15TH ANNIVERSARY OF THE SYNTHESIS OF THE FIRST FLUORINE ANALOGUE OF VITAMIN D_3 CHEMICAL AND BIOLOGICAL ASPECTS OF THE PROBLEM

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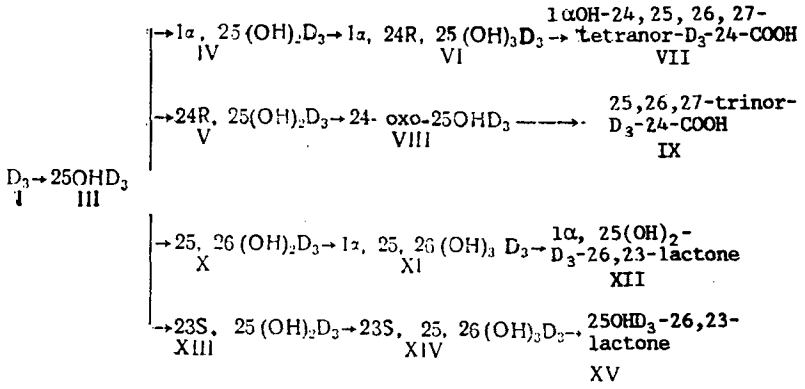
A review of literature information and the results of investigations by the authors on methods of synthesis for, and the biological activity of, fluorine derivatives of vitamin D_3 expands traditional idea on their influence on the regulation of Ca and P metabolism in the organism. The review includes literature sources from 1974 to 1989.

Advances in the interpretation of metabolic pathways and determining the hormonal action of vitamin D_3 (I) [1], and also the vigorous development of organofluorine compounds [2] initiated in the 50s by the synthesis of highly active fluorosteroids [3] served as the impetus for opening up a new and promising direction – the creation and investigation of fluorine derivatives of the vitamins D. We laid its foundation in 1974 by the synthesis and study of the biological activity of the first analogue – 3β-fluorovitamin D_3 ($3\beta FD_3$) (II) [4]. Several years later, intensive investigations in this field were pursued by American and Japanese chemists, when effective methods for introducing fluorine atoms [5-12] and hydroxy groups into various positions of the vitamin D_3 molecule were developed [13-18].

The synthesis of fluorine analogues of vitamin D_3 has permitted not only the preparation of new biologically active compounds but also the revelation of the physiological role of the hydroxy groups in the molecules of vitamin D_3 and its metabolites [6, 12]. The value of the side chain, the trienic system, and the 3β-hydroxy group for the manifestation of the antirachitic activity of the vitamins of the D group had been shown as early as the 30s-50s, but no explanation had been given of their physiological role [14]. The problem was complicated to a still greater degree when the metabolism of vitamin D_3 was studied and it was shown that before it exhibits a stimulating action on the transport of calcium in the intestine, the calcification of bone tissue, the resorption of calcium from bone, and the reabsorption of phosphorus in the kidneys, vitamin D_3 undergoes successive enzymatic hydroxylation in the liver to 25-hydroxy-vitamin D_3 ($25OHD_3$) (III) and then in the kidneys to 1α,25-dihydroxy-vitamin D_3 ($1\alpha,25(OH)_2D_3$) (IV) [1]. The latter is formed intensively under the conditions of hypocalcemia and is the most active vitamin-D compound – the hormonal form of vitamin D_3. Under conditions of normo- and hypercalcemia, $25OHD_3$ (III) is hydroxylated in the kidneys to 24, $25(OH)_2D_3$ (V), which is somewhat inferior in activity to $1\alpha,25(OH)_2D_3$ (IV).



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The further transformations of these metabolites in the organisms can characterized as inactivation. It may take place by various routes. 1α,25(OH)$_2$D$_3$ is hydroxylated in the 24R position to 1α,24R,25(OH)$_3$D$_3$ (VI), which is converted into 1α,OH-24,25,26,27-tetranorvitamin-D$_3$-23-oic acid (VII); 24R,25(OH)$_2$D$_3$ (V) is converted into 24-oxo-25OHD$_3$ (VIII) and then undergoes cleavage to 25,26,27-trinorvitamin-D$_3$-24-oic acid (IX). Furthermore, 25ODH$_3$ (III) is hydroxylated in position 26 to 25,26(OH)$_2$D$_3$ (X), and then to 1α,25,26(OH)$_3$D$_3$ (XI), which is converted into 1α,25(OH)$_2$D$_3$-26,23-lactone (XII). Another route to the last-mentioned compound is initial hydroxylation in position 23 to 23S,25(OH)$_2$D$_3$ (XIII), from which are formed successively 23S,25,26(OH)$_3$D$_3$ (XIV) and then the 25OH-26,23-lactone (XV), which is converted into the 1α,25(OH)$_2$D$_3$-26,23-lactone (XII) [13].

Consequently, on the activation and inactivation of the vitamin D$_3$ molecule a large number of hydroxy groups is introduced, and the necessity arises for elucidating the functional role of each of them, especially in the structure of the main metabolite 1α,25(OH)$_2$D$_3$ (IV). This question could not be solved by introducing into the ogranism synthetic deoxy derivatives of 1α,25(OH)$_2$D$_3$ (IV) (for example, 1αOHD$_3$, 3-deoxy-D$_3$, etc.), since on introduction into the organism they are rapidly hydroxylated in positions 1α and 25, and the main biological activity is determined by the 1α,25(OH)$_2$ derivative [14, 15]. The replacement of the 3βOH group by Cl, Br, I, or a SH group has not been successful, either [15].

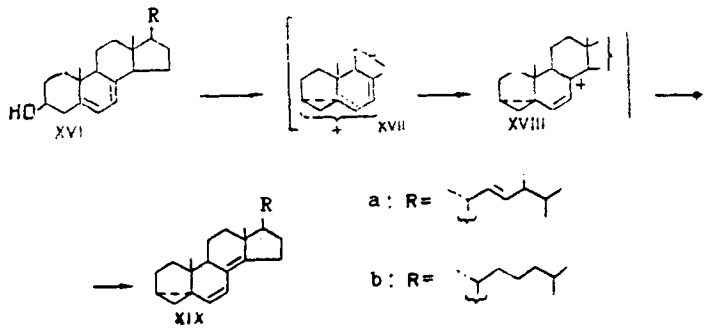
The performance of such transformations is probably connected with a considerable distortion in the structure of the vitamin D$_3$ molecule and leads in almost all cases to a considerable fall in, or the complete loss of, its biological activity [15].

Only the introduction of a fluorine atom (in view of its peculiarity of being able to imitate both H and OH and also its blocking property) into the vitamin D$_3$ molecule has permitted the performance of intensified investigations of the role of hydroxylation for the manifestation of its biological activity and the influence of its metabolites on the occurrence of metabolic processes in the organism. Furthermore, modification of compounds of the vitamin D series has permitted an escape from the framework of their traditional influence on the regulation of the calcium metabolism and a beginning of the study of these compounds as antileukemic [19], antiviral [20], antipsoriatic [21], and immunomodulating [22, 23] drugs.

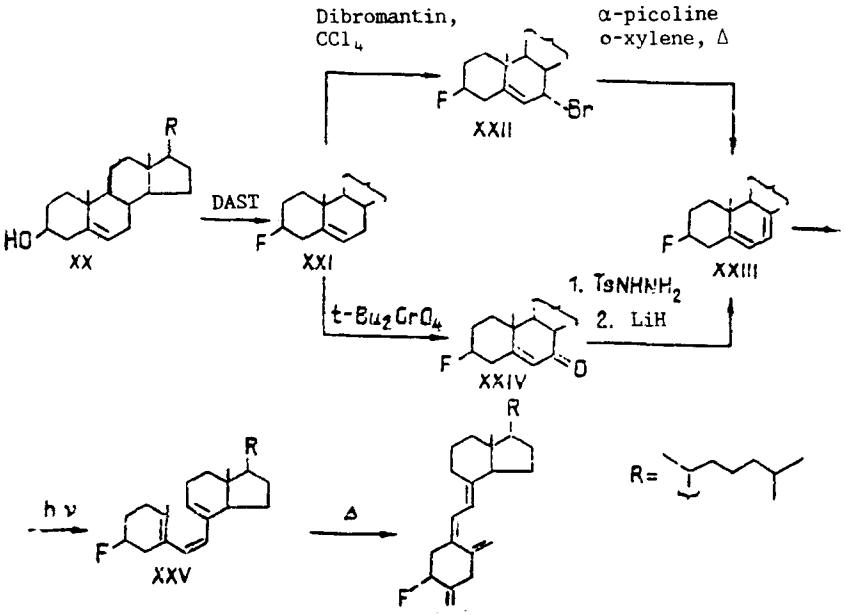
SYNTHESIS OF VITAMIN D$_3$ DERIVATIVES

3βF-DERIVATIVES OF VITAMIN D$_3$

3β-Fluorovitamin D$_3$ (3βFD$_3$) (II) was the first fluorinated analogue of vitamin D$_3$ [4]. The direct fluorination of vitamin D$_3$ did not lead to success: under the action of the Yarovenko reagent (Et_2NCF_2CHFCl) and DAST (Et_2NSF_3) on vitamin D$_3$ and also of metal fluorides on vitamin D$_3$ tosylate an elimination reaction from the homoallyl 3β-position predominated over the substituted reaction [24, 25]. The action of fluorinating agents (the Yarovenko reagent, DAST) on ergosterol (XVIa), 7-dehydrocholesterol (XVIb), and other provitamins D led only to dehydration via the intermediately formed carbocation (XVII), which, because of the presence of the C_7—C_8 double bond, readily isomerizes into (XVIII) and is stabilized by the ejection of a proton from position 14 [26, 27].

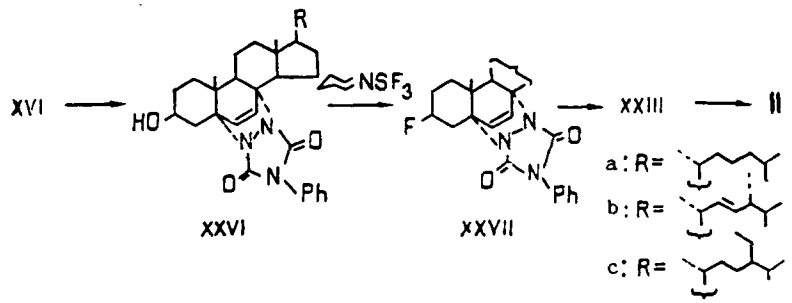


Therefore, to obtain 3βFD$_3$ (II) we first synthesized its provitamin — 3β-fluorocholesta-5,7-diene (XXIII) [28], starting from 3β-fluorocholesterol (XXI) [29] by the usual scheme.

