of Dillon and Nakanishi,[36] and confirmed by a single crystal *X*-ray structural determination of (58). A synthesis of 1α,24(*R*),25-trihydroxycholesterol (59) from (58) has also been achieved[35b] but has not been published at the time of submission of this review.

[35] J. J. Partridge, V. Toome, M. R. Uskoković, (*a*) *J. Amer. Chem. Soc.*, 1976, **98**, 3739; (*b*) in press.
[36] J. Dillon and K. Nakanishi, *J. Amer. Chem. Soc.*, 1975, **97**, 5417.



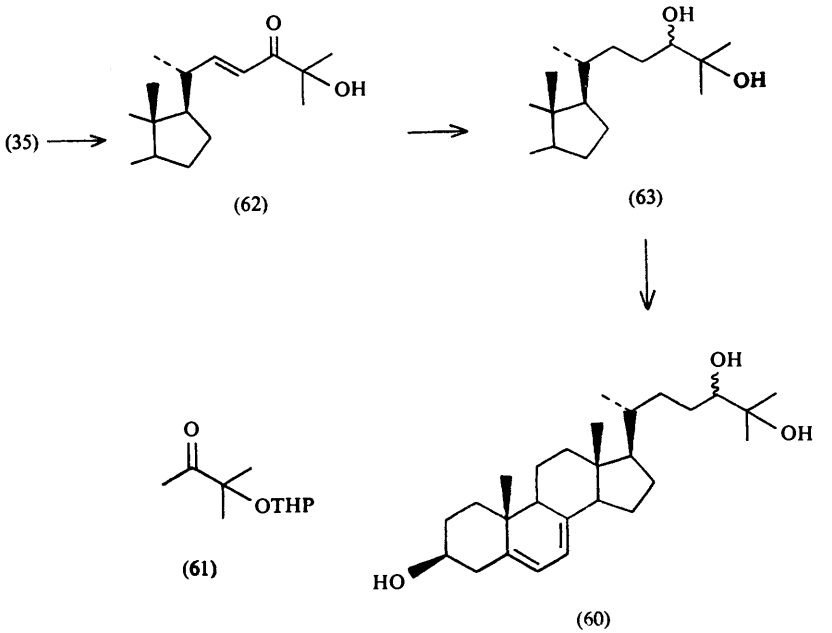
(54) a; $R^1 = R^3 = H$; $R^2 = OH$
$R^1 = OH$, $R^2 = R^3 = H$
b; $R^1 = R^3 = H$; $R^2 = OH$
c; $R^1 = OH$; $R^2 = R^3 = H$

(59) $R^1 = H$; $R^2 = R^3 = OH$

Scheme 12

Eyley and Williams have described[29a] the synthesis of the epimeric procalciferols (60) in an overall yield of 38%, from the noraldehyde (35) (Scheme 13). Aldol condensation of the enolate of (61) with (35), followed by acidic workup

gave the enone (62). Reduction with sodium borohydride in pyridine afforded the epimeric triols (63) which, after removal of the PTAD-protecting group gave (60).



