

Ergosterol Photoisomerization Reaction Scheme

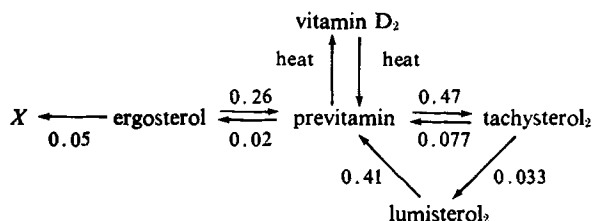
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Abstract □ The kinetics of the various isomers formed at low temperature by UV irradiation of ergosterol were studied experimentally at the wavelength $\lambda = 253.7$ nm. The characteristics of the photostationary state were used to determine the isomer precursor of the toxisterols as well as the quantum transformation yields of one isomer into another.

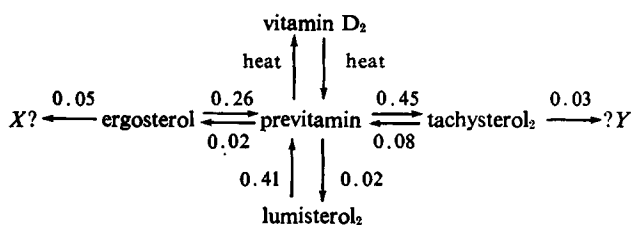
Keyphrases □ Ergosterol photoisomerization—reaction scheme, kinetics, transformation quantum efficiencies between isomers, formation and quantum yields of toxisterols □ Photoisomerization, ergosterol—reaction scheme, kinetics, transformation quantum efficiencies between isomers, formation and quantum yields of toxisterols □ Isomerization of ergosterol by UV irradiation—reaction scheme, kinetics, quantum transformation yields between isomers

Since vitamin D₂ is obtained by submitting ergosterol to UV irradiation, the system of isomers formed under radiation has already been the subject of many works (1–3). The three latest reaction schemes (Schemes I–III), summarizing the experimental results achieved, were successively submitted by Rappoldt in 1958 (4), Sanders and Havinga in 1964 (5), and Sanders in 1967 (6).

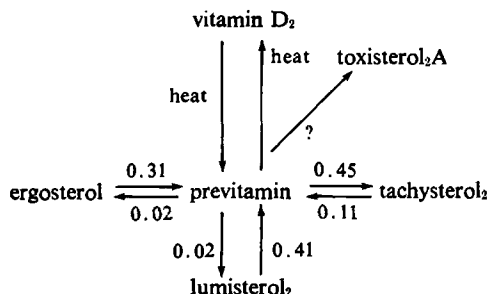
The solely thermal nature of previtamin D₂ ⇌ vitamin D₂ transformations was brought to light by Velluz in



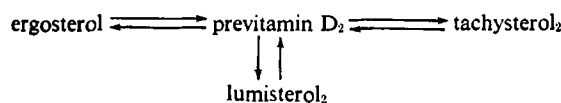
Scheme I—Reaction scheme of ergosterol photoisomerization suggested by Rappoldt (4)



Scheme II—Reaction scheme of ergosterol photoisomerization suggested by Sanders and Havinga (5)



Scheme III—Reaction scheme of ergosterol photoisomerization suggested by Sanders (6)



Scheme IV—Internal structure of the reaction scheme at low temperature suggested by Mermet-Bouvier and coworkers (7–11). In the internal structure, the irreversible transformations do not figure.

1948 (2); all other transformations are photochemical in nature. The values of the corresponding quantum yields are shown in each scheme.

Experimental work recently carried out in this laboratory (7–11), at a sufficiently low temperature for the previtamin D₂ → vitamin D₂ transformation to be negligible, led to the reaction scheme shown in Scheme IV. For this scheme, the present authors attempted to determine: (a) the value of the transformation quantum efficiencies between the various isomers, and (b) the isomers that act in starting the irreversible formation of toxisterols and the corresponding quantum yields.

