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Vitamin D, Example and Challenge*

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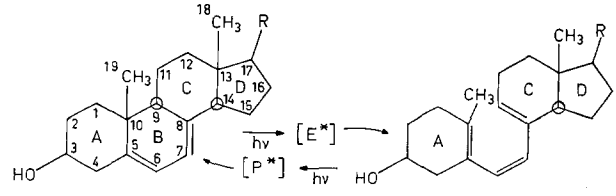
Introduction

There are several reasons why attention is focussed on vitamin D in this lecture, which will deal with rather general aspects of stereochemistry and photochemistry. Of course, there is the historical background that the activities of the Leiden Laboratory in this field started some 20 years ago with a study of the pathway along which vitamin D is formed from its precursors. Also it seemed appropriate in the Paul Karrer lecture to report on developments in an area of the chemistry of vitamins and provitamins, a field in which Professor KARRER himself made a whole series of decisive contributions, which stand out as highlights of chemical research. This motivation is of special relevance for today's lecturer who, like so many students of his generation, was introduced into the field of organic chemistry by using KARRER's impressive textbook, and who in later years had the privilege of knowing him personally and learning from him in many respects. Finally there is a purely scientific reason why vitamin D, selected and produced by nature for specific functions, has been chosen as the centre of this presentation. Vitamin D, its isomers and their characteristic reactions, have been a source of information and inspiration to develop new ideas and hypotheses again and again; and – as I hope to make clear – it continues to be a stimulating object and starting point for fundamental studies up to the present. It forms an example and it remains a challenge to chemistry and chemists.

The name Vitamin D was given half a century ago to the principle which is present in cod liver oil and which could cure or prevent rickets, known in the Netherlands as 'English disease', a term which the British may coolly discard by remarking that it is 'double Dutch' to them. Rickets is characterized by defective ossification and disturbance of Ca uptake, resulting in weakness and deformation of the bones, especially with growing children, who did not get enough sunshine. The disease might be considered a product of antique civilisation, where the human species adopted the habit of residing in caves, later houses, and protecting their bodies and skin with clothes. It may strongly decrease in frequency as a result of trends in modern civilisation, although it remains doubtful whether this aspect is the main drive behind the present fashion of maximizing the area of skin exposed to sunshine. However this may be, for those who get adequate doses of light, vitamin D or 'calciferol' is now known to be formed in the skin under the influence of light; and it might be called a hormone rather than a vitamin. In a sense its formation may represent an interesting relict from primeval times when many organic substances which are essential for the generation of living material, were formed under the influence of radiation energy through a combination of photochemical and thermal reactions.

This brings us to the interesting chemistry and photochemistry of vitamins D, its precursors and its products[1]. The precursors ('provitamin D', ergosterol, 7-dehydrocholesterol) belong to the class of steroids as indicated in Figure 1.



For quantitative description and understanding of the properties of these molecules, it is essential to know their exact geometry and the related electron distribution. Thanks to nowadays computer facilities it has become possible to determine the geometry of

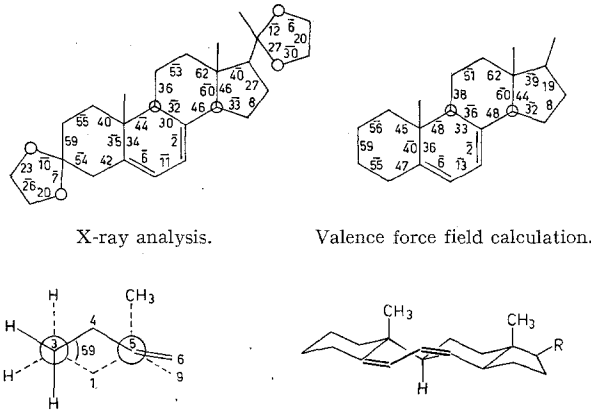
* 14th Paul Karrer Lecture, presented 20 June 1973, in the Aula of the University of Zurich.

[1] For a review see: G. M. SANDERS, J. POT, E. HAVINGA, Fortschr. Chem. org. NatStoffe *27*, 131 (1969).

such 'largish' molecules experimentally, e.g. by X-ray analysis[2], as well as by direct calculation (e.g. by the valence force field method)[3].

Figure 2 represents the values obtained for the internal dihedral angles, that to a great extent define the molecular shape. It is encouraging to see how the calculation satisfactorily reproduces the experimental data, even for ring B containing 2 double bonds.

The capacity to absorb light and undergo photochemical isomerization resides in this conjugated double bond system of ring B, that in accord with theoretical expectation shows strong $\pi \rightarrow \pi^*$ absorption between 250 and 310 nm ($\lambda_{max} = 281$ nm, $\varepsilon = 12000$). Absorption of a lightquant transforms the ground state provitamin D molecule into an excited species (E^x) which may undergo ringopening of the cyclohexadiene system to yield the Z-hexatriene derivative, previtamin D (Figure 1).



X-ray analysis. Valence force field calculation.

Fig. 2. Internal dihedral angles in provitamin D ringsystem.

Figure 4 pictures the conformations of ergosterol and lumisterol. As a significant detail it may be noted that the boatform predicted for ring C of lumisterol on

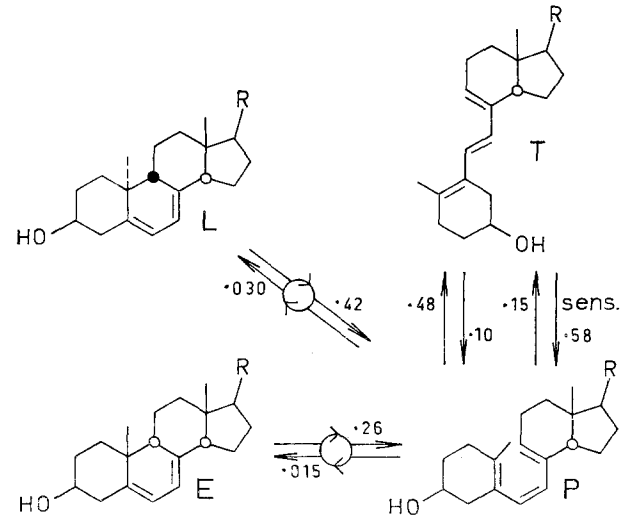


Fig. 3. Photoreactions at ~0 °C.

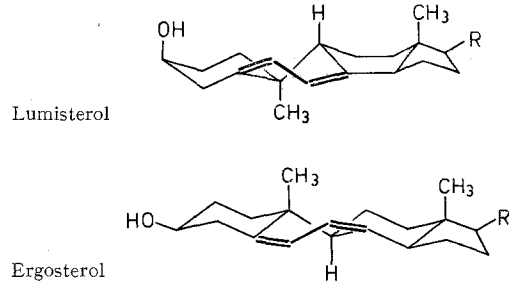


Fig. 4. Conformations of ergosterol (provitamin D) and lumisterol.

The reaction is reversible not in the sense of microreversibility but in the sense that $\pi \rightarrow \pi^*$ excited Previtamin D undergoes ringclosure to form the provitamin back again.

Review of results of older investigations

As an introduction to recent developments, a summary is given of the main results of research and their rationalisations as arrived at some 3 years ago[1].

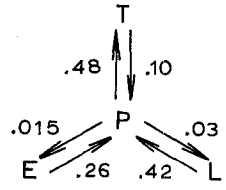
In Figure 3 are presented the photoreactions as they occur in dilute solution at relatively low temperatures (~ 0 °C). Previtamin D undergoes not only a 'conrotatory' ringclosure to ergosterol (7-dehydrocholesterol) but also to the other 9,10 antiisomer lumisterol, having the 9α, 10β configuration. Moreover it shows the well-known $Z \rightleftarrows E$ photoisomerization to the 6–7 E isomer tachysterol. The cyclization reactions are remarkably stereospecific, yielding the 9–10 antiisomeric compounds exclusively.

the basis of conformational considerations corresponds to reality; this could be proved very recently by an X-ray analysis of lumisterol$_3$[4].

The photoreactions presented in Figure 3 could not be effected by irradiation in the presence of a triplet sensitizer (fluorenone), but for the $Z \rightleftarrows E$ isomerization, which then however occurs with strongly different ratio of the quantum yields $\Phi_{Z \rightarrow E}/\Phi_{E \rightarrow Z}$. These new experimental data thus confirm the older experiments indicating that the ringopening and ringclosure reactions, initiated by $\pi \rightarrow \pi^*$ light absorption, start from the first excited singlet and not from the triplet and that probably the same holds to a large extent for the $Z \rightleftarrows E$ isomerization. The values found for the quantum yields[5] are the same at 254 nm and at 313 nm.

