

Forty years ago, a fundamental discovery : precalciferol

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Le précalciférol, le précurseur de la vitamine D, en laquelle il s'isomérisé réversiblement par léger chauffage, a été découvert en 1948 par Velluz et son équipe. La cyclisation du précalciférol d'une part en lumistérol et ergostérol par photolyse, d'autre part en pyrocalciférol et isopyrocalciférol par thermolyse a permis d'établir des corrélations stéréochimiques univoques dont l'importance a été considérable pour l'élaboration ultérieure de la théorie des réactions concertées (réactions péricycliques de Woodward-Hoffmann).

As a consequence of Roussel Uclaf early implication in the production and transformation of various steroids and particularly in the photochemical conversion of ergosterol into vitamin D₂ (also called calciferol or ergocalciferol), a huge amount of experimental observations had accumulated in the files of the firm. However most of the experimental data, thus collected, were not exploited, for quite a while, for lack of a theoretical support that only began around 1950 with the first publication of Pr D.H.R. Barton [1]. Resuming Hassel's concept as to the axial or equatorial orientations of the cyclohexane bonds [2], Barton was able to extract all the information that could be drawn from it, to interpret, in a simple and rational manner, the reactivity of cyclohexane substituent.

A non photochemical step in the formation of calciferol

The lack of theoretical support did not prevent the French firm Roussel Uclaf to provide, in 1948 and in the following years, a fundamental contribution in two domains, still in their early infancy at that time, the one involving the stereochemistry of six membered unsaturated rings and the other the mechanism of thermal and photochemical reactions. The experimental thoroughness of the chemists and their logical mind enabled them to draw a clear picture of the many interrelated compounds which were involved in the intricate chemistry of vitamin D. These results, in turn paved the way to a further conceptual leap forward leading to the theory of concerted reaction and to the selection rules of Woodward-Hoffman [3] and Fukui [4]. Our story really starts in 1948, when first in *Comptes Rendus de l'Académie des Sciences*, then in *Bulletin de la Société Chimique de France*, appear two publications of Velluz and coworkers, the title of which is quite significant : a non photochemical step in the formation of calciferols ("Sur un stade non photochimique dans la formation des calciférols") [5].

An experimental observation at the workshop level, confirmed later in the laboratory, had shown that a slight heating of the resins from the irradiation of ergosterol improved the yield of vitamin D₂. This unexpected observation of an intermediate able to give rise to vitamin D₂ by a purely thermal route, was in contradiction with the scheme shown below proposed earlier by Windaus and his school [6].



According to this scheme, that was to be adopted later like a tenet by the scientific community, the formation of vitamin D₂ from ergosterol and beyond involved, only, a succession of irreversible, photochemical steps.

State of the question in the fifties

In 1948, as far as one can judge from a perusal of the standard book in the field [7], only two structures pertaining to the starting material, ergosterol, and to the end product, vitamin D₂, were known with certainty. The validity of the structures and configurations ascribed to lumistérol and tachystérol were questionable.

The configuration of asymmetric centers at 9 and 10 of ergosterol, involved in the opening of ring B diene, were firmly established, they are shown in *figure 1* : the hydrogen of the center at 9 has a trans relationship to the 19-angular methyl of the center at 10. As to vitamin D₂ structure, it rested on firm ground since it was the result of extensive degradation work performed mainly by two of the groups that contributed most to the structure determination, namely Windaus and his coworkers in Germany [8] and Heilbron and coworkers in Great Britain [9]. At the end of 1948, an X-ray analysis of the crystalline calciferol-iodonitro benzoate performed by Crowfoot and Dünitz [10] provided a complementary confirmation of the structure proposed by both the

preceding groups. The following features of vitamin D₂ structure and configurations were, thus, reliably established, namely the presence of four double bonds, the existence of a conjugated triene, the trans junction of C, D rings and the orientation of ring A hydroxyl group, the configuration of which is the same as that of the hydroxyl at 3 of ergosterol.

Two of the equilibrating forms of vitamin D₂ are represented on figure 1. They only differ between themselves by the spatial arrangement of the double bonds of the conjugated triene [11]. As it will be seen later on, what one may call the "primary form" of vitamin D₂ is the form we name cis-cis on figure 1, being admitted that we are looking at the s-cis or s-trans relationship of two vicinal double bonds two by two and that, by convention, we always start from ring A.