THEORETICAL (12)

Let 1, 2, 3, and 4 be the classes whose elements are molecules of ergosterol, previtamin D₂, tachysterol, and lumisterol, respectively¹

Let f_{ij} be the probability per unit time for a j class element to get into an i class element.

The reaction photoisomerization scheme of ergosterol given in Scheme IV corresponds to a true exotropic graph¹ (13), whose internal structure is shown in Scheme V.

Let external function f_{je} be the exit probability per unit of time of an element of the system, as from class j .

The problem consisted of determining: (a) the value of the internal interaction functions f_{ij} , and (b) the existence and value of the external functions f_{je} .

Evolution Equations—Let $\{N(t)\}$ be the vector whose components:

$$\begin{pmatrix} N_1(t) \\ N_2(t) \\ N_3(t) \\ N_4(t) \end{pmatrix}$$

are the populations of the various classes at time t .

The evolution equation of the system is:

$$\frac{d\{N(t)\}}{dt} = [a]\{N(t)\} \quad (\text{Eq. 1})$$

in which $[a]$ is the interaction matrix whose nondiagonal elements are the internal functions $a_{ij} = f_{ij}$ and whose diagonal elements are the sum of the divergent functions related to a class j bearing the minus sign:

$$a_{jj} = -f_{je} - \sum_k f_{kj} \quad (\text{Eq. 2})$$

The solutions of the differential system (Eq. 1) appear as a sum of four exponential functions:

$$N_j(t) = \sum_{i=1}^4 A_{ij} e^{\lambda_i t} \quad (\text{Eq. 3})$$

where λ_i is the eigenvalues of matrix $[a]$.

¹ A true exotropic graph is a maximum, strongly connected component to which are added boundary functions that are all divergent.

After a sufficiently long irradiation time, Eq. 3 becomes equivalent to:

$$N_j(t) \approx A_{1j}e^{\lambda_1 t} \quad (\text{Eq. 4})$$

where λ_1 is the eigenvalue of the smallest absolute value.

If the evolution of the four isomers is presented as semilogarithmic coordinates, two states may be distinguished as shown in Fig. 1:

1. The transient state during which at least two exponentials enter into each $N_j(t)$ expression.

2. The photostationary state or the permanent evolution state, for which one exponential only directs the evolution of each isomer, that is represented in semilogarithmic coordinates by parallel straight lines of slope λ_1 .

The reaction schemes given in the literature express the experimental results obtained in a transient irradiation state. This report concerns the permanent evolution state characteristics, and the parameters used are: (a) the slope λ_1 , and (b) the relative concentrations of the various isomers:

$$C_j = \frac{N_j(t)}{\sum_j N_j(t)} = \frac{A_{1j}}{\sum_j A_{1j}} \quad (\text{Eq. 5})$$

Relationship between Internal Functions f_{jk} , External Functions f_j , Slope λ_1 , and Relative Concentrations C_j in Permanent Evolution State—The population N_j of class j obeys the following balance equation:

$$\frac{dN_j}{dt} = -X_j N_j + \sum_{k \neq j} f_{jk} N_k \quad (\text{Eq. 6})$$

in which:

$$X_j = f_{j0} + \sum_{k \neq j} f_{kj} \quad (\text{Eq. 7})$$

Now, in the permanent evolution state:

$$\frac{dN_j}{dt} = \lambda_1 N_j \quad (\text{Eq. 8})$$

By identifying Eqs. 6 and 8 and dividing by N_j :

$$\lambda_1 + X_j = \sum_{k \neq j} \frac{f_{jk} C_k}{C_j} \quad (\text{Eq. 9})$$

If N is the total number of elements at time t :

$$N = \sum_j N_j \quad (\text{Eq. 10})$$

The number of elements leaving the system is per time unit:

$$-\frac{dN}{dt} = \sum_j f_{j0} N_j \quad (\text{Eq. 11})$$

Now, in the permanent state:

$$\frac{dN}{dt} = \sum_j \frac{dN_j}{dt} = \lambda_1 \sum_j N_j = \lambda_1 N \quad (\text{Eq. 12})$$

By identifying the relations Eqs. 11 and 12 and dividing by N :

$$\lambda_1 = -\sum_j f_{j0} C_j \quad (\text{Eq. 13})$$

The internal functions f_{jk} and external functions f_{j0} will be determined on the basis of Eqs. 9 and 13.

