undergo a sharp change near or at the CMC of each surfactant, which continues over a narrow concentration range beyond the CMC. This type of behavior has been observed for potassium *n*-octoate (5) and for potassium laurate and lauryl sulfonic acid (8). The concentration dependency of $\bar{v}_1$ and $\bar{v}_2$ is due to the changing ratio of monomeric to micellar surfactant (14, 15). After this transition region, $\bar{v}_1$ and $\bar{v}_2$ begin to become constant again due to the diminishing ratio of the monomeric surfactant to the micellar surfactant.

It also has been suggested (16) that the decrease in the partial specific volume of water with a concomitant rise in that of the surfactant is due to change of water structure upon micelle formation. Below the CMC where the surfactant molecules exist in their monomeric form, the hydrophobic portions of these molecules are tightly surrounded by water molecules (17, 18). Thus, these monomers are subjected to high compression by the strong cohesive field of water molecules. At the same time, by their interposition between water molecules, these monomers effectively reduce the attraction of water molecules for each other which results in less compression on water. Upon aggregation, the area of contact between water and the hydrophobic portions of the surfactant is greatly reduced on account of the large size of the micelle as compared to single molecules. This reduction of the contact area releases most of the water molecules that were originally bound to the surfactant molecules and were the cause of their compression. For these reasons, micelle formation is accompanied by a reduced compression of the surfactant molecules (larger partial specific volume) and by an increased compression of the water molecules (smaller partial specific volume).

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Effect of Wavelength on Production of Previtamin D_2

ELIANE ABILLON[▲] and RENÉ MERMET-BOUVIER

Abstract □ On the basis of a previously established reaction scheme, the formation rate of various isomers produced by UV irradiation of ergosterol at low temperature was calculated for wavelengths between 240 and 320 nm. Particular attention was given to previtamin D_2, the precursor of vitamin D_2; the production optimum and the time required to obtain it were investigated in relation to the irradiation wavelength.

Keyphrases □ Previtamin D_2 photochemical formation from ergosterol—effect of irradiation wavelengths □ Ergosterol photochemical conversion to previtamin D_2—effect of irradiation wavelength □ UV irradiation of ergosterol—conversion to previtamin D_2, effect of various wavelengths

Much work has been done on the photoisomerization of ergosterol (1–3), and various reaction schemes have been proposed (4–6). Only the thermal transformation of previtamin D_2 to vitamin D_2 was reported (2) and is insignificant if the temperature of the irradiated medium is under 10°.

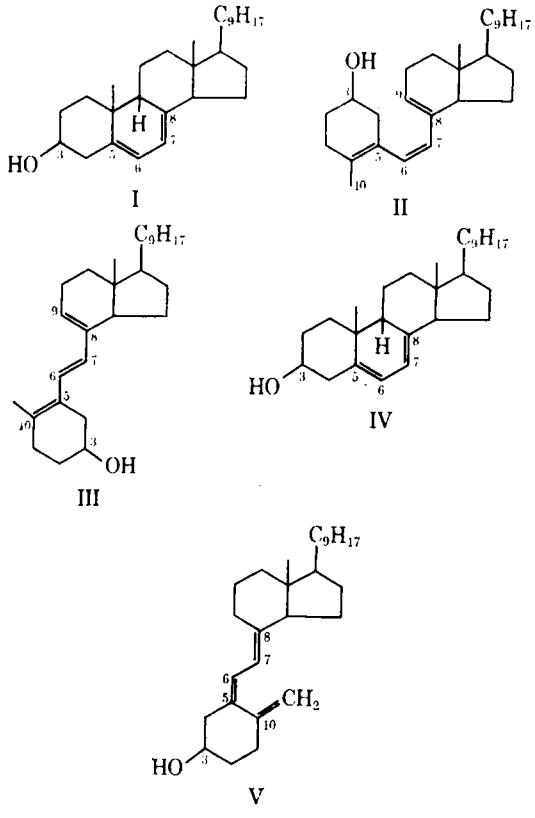
The production of vitamin D_2 is achieved in two steps:

1. UV irradiation of ergosterol (I) at a low temperature, which results in the formation of the following isomers: previtamin D_2 (II), tachysterol$_2$ (III), lumisterol$_2$ (IV), and toxisterols.
2. Separation of previtamin D_2, which becomes vitamin D_2 (V) through thermal transformation.