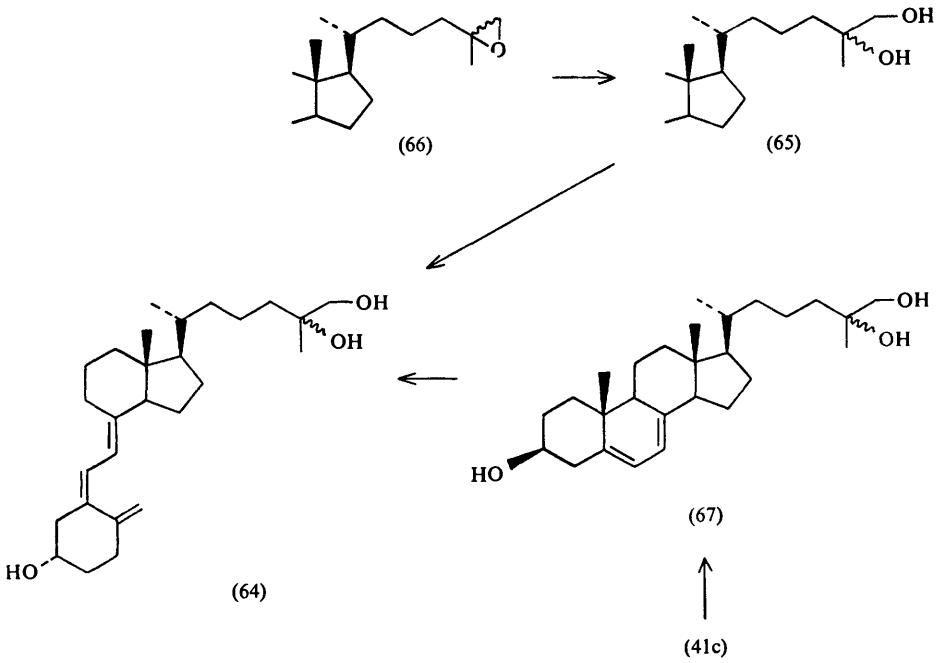
Scheme 13

E. 25ξ,26-Dihydroxycholesterol.—DeLuca's group[37] has isolated another side-chain dihydroxylated cholecalciferol metabolite which possesses biological activity. This metabolite is only produced in minor amounts and has been identified only as 25,26-dihydroxycholecalciferol (64) with the absolute stereochemistry at C-25 as yet to be determined. The exact function of this compound has not been determined either. All that is known is that it stimulates intestinal calcium transport, but that it does not induce bone calcium mobilization. It is also known that 1(*S*)-hydroxylation is as necessary for the onset of the activity of this compound as it is for 25-HCC and 24,25-DHCC.

Syntheses of (64) *via* the corresponding 25ξ,26-dihydroxycholesterols (65) (Scheme 14) have been reported by the groups of Kodicek,[38a] Ikekawa,[38b] and DeLuca.[38c] DeLuca used 26-norcholesten-25-one as starting compound, and

[37] T. Suda, H. F. DeLuca, H. K. Schnoes, Y. Tanaka, and M. F. Holick, *Biochemistry*, 1970, **9**, 4776.

[38] (*a*) J. Redel, P. Bell, F. Delbarre, and F. Kodicek, *Compt. rend.*, 1973, **276**, *D*, 2907; (*b*) M. Seki, J. Rubio-Lightbourn, M. Morisaki, and N. Ikekawa, *Chem. and Pharm. Bull. (Japan)* 1973, **21**, 2783; (*c*) H.-Y. Lam, H. K. Schnoes, H. F. DeLuca, *Steroids*, 1975, **25**, 2.

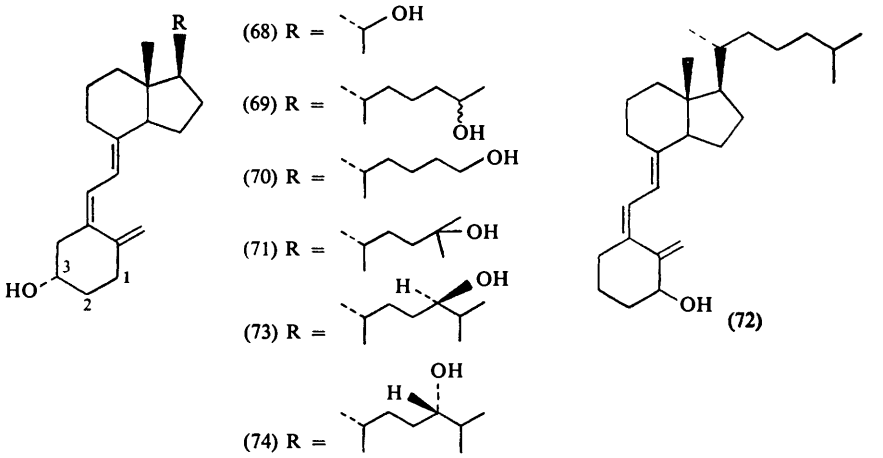


Scheme 14

this was reacted with dimethylsulphonium methylide. The resulting epoxide (66) was treated with potassium hydroxide in DMSO to give (65), as the C-25 epimers. Eyley and Williams[29b] have synthesized the corresponding 25ξ,26-dihydroxyprocholecalciferols (67) directly, by osmylation of (41c), followed by reduction with lithium aluminium hydride.

