# THE PHOTOCHEMICAL INTERCONVERSIONS OF PROVITAMIN D, LUMISTEROL, PREVITAMIN D AND TACHYSTEROL

E. Havinga, R. J. de Kock and M. P. Rappoldt

Laboratory of Organic Chemistry, the University, Leiden; Central Laboratory, N.V. Philips-Duphar, Weesp

**Abstract**- This paper summarizes the results of recent investigations into the photochemical isomerizations in the vitamin D field which were the subject of further research in our laboratories during the last decade. A new scheme is proposed showing the various reactions occurring during irradiation of a provitamin D. The quantum yields of these reactions at 2537 Å were determined. On the basis of these data the effect of the wavelength of the light used on the yields of products is explained. Emission spectra of ergosterol and its photoisomers were measured at 80 K. No phosphorescence was observed. Some aspects of the mechanism of the photochemical cyclizations, ring openings and the *cis/trans* isomerization are discussed.

## (a) *The stoicheiometric reaction pattern*

About five years ago the results of tracer experiments with ^{14}C-labelled provitamin D as well as a theoretical consideration of the quantum yields of the (pre)vitamin D
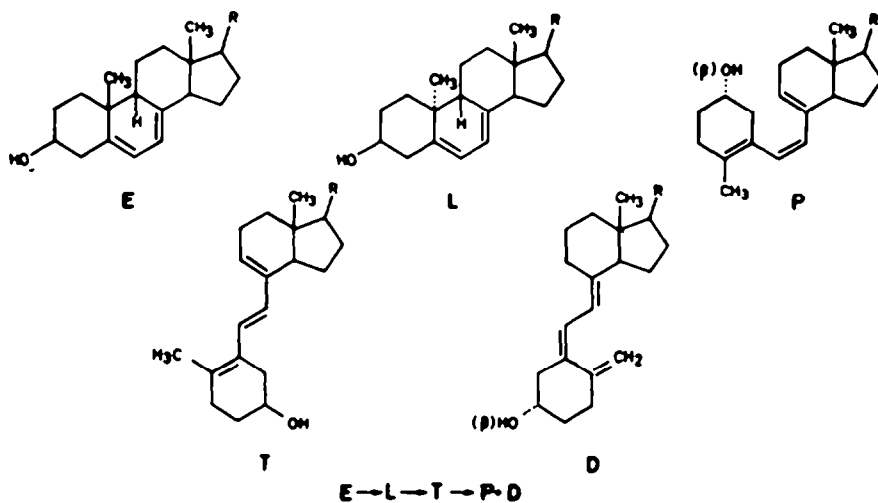


Fig. 1. The Windaus' scheme for the photoisomerization of provitamin D. With R = $C_9H_{17}$:
E = ergosterol; L = lumisterol$_2$; P = pre-ergocalciferol; T = tachysterol$_2$;
D = ergocalciferol.

formation led to the conclusion that the scheme of the photochemical formation of vitamin D from provitamin D via lumisterol and tachysterol, as given by Windaus

[1] E. Havinga, A. L. Koevoet and A. Verloop, *Rec. Trav. Chim.* 74, 1230 (1955); and other papers in this series; For a review see H. H. Inhoffen and K. Irmscher, *Fortschritte der Chem. org. Naturstoffe* 17, 70 (1959).

(Fig. 1) and commonly accepted for twenty years, could no longer be upheld.[1] Even upon incorporation of the discoveries of Velluz concerning the occurrence of pre-vitamin D and the reversibility of at least some of the reactions,[2] the old picture could not give an adequate description of the experimental results. New schemes were suggested but short-cut attempts to prove the correctness of one of these gave no definite results.

It was necessary that a complete analysis of the reaction mixtures obtained with different irradiation times from each of the isomers concerned should be undertaken. In this manner the necessary data for unravelling the pattern of simultaneous and consecutive reactions that occur during prolonged irradiation of, for example, pro-vitamin D solutions could be obtained. This work was started in 1956.

Solutions of each of the four isomers ergosterol (E), lumisterol$_2$ (L), tachysterol$_2$ (T) and pre-ergocalciferol (P) were irradiated with light of suitable wavelength. In each case the composition of the reaction mixture as a function of degree of photo-chemical conversion was determined. The rate of reaction was chosen sufficiently low to avoid the complicating "film effect" resulting from inhomogeneity of the irradiated solution.