Table I—Photochemical Constants of the Four Isomers

	Ergosterol	Previtamin D ₂	Tachysterol ₂	Lumisterol ₂
ϵ_j , liters/mole cm.	4800	8210	9915	4760
ρ_j , molecule/photon absorbed	0.31	0.49	0.11	0.41
X_j , sec. ⁻¹	5.69	15.4	4.17	7.46

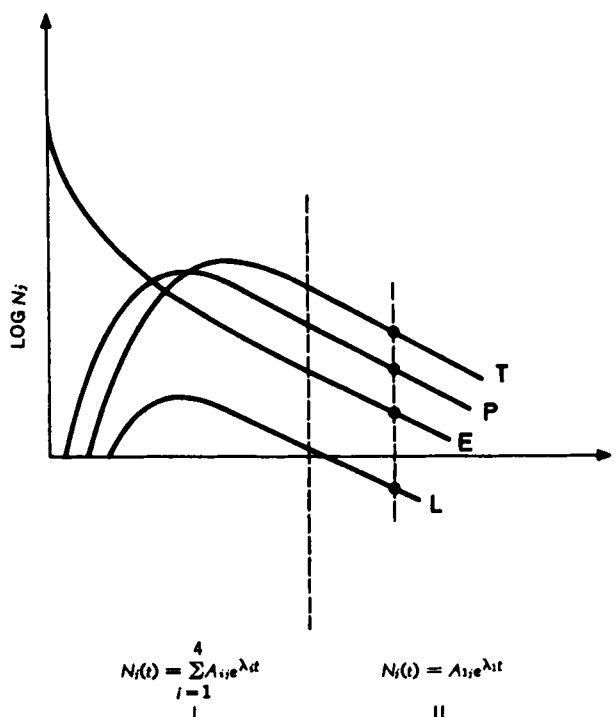


Figure 1—Evolution states of the system. Key: I, transient state; II, permanent evolution state; E, ergosterol; T, tachysterol; L, lumisterol; and P, previtamin D₂.

X_j Values Used—Let ϵ_j be the coefficient of molecular extinction related to the isomer j , expressed in liters per mole centimeter; and let ρ_j be the quantum disappearance yield of the isomer j , expressed in molecules per photon absorbed by the isomer j .

The exit probability per time unit of a class j element is given, in reciprocal seconds, by the expression (8):

$$X_j = 3.822 \times 10^{-3} \epsilon_j \rho_j \quad (\text{Eq. 14})$$

The values given by Havinga *et al.* (14) were taken for ϵ_j and ρ_j . They are summarized in Table I with the corresponding X_j values.

The slope λ_1 and the relative concentrations C_j were the subjects of the following experimental determination.

EXPERIMENTAL

The experimental values of λ_1 and of the relative concentrations C_j in the permanent evolution state at a wavelength of 253.7 nm. were determined by UV spectrophotometry and GC (7), respectively.

Determination of λ_1 —The experimental determination of λ_1 was made with very weak ergosterol solutions ($2 \times 10^{-5} M$) by following the evolution of absorbances of the four substances ergosterol, previtamin D₂, tachysterol, and lumisterol at such a wavelength that the substances outside the true graph (toxisterols) only showed insignificant absorption, since experimental determination techniques do not allow this value to be obtained by determining the slope of the evolution graph of each isomer.

Although the concentration used in the irradiated solutions is low, the 253.7-nm. wavelength incident radiation is slightly absorbed. Therefore, the correcting factor F (8), which reflects the flux decrease in the solutions, was introduced:

$$F = \frac{1 - e^{-A(t)}}{A(t)} \quad (\text{Eq. 15})$$

where $A(t)$ is the absorbance at time t at the incident radiation wavelength (253.7 nm.).