F. Other Hydroxylated Cholecalciferol Analogues.—Many synthetic analogues of the naturally produced hydroxylated calciferol metabolites have been prepared[39] in order to determine structure–activity requirements. However, apart from the metabolites already discussed, the only significant biologically active hydroxylated analogues synthesized to date have been those in which hydroxyl groups at the 1(*S*)-,24(*R*)- or 24(*S*)-, positions, or some combination of these, are present. It is also apparent that part of the side-chain must be present for at least some of the biological activities which are associated with these compounds to be observed. Thus (68) was not active at all, whereas (69), (70), and (71) had about 1—10% of the activity of 25-HCC in initiating intestinal calcium transport and bone mineral mobilization.[40]

[39] For a leading reference to the earlier work see M. Morisaki, N. Koizumi, N. Ikekawa, T. Takeshita, and S. Ishimoto, *J.C.S. Perkin I*, 1975, 1421.
[40] M. F. Holick and H. F. DeLuca, *Adv. Steroid Biochem. Pharmacol.*, 1974, **4**, 111.



Among the most active recently reported synthetic analogues to date are: (i) the 1(*S*)-hydroxycholecalciferol (1c),[17,18a] and 3-deoxy-1(*S*)-hydroxycholecalciferol (72) (these compounds however, require rapid 25-hydroxylation in the liver before the onset of their biological activity[41a,b,42]), and (ii) the 24(*R*)- and 24(*S*)-hydroxycholecalciferols,[43] (73) and (74), respectively, which do not differ in their activity towards the intestinal transport of calcium, and whose activity is similar to 25-HCC itself in the rat. However, in their bone-mobilizing activity, (73) is more potent than (74). For these compounds, prior 1(*S*)-hydroxylation in the kidney is necessary before the onset of biological activity.

5 Conformational Analysis and Structure–Activity Relationships.

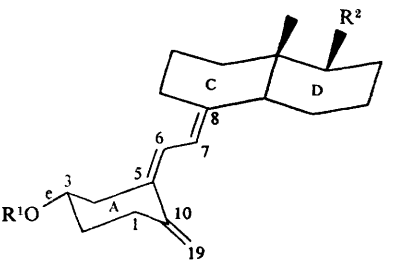
The absolute stereochemistry of a cholecalciferol derivative was determined in 1963 by Crowfoot–Hodgkin, using the 4-iodo-5-nitrobenzoate (75). The bulky C-3 substituent was shown to exist in the equatorial position, as indicated. Ring A was therefore in a conformation in which the C-10—C-19=CH_2 group is situated below the plane of the molecule.

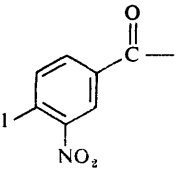
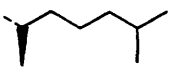
Havinga[4] proposed that in order to account for some of the photoproducts of cholecalciferol in various organic solvents, ring A should exist as a pair of rapidly equilibrating chair conformers, the α- and β-forms (77a) and (77b),

[41] (*a*) M. N. Mitra, A. W. Norman, and W. H. Okamura, *J. Org. Chem.*, 1974, **39**, 2931; (*b*) W. H. Okamura, M. N. Mitra, R. M. Wing, and A. W. Norman, *Biochem. Biophys. Res. Comm.*, 1974, **60**, 179.

[42] H.-Y. Lam, B. L. Onisko, H. K. Schnoes, and H. F. DeLuca, *Biochem. Biophys. Res. Comm.* 1974, **59**, 845.