E was determined by precipitation with digitonin. The concentration of P was found from the increase in ultra-violet absorption after thermal equilibrium P ⇄ D had been set up. For T, a direct spectrophotometric determination at 313 320 m$\mu$ proved to be adequate. L was not determined directly, except in favourable cases where its concentration was large enough to allow of spectro-photometric determination. In one instance the presence of L was established and its amount evaluated by the isolation of lumisterol$_2$-3,5-dinitrobenzoate. Quantum yields were determined using uranyl oxalate actinometry.

Extrapolation of the percentage composition of the reaction mixture to zero time indicated both qualitatively and quantitatively the primary products formed from each of the compounds by photochemical conversion. For example, we represent the data obtained by irradiation of a pre-ergocalciferol solution with light of wavelength ·310 m$\mu$. According to the results, represented in Figs. 2 and 3, the photochemical conversion of pre-ergocalciferol yields more than 90 per cent tachysterol$_2$ and about 5 per cent ergosterol as primary products.[3] In the same way it was established that tachysterol$_2$ gives 70 per cent pre-ergocalciferol and no detectable amount of ergo-sterol, the balance probably consisting of lumisterol$_2$. Lumisterol$_2$ yielded 100 per cent pre-ergocalciferol.[4] Ergosterol was converted to the extent of 85 per cent into pre-ergocalciferol and 15 per cent into other products ("over-irradiation products"?, not lumisterol$_2$).[5]

Quantum yields were determined for the photoconversions of the four isomers using radiation of $\lambda$ 2537 Å.[5] The results lead to the reactions represented in Fig. 4.

On account of its experimental basis the scheme, Fig. 4, may prove to be fairly definite with respect to its major lines. The chief remaining uncertainties are related to the reactions that may lead to lumisterol. As small amounts of this compound are difficult to determine, it is planned to tackle these problems with the aid of tracer

[1] L. Velluz, G. Amiard and A. Petit, *Bull. Soc. Chim. Fr.* 501 (1949); L. Velluz, G. Amiard and B. Goffinet, *Ibid.* 1341 (1955).
[2] M. P. Rappoldt, *Rec. Trav. Chim.* In press.
[3] M. P. Rappoldt, *Rec. Trav. Chim.* 79, 392 (1960).
[4] M. P. Rappoldt and E. Havinga, *Rec. Trav. Chim.* 79, 369 (1960).
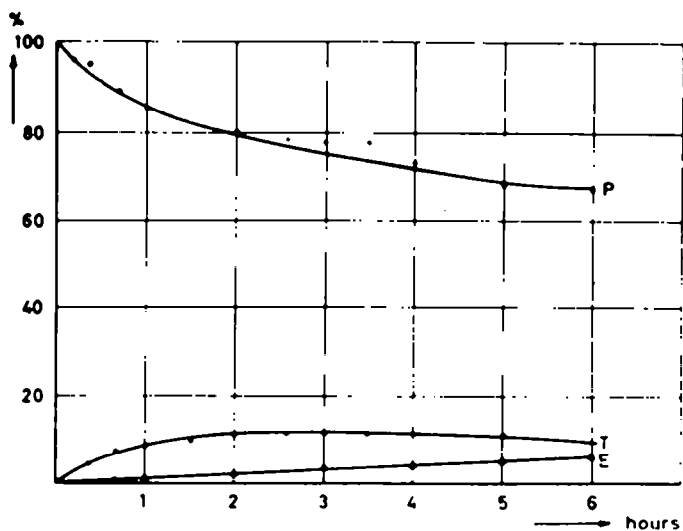[5] M. P. Rappoldt, J. A. Keverling Buisman and E. Havinga, *Rec. Trav. Chim.* 77, 327 (1958).

Fig. 2. Irradiation of a pre-ergocalciferol solution with light of wavelength · 310 mμ. Percentages of P, T and E in the solute plotted against the irradiation time.
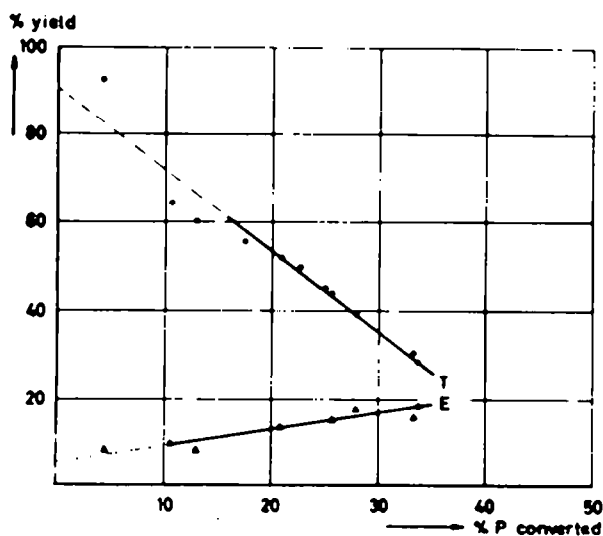