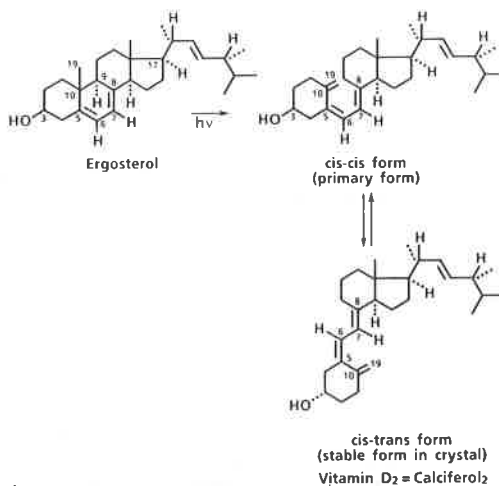


FIGURE 1.

Thus with regard to the cis-cis form of figure 1, the Δ -10-(19), Δ -5-diene is s-cis, the Δ -5, 7-diene is also s-cis, in the cis-trans form of the triene, shown on figure 1, a rotation of ring A, that bears the hydroxyl group, around the 6-7 single bond has taken place and in the latter form Δ -10-(19), Δ -5 still correspond to a s-cis diene but Δ -5, 7 to a s-trans diene. This last cis-trans form of vitamin D₂ may be found in the crystal, for example, as the iodinitrobenzoate derivative of the 3-hydroxyl [10]. The existence of several interconvertible forms of the conjugated trienes of the vitamin D series [12] has been a source of complications for the interpretation of chemical and physical data and specially of UV spectra.

The structures and configurations of the asymmetric centers of ergosterol and vitamin D₂ are, by now, quite secure, what about the other intermediates in Windaus' scheme, namely tachysterol and lumisterol? The latter compound is an isomer of ergosterol and one of the first crystalline compound that can be isolated during the irradiation of ergosterol.

A coherent series of chemical correlations allowed the attribution of the 10- α orientation to the 19 angular methyl of lumisterol but the orientation of the hydrogen of the asymmetric center at 9 remained questionable and, in fact, it was definitely settled only in 1958. Figure 2 gives the correct structure but let us emphasize that the wrong 9- α configuration, ascribed up to 1958 to the hydrogen 9 of lumisterol, contributed to blur, at the beginning of the fifties, the stereospecificity of the photocyclization of precalciferol.

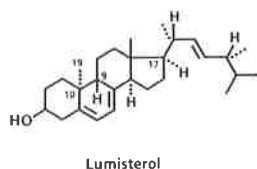


FIGURE 2.

Let us turn now to tachysterol, it may be said that its overall structure was more or less established: it is a conjugated tetraene arising from the opening of ring B between carbons 9 and 10. With respect to vitamin D₂ a distinctive feature is the fact that tachysterol does not bear any methylene. It seemed almost logical for Windaus and his team to ascribe to the supposed product of ring B opening, the structure of a cis-cis triene of the type shown on figure 3. However no decisive evidence was given for the cis or trans configuration of the Δ -6 double bond.



FIGURE 3.

Again, this wrong attribution of the cis configuration to Δ -6 could only add more confusion in a field already complex enough by itself and it delayed for a few years the right attribution of configuration for the precursor or vitamin D₂ found by Velluz and his coworkers.

Let us note that the isolation and purification of tachysterol in the complex mixture of compounds arising from ergosterol irradiation, in particular its separation from vitamin D₂ and lumisterol, takes advantage of its relatively fast rate of reaction with citraconic anhydride (methylmaleic anhydride) and other dienophiles in general, in comparison with the corresponding rates of reaction of these dienophiles with vitamin D₂ and lumisterol. This last observation has allowed, later on, to ascertain the true configuration of the Δ -6 double bond of tachysterol but, before anticipating the events, let us resume their chronological course.

Identification and isolation of precalciferol

In 1949 appears in *Bulletin de la Société Chimique de France* another publication of Velluz, Amiard and Petit [13] very important by its content and its implications as to the origin of vitamin D₂ and of calciferol in general, that is to say of substances that differ from vitamin D₂ only by the nature of the substituent at 17, on the five membered ring. For instance, on figure 4 the formation of vitamin D₃ (cholecalciferol or calciferol 3) from dehydro cholesterol is shown: a distinctive feature with respect to vitamin D₂ is that the side chain at 17 does not contain any unsaturation (compare with figure 1).