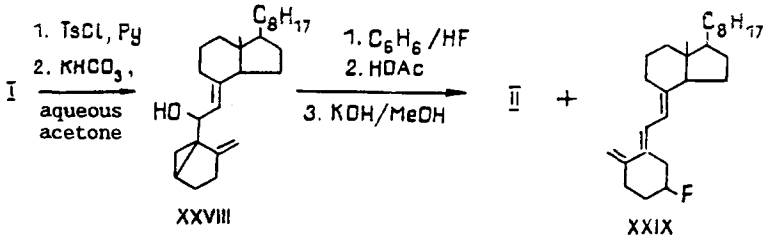


The allyl bromination of (XXI) took place with a high yield of the 7-bromo derivative (XXII), while debromination was accompanied by the partial splitting out of HF, and the yield of the provitamin (XXIII) was extremely low. The oxidation of (XXI) to the 7-keto derivative (XXIV), its conversion into the tosyl hydrazone and then treatment with $LiAlH_4$ enabled the yield of compound (XXIII) to be raised [30, 31].

We later obtained the 3β-fluorovitamins D_2, D_3, and D_5 [27, 28] from the corresponding provitamins after protecting the diene system with 4-phenyl-1,2,4-triazoline-3,5-dione, but in this case, as well, it was impossible to avoid dehydration. These investigations were repeated in 1985 [32] by Kumar et al. who obtained similar results.

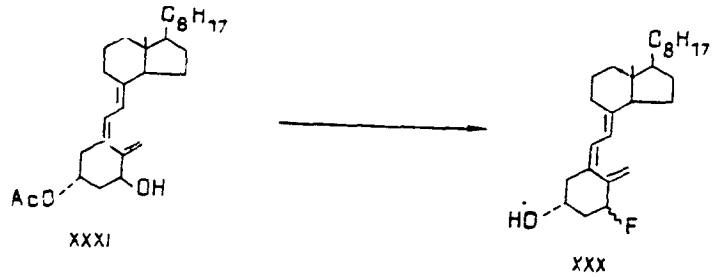


Using protection of the triene system by conversion into the 3,5-cyclovitamin (XXVIII), Mazur et al. obtained $3\beta FD_3$ (II) and vitamin D_3 together with a small amount of the 5,6-trans isomer [25]. The reaction bears a general nature and enables other 3β-halogeno analogues to be obtained. $1\alpha OH\text{-}3\beta FD_3$ and its 5,6-trans isomer were obtained in the same way [25].



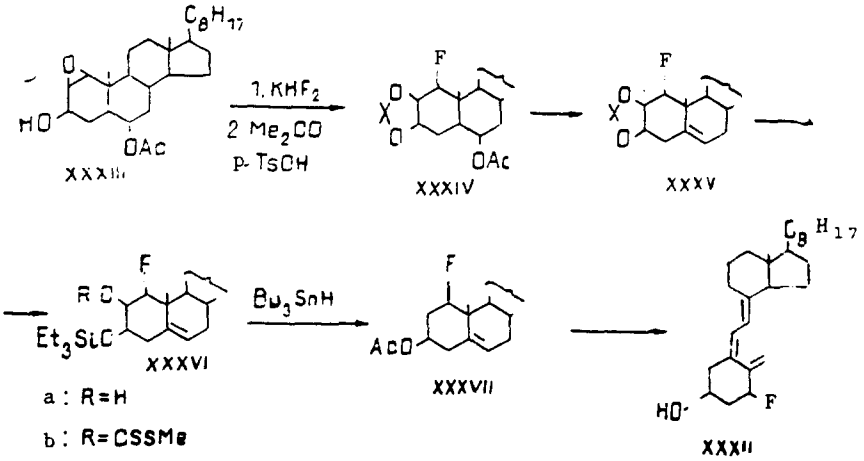
1-Fluoro derivatives of vitamin D_3

1-Fluorovitamin D_3 ($1FD_3$) (XXX) was obtained by DeLuca et al. in 1979 [33] by the action of DAST on a protected 1αOH derivative of vitamin D_3 (XXXI). The configuration of the fluorine in (XXX) was not obtained, in view of the fact that DAST does not cause its inversion.



XXXI XXX

However, five years later DeLuca [34] performed an independent stereospecific synthesis $1FD_3$ and showed that the UV spectrum of the 1F-analogue (XXXII) obtained differed from the spectrum of derivative (XXX). Under the action of KHF_2 on the epoxide (XXXIII) a trans-axial opening of the epoxide ring took place and the fluorine proved to be in the 1α-position; the hydroxy group in (XXXVIa) at C-2 was eliminated in the form of the xanthate, and the F-derivative of cholesterol (XXXVII) was converted by a known method into $1\alpha FD_3$ (XXXII). The α-configuration of the fluorine atom in (XXXII) was confirmed with the aid of PMR spectroscopy [34].



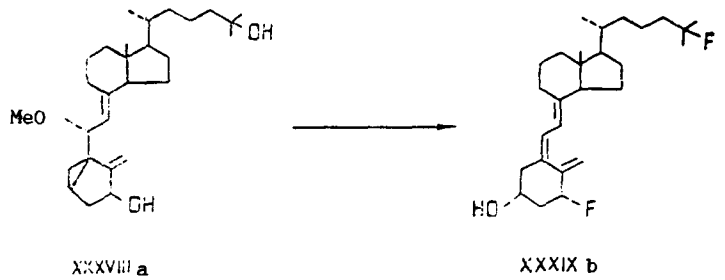
XXXIII XXXIV XXXV

XXXVI XXXVII XXXII

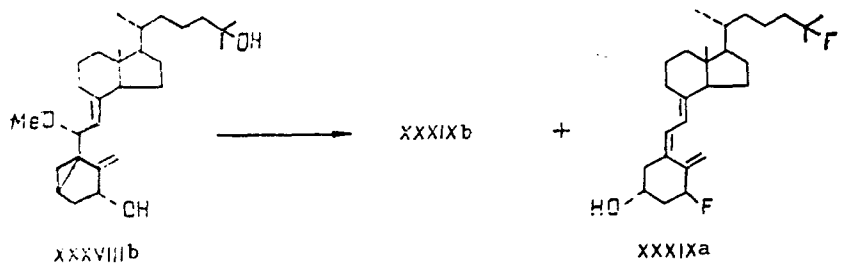
a: R=H

b: R=CSSMe

Analogous characteristics were observed in the fluorination with the aid of DAST of $1\alpha,25(OH)_2$-6MeO-3,5-cyclo-D_3 (XXXVIII): the fluorination of the 1αOH-isomer (XXXVIIIa) took place with complete inversion of the configuration, and the 1βF-derivative (XXXIXb) was formed, while the 1βOH isomer gave a mixture of (XXXIXb) and the 1αF derivative (XXXIXa) with the former predominating [35].

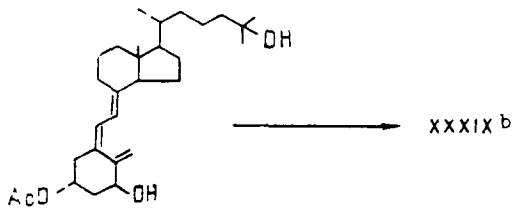


XXXVIII a XXXIX b



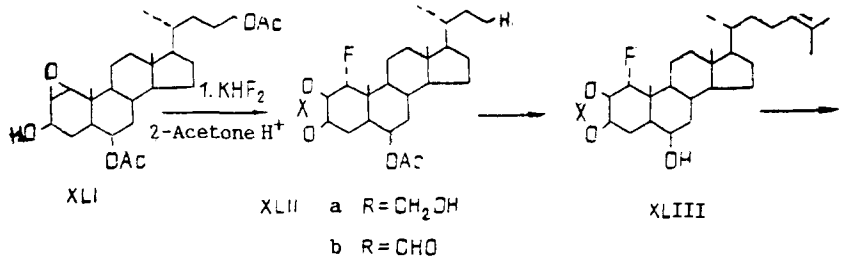
XXXVIIIb XXXIXb XXXIXa

Furthermore, on the basis of these characteristics and also of the results of IR spectroscopy it became possible to determine the configuration of the atom at C-1 as β in the product of the interaction of DAST with the 3β-acetyl derivative (XL), compound (XXXIXb) [36].



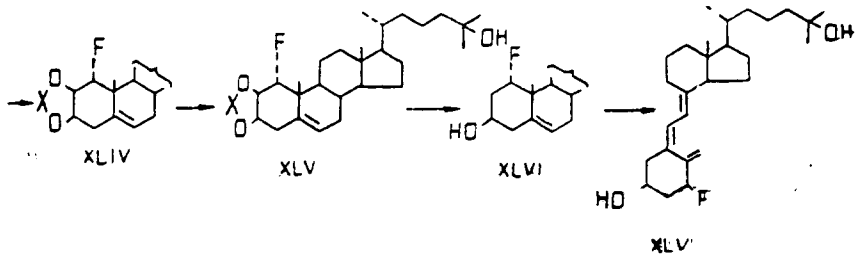
XL

The introduction of a fluorine atom into position 1α of compound (III) was achieved by Kobayashi et al. in 1984 [37] by analogy with the synthesis of (XXXII) [34]), with the simultaneous growth of the side chain and the introduction of a hydroxy group at C-25.



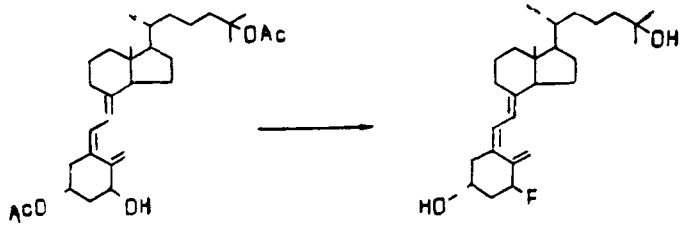
XLI XLII a R=CH2OH XLIII

b R=CHO



XLIV XLV XLVI XLVII

1βF-25OHD$_3$ (XLVII) was also synthesized by the action of DAST on the 3β,25-diacetyl derivative of 1α,25(OD)$_2$D$_3$ (XLIX) [36].