[2] P. B. BRAUN, J. HORNSTRA, C. KNOBLER, E. W. M. RUTTEN and C. ROMERS, Acta crystallogr. *B 29*, 463 (1973).
[3] C. ALTONA, personal communication.
[4] A. J. DE KOK and C. ROMERS, to be published.
[5] Determined by M. R. DAHA at our laboratory (1970).

Upon continued irradiation a 'quasi' stationary state is reached, the composition of which corresponds nicely to what is calculated on the basis of the quantum yields of the various isomerizations and the extinction coefficients of the reaction partners (Figure 5).



	calc.	exp.
T	76	75
P	19.5	20
L	2.5	2.5
E	2	1.5

Fig. 5. Quantumyields and photostationary state of previtamin D_3(D), tachysterol$_3$ (T), 7-dehydrocholesterol (E) and lumisterol$_3$ (L). Solvent: ether; wavelength: 253.7 nm; 5 °C.

At room temperature, body temperature and somewhat higher temperatures (range 20–80 °C) the formation of vitamin D itself, occurring through a remarkably smooth [1–7] H-shift from $(CH_3)_{19}$ to $(CH)_9$, adds a new feature to the scheme, that is, of course, of direct physiological importance (Figure 6). We shall come back to this reaction and to vitamin D itself in a moment.

At still higher temperatures (100–180 °C) a thermal ringclosure of the triene system of precalciferol takes

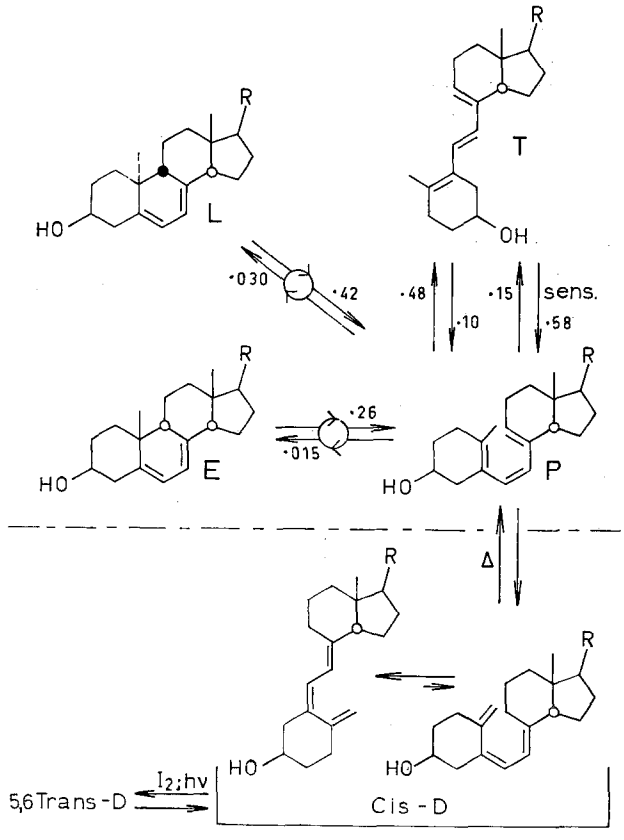


Fig. 6. Reactions at 20°–80 °C.

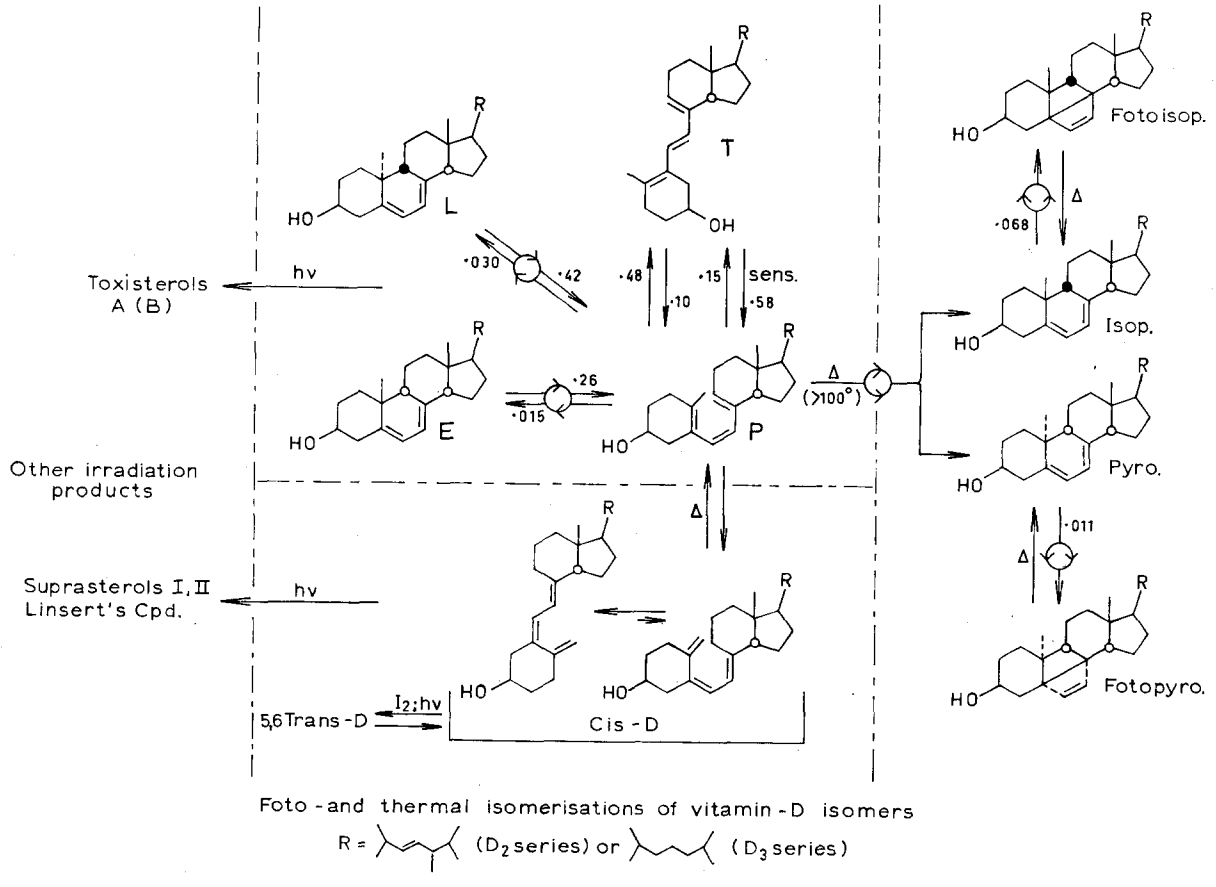


Fig. 7. Reactions at 100°–180 °C.

place yielding the 9–10 syn isomers pyro- and isopyrocalciferol. Evidently these thermal conversions – in striking contrast to the afore-mentioned conrotatory photoinduced cyclization – proceed via a disrotatory pathway, again with complete specificity. This set of reactions formed the classical example of the complementarity of thermal versus photochemical reactions, another striking example of which we found in some classes of photo-induced versus thermal aromatic photosubstitutions[6] (Figure 7).

The conformations of the (9β, 10β) isopyrocalciferol and the (9α, 10α) pyrocalciferol as deduced from conformational considerations are indicated in Figure 8).

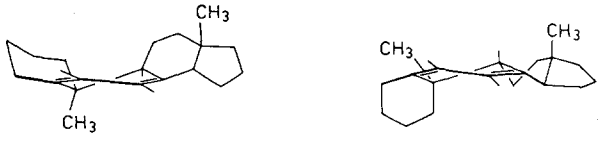


Fig. 8. Conformations of pyro- and isopyrocalciferol.

Here again X-ray analysis recently confirmed the essential features of this picture for pyrocalciferol[7].

With the 9, 10 syn isomers, the wealth of potential reactions in the system is demonstrated by an alternative photoreaction of the cyclohexadiene system, the ringopening now being sterically hindered. The two 9, 10 syn isomers upon irradiation isomerize (with relatively low quantum yields) to the corresponding bicyclo [2,2,0] hexene derivatives, the structure of which was determined by DAUBEN and FONKEN[8]. Curiously this photocyclization can be reversed thermally, probably driven by the release of strain energy from the bicyclic compound. Figure 7, summarizing this short review presents a picture of the thermal and photochemical isomerization reactions centered around previtamin D.

Formation and structure of 7,7' bisteroids

Whereas the scheme of Figure 7 certainly gives a consistent picture of the main processes centered around previtamin D, occurring with such remarkable selectivity and stereospecificity, it is not yet wholly complete. Even at low temperature, and more so at room temperature, upon continued irradiation the extinction in the UV-drops steadily and definitely, due to the formation of so-called 'over-irradiation products'. These are products that are formed with low quantum yields and that upon illumination do not revert to starting material. A new aspect of the photochemistry of the provitamins D was discovered recently in the course of an investigation into the nature of these compounds.