[43] P. H. Stern, H. F. DeLuca, and N. Ikekawa, *Biochem. Biophys. Res. Comm.*, 1975, **67**, 965.



(75) R^1 =  , R^2 = 

(76) R^1 = H, R^2 = H

respectively. This prediction has been shown to be correct[44] by a study of the 300 MHz ^{1}H n.m.r. spectra of cholecalciferol in chloroform and carbon tetrachloride using lanthanide shift reagents, together with a computer-assisted analysis of the spectra. By analysing the proton couplings to the C-3 proton, it was clear that the A-ring was an approximately equimolar mixture of rapidly equilibrating α- and β-conformers. Similar results for ergocalciferol have also been obtained.[45]

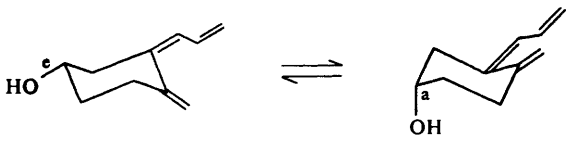
A comparison of the ^{1}H n.m.r. spectra of cholecalciferol with those of 1,25-DHCC, (1c), and the octa-nor derivative (76) showed that the conformations of ring A were unaffected by the nature of the side-chain. The introduction of a 1(*S*)-hydroxy group however, does shift the conformational equilibrium between (78a) and (78b). Thus, the β-form (78b), in which the 1(*S*)-hydroxy group is equatorial, is slightly favoured, despite the fact that both conformers possess one axial and one equatorial hydroxy substituent. Presumably, hydrogen bonding between the hydroxy and the C-10—C-19=CH_2 groups in the β-form could account for this.

Okamura and co-workers have proposed[46,12b] that the biological activity of calciferol-like molecules is dependent on the conformational equilibrium bias towards that conformer in which the substituent on the C-1 position (or its 'pseudo' equivalent) is in the equatorial position. This is based on the recognition

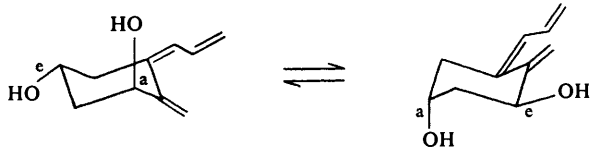
[44] (*a*) R. M. Wing, W. H. Okamura, M. R. Pirio, S. M. Sine, and A. W. Norman, *Science*, 1974, **186**, 939; (*b*) R. M. Wing, W. H. Okamura, A. Rego, M. R. Pirio, and A. W. Norman, *J. Amer. Chem. Soc.*, 1976, **98**, 4980.

[45] G. N. LaMar and D. L. Budd, *J. Amer. Chem. Soc.*, 1974, **96**, 7317.

[46] W. H. Okamura, A. W. Norman, and R. M. Wing, *Proc. Nat. Acad. Sci. U.S.A.*, 1974, **71**, 4194.



'α' conformer (77a) ⇌ 'β' conformer (77b)

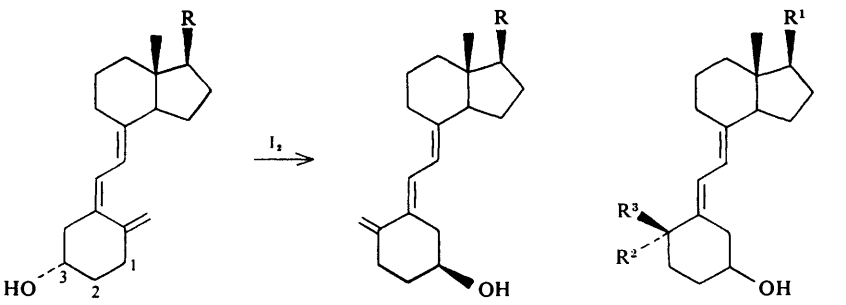


'α' conformer (78a) ⇌ 'β' conformer (78b)