Fig. 3. Irradiation of a pre-ergocalciferol solution with light of wavelength · 310 mμ. Percentages of T and E in the conversion product plotted against percentages of P converted.

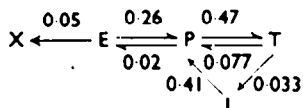

Fig. 4. The essential photochemical reactions of ergosterol and its isomers with quantum yields at 2537 Å.

experiments. In accordance with the view expressed in an earlier paper,[6] the excited states of the various isomers, formed by absorption of light, appear to differ from each other. This is especially noteworthy in the case of the *cis/trans* isomers P and T; on photoactivation, these compounds undergo—besides interconversion—ring closure

to form different products (E and L, respectively). Later in this paper, the mechanistically important difference between excited *cis* and *trans* isomers, also observed in the case of stilbene,[7] is referred to.

### (b) *The photostationary state*

If the scheme represented in Fig. 4 is correct, prolonged irradiation should give rise to a quasi-stationary state. For a given wavelength the composition of this state would

TABLE 1. PERCENTAGES OF P, T AND E IN THE SOLUTE AFTER
PROLONGED IRRADIATION, $\lambda$   2537 Å

| Starting material | % in the solute | | |
|---|---|---|---|
| | T | P | E |
| Ergosterol | 68 | 14 | |
| Tachysterol$_2$ | 72·5 | 15·5 | 1·4 |
| Lumisterol$_2$ | 71 | 14·5 | 1·5 |

TABLE 2. THE CALCULATED COMPOSITION OF THE PHOTO-
STATIONARY STATE AT VARIOUS WAVELENGTHS

| $\lambda$ in m$\mu$ | % E | % P | % T | % L |
|---|---|---|---|---|
| 253·7 | 2 | 19 | 68 | 11 |
| 280 | 1·5 | 47 | 41 | 10 |
| 296·5 | 1·4 | 60 | 27 | 12 |
| 302 | 3·4 | 53 | 26 | 17 |
| 313 | 26 | 19 | 8 | 47 |

be the same, irrespective of the isomer used as starting material. The formation of "over-irradiation" products does not give rise to serious complications; it proceeds slowly enough to assure constant concentration during a considerable period. Ergosterol, tachysterol$_2$ and lumisterol$_2$ were irradiated with light of wavelength 2537 Å until constant concentration was reached; the results of these experiments are summarized in Table 1. The same stationary state is reached by prolonged irradiation of each of the isomers considered.

The data represented in Fig. 4, together with the extinction coefficients of the various isomers at a given wavelength, enable us to predict by calculation the composition of the (quasi) photostationary state at that wavelength. Adhering to the assumption that the quantum yields do not vary appreciably within the wavelength region (250–320 m$\mu$) concerned, the results represented in Table 2 were obtained for the composition of the stationary state mixtures at varying wavelengths. These figures have only semiquantitative value.

The first conclusion to be drawn from the figures is, within the limits of error, that there is agreement with the experimental data obtained with $\lambda$   2537 Å (Table 1). This supports the validity of the scheme, Fig. 4, and the assumption of the constancy of the stoicheiometric pattern with varying wavelength that formed the basis of the evaluation leading to Table 2. It must be pointed out, that whereas quantum yields of

[7] G. Riezebos, Thesis, Leiden (1959).

conversion were measured at 2537 Å, the stoicheiometric yields of formation of the various reaction products were mostly determined at longer wavelengths.

Table 2 clarifies the situation with respect to the seemingly contradictory remarks in the literature about the yields of photo-products. Harris et al.[8] reported that "the photochemical activation of ergosterol by ultra-violet light of 2537-3025 Å is substantially uniform per quantum of energy applied". It has been reported that light of 270–310 m$\mu$ gives a high yield of vitamin D, light of shorter wavelength yields mainly tachysterol and light of longer wavelength causes production of predominantly lumisterol.[9] The data of Table 2 demonstrate clearly the shift in the yields of products to be expected from long-term irradiation experiments with light of varying wavelengths.