If one irradiates 7-dehydrocholesterol in not too low concentration in ethanol at 0 °C, a precipitate slowly forms which is insoluble in most solvents but can be dissolved upon acetylation. Spectroscopic analysis and NMR-data suggested that it is a long known bisteroid, 7.7'-dehydrobischolestadienol. This compound had formerly been obtained by eosine-sensitized oxidation of 7-dehydrocholesterol. Actually the product consists of 3 isomers. However, these are not rotational isomers nor configurational isomers as thought previously[9,10], but the 3 structural isomers with different location of the double bonds represented in Figure 9[11].

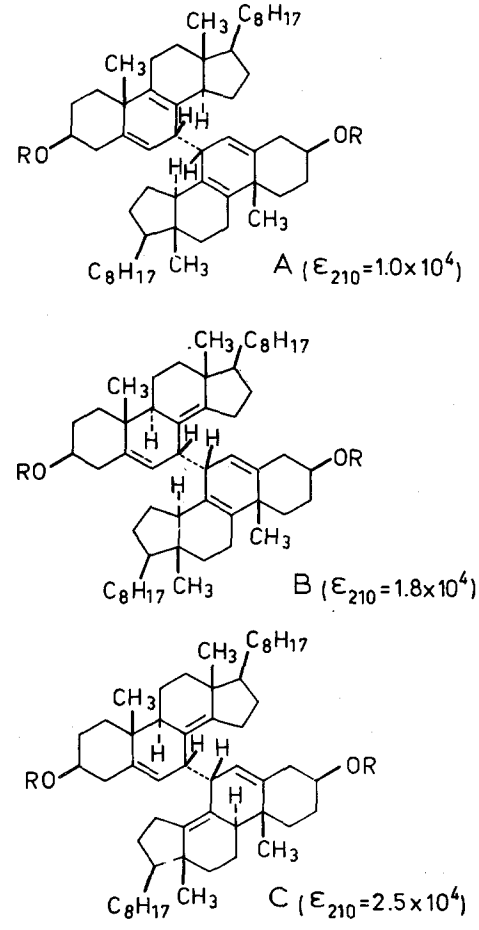


Fig. 9. 3 isomeric 7,7'*bis*-cholestadienols.

[6] For a review see: E. HAVINGA, R. O. DE JONGH, M. E. KRONENBERG, Helv. chim Acta *50*, 2550 (1971).
[7] C. ROMERS, personal communication.
[8] W. G. DAUBEN and G. J. FONKEN, J. Am. chem. Soc. *81*, 4060 (1959).
[9] P. J. FLANAGAN and J. B. THOMSON, Tetrahedron Lett. *22*, 1671 (1965).
[10] P. CRABBÉ and K. MISLOW, Chem. Commun., *1968*, 657.
[11] F. BOOMSMA, H. J. C. JACOBS, E. HAVINGA, A. VAN DER GEN, Recl. Trav. chim. Pays-Bas Belg., in press.

The ultraviolet extinction of B (ε = 18000) is almost exactly the average of that of A (10000) and C (25000). All physical data are consistent with the newly proposed structures. A closer study of other irradiation products of previtamin D (toxisterols etc.) is underway to clarify the nature of the remaining but still unknown photoreactions and thus complete the scheme, which in its main lines now seems well established (Figure 7).

The previtamin D ⇄ vitamin D equilibrium

Subsequent to this story around the pro- and previtamins D, it seems appropriate to focus attention now on the reaction that yields the biologically active vitamin D itself: the thermal equilibrium pre D ⇄ vit D (Figure 10).

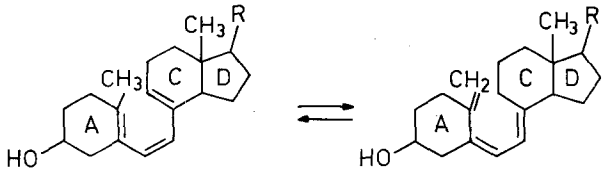
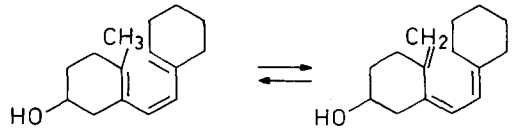


Fig. 10. Previtamin D (263 nm) ~ 20%, Vitamin D ~ 80%.



P-model compound (241 nm) ~ 95%. D-model compound ~ 5%.

There are two remarkable features in this reaction:

a) This first example of a [1–7] H shift (now called 'sigmatropic') occurs smoothly even at relatively low temperature. Studies with molecular models, as well as the kinetic parameters (ΔH = 19 kcal/mole, ΔS = —12 e.u.) suggested that this shift of hydrogen and double bonds could take place via an antarafacial transition, favourable on steric grounds (and – as became clear later – on orbital symmetry relationships as well).

b) The other pecularity of the pre D ⇄ vit D that we will consider more closely, is related to the position of the equilibrium. From a physiological point of view, it seems appropriate that this equilibrium should be on the side of the biologically active vitamin D (~ 80%). However, from a chemical viewpoint one begins to wonder, since double bonds exocyclic to a cyclohexane ring are energetically unfavourable. And indeed with related compounds, e.g. those lacking the D-ring and also those having the D-ring attached in *cis*-configuration (13α-isomer), the 'previtamin D' form strongly predominates (> 95%).

There is another characteristic difference between previtamin D and the model compounds lacking the D-ring in that its UV-absorption band lies at significantly longer wavelength (263 versus 241 nm).

Conformational analysis gives us a lead to recognize Nature as an experienced, very senior chemist, making delicate use of the influence of the trans-attached 5 membered D-ring (Figure 11)[12]. We follow here a line of reasoning for the hydrindane system initiated by DREIDING[13].

A Newman-projection along the C_{14}-C_{13} bond shows that this *trans* 5-membered ring, which itself prefers to have a small internal dihedral angle (~ 40°), tends to make the dihedral angle Φ in ring C larger than in unsubstituted cyclohexane (55°)[13–16]. X-ray analyses (cf. Figure 2) show that in normal steroids Φ is ~60°.

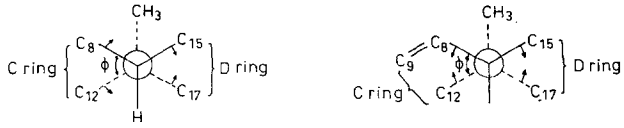


Fig. 11. Newman projection along C_{14}-C_{13}. 5-membered trans D-ring effects increase of Φ to 60° C_8–C_9 double bond tends to make Φ smaller. Opposing influences of D ring and double bond give rise to strain in Pre-D.

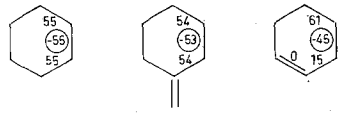


Fig. 12. Internal dihedral angles in cyclohexane, methylene cyclohexane and cyclohexene system.

If, however, a double bond is introduced at position 8–9 in ring C, this tends to decrease Φ (Φ = 45° in cyclohexene itself, as can be seen from electron diffraction data (Figure 12).

The result of the opposing influences of the double bond C_8-C_9 and the *trans* attached D-ring is a strained molecule, with an excess of energy that can be evaluated to amount to several kcal/mole. Since an exocyclic bond (as present in vitamin D) causes much less strain (see diffraction data of Figure 12), the energy balance is shifted in favour of the vitamin D.

This explanation could be checked using an approach that is characteristic for the synthetic chemist: systematic variation of molecular structure[12]. In this case the relevant variation was brought about by changing the size of the *trans*-attached ring D, and thereby varying the conformational strain about bond

[12] H. J. TAKKEN, Thesis Leiden (1971).
[13] A. S. DREIDING, Chemy. Ind., *1954*, 992.
[14] G. QUINKERT, Experientia *13*, 381 (1957).
[15] E. J. COREY and R. A. SNEEN, J. Am. chem. Soc. *77*, 2505 (1955).
[16] R. BUCOURT, Bull. Soc. chim. Fr. *1963*, 1262.

C_{13}-C_{14}. In agreement with the rationalization offered, we found that going from no D-ring or a *cis* D-ring to a 6-ring, a 5-ring and to a 4-membered ring, the equilibrium gradually shifts away from the previtamin D side (~100%, 65%, 20%, ~0%; Figure 13).