by several groups of the following factors: (i) all biologically active calciferols in anephric animals possess a 1(*S*)-hydroxyl group *e.g.* (8), (1c), and (72), or its 'pseudo' equivalent *e.g.* 5,6-*trans*-cholecalciferol [5*E*,7*E*-cholecalciferol, or 5*E*,7*E*-CC, (79)], dihydrotachysterol$_3$ [DHT$_3$, (80)]. These latter compounds were the first active analogues[3] of cholecalciferol to be prepared. The 'pseudo' 1(*S*)-hydroxy group is merely derived by geometrical transposition of the C-3 hydroxy group as a result of isomerization of the $\Delta^{5,6}$-olefin. Thus (79) is obtained by iodine-catalysed isomerization of cholecalciferol (1b), and (80) is obtained as a major photoproduct from the precholecalciferol (6). (ii) Active analogues do not require a hydroxy group in what geometrically corresponds to the C-3 hydroxy group in (1a). These include (72), (79), and (80); (iii) The C-10—C-19=CH_2 group is not required for activity. (iv) The nature of the side-chain does not affect the ring A conformational equilibrium, although some part of it is necessary, as seen previously, for biological activity. As yet, however the side-chain requirements have not been fully established.

An empirical conformational analysis of the synthetic analogue (72) predicts[12b] that the conformer in which the 1(*S*)-hydroxyl group is in the equatorial position should be favoured by *ca.* 0.4 kcal mol^{-1}. Thus, the β-conformer should favour the α-conformer by a ratio of *ca.* 66 : 34. This compares with the n.m.r.-determined ratio of *ca.* 55 : 45 respectively for the β- and α- conformers of 1,25-DHCC. The compound (72) in fact, shows greater activity towards inducing intestinal calcium transport than does 1,25-DHCC. [It seems likely however, that (72) is first metabolized to its corresponding 25-hydroxy-derivative before the onset of activity.]

Further empirical conformational analyses carried out in the same manner



(1b) R = C_8H_{17} (79) R = C_8H_{17} (80) R^1 = C_8H_{17}, R^2 = CH_3, R^3 = H

(81) R^1 = C_9H_{17}, R^2 = H, R^3 = CH_3

on 5*E*,7*E*-CC, DHT_3, and the dihydro-ergocalciferol (81) all of which possess the 'pseudo' 1(*S*)-hydroxyl group, are summarized in the Table.

Table

Compound	Predicted percentages[12b]	
	β-conformer	α-conformer
DHT_3 (80)[a]	97	3
5*E*,7*E*-CC (79)	66	34
'dihydroergocalciferol' (81)	9	91

[a] Relative conformational populations confirmed by n.m.r.

Thus it is predicted that the general order of biological activity for these compounds should be DHT_3 > 5*E*,7*E*-CC > (81). In fact, (81) is completely inactive. Towards intestinal calcium transport, DHT_3 is more active than 5*E*,7*E*-CC. Furthermore, the 25-hydroxy derivative of the latter compound is even more active than 25-HCC itself, in stimulating intestinal calcium transport,[47] in anephric animals.

To further delineate this conformational structure–activity hypothesis, the following compounds have been synthesized but their relative biological activities have not been reported yet: 1(*S*)-hydroxy-3(*R*)-cholecalciferol,[48a,b] 3(*R*)-cholecalciferol[49] and 5*E*,7*E*-3(*R*)-cholecalciferol.[49] In addition, work on the homo-ring A analogues is in progress.[50]

[47] M. I. Holick, M. Garabedian, and H. F. DeLuca, *Science*, 1972, **176**, 1247.
[48] (*a*) M. Sheves, F. Berman, D. Freeman, and Y. Mazur, *J.C.S. Chem. Comm.*, 1975, 643; (*b*) W. H. Okamura and M. R. Pirio, *Tetrahedron Letters*, 1975, 4317.
[49] D. J. Aberhart, J. Y.-R. Chu, and A. C.-T. Hsu, *J. Org. Chem.*, 1976, **41**, 1067.
[50] S. M. Sine, T. E. Conklin, and W. H. Okamura, *J. Org. Chem.*, 1974, **39**, 3797.