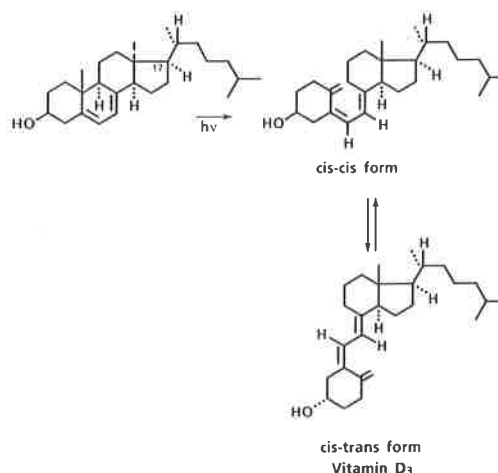


FIGURE 4.

Velluz, Amiard and Petit confirm, in 1949, the existence of a substance able to give rise to calciferol merely by gentle warming without the help of any light ("une substance capable de donner naissance au calciférol par un léger chauffage sans apport d'énergie lumineuse"). It is possible to follow the formation of the labile intermediate involved in the formation of vitamin D either by polarimetric readings at the sodium D line or by UV absorption. Starting from ergosterol (λ max 282 nm, ϵ 11900) there is an intermediate (λ max 262, ϵ 9000) converted by moderate warming to vitamin D₂ (λ max 265, ϵ 18300). This intermediate is given the name precalciferol to characterize it as the precursor of vitamin D₂.

Besides the spectroscopic observations the evidence for the existence of this intermediate is provided by the isolation and characterization of the crystalline dinitrobenzoate of precalciferol. In the absence of oxygen and light at 60 °C in benzene solution, precalciferol dinitrobenzoate yields 65 % of vitamin D₂ dinitrobenzoate. A similar sequence of steps takes place starting from dehydrocholesterol (figure 4) and provides vitamin D₃ through the intermediacy of previtamin D₃ (precalciferol 3).

The thermal equilibrium precalciferol-calciferol

In the same publication, the French chemists demonstrate the existence of an equilibrium between precalciferol and calciferol : the thermal reaction is reversible even if the position of the equilibrium, for conformational reasons which will be explicitly stated later on, is strongly shifted towards the vitamin side



This equilibrium opens a new access to precalciferol since heating pure vitamin D₂ in the absence of air and light, yields a mixture of vitamin and precalciferol which are separated by known, described methods. Many laboratories were thus able to get pure precalciferol allowing them to study its structure and properties.

To sum up, the French publication of 1949 put to light the following points :

- precalciferol is the immediate precursor of vitamin D₂,
- the conversion of precalciferol into vitamin D₂ is a purely thermal, reversible reaction,
- the photochemical generation of precalciferol and its thermal conversion into vitamin D₂ seem to be general for steroidal Δ -5, 7 dienes, independently on the nature of the substituent at 17.

On account of these results the scheme of Windaus could be looked at suspiciously and two questions arose :

- what is the structure of precalciferol ?
- where to insert the precalciferol in the sequence of photochemical steps starting from ergosterol ?

Structural determinations

With respect to the first question it was obvious that the remarkable ease of thermal conversion of precalciferol into vitamin D₂ and vice versa ought to correspond to a strong structural analogy between both these substances, probably linked by "chemical isomerization" [5]. Although the structure of calciferol seems to us, now, fairly evident, this problem was solved only after a few years had elapsed. Why did it take so much time ? We

have already mentioned the perverse effect of the wrong attribution of configurations. Moreover one has to realize that the by now routine recording equipment of IR spectra did not appear in Europe before 1953. In the absence of such IR spectra, structural determinations were mainly based on combination of analytical and degradation reactions, like the oxidative cleavage of double bonds. For example microhydrogenation of double bonds allowed, in principle, the dosage of double bonds and thus their numbering ; the oxidative cleavage of a double bond by chromic acid or by lead tetraacetate, after a preliminary glycolation of the double bond, was necessarily followed by the isolation and identification of all the degradation products (for instance formaldehyde from the oxidative cleavage or ozonolysis of a methylene).

Collecting all these informations and using them purposely it was possible to reconstitute the structure of the starting material from its various fragments of degradation. Needless to say, these degradative methods were very lengthy and time consuming, leading sometimes to ambiguous results. In the case of compounds as sensitive to oxygen, light and heat as the trienes of the D and pre-D series it was a strong requirement of any degradative work, to proceed at a temperature as low as possible, in order to minimize interconversions and thus to get reliable results. Indeed any thermal isomerization of precalciferol into vitamin D₂ would impair more or less deeply the results.