These new results seem in strong support of the hypothesis that the predominance of vitamin D in the pre D ⇄ D equilibrium (as well as the bathochromic shift of the previtamin D UV-absorption) compared to that of model compounds lacking the D-ring are caused by conformational strain due to the combination of the endocyclic C_8-C_9 double bond and the *trans* attached D-ring. The strain increases with decrease of the size of ring D.

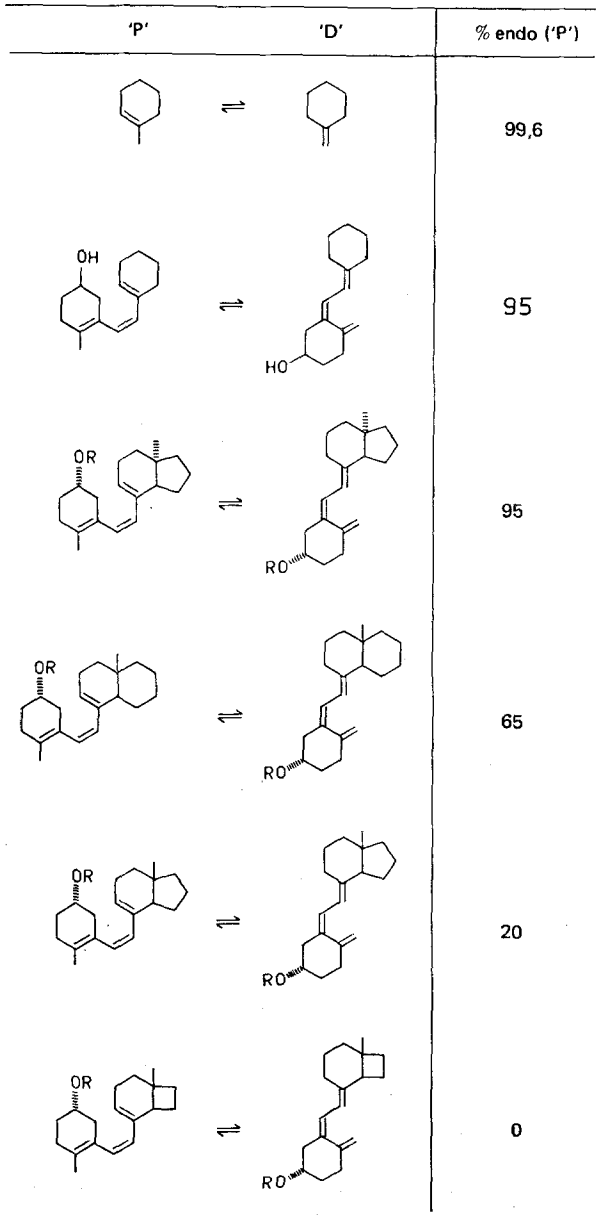


Fig. 13. The Pre D ⇄ D equilibrium as a function of structure and configuration (variation of conformational strain).

Photochemistry of vitamin D

After this discussion of the thermal reaction by which vitamin D is formed, let us now turn to the photochemistry of vitamin D. Two irradiation products, denoted in the literature as Suprasterol I and Suprasterol II, were known for some time and were identified as bicyclohexene [3, 1, 0] derivatives by DAUBEN et al.[17], whilst an X-ray anaylsis of Suprasterol II was made by SAUNDERSON and CROWFOOT-HODGKIN[18]. As will be explained later in this lecture, we had reason to search for isomers of another type as photoproducts from vitamin D. Recently, at our laboratory, 4 new compounds could be detected and isolated[19]. Upon heating they revert to vitamin D (or to [Δ^5Z]-vitamin D). Oxidation gives 'Grundman' ketone and the UV-, IR- and NMR-data are all consistent with the structures proposed in Figure 14. The NMR-data allow one also to determine the configuration of each of the two cyclobutene derivatives.

In Figure 14 the whole family around vitamin D is pictured, as we now think completely. This, then, more or less rounds off the review of what has been found experimentally. I hope that it may have served the purpose of giving an impression of the variety and – still more important – of the specificity of the thermal and the photoinduced reactions in the vitamin D field.

Three principles of rationalisation

It is no wonder that we began to speculate on the mechanism and tried to find explanations of these stereospecific reactions at an early stage. About 12 years ago a first rationalization was published which invoked essentially 3 lines of reasoning or 3 categories of effects for explaining the experimental facts[20]. There was an old one:

a) *non-bonding interactions* (steric hindrance) (accounting i.a. for the absence of ringopening of excited pyro- and isopyrocalciferol);
and two new ones:
b) *orbital symmetry* (introduced as a rationale for understanding the stereochemistry and the complementarity of the thermal and photoreactions mentioned above). This line of reasoning suggested to us by L. J. OOSTERHOFF can now be considered 'classical' also, owing to the studies of R. B. WOODWARD and R. HOFFMANN and many others.

[17] W. G. DAUBEN and P. BAUMANN, Tetrahedron Lett. *1961*, 565. – W. G. DAUBEN, quoted in R. B. WOODWARD and R. HOFFMANN, *The Conservation of Orbital Symmetry* (Verlag Chemie and Academic Press, New York 1970), p. 80.

[18] C. P. SAUNDERSON and D. CROWFOOT HODGKIN, Tetrahedron Lett. *1961*, 573.

[19] S. A. BAKKER, J. LUGTENBURG, E. HAVINGA, Recl. Trav. chim. Pays-Bas *91* 1459 (1972).

[20] E. HAVINGA, J. L. M. A. SCHLATMANN, Tetrahedron *16*, 146 (1961).

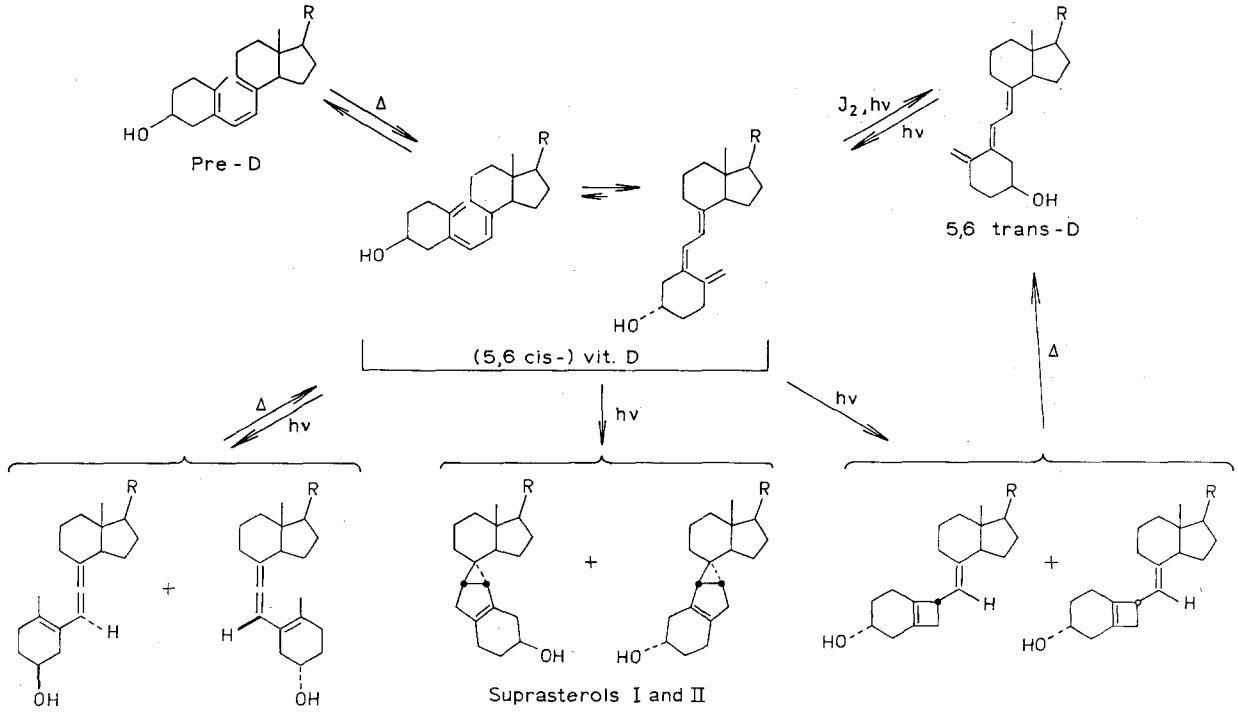


Fig. 14. Isomerization reactions and photoproducts of vitamin D.

c) *influence* of ground-state *conformational equilibrium* on product-composition of photoreactions.

This third principle, that in the 1961 article was used i.a. to rationalize the low quantum yield of cyclization of previtamin D, received relatively little attention. Moreover, its theoretical implications for the description of what changes may occur during thermal relaxation have hardly been discussed. To elucidate this principle in a direct way, we shall not follow the historical development but rather discuss some recent results obtained with simple model compounds.