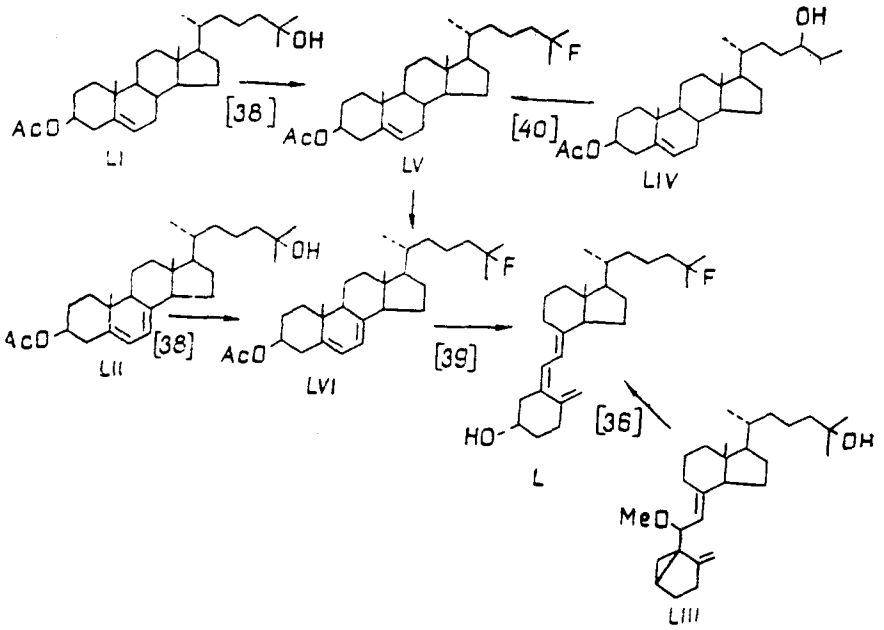


XLIX XLVIII

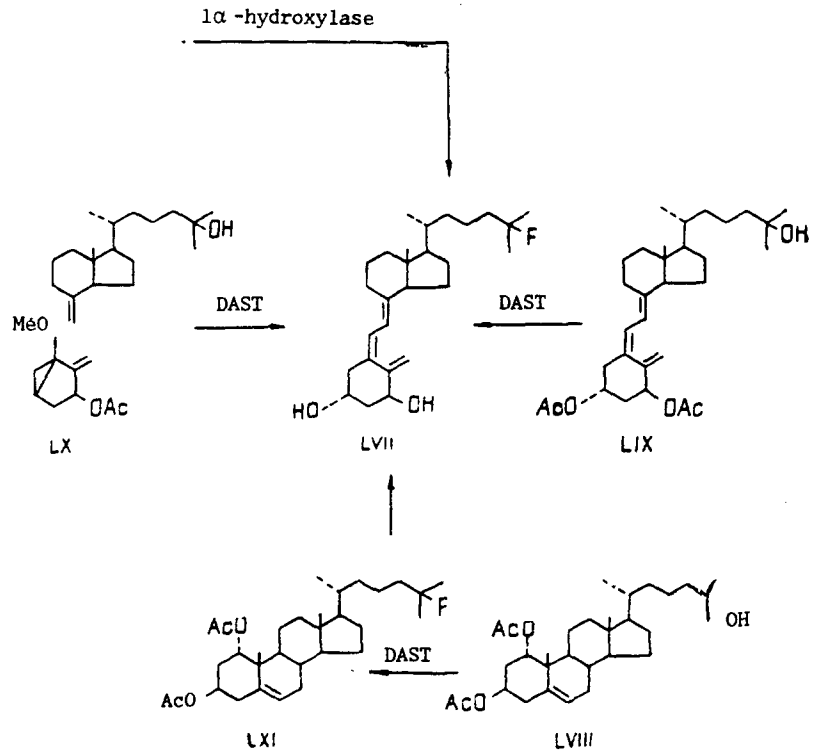
25-FLUORO DERIVATIVES OF VITAMIN D$_3$

The synthesis of 25-fluorovitamin D$_3$ (25FD$_3$) (L) has been carried out by various routes. Started from the 25-hydroxy derivative of cholesterol (LI) or the provitamin (LII), DeLuca et al. in 1977 (38] were the first to obtain (L) with the aid of DAST. Analogously and independently from them, compound (L) was synthesized by Jones [39]. In 1980, DeLuca et al. showed that (L) can be obtained by the action of DAST from the cyclovitamin (LIII), as well [36]. In addition, Kobayashi et al. in 1982 [40], in an attempt to replace the OH at C-24

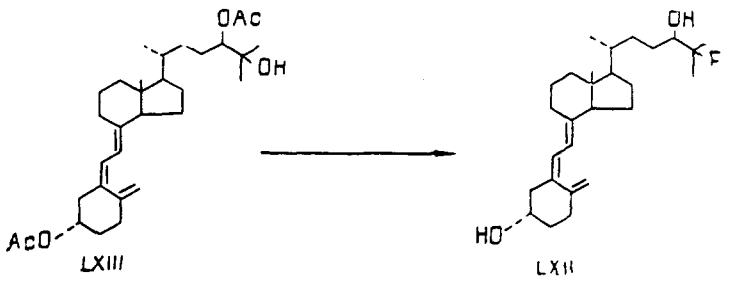
in compound (LIV) by the action of $PhPF_4$, unexpectedly obtained the 25F-derivative (LV), which was also converted into compound (L).



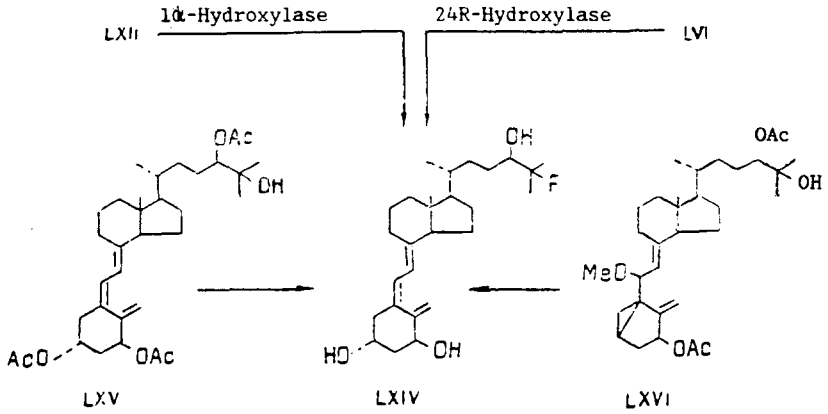
1α-Hydroxy-25-fluorovitamin D_3 ($1\alpha OH\text{-}25FD_3$) (LVII) was obtained enzymatically from (L) [41], and a chemical synthesis of the vitamin (LIX), and of the cyclovitamin (LX) was carried out from the 25-hydroxy-1α-acetoxy derivative of cholesterol (LVIII) [36, 42].



24R-Hydroxy-25-fluorovitamin D_3 ($24ROH\text{-}25FD_3$ (LXII) was synthesized by DeLuca [43] and Uskokovic [44] independently of one another in 1979 by the action of DAST on the protected hydroxy derivative (LXIII).

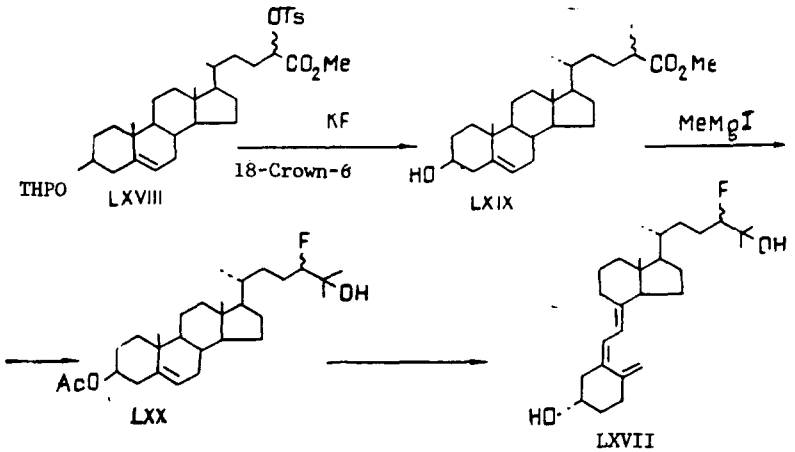


1α,24R-Dihydroxy-25-fluorovitamin D_3 (1α,24R$(OH)_2$-25FD_3) (LXIV), obtained enzymatically from (LXII) and (LVII) [45], has also been synthesized by the action of DAST on the corresponding hydroxy derivatives of the vitamin (LXV) and the cyclovitamin (LXVI) [44].

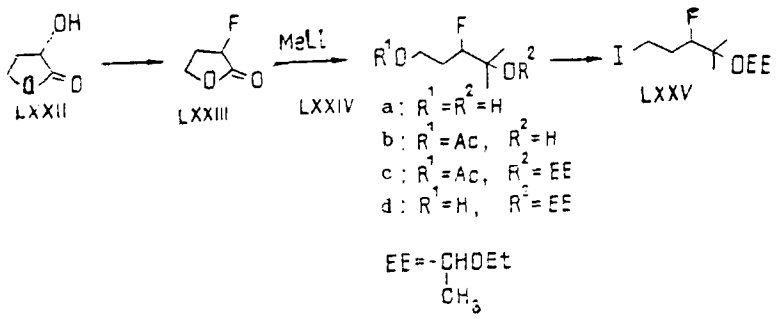


24-FLUORO DERIVATIVES OF VITAMIN D_3

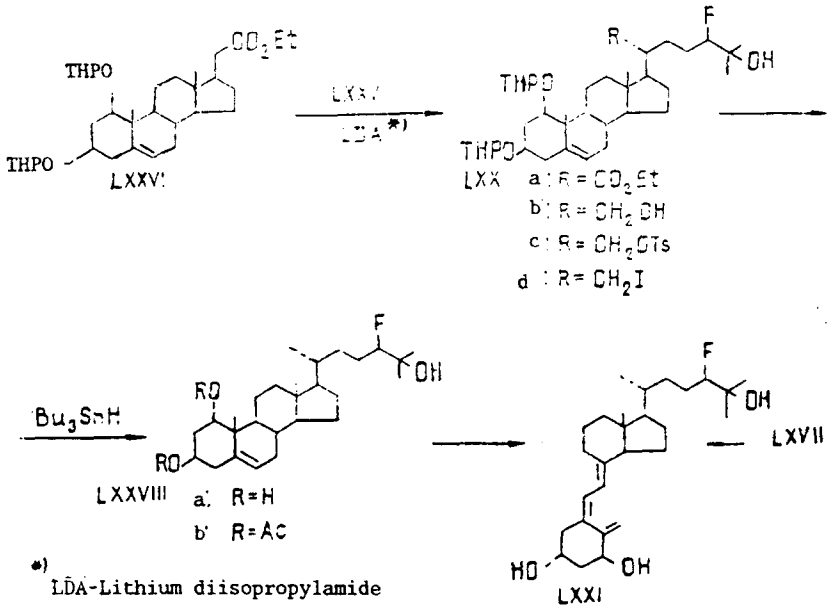
24ξ-Fluoro-25-hydroxyvitamin D_3 (24ξ-F-25OHD_3) (LXVII) was obtained by Kobayashi et al. in 1979 [40, 46]. The introduction of the fluorine atom was carried out by the nucleophilic replacement of the OTs group under the action of KF in homocholenic acid derivative (LXVIII).