The steps in the structural determination of precalciferol

A reinterpretation of chemical data and UV absorption spectra first allowed to correct the configuration ascribed to the Δ -6 double bond of tachysterol and to rightly attribute to it the trans Δ -6 configuration (figure 5) : this trans configuration of Δ -6 had already been contemplated in the first publication of Velluz, Petit and Amiard in 1948 [14] but it was left aside without any apparent reason.

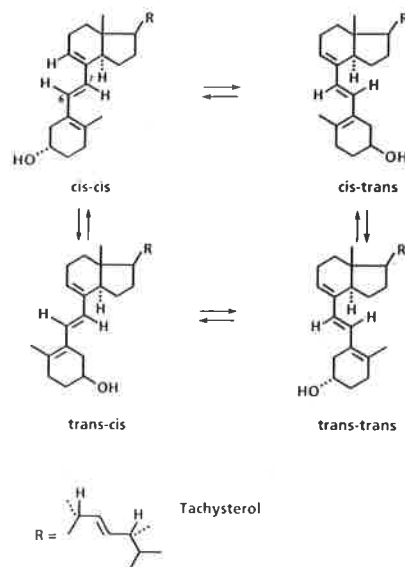


FIGURE 5.

During a general survey of the reactivity of conjugated dienes towards dienophiles, Alder and Schumacher pointed out in 1953 [15] that the fast rate of reaction of tachysterol with citraconic anhydride and other dienophiles, in comparison with the rates of vitamin D₂ and precalciferol, was hardly compatible with a cis-cis triene of the type shown on figure 3 which could not explain the rate difference. However the greater reactivity of tachysterol

towards dienophiles could be explained if the Δ -6 bond, instead of having the cis configuration had the trans Δ -6 configuration (figure 5). Were this the case, dienophile addition to the cis-cis or the cis-trans form of the conjugated triene (figure 5) should indeed be much easier than for vitamin D₂ (cf. figure 1).

In 1954, Inhoffen, Brückner, Gründel and Quinkert stressed the theoretical importance of the experimental results, reported by Velluz and his team, after having reproduced them [16]. Furthermore, using model triene compounds, they underscored that the UV absorption of tachysterol is consistent only with the Δ -6 trans double bond whilst the UV absorption of precalciferol corresponds rather to a triene of the type of figure 3, that is to say, with the cis Δ -6 isomer of tachysterol.

By 1954, it looked quite likely that precalciferol, the precursor of vitamin D₂ was the cis-cis triene of figure 3 (other forms of the triene are shown on figure 6) a structure that was wrongly ascribed to tachysterol, itself the trans Δ -6 isomer of precalciferol.

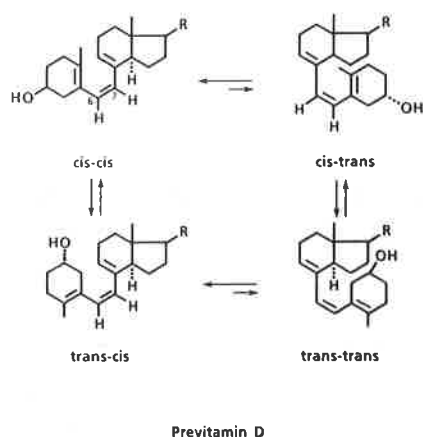


FIGURE 6.

These assumptions arising from considerations of chemical reactivity and from the analysis of spectroscopic data were able to account for almost all of the experimental results of this series.

An experimental confirmation of the rightness of these hypotheses was provided in 1955 by chemical means. These chemical studies, delicate to implement on account of the thermal sensitivity of precalciferol, were performed by both the groups mainly involved in the structure determination, in Romainville by Velluz and his group [17] and, in Leiden, by Havinga and his group [18]. From the chemical standpoint the most relevant results, as to the structure determination of precalciferol, resulted from the ozonolysis that yielded ten times less formaldehyde than that of calciferol, implying the absence of any methylene group in precalciferol [17a]. Infra red spectra confirmed the absence of a methylene in precalciferol. If precalciferol and tachysterol do differ only through cis-trans isomerism at the Δ 6 double bond [19] a conversion into one another should be feasible.

Indeed, this isomerization was effected, nearly at the same time in 1955 by Velluz, Amiard, Goffinet in France [20] and by Verloop, Koevoet, Havinga [18] in the Netherlands.