Spectroscopy, conformation and photochemistry of simple hexatrienes

A detailed study was made of (E) and (Z) 1,3,5-hexatriene and a series of alkylhexatrienes[21,22]. We considered it essential to start this study with the spectroscopic exploration of the compounds since this may procure a basis for the discussion of the photochemical phenomena. The low molecular model compounds here have the advantage that they can be studied in the gas phase as well as in concentrated solution of course.

The first item enables one to obtain well structured singlet → singlet UV-absorption spectra. The second property is even more useful, since in solvents like bromoform and methylene iodide the formally forbidden S → T absorption acquires an oscillator strength sufficient to make possible direct S → T absorption spectroscopy (and ensuing photochemistry!)

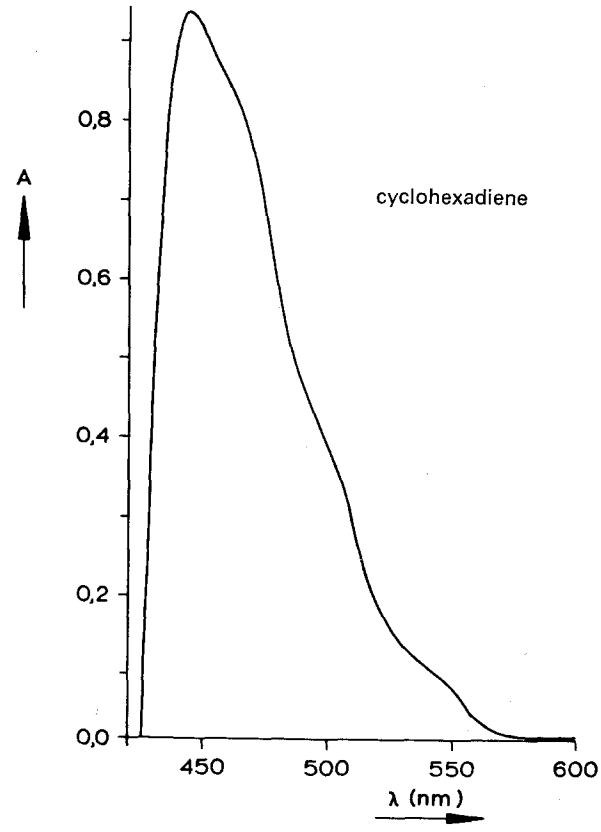


Fig. 15. Absorption spectrum of 3 cm layer of a 1:1 mixture of cyclohexadiene and methylene iodide.

[21] N. G. Minnaard, Thesis Leiden (1970). – N. G. Minnaard and E. Havinga, Recl. Trav. chim. Pays-Bas Belg., in press.

[22] P. J. Vroegop, Thesis Leiden (1972). – P. J. Vroegop, J. Lugtenburg and E. Havinga, Tetrahedron *29*, 1393 (1973).

Figures 15, 16 and 17 exemplify the direct S → T absorption spectra of cyclohexadiene, (Z) and (E) 1,3,5- hexatriene, showing a vibrational structure that surpassed our optimistic dreams. Figure 18 summarizes the results of the analysis of the singlet and triplet absorption spectra, producing reliable values for the energy levels of the compounds under investigation. It proved possible to do 'triplet' photochemistry with the molecules brought into the triplet state by absorption of visible light (S → T transition). One thus avoids uncertainties and complications that may arise by addition of sensitizers. This method directly confirmed that triplet reaction of 1,3,5-trienes lead to Z ⇄ E isomerization and to dimerization but not to cyclization.

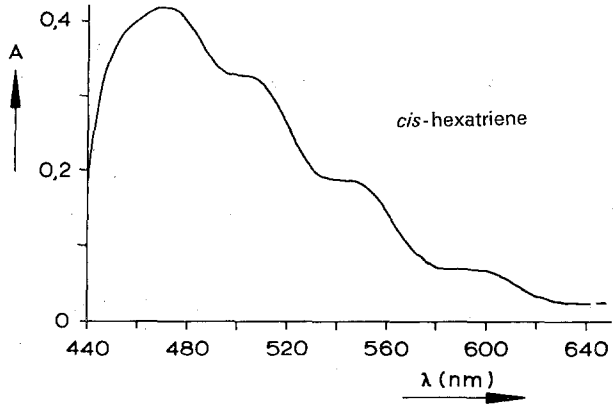


Fig. 16. Absorption spectrum of 3 cm layer of a 1:1 mixture of (Z)-hexatriene and methylene iodide.

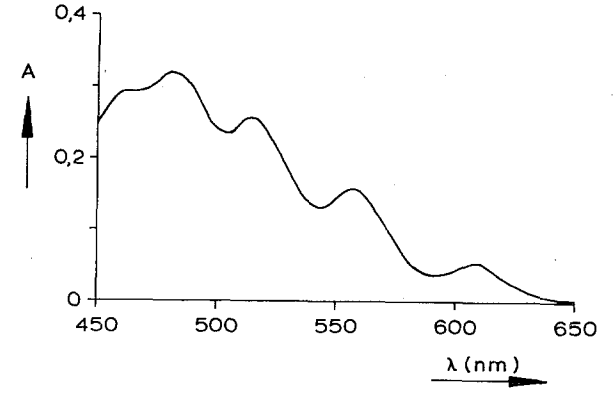


Fig. 17. Absorption spectrum of 3 cm layer of a 1:1 mixture of (E)-hexatriene and methylene iodide.

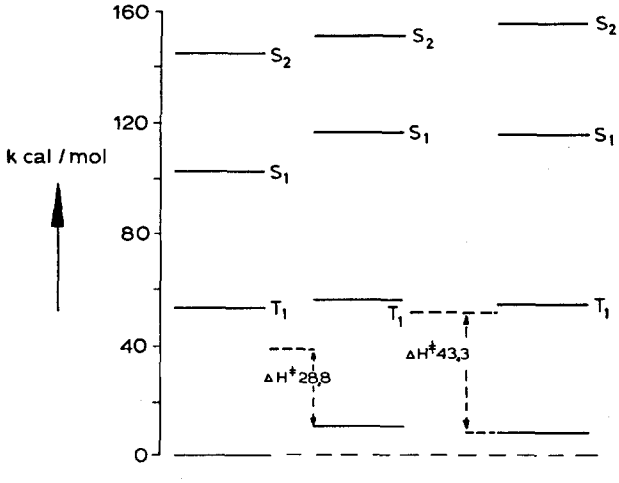


Fig. 18. Energy levels of cyclohexadiene, (E)-hexatriene and (Z)-hexatriene.

Now with the simplest possible compound, (Z)-hexa-1,3,5-triene itself formation of cyclohexadiene was not observed under UV-irradiation in the singlet absorption band either, whereas cyclization by heating proves completely feasible. Here our third principle (c) provides a straightforward explanation.

In Figure 19 are represented the three more or less planar conformations of (Z)-hexatriene (cZc, tZc and tZt). The high value of the extinction coefficient ($\varepsilon = 41{,}000$) (and the NMR-data) tells us that the tZt form strongly predominates in the conformational equilibrium. This is no great difficulty for a thermal cyclization since the cZc form – or better the corresponding transition state – can be realized through rotation about the single bonds. We assume that the situation is essentially different for a cyclization in the (singlet) excited state. There interconversion between the three forms will not be possible as a result of the high rotational barrier about the bonds that now have considerable double bond character and the very short

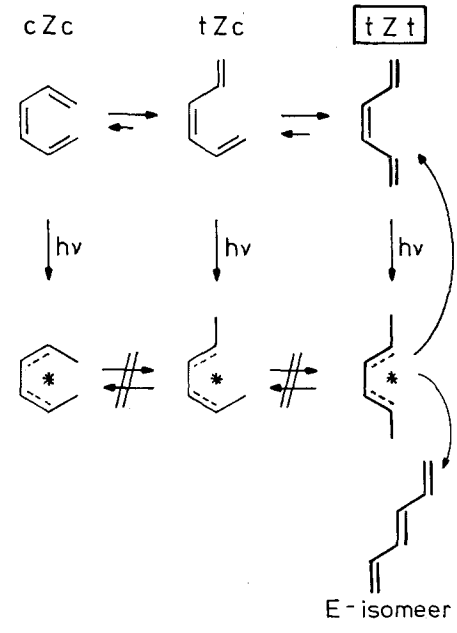


Fig. 19. (Z)-hexa-1,3,5 triene; $\varepsilon = 41{,}000$ (254 nm) tZt conformation predominates. Conformational equilibrium in ground state determines product of photoreaction (here Z → E isomerization).

lifetime (10^{-8} sec) during which such barriers cannot be overcome. This is the characteristic element of our 'third effect' (c). Our line of thought leads us to predict that practically the only form that will exist in the excited state is the stretched tZt form. This form can only revert to the ground state or – since the central bond has less double bond character – transform into (E)-hexatriene, as is observed experimentally.