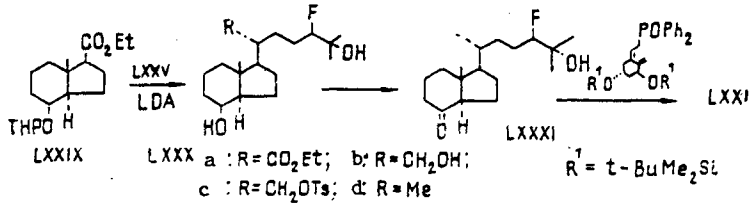
1α,25-Dihydroxy-24R-fluorovitamin D_3 (1α,25$(OH)_2$-24RFD_3) (LXXI) was synthesized by Uskokovic et al. [47, 48] in 1981 by a convergent scheme. The fluorine was introduced by the action of DAST on the optically pure hydroxybutyrolactone (LXXII), and the iodide (LXXV) was obtained in several stages.



Then the iodide (LXXV) was condensed in the presence of $(i\text{-}Pr)_2NLi$ with the ethyl ester of 1α,3β-bis(tetrahydropranyloxy)-21-norchol-5-en-22-oic acid (LXXVI). The CO_2Et group at C-21 in (LXXVIIa) was converted by a number of successive reactions into CH_3, and the vitamin (LXXI) was obtained from the diacetate (LXXVIIIb) in the usual way [47].

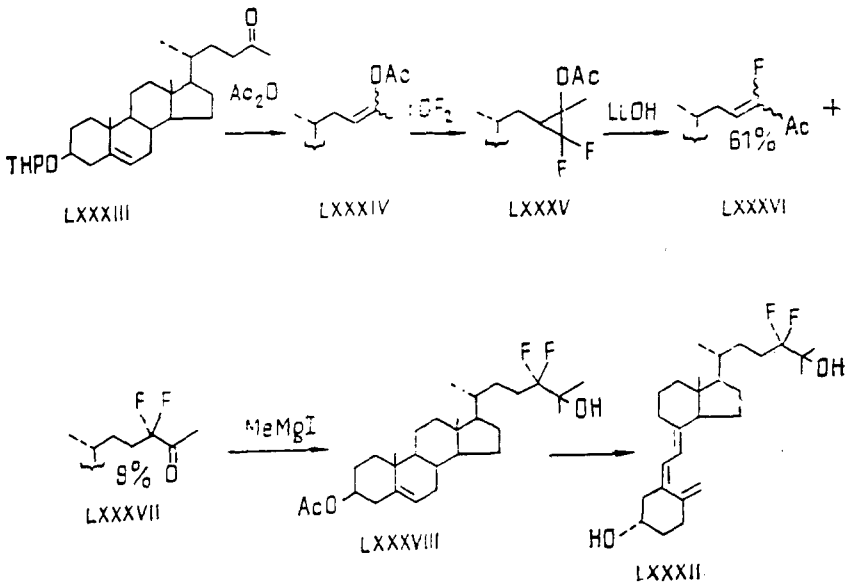


Uskokovic et al. [48] have also performed the convergent synthesis of (LXXI) from the iodide (LXXV) by a AB + CD scheme.

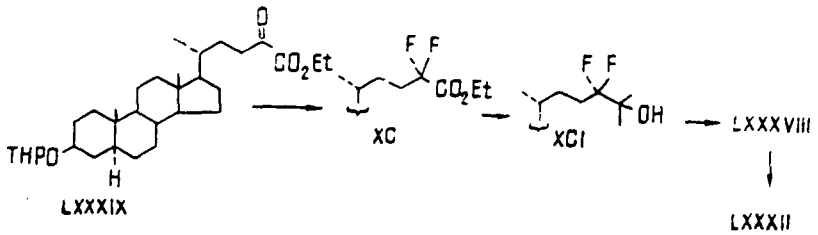


In addition, compound (LXXI) has been obtained enzymatically from the fluoride (LXVII) [49].

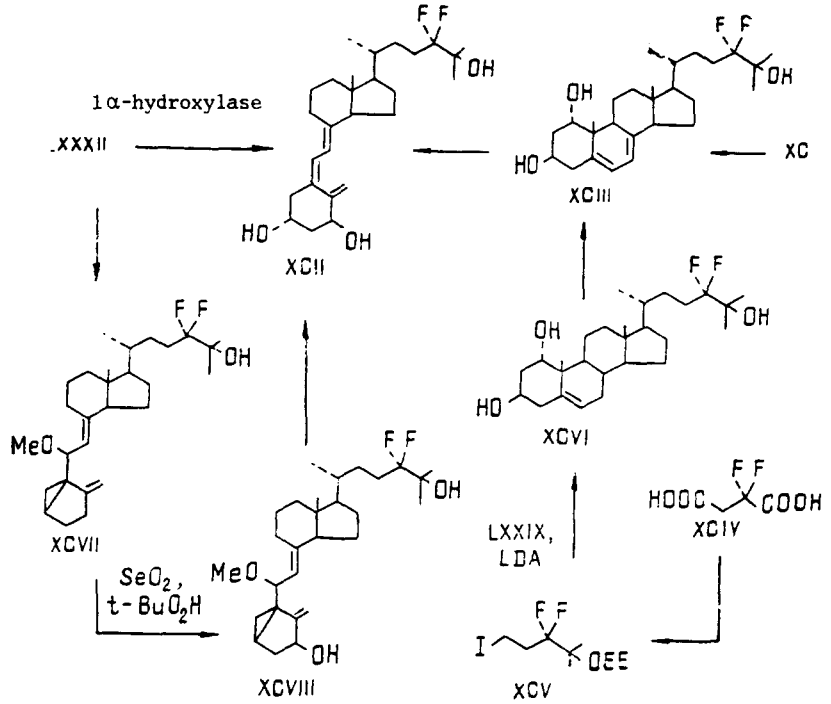
24,24-Difluoro-25-hydroxyvitamin D_3 ($24,24F_2$-$25OHD_3$) (LXXXII) was synthesized in 1979 by two groups of Japanese chemists independently of one another. To introduce the fluorine atoms, Kobayashi et al. [40] used the addition of difluorocarbene to the enol acetate (LXXXIV), followed by the opening of the cyclopropane ring in (LXXXV) under the action of the hydroxide ion.



Ohmori et al. [50] introduced the fluorine under the action of DAST on the α-keto ester (LXXXIX).



1α,25-Dihydroxy-24,24-difluorovitamin D_3 (1α,25(OH)$_2$-24,24-F_2D_3) (XCII) was obtained by DeLuca et al. in 1979 under the action of a kidney homogenate on compound (LXXXII) [50]. The chemical synthesis of (XCII) has been achieved by several other groups of chemists.

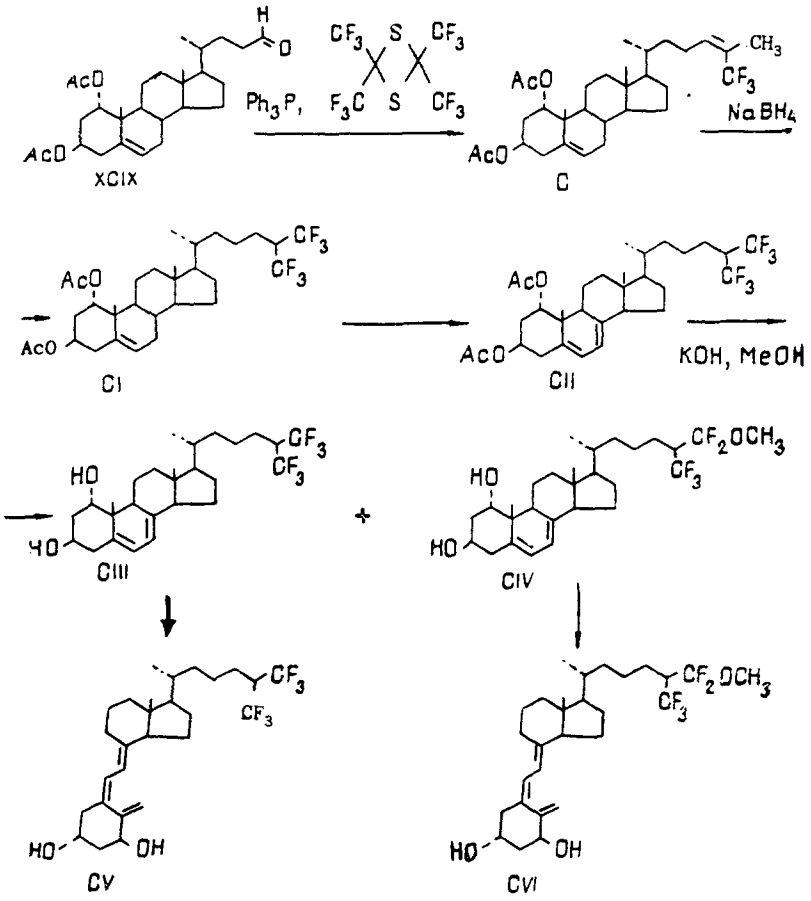


For the synthesis of the provitamin (XCIII), Ohmori et al. [51] started from the difluoro derivative of cholestane (XC). Uskocovic et al. [52] used a convergent scheme analogous to the synthesis of (LXXI) – 2,2-difluorosuccinic acid (XCIV) was converted into the