The isomerization of precalciferol, dissolved in ether, into tachysterol was performed by the French team using UV light at room temperature and treating the resins from the irradiation with citraconic anhydride in order to separate the tachysterol and identify it. Even if the isomerization yield, in the latter case, is about 30 % it is quite significant if one takes into account the recovered precalciferol, characterized by its thermal conversion into calciferol. The Dutch team isomerized, in nearly quantitative yields, precalciferol into tachysterol with iodine and daylight in an apolar solvent, also at room temperature.

Photochemical reactions : their reversibility

In 1955, a very important experimental observation, that was to deeply influence the further conceptual development was made by Velluz, Amiard and Goffinet [21]. They found that UV irradiation of precalciferol into tachysterol was accompanied by the formation of small quantities of ergosterol (3 %) and lumisterol (2 %). This unexpected observation implies that lumisterol is not necessarily a precursor of precalciferol but rather that it draws its origin from the photocyclization of precalciferol. Precalciferol appears, thus, as the immediate precursor of all the observed transformation products. The presence of ergosterol besides lumisterol may be taken as evidence that all these photochemical reactions are, at least partly, reversible. From the new data we may now rectify the reaction sequence leading to vitamin D₂ as shown of figure 7.

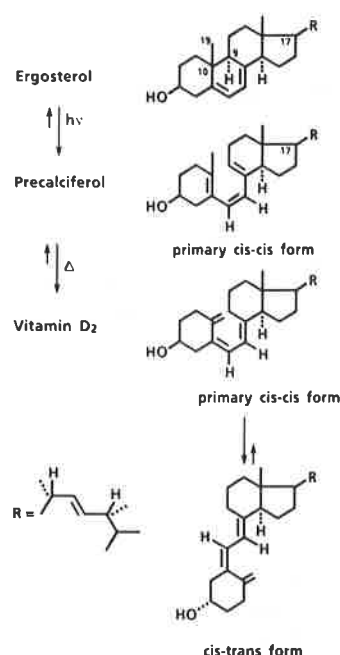


FIGURE 7.

Vitamin D formation involves one reversible photochemical step, implementing the opening of ring B of ergosterol into precalciferol, and another reversible step of thermal isomerization converting precalciferol into vitamin D₂. The discovery of precalciferol, the key intermediate has thus brought a simplification of the reaction scheme. Moreover it enabled the chemists to precisely correlate the various products of either photolysis or thermolysis.

Stereochemistry of the photocyclization

The steric aspect of precalciferol photocyclization into ergosterol and lumisterol also deserves our attention even if this stereochemical feature was missed by the chemists at the time, owing to the prevalence of the wrong α configuration ascribed to the hydrogen at 9 of lumisterol. Of course, already by 1958 the striking stereospecificity of the photochemical ring closure of precalciferol into ergosterol and lumisterol could no longer be ignored : indeed in both compounds, substituents of the asymmetric centers at 9 and 10 are trans to each other (figure 8) namely 9- α -H, 10- β -CH₃ in ergosterol, 9- β -H, 10- α -CH₃ in lumisterol.

Stereochemistry of thermal cyclization of trienes

Let us recall, now, an observation that was made in 1933 by Busse at Göttingen [22]. Heating calciferol at 188 °C for four hours in a sealed tube with exclusion of oxygen, he found that vitamin D₂ was converted in 52 % yield into a mixture of equal parts of pyrocalciferol and isopyrocalciferol, two other diastereoisomers of ergosterol and lumisterol.

In view of the ready reversibility of the thermal isomerization of precalciferol into calciferol one could surmise that the thermolysis of calciferol as reported by Busse, involves precalciferol, a point which was later indirectly confirmed by Havinga and coworkers [23].

As shown on figure 8, it can be seen that precalciferol gives rise, through photolysis to ergosterol and lumisterol and through thermolysis to pyrocalciferol and isopyrocalciferol. All these compounds are stereoisomers and may be connected two by two by chemical correlations: by mercuric acetate dehydrogenation lumisterol and pyrocalciferol provide the same Δ -5, 7, 9 (11)-triene whilst ergosterol and isopyrocalciferol provide a single Δ -5, 7, 9 (11)-triene, isomeric with the former one. Therefore, the difference between ergosterol and isopyrocalciferol involves only the configuration at 9: isopyrocalciferol is necessarily the 9- β -H isomer of ergosterol. On account of the wrong configuration ascribed to the hydrogen at 9 of lumisterol, the configuration at 9 of pyrocalciferol was also wrongly assigned. Only, in 1958, a rectification of these configurations could be made, allowing to correctly ascribe the 9- β -H configuration to lumisterol and the 9- α -H configuration to pyrocalciferol.