This factor enables the mean intensity $\bar{I}(t)$ in the irradiated solution to be known, since the incident intensity I_0 can only be used, strictly, in a very shallow system. The values $A(t)$ are determined by the experimental measurements of the change in absorbance as a function of the irradiation time at the incident radiation wavelength ($\lambda = 253.7$ nm.).

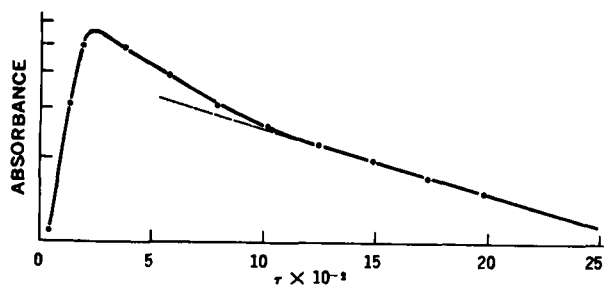


Figure 2—Changes of absorbance with the variable $\tau = \int_0^t [I(t)/I_0] dt$. The slope in the rectilinear part of the curve is the slope λ_1 of the permanent evolution state.

The mean intensity is then calculated, from the experimental values obtained, by the formula:

$$\bar{I}(t) = I_0 \times F \quad \text{or} \quad \bar{I}(t) = I_0 \times \frac{1 - e^{-A(t)}}{A(t)} \quad (\text{Eq. 16})$$

The transition from variable t , irradiation time that could be used in a very thin system, to the variable τ , which is necessary to make the system used linear (this system was approximately but not really a very thin system since there was an attenuation of the incident flux in the irradiation medium), is given by the formula:

$$\tau = \int_0^t \frac{\bar{I}(t)}{I_0} dt \quad (\text{Eq. 17})$$

Figure 2 shows the experimental results obtained with an ergosterol solution as the initial product; τ is on the abscissa, and the changes in absorbance with irradiation time, for a wavelength $\lambda = 290$ nm, where the substances outside the true graph have no input, are on the ordinate.

The value of slope λ_1 is calculated with the following formula:

$$\lambda_1 = \frac{\log A_1 - \log A_2}{\tau_1 - \tau_2} \quad (\text{Eq. 18})$$

where A_1 and A_2 are the measured absorbances corresponding to τ_1 and τ_2 , respectively.

Measurements made with previtamin D_3 , lumisterol₂, and tachysterol₂ as starting products gave, within experimental errors, the same value of slope λ_1 . It was thus checked that the elements belonging to a same true graph have, in fact, the same slope λ_1 (12) in the same permanent evolution state, irrespective of the initial conditions.

The experimental value of slope λ_1 equals $-0.27 \pm 0.01 \text{ sec.}^{-1}$.

Determination of Relative Concentrations C_i .—The very small amount of substance obtained after UV irradiation of a low con-

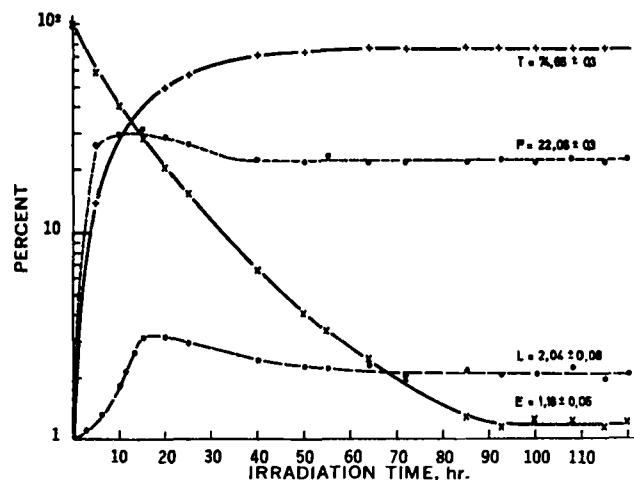
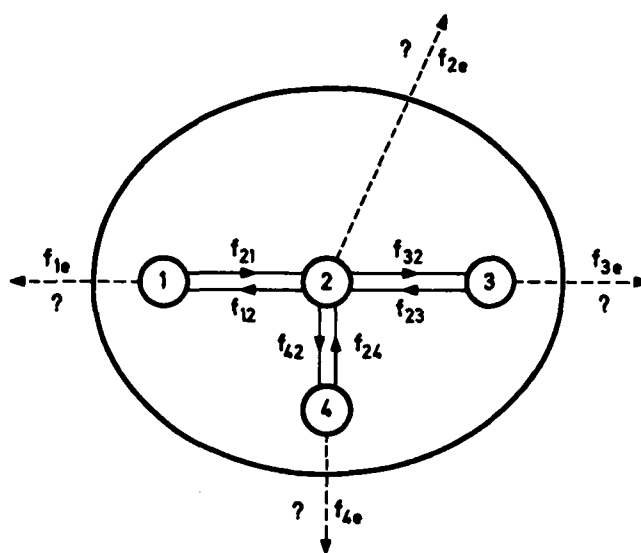