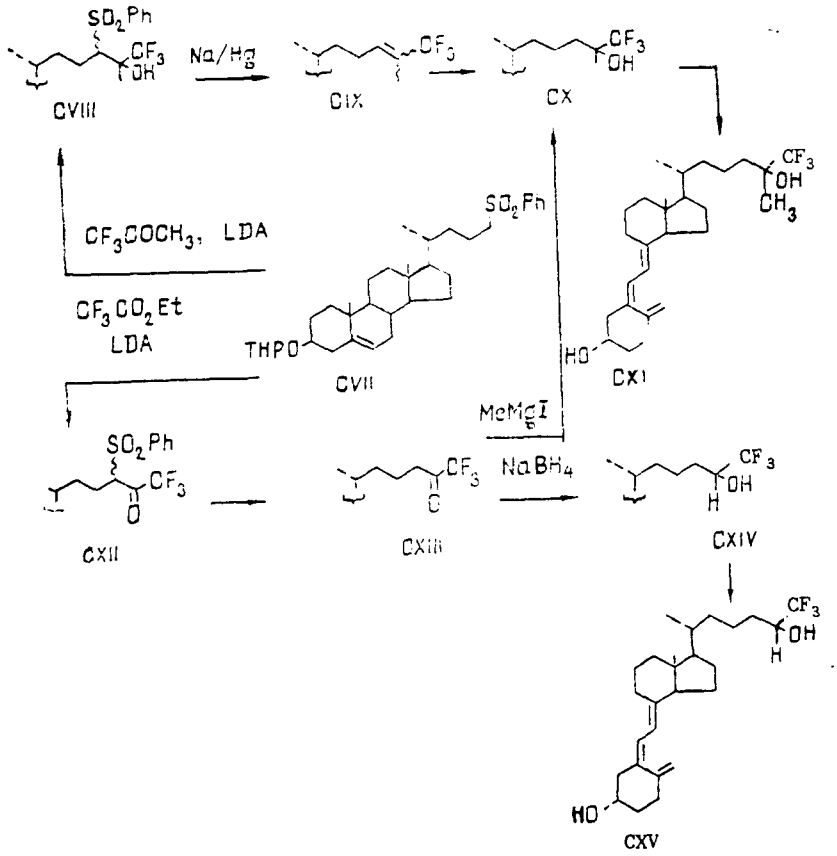
iodide (XCV), which was condensed with the ester (LXXVI). To introduce the OH group at C-1, Kobayashi et al. [53] used cyclovitamin protection and oxidized the cyclovitamin (LCVII) so obtained with the aid of SeO_2 to (XCVIII).

26- AND/OR 27-FLUORO DERIVATIVES OF VITAMIN D_3

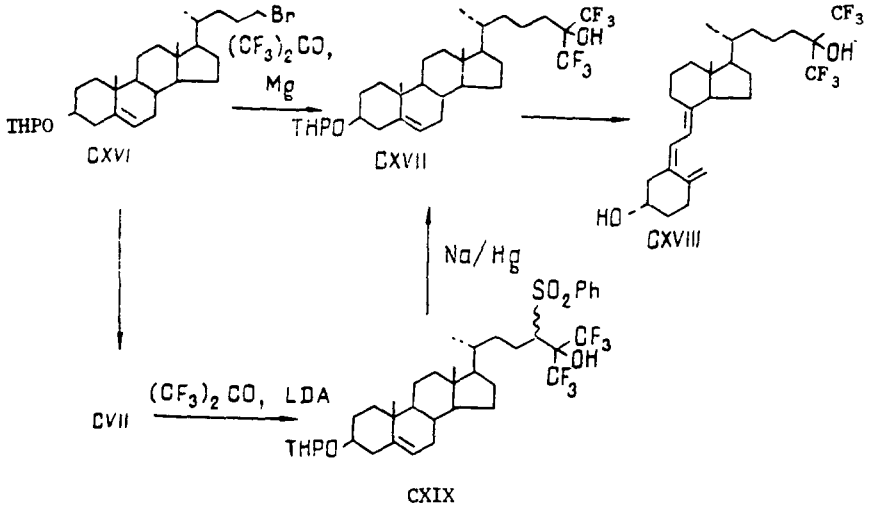
1α-Hydroxy-26,26,26,27,27,27-hexafluorovitamin D_3 (1αOH)-26,26,26,27,27,27F_6D_3) (CV) was obtained in 1987 [54] from the 1α,3β-diacetoxy derivative of 25,26,27-trinorcholest-5-en-24-al (XCIX). The Wittig condensation of the aldehyde (XCIV) with Ph_3P and the dimer of hexafluorothioacetone, followed by reduction of the side chain of (C), gave the hexafluoro derivative of cholesterol (CI), which was converted into the acetyl derivative of the previtamin (CII). It is interesting to note that when the acetyl protection was removed in (CII), in addition to the formation of the provitamin (CIII), nucleophilic replacement of the fluorine by a OCH_3 group was observed, and a certain amount of compound (CIV) was obtained, which, after isomerization under the usual conditions, gave 1α-hydroxy-26-methoxy-26,26,27,27,27--pentafluorovitamin D_3 [54].



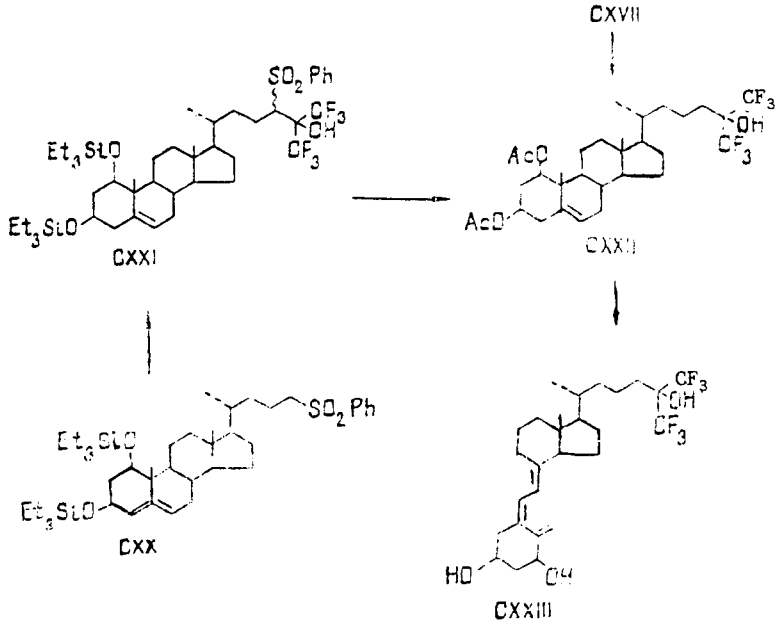
25-Hydroxy-26,26,26-trifluorovitamin D_3 (25OH-26,26,26FD_3) (CXI) and 25-hydroxy-26,26,26-trifluoro-27-norvitamin D_3 (25OH-26,26,26F_3-27-nor-D_3) (CXV) were synthesized in 1981 by Kobayashi et al. [56]. The CF_3 group was introduced into the phenylsulfonyl derivative of 25,26,27-trinorcholesterol (CVII) by treating its carbanion with trifluoroacetone, followed by reduction and the appropriate transformation of the fluorine derivative of cholesterol (CX) into the vitamin (CXI).



The interaction of (CVII) with CF_3CO_2Et and Al(Hg) followed by reduction of the ketone (CXIII) led to the vitamin (CXV) [56].

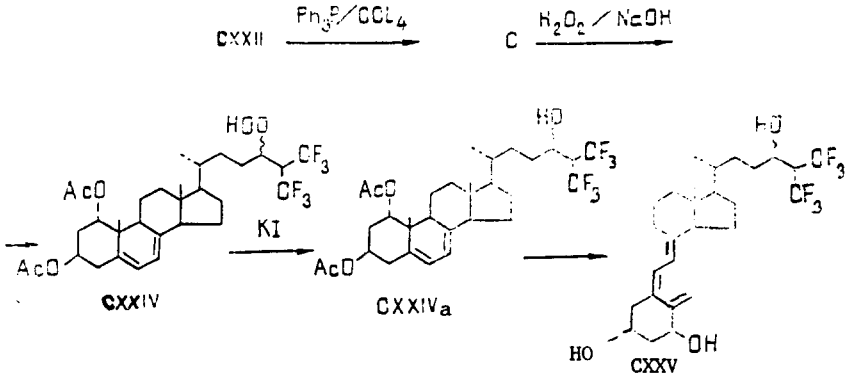


25-Hydroxy-26,26,26,27,27,27-hexafluorovitamin D_3 (25OH-26,26,26,27,27,27F_6D_3) (CXVIII) was obtained by Kobayashi et al. [57] in a similar way with the aid of hexafluoroacetone. The direct interaction of $(CF_3)_2CO$ with the organomagnesium compound obtained from the bromide (CXVI) led to (CXVII) in low yield. The route through the phenylsulfonyl derivative (CVII) was more successful.