In order to effect photochemical cyclization to an appreciable extent, we should make the tZt form (and the tZc form) unfavorable and thus increase the percentage of the cZc form. This we effected by alkyl-substitution at positions 2 and 5 of hexatriene (Figure 20). Even with 2,5 dimethyl-Z-hexatriene, the cZc form was found to predominate ($\varepsilon = 12{,}000$). As is consistent with the adopted line of reasoning, this compound gave – on top of the E isomer and the cyclobutene isomer – a substantial yield of 1,4 dimethyl cyclohexadiene. In this way the photocyclization observed originally with previtamin D could be reproduced with the simplest possible model compound.

Let us finally consider an example in which the third type of conformer (cZt) is the preferred one: 2 methyl-(Z)-hexa-1,3,5-triene (Figure 21). We now find – according to expectation – a relatively good yield of the methyl bicyclohexane (3,1,0), the methyl-vinyl cyclobutene and the 5-methyl hexa-1,2,4-triene (that were very minor products with hexatriene itself). All products were identified by (lack of) UV-absorption and by NMR-analysis, including double resonance techniques. Figure 22 summarizes the UV-data and NMR that consistently indicate the preferred conforation of the compounds studied thus far; Figure 23 shows the correlations between the conformations and the various products.

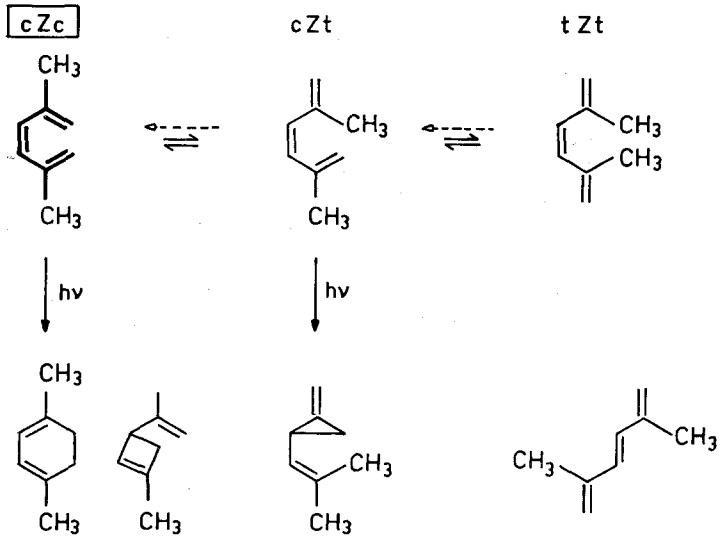


Fig. 20. 2,5-dimethyl-(Z)-hexa-1-3,5-triene; ε = 12,300 (237 nm); conformations and photoproducts.

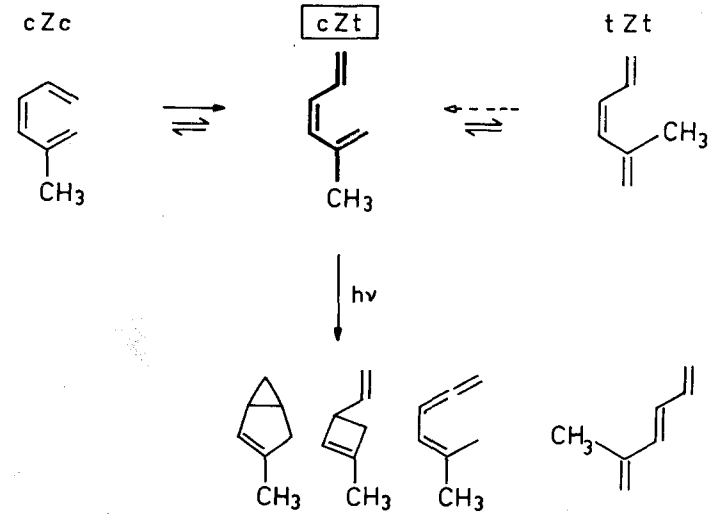


Fig. 21. 2-methyl-(Z)-hexa-1,3,5-triene; ε = 22,400 (259 nm); conformations and photoproducts.

Conformations and photoproducts of vitamin D

We now have the essential data to understand what we were looking for in the case of vitamin D. It has an $\varepsilon = 19{,}000$ at 260 nm that exactly corresponds to those of the substituted hexatrienes in the cZt conformation. This is nicely confirmed and defined in more detail by the X-ray work on steroids and vitamin D derivatives at Leiden (ROMERS and KNOBLER[23]) as well as by an older X-ray investigation of vitamin D 4-iodo-5-nitrobenzoate by HODGKIN-CROWFOOT, WEBSTER and DUNITZ[24] (Figure 24).

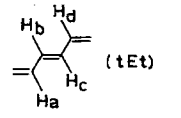
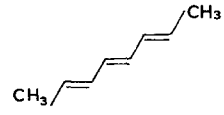
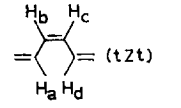
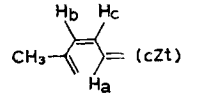
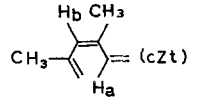
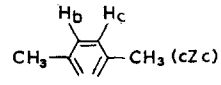
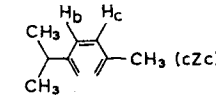
	main conformation	$\varepsilon(\lambda_m)$	$\delta H_a, \delta H_d$	δH_b, δH_c
[E]hexa-1,3,5-triene	 (tEt)	51700 (256)	6.0-6.5	
[E.E.E.] okta-2,4,6- triene		55000 (263)	5.7-6.2	
[Z]hexa-1,3,5-triene	 (tZt)	41000 (254)	6.3-7.1	5.6-6.2
2 methyl-[Z]-hexatriene	 (cZt)	22400 (259)	6.7-7.2	5.7-6.2
2,4 di methyl-[Z]-hexatriene	 (cZt)	21900 (263)	6.7-7.2	5.73
2,5 di methyl-[Z]-hexatriene	 (cZc)	12300 (237)	—	5.87
2 methyl-5- i.propyl-[Z]-hexatriene	 (cZc)	8700 (245)	—	6.0

Fig. 22. UV-extinction coefficients (at λ_{max}), characteristic NMR-data and the preferred conformation of some hexa-1,3,5-trienes.

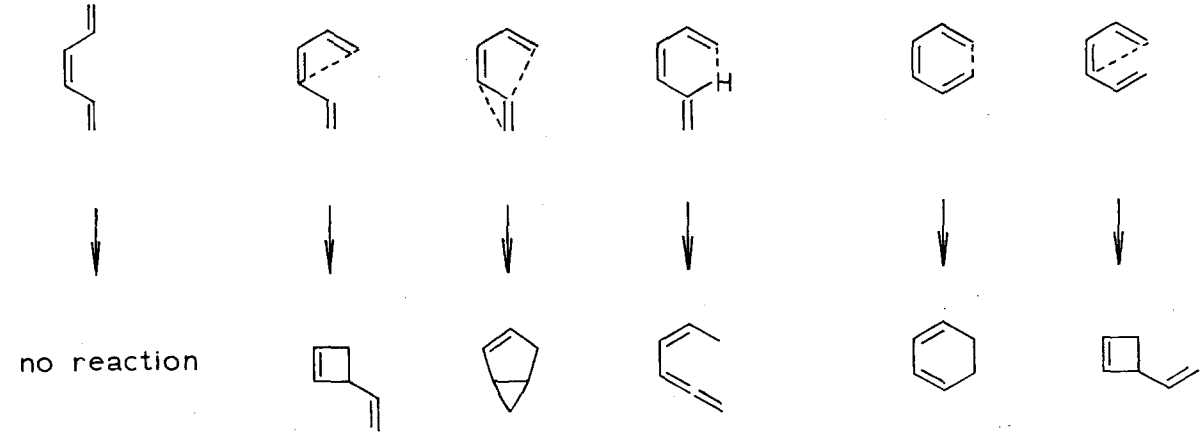


Fig. 23. The 3 planar conformations of the (Z)-hexa-1,3,5-triene system and the corresponding photoproducts. Formation of the Δ^3(E)-isomer is omitted from the representation.