The purpose of this work was to determine the effect of the irradiation wavelength on the percentage of the previtamin formed. The absorption properties were investigated as a function of wavelength for the isomers concerned in accordance with a recently determined reaction scheme representing the different photochemical transformations.

PHOTOCHEMICAL REACTION SCHEME OF ERGOSTEROL AT LOW TEMPERATURE: CORRESPONDING KINETICS

The reaction scheme (7, 8) used in this study is shown in Scheme I. The quanta transformation yield values are indicated and are ex-



pressed in number of molecules per photon absorbed by the starting isomer.

The kinetic reaction of the various isomers (9) was studied. The isomer system investigated corresponds to the interaction graph in Scheme II. This is a true exotropic graph (10) in which: (*a*) summits ①, ②, ③, and ④ represent the classes of ergosterol, previtamin D_2, tachysterol$_2$, and lumisterol$_2$ molecules, respectively; (*b*) the f_{kj} functions represent the probabilities, per unit of time, of transforming the isomer (*j*) into the isomer (*k*); and (*c*) the f_{2e} function represents the probability, per unit of time, of the exit of previtamin D_2 molecule from the system concerned.

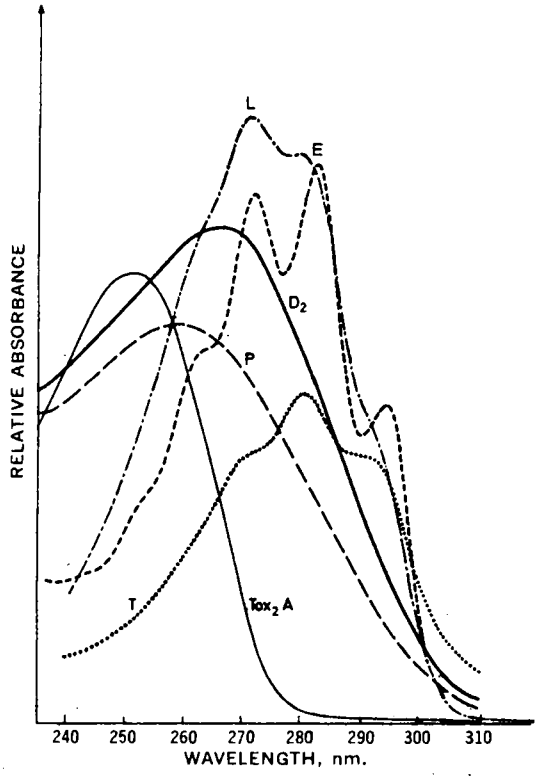
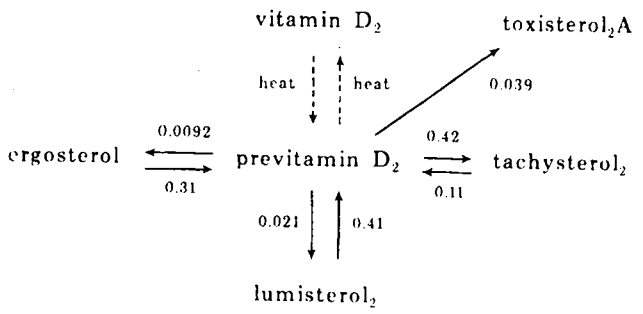


Figure 1—*UV absorption graphs. Key: E, ergosterol; L, lumisterol$_2$; P, previtamin D_2; T, tachysterol$_2$; D, vitamin D_2; and Tox$_2$A, toxisterol$_2$A.*



Scheme I—Reaction scheme of ergosterol photoisomerization at low temperature

Let $N_j(t)$ be the number of molecules in isomer (*j*) at time *t*. The equations governing the change in the system are:

$$\frac{dN_1(t)}{dt} = f_{12}N_2(t) - f_{21}N_1(t) \qquad \text{(Eq. 1)}$$

$$\frac{dN_2(t)}{dt} = f_{21}N_1(t) + f_{23}N_3(t) + f_{24}N_4(t) - (f_{12} + f_{32} + f_{42} + f_{2e})N_2(t) \qquad \text{(Eq. 2)}$$