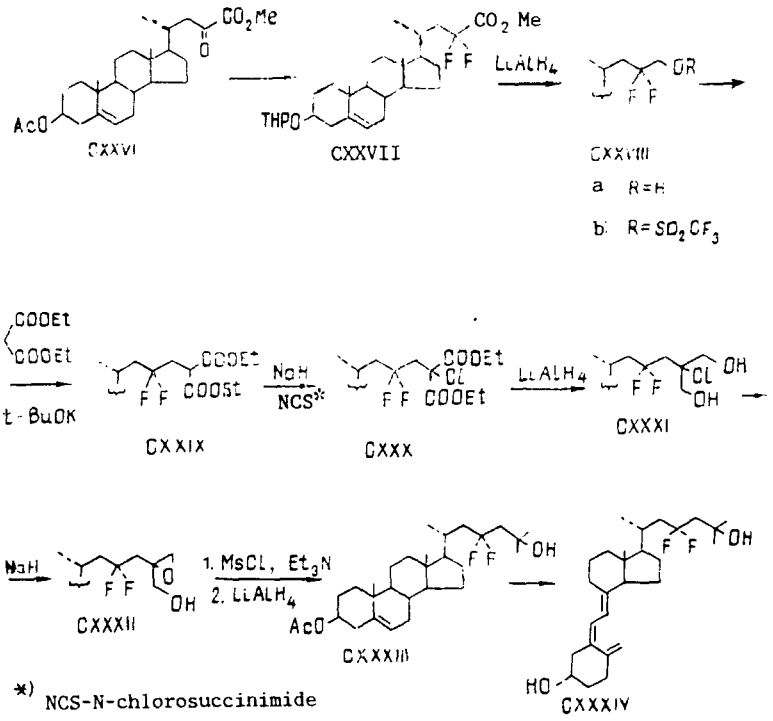
1α,25-Dihydroxy-26,26,26,27,27,27-hexafluorovitamin D_3 (1α,25(OH)$_2$-26,26,26,27,27,27F$_6$D$_3$) (CXXIII) was synthesized by Kobayashi et al. [58] in two ways. In the first case, $(CF_3)_2CO$ was condensed with the stable anion obtained from the sulfone (CXX), already containing a 1αOH group; and in the second case, the 1α-hydroxy group was introduced into the hexafluoro-substituted cholesterol (CXVII) by known methods.

1α,24ξ-Dihydroxy-26,26,26,27,27,27-hexafluorovitamin D_3 (1α,24ξ(OH)$_2$26,26,26,27,27,27F$_6$D$_3$) (CXXV) was synthesized from compound (CXXII), which, after dehydration, was converted into (C), from which, on oxidation with H_2O_2 followed by reduction of the hydroperoxide (CXXIV), the 24-hydroxy derivative (CXXIVa) was obtained [59, 60].



23-FLUORO DERIVATIVES OF VITAMIN D_3

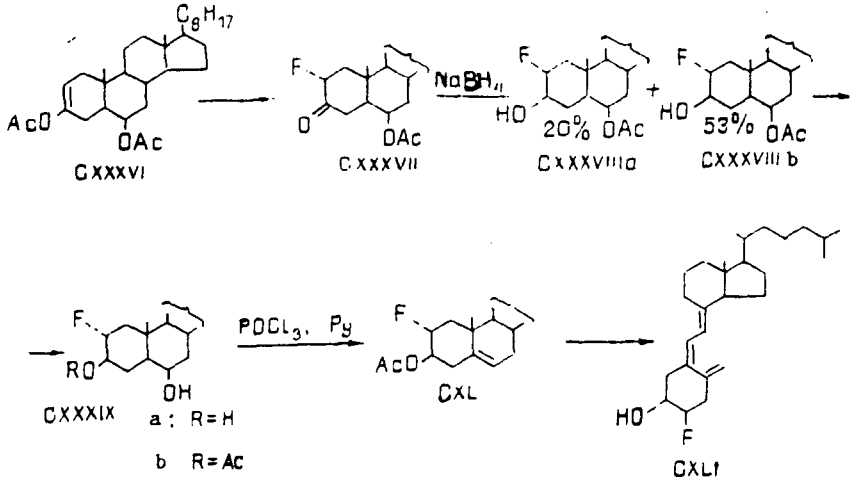
23,23-Difluoro-25-hydroxyvitamin D_3 (23,23F$_2$-25OHD$_3$) (CXXXIV) has been synthesized [61-63] by the action of DAST on the methyl ester of the α-keto acid (CXXVI), which was followed by its reduction to the alcohol (CXXVIIIa), and this was converted into the trifluoromethylsulfonyl derivative (CXXVIIIb). Condensation of the latter with the carbanion of malonic ester gave the diester (CXXIX) into which a chlorine atom was introduced at C-25, and the ester groupings of compound (CXXXI) were reduced to alcohol groups. The action of a base on the chlorohydrin (CXXXI) gave the enoxide (CXXXII), and then the dimesyl derivative was reduced to the 25-hydroxy derivative of cholesterol (CXXXIII).



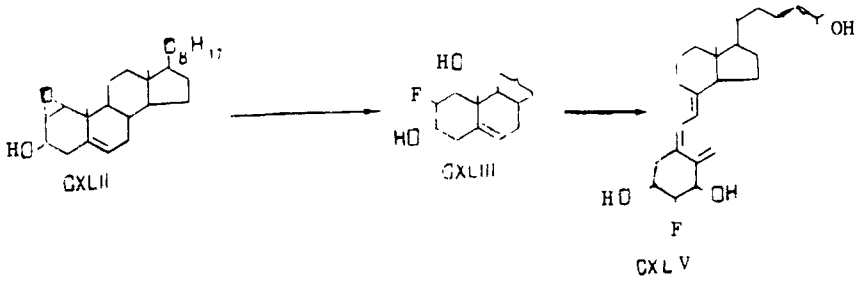
1α,25-Dihydroxy-23,23-difluorovitamin D_3 (1α,25(OH)$_2$-23,23F$_2$D$_3$) (CXXXV) was obtained by Ikekawa et al. [64-66] by the action of a kidney homogenate on (CXXXIV).

2-FLUORO DERIVATIVES OF VITAMIN D_3

2α-Fluorovitamin D_3 (2αFD$_3$) (CXLI) was synthesized by Japanese chemists in 1986 [67] by the action of cesium fluoroxysulfate on the enol acetate (CXXXVI). Under these conditions, the fluorine atom enters the 2α-position exclusively. Reduction of the ketone (CXXXVII) and regeneration of the Δ^5-double bond gave the 2α-fluorocholesterol ester (CXL).



To synthesize 1α-hydroxy-2β-fluorovitamin D_3 (1αOH-2βFD$_3$) (CXLIV) Ikekawa et al. [68] performed the trans-diaxial opening of the epoxide ring by the action of KHF_2 on (CXLII).

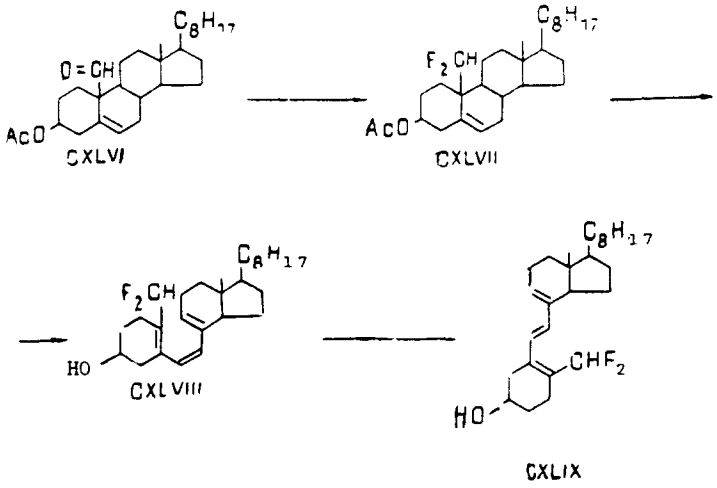


CXLII CXLIII CXLV

1α,25-Dihydroxy-2β-fluorovitamin D_3 (1α,25-$(OH)_2$-2βFD_3) (CXLV) was obtained by DeLuca et al. [69] enzymatically from (CXLIV).

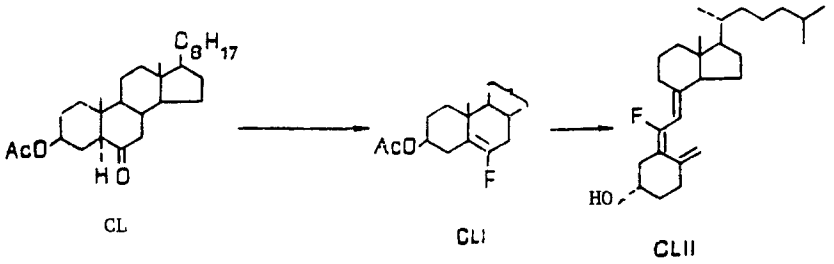
VITAMIN D_3 DERIVATIVES WITH A FLUORINE ATOM IN THE TRIENIC SYSTEMS

Attempts to synthesize 19,19-difluorovitamin D_3 proved to be unsuccessful [70], since 19,19-difluoroprevitamin D_3 (CXLVIII), obtained from the aldehyde (CXLVI) with the aid of DAST and the usual transformations, was converted on thermal isomerization not into the corresponding vitamin but into 19,19-difluorotachysterol (CXLIX).



CXLVI CXLVII CXLVIII CXLIX

6-Fluorovitamin D_3 (6FD_3 (CLII) was synthesized by Dauben in 1985 [71] by introducing a fluorine atom into position 6 of the ketone (CL) under fairly severe conditions by means of $C_5H_{11}NSF_3$, with the simultaneous formation of a small of the 6,6-difluoro derivative. Compound (CLII) proved to be extremely sensitive to oxygen.

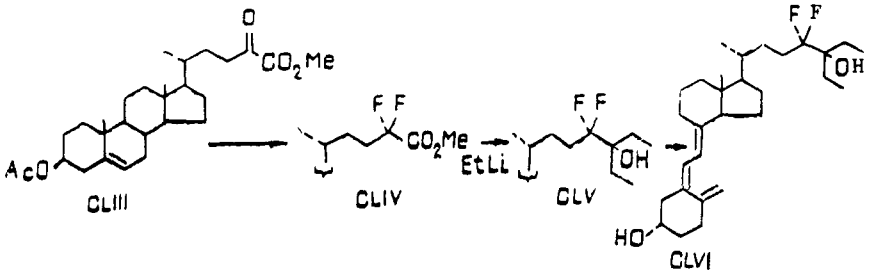


CL CLI CLII

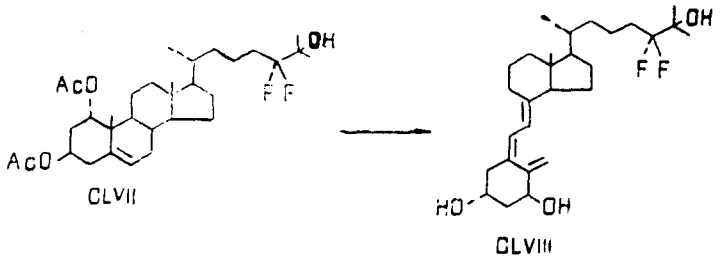
HOMO ANALOGUES OF VITAMIN D_3

In addition to the "normal" analogues of vitamin D_3 in which the carbon skeleton is retained, a number of fluorine derivatives with a changed skeleton have been synthesized. Characteristic for them is an increased number of carbon atoms.