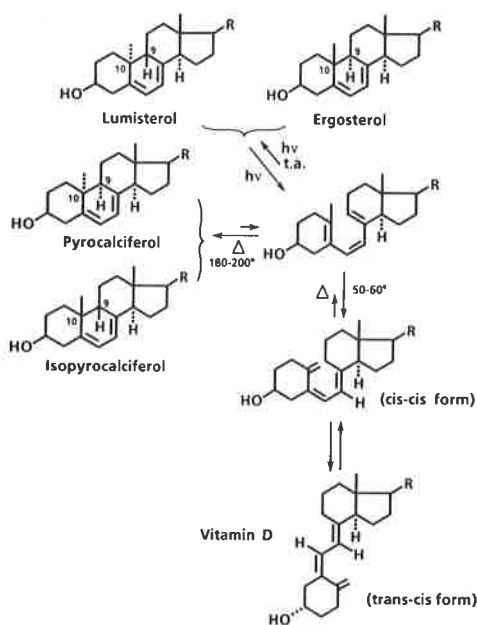


FIGURE 8.

By 1959, the correlations, appearing on figure 8, were firmly established and they were bound to attract theoretical interpretation. The difference in the steric course of the ring closure of precalciferol either by photochemical or by thermal cyclization was striking enough to draw the attention and arouse the reflexion as to the reasons of such different behaviors.

The thermal isomerization precalciferol-vitamin D

Any properly performed, experimental study provides more or less precise answers to the questions that have been asked but, always, raises many other questions and further enlarges the unexplored part in the field of knowledge.

The studies in the field of vitamin D do not escape to this general rule and the extreme readiness of the thermal isomerization of precalciferol into vitamin D could only attract the curiosity of the chemists and theoreticians. It is not surprising, under these conditions, that its mechanism has been the subject of investigations by many groups.

Velluz, Amiard, Goffinet in 1955 [17], Legrand and Mathieu in 1957 [25] had interpreted this thermal isomerization of precalciferol into calciferol as an intramolecular reaction of the ene-synthese type, taking place through the migration of an hydrogen atom with concomitant shift of the double bonds of the conjugated triene. This reaction, occurring already at room temperature in neutral solvent may be considered neither as nucleophilic nor as electrophilic, taking into account its insensitivity to acid-base catalysis. It may neither be taken as proceeding through a free radical since it is not influenced by promoters or inhibitors ("La réaction, qui a lieu dès la température ambiante en solvant neutre, ne peut être tenue ni comme nucléophile ni comme électrophile étant donné son insensibilité à la catalyse acide-base. On ne peut pour autant la considérer comme radicalique, car elle n'est pas non plus influencée par les promoteurs ou les inhibiteurs").

Legrand and Mathieu in 1957 assumed that the reaction was a concerted circular transfer of the type shown on figure 9a and to support their proposal they measured a few thermodynamic constants of the reaction. For the entropy of activation they found weak, negative values consistent with a reaction involving a cyclic transition state.

Similar measures were performed nearly at the same time by the Dutch team [26, 27], they found a value of 18 kcal/mol for the energy of activation and of 20 e.u. for the entropy of activation. They noted also the absence of deuterium incorporation when calciferol acetate is kept at 70 °C for a few hours in a mixture of benzene and deuterated methanol (CH₃OD). Finally they were able to conclude, as did Legrand and Mathieu, that it is an intramolecular reaction with a rigid transition state involving simultaneous formation and breaking of the bonds concerned by the isomerization (figure 9b). The position of the equilibrium and the reasons of the unusually facile concerted hydrogen transfer occurring through a eight membered quasi-ring remained mysterious.

Still in 1988 the low temperature required to cause the sigmatropic 1.7 shift, remains mysterious, especially in comparison with the experimental condition in which are implemented thermal reactions such as that of Claisen.