Figure 3—Evolution with the irradiation time of the relative concentration of the four isomers. Key: E, ergosterol; T, tachysterol; L, lumisterol; and P, previtamin D_3 .



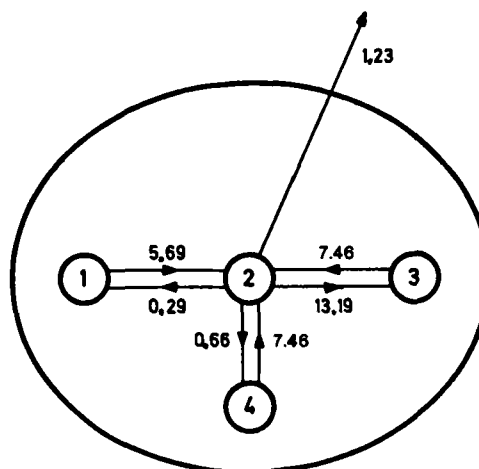
Scheme V—Internal structure of the true exotropic graph of the photoisomeric system of ergosterol. The possible irreversible transformations are represented by external functions f_{ie} .

centration ergosterol solution ($2 \times 10^{-5} M$) in ether does not allow the various isomers formed to be determined separately. Stronger solutions were, therefore, used ($1.25 \times 10^{-2} M$). The F factor, characterizing the flux decrease, becomes zero, and the transition through the variable τ is no longer possible. But the system then tends to an asymptotic state whose relative compositions are the same as those of the permanent evolution state defined previously. Therefore, the determinations were made as soon as this asymptotic state was reached (7).

The separation methods used for the qualitative analysis (9–11) showed that for the long irradiation times needed to reach the asymptotic state the previtamin and one of the toxisterols were not separated; only GC, with 3.04-m. (10-ft.) long and 2-mm. \times 0.6-cm. (0.25-in.) diameter Pyrex columns filled with 3% XE-60 on Gas Chrom Q, 100–120 mesh, permitted adequate separation of all isomers.

Before being used, the column was placed in an oven heated to 230° for 2 days, with a convection gas (helium) flow of 20 ml./min. The temperature of the oven was then regulated to 150° , and several injections of $10 \mu\text{l.}$ each of hexamethyldisilazane were made.

When the baseline of the recorder was stabilized, the temperature of the oven was again set at 230° . As soon as the flame-ionization detector gave a stable response, several injections of $2 \mu\text{l.}$ each of an irradiated ergosterol solution were made. This procedure reduces the absorption effects on the support, which are frequently encountered with new columns, and allows aging of the column.



Scheme VI—Interaction graph of the system corresponding to experimental results

Table II—Quantum Transformation Yields of Previtamin D₂

Previtamin D ₂ →	Ergosterol	Tachysterol ₂	Lumisterol ₂	Toxisterol ₂
Sanders (6)	0.02	0.45	0.02	—
This study	0.0092	0.42	0.021	0.039

Finally, it was necessary to make sure that the GC, owing to the high temperature used, did not cause any distortion of the structure of the eluted isomers. After trapping the various eluates in the oven and directly at the exit of the column and analyzing them by UV spectrophotometry, UV spectra of each isomer were obtained identical to those obtained at low temperature.