24,24-Difluoro-25-hydroxy-26,27-dimethylvitamin D_3 ($24,24F_2$-25OH-$26,27Me_2D_3$) (CLVI) has been obtained by the action of DAST on the α-keto ester (CLIII) [72].

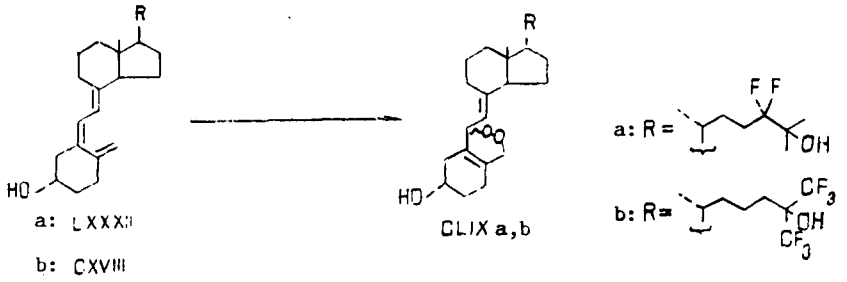


24a,24a-Difluoro-1α,25-dihydroxy-24-homovitamin D_3 (24a,24a-F_2-1α,25$(OH)_2$-24-homo-D_3) (CLVIII) was synthesized in 1987 by Ikekawa et al. [73] from the corresponding difluoro-substituted homocholesterol (CLVII) hydroxylated at C-1.



6,19-ENDOPEROXIDES OF VITAMIN D_3 DERIVATIVES

6,19-Epidioxy-6,19-dihydro derivatives of vitamin D_3 containing fluorine atoms at C-24 (CLIXa) and C-26,C-27 (CLIXb) were obtained by Suda et al. in 1985 [74] in the form of a R, S-epimeric mixture on photochemical oxygenation (addition of singlet oxygen) in the presence of a sensitizer.



BIOLOGICAL ACTIVITY OF FLUORINE DERIVATIVES OF VITAMIN D_3

3β-FLUORO DERIVATIVES

The introduction of a fluorine atom in place of the 3β-hydroxy group has enabled additional information to be obtained on the rule of the latter in the vitamin D_3 molecule. This question was raised as early as 1939 by Windaus [75] and has remained open up to the present time. Investigations performed in the 30s–60s showed that the replacement of the 3β-hydroxy group by a 3α- group lowers the antirachitic activity by a factor of 10-20 [75]; the introduction of a keto group in place of the hydroxyl decreases the activity 300 times [76]; the replacement of the 3βOH by a Cl, Br, or I atom or a SH group completely deprives the compound of activity [25, 77]; and on the introduction of a methyl group into the 3α- position the activity falls to 1/80 of that of D_3 [76].

These facts indicate the physiological significance of the hydroxy group at C-3 in the vitamin D_3 molecule and the impermissibility of structural changes in this position. But what is its concrete role?

Investigations of the 70s–80s connected with the study of active metabolites and analogs of vitamin D_3 and the expansion of criteria for evaluating the biological action of com-

pounds of the vitamin D series provide the possibility of suggesting a dual role of the 3βOH in the molecule.

The absence of the 3β-hydroxy group (3-deoxyvitamin D_3) leads to a fall in activity in the stimulation of the transport of Ca in the intestine as compared with vitamin D_3 in vivo and the loss of its capacity for binding with the cytoplasmic receptor in vitro [32]. At the same time, in the presence of a 1αOH group (3-deoxy-1αOHD_3), a high activity is observed in the stimulation of the transport of Ca in the intestine, comparable with that of 1α,25$(OH)_2D_3$, with a slight influence on the bone tissue. The necessity for larger doses of 3-deoxy-1αOHD_3 and of a longer time to achieve the maximum level of response as compared with 1α,25$(OH)_2D_3$ is possibly explained by the necessity for preliminary 25-hydroxylation. Synthesized 3-deoxy-1α,25$(OH)_2D_3$ proved to be identical with 1α,25$(OH)_2D_3$ with respect to the stimulation of the transport of Ca in the intestine [32]. Consequently, in the absence of the 3βOH group metabolic hydroxylation is apparently hindered, and this also explains the low activity of 3-deoxy-D_3 and the high activity of 3-deoxy-1α,25$(OH)_2D_3$.

Investigations to explain the capacity of metabolites and synthetic analogues binding with specific cytosolic receptors have shown that the elimination of the 3β-hydroxy group from 1α,25$(OH)_2D_3$ lowers this capacity 8-fold, while the elimination of the 1α- or the 25-OH group lowers it 900-fold and that of the 3β- and 25-OH groups 5000-fold, and the simultaneous elimination of the 1α- and 25-OH groups (vitamin D_3 itself) leads to the practically total loss of binding capacity (100,000-fold) [78]. It is possible that the fall in activity of 3-epi-1αOHD_3 and of 3-epi-1α,25$(OH)_2D_3$ is due to an interference in the formation of the complex with the receptor with a change in the configuration of the 3β-hydroxy group [75], just as on the introduction of voluminous substitutents (Cl, Br, I) [25] into the C-3 position. This interference is possibly a consequence of conformational changes and/or steric hindrance in the formation of the hormone-receptor system.

What has the replacement of the 3βOH group by a fluorine atom led to? In experiments on chicks to determine antirachitic activity, 3βFD_3 proved to be comparable with, and in the calcification of bone tissue, 1.5 times superior to, vitamin D_3 [4]. Kumar [32], on rats, showed a lower activity of 3βFD_3 than of vitmain D_3 but a higher activity than of 3-deoxy-D_3 in the stimulation of the transport of Ca in the intestine and its mobilization from bone. In the test for the induction of the synthesis of the vitamin D-dependent calcium-binding protein (CaBP) using a culture of tissue from the chick embryo duodenum, 3βFD_3 was approximately 1000 times less active than 1α,25$(OH)_2D_3$ but was equal to vitamin D_3, while 3-deoxy-D_3 showed no activity in this test.

In in vitro experiments on the capacity of analogous for displacing tritium-labeled 1α,25$(OH)_2D_3$ from its complex with the receptor of chick intestine cytosol, and also of labeled 25OHD_3 from the complex with the vitamin D-binding protein, both 3βFD_3 and 3-deoxy-D_3 exhibited extremely weak activity as compared with vitamin D_3 [32].

Thus, the replacement of the 3β-hydroxyl by a fluorine atom does not lead to a loss of activity, as in the case of the Cl, Br, and I analogues. At the same time, the introduction of a fluorine atom imparts a certain peculiarity into the manifestation of the biological activity of the compounds obtained. 3βFD_3 exhibits a capacity equal to that of vitamin D_3 for the synthesis of CaBP (imitation of a hydroxy group) with an absence of a capacity for binding with the receptor, as also for 3-deoxy-D_3 (imitation of hydrogen).

Numerous investigations of the role of the hydroxy group have shown that as yet it is impossible to determine it unambiguously. The contributions of this group to the manifestation of activity at various stages of metabolism are not identical, which explains all the diverse behaviour of the compounds studied.

25-FLUORO DERIVATIVES

The replacement of the hydroxy group at C-25 by a fluorine atom has enabled its role in the metabolism and biological activity of vitamin D_3 derivatives to be determined to a considerable degree. The activity of 25FD_3 in the induction of the synthesis of CaBP is 45 times that of vitamin D_3 [79], with retention of a weak activity in vivo [80]. The capacity of 25FD_3 for stimulating the transport of Ca in the intestine and for mobilizing it from bone is 100-1,000 times smaller than for vitamin D_3 [80]. 25FD_3 has proved to be an inhibitor of 25-hydroxylase without at the same time exhibiting antivitamin properties, like the 25-aza

analogue [6, 41]. It has been established that $25FD_3$ is hydroxylated in the organism to $1\alpha OH\text{-}25D_3$. The capacity of this fluorine analogue for binding is 315 times smaller than that of $1\alpha,25(OH)_2D_3$. At the same time, in vivo the capacity of $1\alpha OH\text{-}25FD_3$ for stimulating the transport of Ca in the intestine and for mobilizing it from bone is 50 times smaller than for $1\alpha,25(OH)_2D_3$. The existence of a considerable lag period in the biological response to $1\alpha OH\text{-}25FD_3$ is a consequence of its metabolism to $1\alpha,24R(OH)_2\text{-}25FD_3$ [42, 81].

The introduction of a fluorine atom into position 25 when a 24ROH group is present in the molecule ($24ROH\text{-}25FD_3$) led in vitro to a fall in the degree of binding with the specific cytosolic receptor by a factor of 530 as compared with $1\alpha,25(OH)_2D_3$, i.e., to the level characteristic for $25OHD_3$ and $24ROHD_3$ [43]. The activity of $24ROH\text{-}25FD_3$ in the transport of Ca in the intestine and its mobilization from bone was likewise equal to that of $25OHD_3$. Both compounds were inactive in nephrectomized animals and had the same time dependence of the biological response [45]. The metabolism of $24ROH\text{-}25FD_3$ in the organism, like that of $1\alpha OH\text{-}25FD_3$, leads to $1\alpha,24R(OH)_2\text{-}25FD_3$ [44]. The activity of the latter in the mobilization of calcium from bone is equal to that $1\alpha,25(OH)_2D_3$ and twice as great as that of $1\alpha,24R,25(OH)_3D_3$. In its influence on the calcification of bone tissue, $1\alpha,24R(OH)_2\text{-}25FD_3$ is 100 times better than 1α, 24R, $25(OH)_3D_3$ while being 5 times less active than 1α, $25(OH)_2D_3$. The antirachitic activity of $1\alpha,24R(OH)_2\text{-}25FD_3$ is 20 times less than that of $1\alpha,25(OH)_2D_3$ [44].

The facts presented show that a study of the role of the 25OH group with the aid of fluorine derivatives has permitted an indirect estimation of the significance of the hydroxylation of the molecule in the 1α and 24R positions. It is obvious that the 24ROH derivatives can regulate the calcium metabolism in the organism in the absence of a 25-hydroxy group and, consequently, such a group is not essential in this case. Compounds with a 1αOH group are more active than derivatives with 24ROH when a fluorine atom is present at C-25.