$$\frac{dN_3(t)}{dt} = f_{32}N_2(t) - f_{23}N_3(t) \qquad \text{(Eq. 3)}$$

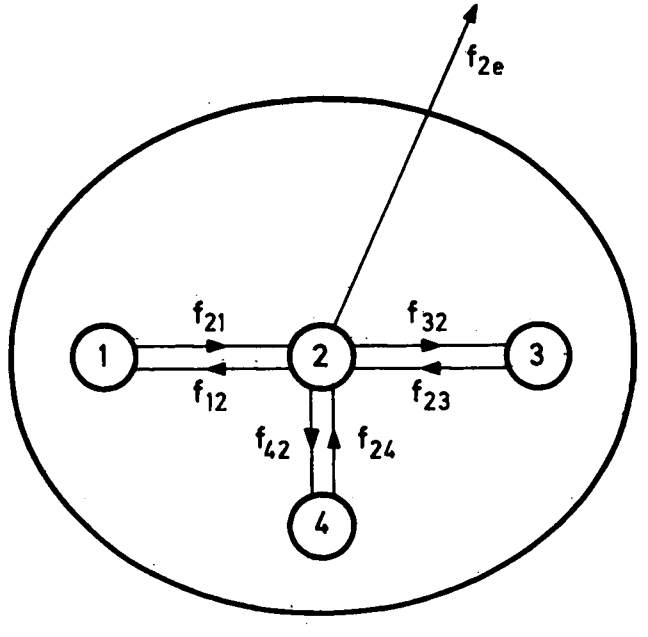
$$\frac{dN_4(t)}{dt} = f_{42}N_2(t) - f_{24}N_4(t) \qquad \text{(Eq. 4)}$$

The expression of the transfer function (in sec.$^{-1}$) is (11):

$$f_{kj} = 0.38 \times 10^{-20}\,\Phi_0 \cdot \varphi_{kj} \cdot \epsilon_j(\lambda) \qquad \text{(Eq. 5)}$$

where Φ_0 is the incident photon flux, expressed in photons/cm.^{2} sec.; φ_{kj} is the quantum transformation yield of the isomer (*j*) into the isomer (*k*), expressed in molecules per absorbed photon; and $\epsilon_j(\lambda)$ is the molar absorptivity of isomer (*j*) at wavelength λ.

The differential system (Eqs. 1–4) is valid for a thin-layer irradiation. It has been shown (11) that this system also holds for thick-



Scheme II—True exotropic graph of the ergosterol photochemical system at low temperature. (A true exotropic graph is a maximum strongly connected component to which are added boundary functions that are all divergent.)

Table I—Molar Absorptivities[a] of the Four Isomers

λ, nm.	Ergosterol	Previtamin D_2	Tachysterol$_2$	Lumisterol$_2$
253.7	4800	8220	9930	4760
265	8305	8250	19050	8020
270	11100	7550	23200	9300
280	11700	5600	28000	8970
296.5	5240	1905	18400	3260
302	1110	1110	9740	1190
313	15.9	342	3580	47.6
316	11.9	222	2860	31.7
320	7.9	127	1985	23.8

[a] From data of Havinga and coworkers (12, 13).

layer irradiation—the medium being kept homogeneous—providing that the time t variable is replaced by the variable:

$$\tau = \int_0^t \frac{\bar{\Phi}_{(t)}}{\Phi_0}\, dt \qquad \text{(Eq. 6)}$$

where $\Phi_{(t)}$ is the mean photon flux in the irradiated medium at time t.

EXPERIMENTAL

Data Used—The differential system (Eqs. 1–4) was solved by computer with the following data.

1. The incident photon flux Φ_0 was assumed to be equal to 10^{18} photons/cm.^{2} sec.
2. The quanta yields φ_{kj} used are indicated in Scheme I.
3. The initial conditions required that only the ergosterol was present at the beginning and the number of molecules $N_1 (t = 0) = 1$.
4. Two series of molar absorptivities, $\epsilon_j(\lambda)$, were used. The first series was taken from the data of Havinga and coworkers (12, 13) and is given in Table I. The second series was obtained from UV spectra determinations as explained in the *Procedures* section. The absorption curves were obtained in relative values (Fig. 1), and it was necessary to normalize them to obtain the molar absorptivities in absolute values. To make this normalization, the values given by Havinga and coworkers at the wavelength of 253.7 nm. were retained and yielded:

$$\underset{\text{absolute value}}{\epsilon_j(\lambda)} = \underset{\text{relative value}}{\epsilon_j(\lambda)} \times \left(\frac{\epsilon_j\,(253.7)_{\text{Havinga}}}{\epsilon_j\,(253.7)_{\text{relative value}}} \right) \qquad \text{(Eq. 7)}$$

Procedures—The various isomers were obtained by irradiating an ergosterol solution in ether (10 g./l.) at 5° with two UV sources:

1. A germicide lamp[1] which, with its 253.7-nm wavelength emission, furthered the production of tachysterol$_2$.
2. A high pressure mercury vapor lamp[2] fitted with two filters. One filter consisted of a quartz tank filled with distilled water to reduce the intensity of IR radiation, and the other was a filter[3] that absorbed the short wavelength UV radiation, thereby producing lumisterol$_2$.