The internal standardization procedure was used for the quantitative analysis, and the measurement of the areas of the peaks representing the four isomers of the true graph (ergosterol, previtamin D₂, tachysterol, and lumisterol) was made with an electronic digital integrator².

The results obtained with a chromatograph³ are given in Fig. 3. The ergosterol solutions in ether had a concentration of 1.25×10^{-2} M. They were irradiated with an incident flux of 1.24×10^{-8} photon/cm.² sec.

The experimental values of λ_1 and of the relative concentrations were: $\lambda_1 = -0.27 \pm 0.01$ sec.⁻¹, $C_1 = 1.18 \pm 0.05\%$, $C_2 = 22.06 \pm 0.3\%$, $C_3 = 74.66 \pm 0.3\%$, and $C_4 = 2.04 \pm 0.08\%$.

RESULTS AND DISCUSSION

Values of Internal Divergent Functions Relative to Class 2—Let Eq. 9 be applied in turn to classes 1, 3, and 4:

$$f_{12} = (\lambda_1 + X_1) \frac{C_1}{C_2} = 0.29 \text{ sec.}^{-1} \quad (\text{Eq. 19})$$

$$f_{32} = (\lambda_1 + X_3) \frac{C_3}{C_2} = 13.19 \text{ sec.}^{-1} \quad (\text{Eq. 20})$$

$$f_{42} = (\lambda_1 + X_4) \frac{C_4}{C_2} = 0.66 \text{ sec.}^{-1} \quad (\text{Eq. 21})$$

Value and Unicity of External Function f_{2e} —The value of f_{2e} is given by:

$$f_{2e} = X_2 - (f_{12} + f_{32} + f_{42}) \quad (\text{Eq. 22})$$

giving $f_{2e} = 1.229 \text{ sec.}^{-1}$. This external function is unique because, using Eq. 13, the term corresponding to class 2—allowing for the value of f_{2e} , previously determined—is:

$$-f_{2e}C_2 = 0.271 \quad (\text{Eq. 23})$$

The value of λ_1 being $\lambda_1 = -0.27$, the term $-f_{2e}C_2$ suffices to express it and the three other terms are, therefore, zero. Thus, $f_{1e} = f_{3e} = f_{4e} = 0$.

Value of Converging Functions Related to Class 2—Classes 1, 3, and 4 were not given external functions:

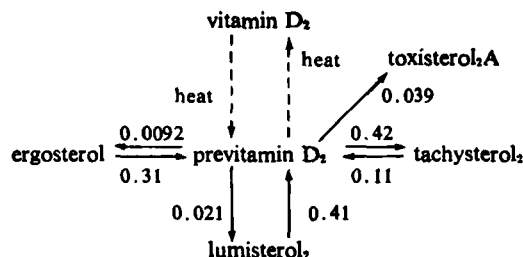
$$f_{21} = X_1 = 5.69 \text{ sec.}^{-1} \quad (\text{Eq. 24})$$

$$f_{23} = X_3 = 7.46 \text{ sec.}^{-1} \quad (\text{Eq. 25})$$

$$f_{24} = X_4 = 4.17 \text{ sec.}^{-1} \quad (\text{Eq. 26})$$

² Varian Aerograph model 480.

³ Varian Aerograph model 2100.



Scheme VII—Suggested reaction scheme for the photoisomerization of ergosterol

Proposed Reaction Scheme—All these results are summarized in Scheme VI in which the transfer functions are expressed in reciprocal seconds.

As the quantum yield ρ_{ij} of the transformation of isomer j into isomer i is obtained, as from the transfer function f_{ij} by the relation (8):

$$\rho_{ij} = 2.616 \times 10^3 \frac{f_{ij}}{\epsilon_j} \quad (\text{Eq. 27})$$

the reaction scheme shown in Scheme VII is obtained. This scheme is structurally identical to those proposed by Velluz (2) and Sanders (6) (Scheme III). It differs from them by the value of the quantum transformation yields of previtamin D₂.

The values submitted by Sanders (6) and the values from the present study are summarized in Table II.

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