Figure 24 gives the conformation of a vitamin D analogue, the 3,20 *bis* (ethylene dioxy)-9,10-seco-pregna-5,7,10(19)-triene as deduced by KNOBLER and ROMERS[23]. For those interested in conformational details, attentions is drawn to:

6-rings: in chair forms, 5-rings: D in half chair, dioxolane rings in envelope form.The triene system – according to expectation from molecular models – is not planar as shown by the value of the dihedral angles. In solution there will be rapid equilibrium between 2 forms the β (CH_2 above plane) and α form (CH_2 below) (Figure 25).

HODGKIN-CROWFOOT, WEBSTER and DUNITZ[24] studied a derivative – the 4-iodo-5-nitrobenzoate – that has the α-form allowing the bulky substituent at C_3 to take the energetically favoured equatorial position. With the diketale no such directive influence is present; it happens to be in the β-form.

If we look again at Figure 14, we see that the photoproducts of vitamin D are the isomers one would expect from a triene in cZt conformation.

Concluding remarks

On the one hand, of course, it is gratifying that all these recent results follow the simple pattern proposed in 1961 to explain the order of magnitude of certain quantum yields of isomerization in the vitamin D field.

[23] C. KNOBLER, C. ROMERS, P. B. BRAUN and J. HORNSTRA, Acta crystallogr. *B 28*, 2097 (1972).
[24] D. CROWFOOT HODGKIN, M. S. WEBSTER and J. D. DUNITZ, Chemy Ind. *1957*, 1148.

Figure 26 pictures the hypothesis that the various conformers of the ground state equilibrium upon absorption of UV-light yield species which do not

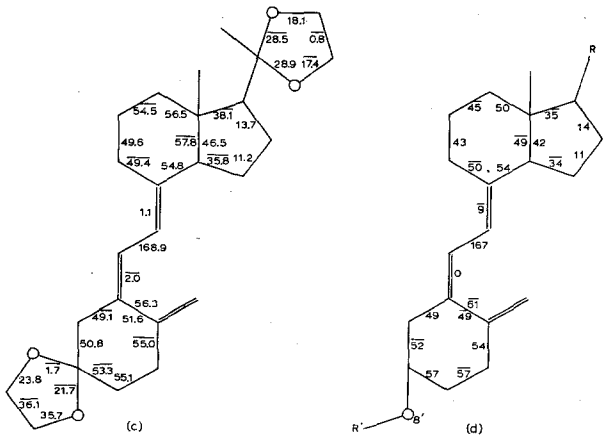


Fig. 24. Conformations and internal dihedral angles of vitamin D-like compounds (X-ray analyses).

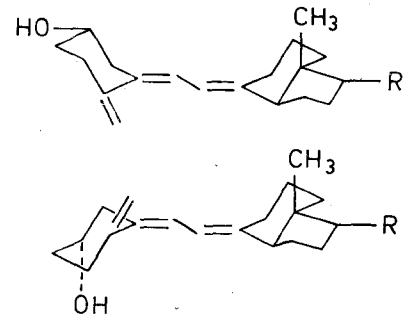


Fig. 25. 2 conformations of vitamin D.

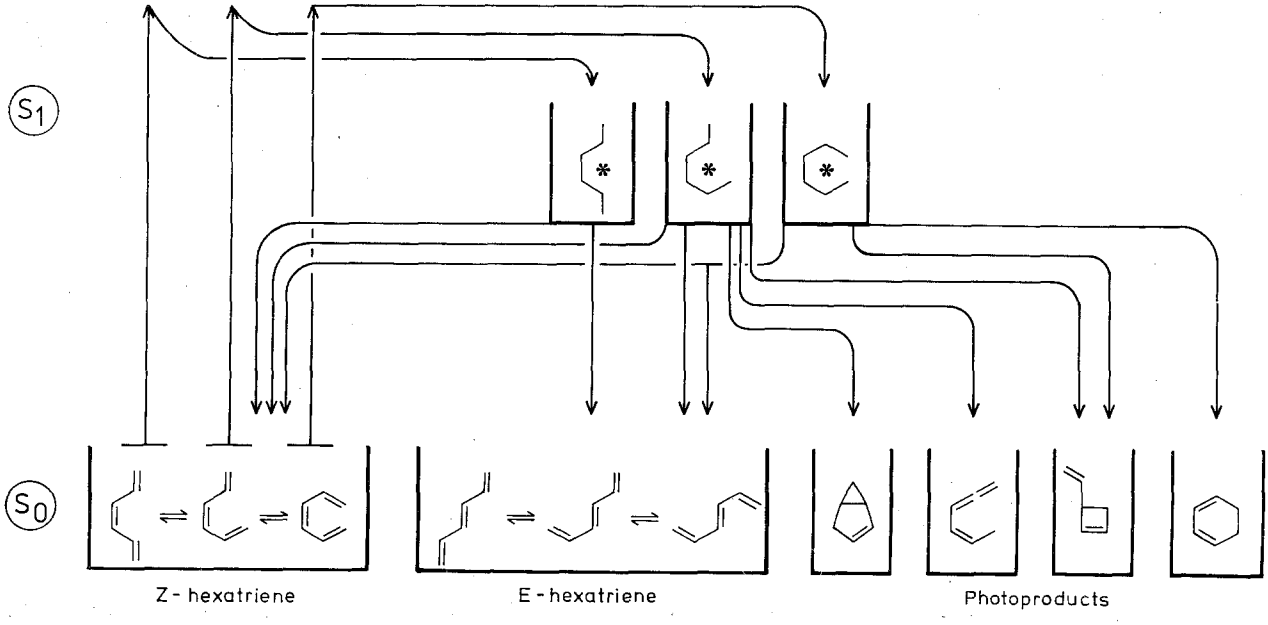


Fig. 26. Formation of photoproducts from conformations of (Z)-hexatriene.

interconvert during their short singlet excited lifetime. Each of these excited species then transforms into specific products when returning to a ground state. The compounds are expected rigorously to follow the US primary law of traffic: 'keep your lane'. However, this simple picture that evidently has good predictive value in practice, gives rise to a series of questions when considered from a theoretical point of view. Why should a molecule, brought into a vibrationally excited S_1 state by absorption of a short wavelength quantum, not be able to overcome the rotational barriers separating it from the other excited species? We might consider that perhaps the solvent cage will – within the extremely short period of vibrational relaxation (10^{-12} sec) – oppose gross changes of molecular form and orientation. And subsequently upon arrival in the low vibrational levels of the S_1 state, the double bond character of the (originally single) bonds will endow the molecule with resistance against rotation sufficient to withstand thermal activation during singlet state lifetime ($\sim 10^{-8}$ sec). However, there is not much experimental data to argue that such an effect of the solvent cage might be of real importance. We should therefore not overlook an alternative assumption, i.e. that conformational equilibration may occur in the excited state and that the favoured form in the exicted state could show overall similarity with the most stable conformation in the ground state. A highly interesting and important field for future research lies in the conformational analysis of molecules in the excited state (flash techniques, etc.). In our particular problem we hope to arrive at an answer by studying the product composition as a function of the wavelength of the light used for excitation and as a function of temperature. At this moment indications remain somewhat in favour of the original simple alternative: non-interconversion of excited conformations. However this may be, it is clear that a world of exciting new chemistry is awaiting us if we follow the example and the challenge that the wonderful molecules of Nature like vitamin D continue to offer to Science.

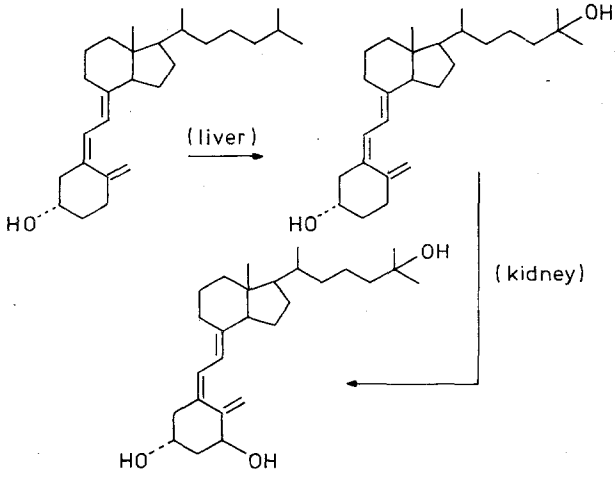


Fig. 27. 1α, 25-dihydroxycholecalciferol, directly active derivative of vitamin D_3.