These isomers were separated by column chromatography (14). The column (60 × 1 cm.) was filled with a mixture of silica gel (88%) and alumina (12%). The elution was made with the same solvent (ether) used in the UV irradiation medium of ergosterol. With this method, five isomers could be separated and the eluted fractions corresponding to each isomer were analyzed by UV spectrometry. The UV spectra for these isomers are shown in Fig. 1.

RESULTS

Previtamin D_2 Production—During irradiation, the formation of previtamin D_2 began, rose to a maximum, and then decreased to zero.

[1] Mazda TG-16 Lampe Mazda, Paris 8e, France.
[2] Bausch & Lomb, Rochester, N. Y.
[3] MTO-J280a, MTO filter, Massy, France.

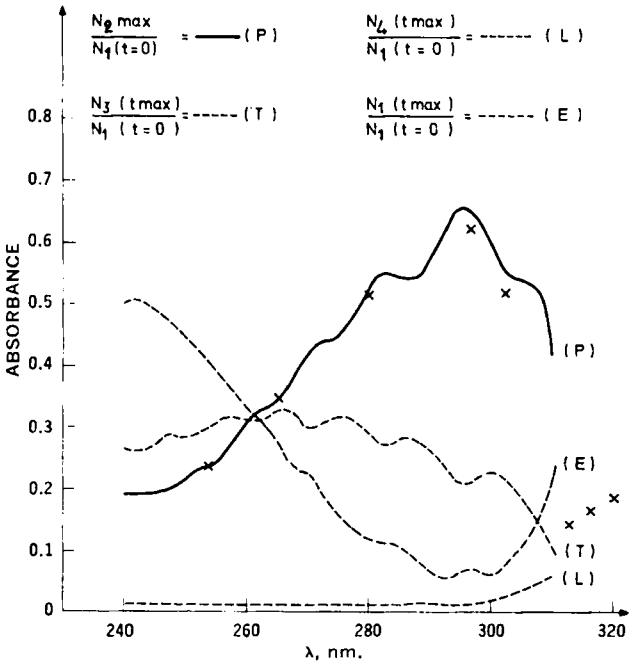


Figure 2—*Effect of wavelength on the maximum previtamin D_2 production and the simultaneous production of isomers. Key: (P), maximum previtamin D_2 production; (E), simultaneous ergosterol production; (T), simultaneous tachysterol$_2$ production; (L), simultaneous lumisterol$_2$ production; and ×, maximum previtamin D_2 production obtained with molecular extinction coefficients (12, 13).*

If the maximum number of previtamin molecules formed is called N_2 max and the number of ergosterol molecules before the irradiation is called $N_1(t = 0)$, the variation in the maximum number N_2 max/$[N_1(t = 0)]$ of previtamin D_2 molecules formed, brought down to an initial ergosterol molecule, according to the wavelength, is given in Fig. 2. The results obtained with the data of Havinga and coworkers (12, 13) (Table I) are indicated by ×'s. This graph (Fig. 2), plotted point by point every 2.5 nm., shows that maximum previtamin D_2 production occurs at the 295-nm. wavelength for which the percentage of ergosterol molecules transformed into previtamin D_2 is:

$$\frac{N_2 \text{ max}}{N_1(t = 0)} = 65\% \qquad \text{(Eq. 8)}$$

This result disagrees with Deribere (15) who recommended UV radiation at approximately 280 nm. corresponding to the maximum absorption of ergosterol and tachysterol$_2$. At this wavelength there is, in fact, a percentage of:

$$\frac{N_2 \text{ max}}{N_1(t = 0)} = 52.5\% \qquad \text{(Eq. 9)}$$

The t_{max} irradiation times required to obtain the maximum previtamin D_2, according to the wavelength, are given in Fig. 3. These times correspond to an incident photon flux $\Phi_0 = 10^{18}$ photons/cm.^{2} sec. For a different Φ_0' flux, the interaction functions become equal to:

$$f_{kj}' = \frac{\Phi_0'}{\Phi_0} f_{kj} \qquad \text{(Eq. 10)}$$

The differential system (Eqs. 1–4) remains the same if:

$$t' = \frac{\Phi_0}{\Phi_0'} t \qquad \text{(Eq. 11)}$$

is submitted. The yield values are therefore identical and only the time required to obtain them varies.