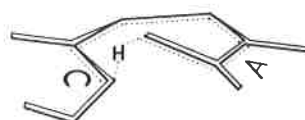
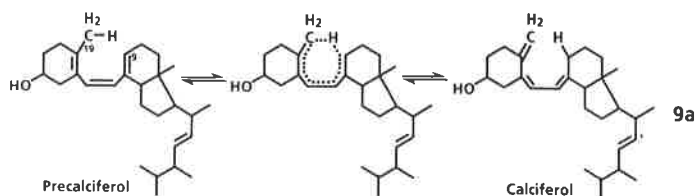


FIGURE 9.

Interpretation of the position of equilibrium

At first sight, it may appear somewhat paradoxical that the equilibrium precalciferol-calciferol is so shifted towards the vitamin (20:80). From the literature it is known that a double bond exocyclic to a cyclohexane is energetically less favorable than the corresponding endocyclic double bond. Therefore one could expect the equilibrium to be shifted to the opposite side. In the case of precalciferol and despite two double bonds exocyclic to cyclohexane, the equilibrium is shifted towards the vitamin. A correct conformational explanation was supplied by Schlatmann, Pot and Havinga, in 1964, [27] taking into account the deformations and strain introduced by the endocyclic Δ -8 double bond on the neighboring pentagonal D ring and the resulting tension that arises at the level of CD rings.

Applying Bucourt's [28] notions of conformational transmission to the cis or trans junction of hydrindane rings, the following explanation can be proposed. First, let us remind to the reader that the closure of a torsion angle is the decrease, in absolute value of that angle with respect to a reference value, taken usually as the value of the cyclohexane ring 55° . Conversely the opening of a torsion angle is the increase in absolute value, of a torsion angle with respect to the same reference value.

In bicyclic compounds of the trans hydrindane type, that is to say with a trans junction as in the case of CD steroidal rings, deformations of torsion angles at ring junction are opposite : to a closure of a torsion angle of junction corresponds an opening of the other torsion angle of junction and vice versa. As the usual, average values of the torsion angle of a saturated cyclopentane are below 50° , it means that these torsion angles are already "closed" with respect to the reference value. Therefore the weak value of the torsion angle of junction of ring D, in a trans hydrindane system is likely to favor an increase of the absolute value (an opening) of the torsion angle of junction of ring C with ring D and it will oppose anything that tends to decrease the absolute value (any closure) of this torsion angle. Introducing the Δ -8 double bond into ring C tends to close the torsion angle of junction of ring C. To avoid an added strain in the bicyclic system with a trans junction of the ring, the double bond remains exocyclic and, thus, the strain that would be experienced by the trans bicyclic system controls the position of the equilibrium.

In support of this explanation it is clear that if one decreases the absolute value of the torsion angle of junction of ring D, with respect to the value of cyclopentane, one has to expect a near complete shift of the equilibrium to the side of the exocyclic double bond, that is, to the side of the vitamin [29]. That is precisely what happens when the cyclopentane is replaced by a cyclobutane D ring, the junction of the CD rings remaining trans (figure 10 a). In cyclobutane the value of the torsion angle is at most around 25° [28]. A similar result would have been observed if a Δ -16 double bond had been introduced in the pentagonal D ring of the steroid but, so far this does not appear to have been realized.

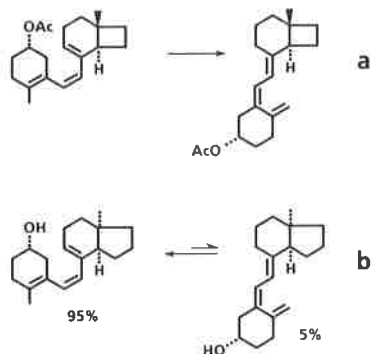


FIGURE 10.

If instead of a trans CD junction of the rings we are dealing with a cis junction, we can expect, on the contrary, a stabilization of the precalciferol form for the following reason. In cis hydrindane systems, there is a similar transmission of deformation on both sides of the ring junction : to a closure of a torsion angle of junction corresponds a closure of the torsion angle of junction of the other ring and to an opening and opening. Consequently, if there is a cis junction of CD rings [29] the introduction of the Δ -8 double bond into ring C, closes the torsion angle of junction of ring C and causes a closure of the angle of torsion of the junction in ring D that is energetically favorable, thus, in the last case, the endocyclic Δ -8 double bond is favored over the exocyclic Δ -7 and the equilibrium is strongly shifted to the precalciferol side (10b).