6 Summary and Conclusions

The discovery that 'vitamin D' could cure and prevent rickets has led to the discovery of a new endocrine system. In this system, the kidney functions as the secretory organ and regulates the production of 1,25-DHCC in response to various physiological signals. The behaviour of 1,25-DHCC is typical of other steroid hormones, and its major function is to maintain calcium and phosphate homeostasis. It does this in conjunction with another hormone, PTH. Whether or not 1,25-DHCC is first metabolized to a more active hormonal form has not yet been established. All that is known is that some part of the side-chain is needed for biological activity and that the side-chain is oxidatively degraded rapidly. However, the metabolite which is produced has not yet been isolated or identified nor has its site of synthesis been determined. The use of 1,25-DHCC with additional radioactive labelling either at specific positions in the side-chain or at C-6,[51] would be of help in this regard.

Okamura and Norman's conformational-equilibrium hypothesis for biological activity related to ring A is at the least an attractive working model. The initial competition studies[12b] reported by these authors generate many unanswered questions, and clearly much more investigation needs to be conducted in this area. For instance, in terms of intestinal calcium transportation relative to 1,25-DHCC, it is more important to compare synthetic analogues which contain a C-25-hydroxyl group than those that do not. Access to these derivatives and other potentially important analogues, of course provide many synthetic challenges to the chemist. Finally, a re-thinking of 'vitamin D' is required. It has been suggested that the true antirachitic 'vitamin' is the light photon that converts the endogenous source of the procholecalciferol in the skin into the precursors of hormonally active compounds.

The author wishes to thank Professor G. Just of McGill University for initially stimulating his interest in this area. Professors J. Orr and A. Fallis of Memorial University are thanked for many useful suggestions.

[51] D. Harnden, R. Kumar, M. F. Holick, and H. F. DeLuca, *Science*, 1976, **193**, 493.

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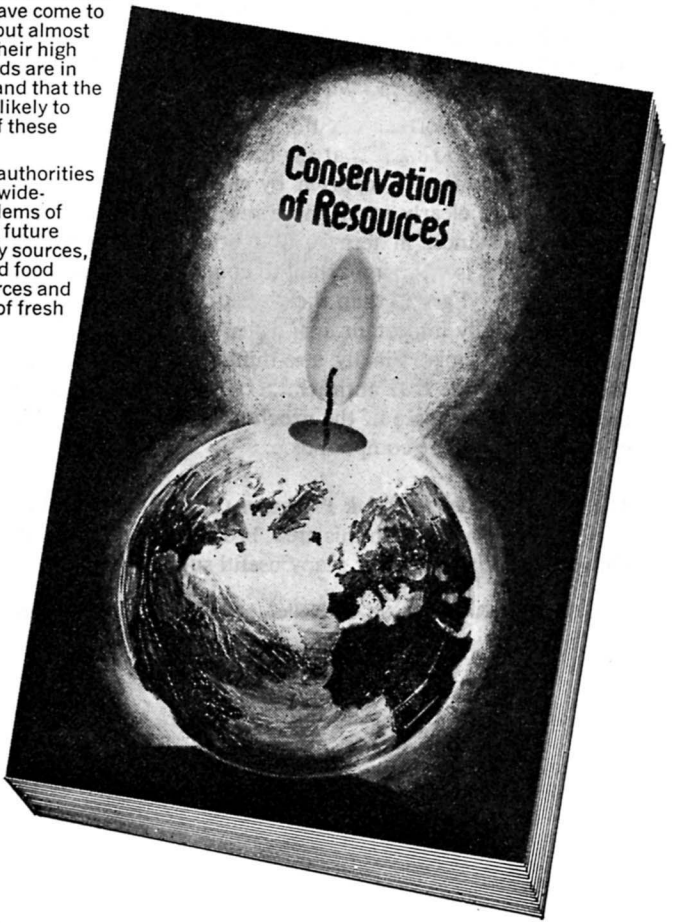

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