Formation of Other Isomers—When investigating the production of previtamin D_2, it is important to know the evolution of the other photochemical isomers: ergosterol, lumisterol$_2$, and tachysterol$_2$.

The concentrations $N_1(t_{max})$, $N_4(t_{max})$, and $N_3(t_{max})$ of these

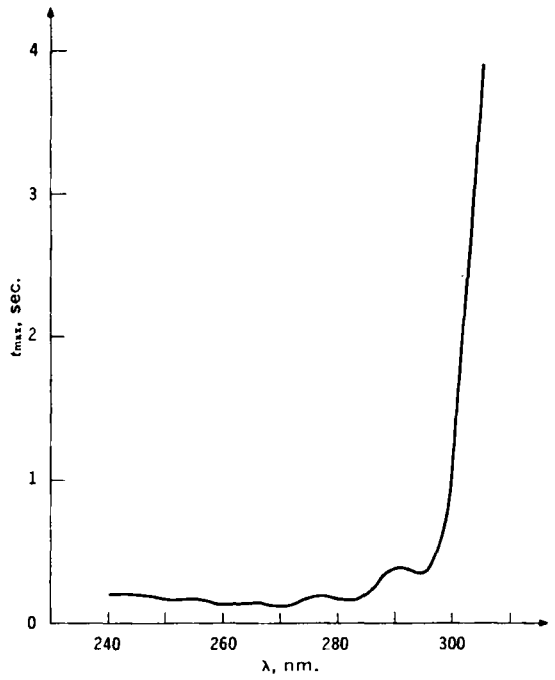


Figure 3—*Time needed to reach maximum previtamin D_2 production, with intensity of irradiation $\theta_0 = 10^{18}$ photons/cm.^{2} sec.*

isomers, when the previtamin has reached its maximum, are shown in Fig. 2. For the maximum of previtamin D_2 at the 295-nm. wavelength, there is a minimum of the three other isomers. The $N_2(t_{max})/N_3(t_{max})$, $N_2(t_{max})/N_1(t_{max})$, and $N_2(t_{max})/N_4(t_{max})$ ratios are, respectively, equal to 3.1, 10.2, and 47.5 at the 295-nm. wavelength and 1.8, 4.4, and 38.8 at the 280-nm. wavelength. This is an additional argument for seeking an irradiation source as rich as possible in UV radiation near to 295 nm.

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Quantitative Determination of Thebaine in Poppy Plants Using High Speed Liquid Chromatography

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Abstract □ A method for the quantitative determination of thebaine in poppy plants using high speed liquid chromatography is described. A nonionic polymeric adsorbent resin column cleanup of the sample is used to eliminate interferences from nonalkaloidal plant components. The separation is effected on a high efficiency adsorption column using *n*-hexane–chloroform–methanol–diethylamine (900:75:25:0.1) as the mobile phase. The column effluent is continuously monitored with a UV photometric detector (254 nm.). The thebaine fraction is well resolved from the isothebaine and orientalidine fractions. With 1-g. poppy samples, 0.01% thebaine is readily determined (detection limit of less than 25 ng.).

Keyphrases □ Poppy plants—analysis of thebaine using high speed liquid chromatography □ *Papaver bracteatum*—analysis of thebaine using high speed liquid chromatography □ Thebaine in poppy plants—analysis, high speed liquid chromatography □ Liquid chromatography, high speed—analysis, thebaine in poppy plants

A sensitive and rapid method was sought for the analysis of thebaine in small samples of poppy plants and parts thereof. The classical procedures of solvent extraction or column chromatography combined with gravimetric or titrimetric estimation of the isolated alkaloids were precluded (1, 2). Extraction methods for isolating the alkaloids from poppy plants and their subsequent separation for biosynthetic studies were previously described (3, 4). Adsorption chromatography on alumina followed by partition chromatography on