Photochemical formation of precalciferol and structure

In order to determine the scope of the photochemical formation of precalciferol Velluz, Amiard and Goffinet prepared a series of Δ -5, 7 dienes in the normal and in the 19 nor series and furthermore they analyzed the influence of substituents of the rings [30]. From these studies it appears that a substituent close to the 9-10 bond, a 11- α hydroxy for instance, yields a very deep influence on the formation of the corresponding precalciferol, since it is able to prevent it. However a remote substituent, at 17 for instance, has a negligible influence on precalciferol formation. Thus precalciferol formation occurs in the absence of any substituent at 17 or when the substituent is a 17- β hydroxyl group. In this case, the French investigators succeeded in obtaining the corresponding precalciferol, namely the 17- β hydroxy-etio-precalciferol in its free crystalline state. The 19 angular methyl is required for the formation of precalciferol and in its absence, irradiation of the Δ -5, 7 dienes directly leads to the corresponding tachysterols.

Pr L. Velluz in a conference delivered at Leiden in 1959, devoted to precalciferol and photoreactions in steroid series [31], had suggested to theoretical chemist to pay attention to the peculiar hydrogen transfer that takes place so readily in the thermal conversion of precalciferol to calciferol ("singulier transfert d'hydrogène que pose l'extrême facilité de la réaction précalciférol calciférol"). He had, moreover, stressed a few possible biological implications of that equilibrium in living organisms.

Theoretical interpretations

The Dutch team, the most involved in the field was the first to provide a theoretical answer to the questions raised by the stereochemical relationships existing among precalciferol and the various products of ring closure, either photochemical (ergosterol and lumisterol) or thermolytic (pyro- and isopyrociferol) or by thermal isomerization (vitamin D). In a publication, appearing in *Tetrahedron* in 1961, Havinga and Schlatmann made a general survey of the stereospecificity of thermal and photochemical transformations in the vitamin D field [32]. They explicitly proposed a novel interpretation, involving the conservation of orbital symmetry, indeed they wrote [33] : "As Pr Oosterhoff pointed out, another factor that possibly contributes to the stereochemical differences between the thermal and the photoinduced ring closure may be found in the symmetry characteristics of the highest occupied orbital of the conjugated hexatriene system. In the photoexcited state this highest occupied orbital is antisymmetric with regard to the plane that is perpendicular to the bond 6,7 making "syn approach less favourable".

In 1965 are published the preliminary communications of Woodward and Hoffmann, relative to the stereochemistry of

electrocyclic reactions [3] and the selection rules for sigmatropic reactions [34].

In the first communication, reference is made to the thermal disrotatory ring closure of precalciferol to pyro- and isopyrocalfiferol and to the photochemical conrotatory ring closure of precalciferol into ergosterol and lumisterol. Only the Dutch team is credited for these interconversions and there is no mention of the studies of Velluz and coworkers which is, at the least, unfair. The theoretical anteriority of Pr Oosterhoff is also recognized by Woodward and Hoffmann which seems fair.

The second communication explicitly makes reference to the vitamin D series : "An important (1.7) thermally induced hydrogen shift in an open chain system has been known for some time in the precalciferol-calciferol equilibrium". Again the sole reference is that of the Dutch chemists who, of course, are not at all responsible for the omission of the fundamental paper of Velluz and his team. Such an omission, by the way, is astonishing coming from such a prominent scientist who had no difficulty to read French. Let us hope that our short account of the discovery of precalciferol and its properties may contribute to a better appreciation of the pioneering work of Velluz and his team in the field.

Conclusion

The discovery of precalciferol by Velluz and his team, in 1948, remains a milestone in the history of organic chemistry and its development (already 40 years !). On the purely chemical level, this discovery, that of the properties of precalciferol, the thermal, photochemical interconversions have contributed to open a few new chapters with respect to the stereochemistry of concerted reactions and of hydrogen transfer in polyenes.

The intrication of steric, electronic and conformational factors is another reason to take interest in these peculiar series. From this point of view, vitamin D chemistry may be said to be exemplary even if many dark corners still subsist.

With regard to biology and in spite of the studies performed up to now in these series, the problems have by far not been completely cleared up and they deserve complementary investigations : calcium mobilization in living organisms still raises many fundamental questions and the consequences have not yet been completely evaluated.

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- [12] Tachysterol (*fig. 5*) may adopt four "planar" forms, independently of the "gauche" forms ; precalciferol (*fig. 6*) may also adopt two "planar" and a few "gauche" forms : see W.G. Dauben, P.E. Share, R.R. Ollmann, *J. Amer. Chem. Soc.*, **1988**, *110*, 2548-2554.
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