Within this perspective it seems appropriate to indicate another novel nucleus of development that originated in recent years through the investigations of the groups of KODICEK, DELUCA and NORMAN[25] in particular. They were able to establish that vitamin D in the body is metabolized to the 25 hydroxy derivative in the liver and then to the physiologically directly active 1α,25 dihydroxycalciferol in the kidney (Figure 27). This makes clear, on the one hand, the relationship between dysfunctions of the kidney and certain types of rickets that until now were refractory to standard vitamin D therapy. On the other hand, it opens a new field of research on the mechanism of hydroxylation of vitamin D, on the relation between molecular structure and activity of these compounds and on the mode of action of 1α, 25 dihydroxycalciferol. With regard to this latter class of problems, one could rightly quote from the Nobel lecture (1937) of PAUL KARRER: 'It is the task of physiology today to explain the intervention of these agents in the cell process. As this is, however, a matter of chemical processes these will in the end probably have to be elucidated by the chemist once again'.

Zusammenfassung

Vitamin D ist eine in verschiedener Hinsicht interessante Verbindung, deren Untersuchung zu neuen Erkenntnissen auf dem Gebiete der Stereochemie, der Photochemie und der Reaktionsspezifität beigetragen hat. Insbesondere die damals intrigierende Entstehungsweise unter dem Einfluss von Ultraviolettstrahlung (antirachitische Wirkung des Sonnenlichtes) hat frühzeitig die Aufmerksamkeit von Chemikern und Physikern erregt. Es sind dann die sehr spezifisch verlaufenden thermischen und photochemischen Isomerisierungen gewesen, die zum Verständnis des Reaktionsmechanismus und zu den dem Reaktionsverlauf zugrunde liegenden Gesetzmässigkeiten geführt haben. Auch bieten die Moleküle der unterschiedlichen Vitamin-D-Isomeren mit deren Chiralitätszentren und dem polyzyklischen Aufbau nach wie vor ein fruchtbares Gebiet zur Prüfung der Reichweite der modernen Konformationsanalyse und von chiroptischen Betrachtungen.

Es wird eine Übersicht über die verschiedenen experimentellen Ergebnisse und die daraus entwickelten theoretischen Spekulationen gegeben. Als Grund-

[25] D. E. M. LAWSON, D. FRASER, E. KODICEK, H. R. MORRIS, D. H. WILLIAMS, Nature, Lond. *230*, 228 (1971). – M. F. HOLICK, E. J. SEMMLER, H. K. SCHNOES and H. F. DELUCA, Science *180*, 190 (1973). – A. W. NORMAN, J. F. MYRTLE, R. J. MIDGETT and H. G. NOWICKI, Science *173*, 51 (1971).

lage wird immer wieder die genaue Kenntnis der molekularen Geometrie und der Bindungsverhältnisse angestrebt. Diese lässt sich heutzutage mittels Konformationsanalyse,«Valence Force Field»-Kalkulationen und insbesondere auch Röntgenanalyse in konsistenter Weise gewinnen. Bei der weiteren Diskussion der (photo)chemischen Prozesse zeigen sich 3 Faktoren zum Verständnis und zur Voraussicht des Ablaufs wichtig. Es ist die schon längst bekannte sterische Hinderung und zwei weitere, beim Studium der Reaktionen im Vitamin-D-Gebiet zuerst formulierte Faktoren: der Einfluss der Orbitalsymmetrie und (für Photoreaktionen) die Lage des Konformationsgleichgewichtes im Grundzustand. Insbesondere das letztgenannte Prinzip hat sich bei der Auffindung von bisher unbekannten Photoprodukten des Vitamins D als sehr wichtig erwiesen.

Auch in der Zukunft werden die von der Natur sorgfältig selektionierten Moleküle von Vitamin D und dessen Isomeren ein anregendes Beispiel und Untersuchungsobjekt beim Studium der detaillierten Mechanismen von Photoisomerisierungen und bei der Konformationsanalyse von Molekülen in aktiviertem Zustand darbieten.

SPECIALIA

Δ^6-Tetrahydrocannabinol-7-oic Acid, a Urinary Δ^6-THC Metabolite: Isolation and Synthesis

The metabolism of Δ^1-tetrahydrocannabinol (Δ^1-THC) (I) and Δ^6-THC (IIa) has been investigated by numerous groups in recent years[1,2]. The major primary metabolic process is considered to be mono-oxygenation, mostly on the C-7 position to give, in the case of Δ^1-THC, the biologically active 7-hydroxy-Δ^1-THC (III). Recently BURSTEIN et al.[3] reported the isolation from rabbit urine of 2 major acidic metabolites of Δ^1-THC. They were shown to possess structures IVa and IVb. The isolation of these acids poses two important questions: Are they formed from 7-hydroxy-Δ^1-THC (III)?; Are these acids, and/or their possible aldehydic precursors, also biologically active? Hence, it seemed of considerable importance and interest to synthesize and test the biological activity of these acids and the respective 7-aldehydo cannabinoids and to investigate the metabolism of 7-hydroxy-Δ^1-THC. However, practical methods for the preparation of Δ^1-THC derivatives oxygenated at C-7 have not yet been developed. As an alternative, we decided to synthesize the corresponding Δ^6-THC derivatives. Since the biological properties and usually also the chromatographic behaviour in the Δ^1 and Δ^6-THC series are closely parallel[4,5], we assume that results obtained in one of the series will also apply to the other. Concurrently we investigated the urinary metabolites of 7-hydroxy-Δ^6-THC (V), a major physiologically active metabolite of Δ^6-THC, for the possible presence of Δ^6-THC-7-oic acid (VIa).

Synthesis. Δ^6-THC acetate (IIb) was oxidized with selenium dioxide in ethanol for 24 h. On chromatography yields of 27–33% of Δ^6-THC-7-al acetate (VII) were obtained. This oily compound, $[\alpha]_D$ (in ethanol)-271°, was identified on the basis of its IR-spectrum (peaks at 1690 and 1775 cm^{-1} for the aldehydic and acetate groups respectively); its UV-spectrum (peaks at 281 nm, ε, 2680; 274 nm, ε, 2620; shoulder at 220 nm, ε, 15200), and its NMR-spectrum (δ, in $CDCl_3$, 0.90, 1.20, 1.42, methyl groups; 2.26, s, acetate methyl group; 6.46, 6.55, d, aromatic protons; 6.80, m, vinylic proton; 10.22, s, aldehydic proton).

The aldehyde VII, was oxidized in a 59–63% yield to Δ^6-THC-oic acid methyl ester (VIb) by reaction with manganese dioxide and sodium cyanide in methanol for 12 h[6,7]. The oily VIb, $[\alpha]_D$ (in ethanol) −302°, was identified by its IR-spectrum (peak at 1695 cm^{-1} of the carbomethoxyl group), and its NMR-spectrum (δ, in $CDCl_3$, 0.88, 1.10 and 1.38, methyl groups; 3.73, s, carbomethoxyl group; 6.15 and 6.20, aromatic protons; 7.02, m, olefinic proton). On hydrolysis, Δ^6-THC-7-oic acid (VIa) was obtained, $[\alpha]_D$ (in ethanol) −287°; IR-spectrum, peak at 1690 cm^{-1}, and typical wide carboxylic acid band in the 3000 cm^{-1} region; UV-spectrum, peaks at 274 nm, ε, 1454; 281 nm, ε, 1454 and shoulder at 230 nm, ε, 10530; molecular weight (mass spectrum) 344; NMR-spectrum, δ (in CCl_4), 0.95, 1.25 and 1.30 (methyl groups); 3.65 (C-2 proton); 5.90, 6.07 (aromatic protons), 7.05 (olefinic proton). The methyl ester acetate of Δ^6-THC-7-oic acid (VIc) was prepared by acetylation of VIb.

Biological tests. The free acid (VIa), its methyl ester acetate (VIc), and the aldehyde (VII) were tested in rhesus monkeys as described before[4]. Neither VIa, nor VIc showed any activity in doses up to 10 mg/kg. These observations contrast sharply with the activity recorded

[1] W. D. M. PATON and J. CROWN, *Cannabis and its Derivatives* (Oxford University Press, London 1972).
[2] R. MECHOULAM, *Marijuana. Chemistry, Pharmacology, Metabolism and Clinical Effects* (Academic Press, New York 1973).
[3] S. BURSTEIN, J. ROSENFELD and T. WITTSTRUCK, Science *176*, 422 (1972).
[4] H. EDERY, Y. GRUNFELD, Z. BEN-ZVI and R. MECHOULAM, Ann N.Y. Acad. Sci. *191*, 40 (1971).
[5] M. E. WALL, Ann. N.Y. Acad. Sci. *191*, 23 (1971).
[6] E. J. COREY, N. W. GILMAN and B. E. GANEM, J. Am. chem. Soc. *90*, 5616 (1968).
[7] J. L. G. NILSSON, I. M. NILSSON, S. AGURELL, Z. BEN-ZVI and R. MECHOULAM, Acta pharmac. Suecica *9*, 215 (1972).