Thus, investigations of 25-fluoro derivatives have confirmed the importance of the 25OH group in the manifestation of the various forms of biological activity of vitamin D_3 metabolites both in vivo and in vitro. This group obviously participates in binding with the receptor in processes of the further hydroxylation of the molecule, in affecting the calcification of bone, in the manifestation of a complex antirachitic action, and, to a somewhat smaller degree, in the mobilization of calcium from bone. A fluorine atom at C-25 imitates hydrogen and not hydroxyl, showing some specificity of action, as in the case of the 3β-fluoro derivatives.

1-FLUORO DERIVATIVES

According to a modern concept [1], the highest level of vitamin D activity is connected with metabolic hydroxylation at C-1 in the α-position. A change in the 1αOH configuration to β leads to the complete loss of activity [82].

The activity of $1\alpha FD_3$ has not hiterto been studied. The $1\beta FD_3$ obtained by De Luca et al. (initially identified as $1\alpha FD_3$) was 100 times less active in the stimulation of the transport of Ca in the intestine and 10-15 times less in its mobilization from bone as compared even with vitamin D_3 itself [33]. This compound caused the maximum response considerably later than $1\alpha OHD_3$. This fact, and also the absence of a response in nephrectomized animals (in contrast to $1\alpha OHD_3$) shows the probability of the preliminary metabolism of $1\beta FD_3$ in vivo into a more active compound.

The simultaneous blocking of a hydroxy group at C-1 in the β-position and one at C-25 leads to the complete disappearance of the capacity for stimulating the transport of Ca and its mobilization form bone in vivo. $1\beta,25D_2D_3$ can be considered as an inert analogue [79]. At the same time, both 1βF analogues exhibit considerable activity in the induction of the synthesis of CaBP in a tissue culture of the chick duodenum as compared with vitam D_3 [37]:

D_3	$<1\beta, 25F_2D_3$	$<1\beta FD_3$	$<1\alpha, 25(OH)_2D_3$
8.6	570	3850	10.000

The introduction of a fluorine atom into the α-position at C-1 even when a hydroxy group is present at C-25 ($1\alpha F\text{-}25OHD_3$) leads to the disappearance of activity in vivo in relation to the transport of Ca in the intestine and its mobilization from bone (in doses 5 times exceeding those of $25OHD_3$), while in vitro $1\alpha F\text{-}25OHD_3$ binds with the specific cytosolic receptor 30 times better than $25OHD_3$ [37] binds with the specific cytosolic receptor.

The facts given confirm the exceptional role of the 1α-hydroxy group; however, they are insufficient to explain the activity of the 1β-fluoro derivatives.

24-FLUORO DERIVATIVES

Starting from the specific action of $24,25(OH)_2D_3$ on the mineralization of bone tissue, some workers consider that hydroxylation at C-24 is important for the regulation of the calcium metabolism [1]. The considerable interest in the biological activity of some 24-fluoro analogues of vitamin D_3 has raised the question of the role of hydroxylation at C-24. Both the monofluoro derivative – $24F\text{-}25OHD_3$ and the difluoro derivative – $24,24F_2\text{-}25OHD_3$ have antirachitic activities and capacities for stimulating the transport of Ca in the intestine and for mobilizing it from bone that are equal to those of $25OHD_3$ [53, 83-85]. It is likely that in this case the fluorine at C-24 imitates hydrogen. Nephrectomized animals give no response to $24,24F_2\text{-}25OHD_3$, which shows the necessity for its preliminary metabolic activation at C-1 in order to manifest biological action.

A difluoro derivative at C- 24 – $1\alpha,25(OH)_2\text{–}24,24F_2D_3$ – is the first synthetic analogue the activity of which exceeds the most active hormonal form of vitamin D_3 – $1\alpha,25(OH)_2D_3$ [53, 86, 87]. $1\alpha,25(OH)_2\text{-}24,24F_2D_3$ is 5-10 times more effective than $1\alpha,25(OH)_2D_3$ in its capacity for stimulating the transport of Ca in the intestine and mobilizing it from bone, in increasing the level of phosphorus in the blood and healing rickets, and 4 times more effective in the induction of the synthesis of CaBP in vitro. These compounds are equal with respect to their capacity for binding with the specific cytosolic receptor. Furthermore, the fluorine analogue exhibits a more prolonged action than the nonfluorinated compound [88, 89].

With respect to rickets, the 24-monofluoro derivative $1\alpha,25(OH)_2\text{-}24RFD_3$ is also superior to $1\alpha,25(OH)_2D_3$ and even to the difluoro analogue $1\alpha,25(OH)_2\text{-}24,25F_2D_3$ [47, 90].

Consequently, the blocking by fluorine of position 24 probably prevents inactivation by 24R-hydroxylation. The result of this is a higher activity and prolongation of the action of the 24-fluoro derivatives of vitamin D_3 hydroxylated at C-1 and C-25.

26- AND/OR 27-FLUORO DERIVATIVES

Hydroxylation at C-26 and C-27 represents an independent direction of the catabolism of vitamin D_3 and is also a stage of inactivation following 24R-hydroxylation. When positions 26 and 27 are blocked, both directions of the degradation of vitamin D_3 cease, which leads to an increase in the efficacy and a prolongation of the action of the compounds.

The hexafluoro analogue $26,26,26,27,27,27F_6\text{-}25OHD_3$ is identical with $25OHD_3$ with respect to the stimulation of the transport of Ca in the intestine, its mobilization from bone, the mineralization of bone tissues, and increasing the level of serum phosphorus; it is inactive in nephrectomized animals. $26,26,26,27,27,27F_6\text{-}25OHD_3$ possesses a prolonged action in comparison with $25OHD_3$, which presupposes a preliminary activation to the 1αOH derivative and retardation of the inactivation stage [91].

$26,26,26,27,27,27_6\text{-}1\alpha25(OH)_2D_3$ is at least 10 times more active than the natural hormone $1\alpha25(OH)_2D_3$ in tests on the transport of Ca in the intestine and its mobilization from bone, and also 5 times more effective in the treatment of rickets and raising the level of phosphorus in the blood [92]. The hexafluoro analogue of $1\alpha,25(OH)_2D_3$, in contrast to $1\alpha,25(OH)_2D_3$ itself [93], is active both on subcutaneous and on peroral administration and its effect is more prolonged (by a factor of 2-3), particularly in raising the level of phosphorus and in the mineralization of bone. At the same time, the fluorine analogue is somewhat less effective in binding with the rat vitamin-D_3-binding protein and 3 times as active in binding with the specific cytosolic receptor of the chick intestine.

Thus, $26,26,26,27,27,27F_6\text{-}1\alpha,25(OH)_2D_3$ is the most active of the metabolites and synthetic analogues of vitamin D_3.

23-FLUORO DERIVATIVES

The oxidative degradation of the vitamin D_3 molecule takes place readily at position 23 [1]. Protection of the C_{23} carbon atom by the introduction of two fluorine atoms into the $25OHD_3$ molecule leads to $23,23F_2\text{-}25OHD_3$, which is 5-10 times less active than $25OHD_3$ in general antirachitic tests [63, 94]. $23,23F_2\text{-}25OHD_3$ was converted with the aid of a kidney homogenate into $23,23F_2\text{-}1\alpha,25(OH)_2D_3$, the activity of which was 1/7 of the activity of

$1\alpha,25(OH)_2D_3$ in binding with the sepcific cytosolic receptor [63, 65]. The reasons for the lowered activity of the 23-fluoro analogue of vitamin D_3 are unknown.

OTHER FLUORINE DERIVATIVES

It is known that the presence of a 2α-hydroxyl in the vitamin D_3 molecule inhibits its metabolism or, at least, its 1α-hydroxylation in the kidneys [95, 96]. $2\alpha FD_3$ does not differ from vitamin D_3 with respect to its influence on the transport of Ca in the intestine and its mobilization from bone [67] and is 1000 superior to $2\alpha OHD_3$ [95].

A fluorine atom present in the 2β-position between hydroxy groups at 1α and 3β is also similar to hydrogen, since $1\alpha OH\text{-}2\beta FD_3$ is practically identical with $1\alpha OHD_3$ with respect to its stimulation of the transport of Ca and its mobilization from bone and with respect to antirachitic activity [97]. Similarly, $1\alpha,25(OH)_2\text{-}2\beta FD_3$ does not differ from the natural hormone $1\alpha 25(OH)_2D_3$ with respect to its capacity for stimulating the transport of Ca in the intestine and its mobilization form bone [69].

Consequently, a fluorine atom present in the 2α- and 2β-positions imitates hydrogen.

$6FD_3$ behaves as an antagonist of the binding with the specific receptor of vitamin D_3 metabolites on combined administration with $1\alpha,25(OH)_2D_3$ in vitro [71].

ANTILEUKEMIC ACTION OF FLUORINATED COMPOUNDS OF THE VITAMIN D SERIES

A more profound investigation of the role of $1\alpha,25(OH)_2D_3$ in the regulation of calcium homeostasis led two groups of workers to the discovery in 1981 of the participation of this hormone not only in the inhibition of the proliferation [98] but also in the induction of the differentiation [99] of cells.

In 1983-1984 it was estabished that antileukemic action was characteristic not only of $1\alpha,25(OH)_2D_3$ but also, to a considerable degree, of fluorine derivatives of vitamin D_3 — $24,24F_2\text{—}1\alpha,25(OH)_2D_3$ [100] and $1\alpha F\text{-}25OHD_3$ [37], and also $26,26,26,27,27,27,F_6\text{-}1\alpha OHD_3$ [101].

Subsequent searches for new antileukemic agents were performed purposefully with the imparting to them of specific properties in relation to the leukotic cells while suppressing their antirachitic action. For example, the introduction of an additional CH_2 group at C-24 accompanied by a sharp decrease in the capacity for regulating Ca-P metabolism led to the creation of $24,24F_2\text{-}24\text{-homo-}1\alpha,25(OH_2)D_3$. This compound induced the differentiation of human promyelocytic leukemia HL-60 cells into monocytes/macrophages while scarcely affecting Ca homeostasis [73, 102].

The 6,19-endoperoxides of $24,24F_2$ — and $26,26,26,27,27,27F_6\text{-}25OHD_3$ also possess antileukemic activity [74].

The development of these investigations may prove to be promising for medical practice, since the compounds studied possess high antileukemic activity in vitro in vanishingly small concentrations (2-200 pM) [103].

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