(c) *Some investigations into the nature of the photochemical intermediates*

While the knowledge of the paths of reaction and of the stoicheiometry of the photo-isomerizations in the vitamin D field has substantially increased in recent years, the study of the reaction mechanisms is still in the orientation stage. In a previous publication,[10] it was mentioned that there is the possibility that illumination of provitamin D (and other isomers) may give rise to a triplet state that could function as a not too short lived intermediate. Efforts have been made to investigate this possibility. With this in mind, the luminescence spectra were measured. During irradiation of solid solutions of ergosterol or 7-dehydrocholesterol in EPA* at 80°K, no indications of phosphorescence were found. From these experiments, the occurrence of bi-radicals or triplet states seems rather improbable. Solutions of ergosterol in EPA or ether showed no luminescence upon irradiation at room temperature with ultra-violet light of $\lambda$ = 230–300 m$\mu$. As the quantum yield of the photochemical conversion of ergosterol in ethereal solution is about 0·3, 70 per cent of the excited ergosterol molecules seem to lose, under these conditions, their surplus energy by radiationless transitions. Characteristic fluorescence was, however, observed during illumination of solid solutions in EPA of ergosterol, lumisterol and tachysterol and also of pyrocalciferol (9$\alpha$-lumisterol) and isopyrocalciferol (9$\beta$-ergosterol). The shape of the spectra (cf. Fig. 5, 6, 7 and 8), having mirror symmetry with the corresponding ultra-violet absorption spectra, together with other criteria, identify these fluorescence spectra as due to transitions from the lowest level of the first excited singlet state to the lower levels of the ground state. It should be noticed that the *cis* isomer pre-ergocalciferol does not show fluorescence nor phosphorescence.

On the evidence now available, it would appear that the photo-isomerizations of the pro- and pre-vitamins D occur via the excited singlet states of the various molecules. In order to arrive at a more positive conclusion, it is necessary to know both the quantum yields of the photochemical conversions and the fluorescence yields obtained with solid solutions (at $\sim 80°$K).

(d) *Discussion*

It is not difficult to conceive that in the excited singlet state the atoms 9 and 10 of

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* A mixture of 5 parts ether, 5 parts isopentane and 2 parts ethanol.

[8] R. S. Harris, J. W. M. Bunker and L. M. Mosher, *J. Amer. Chem. Soc.* **60**, 2579 (1938).

[9] E. H. Reerink and A. van Wijk, *Strahlentherapie* **40**, 728 (1931); A. Windaus, K. Dithmar and E. Fernholz *Liebigs Ann.* **493**, 259 (1932); Cf. H. Vogel and H. Knobloch, *Chemie und Technik der Vitamine* p. 218. Ferdinand Enke Verlag, Stuttgart (1950).

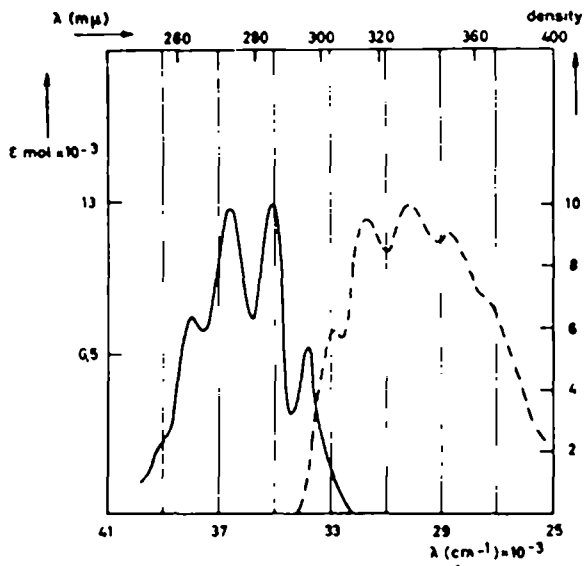[10] E. Havinga, A. Verloop and A. L. Koevoet, *Rec. Trav. Chim.* **75**, 371 (1956).

FIG. 5. — — Ultra-violet absorption spectrum of ergosterol at 80 K. – – – Fluorescence spectrum of ergosterol in EPA at 80°K.
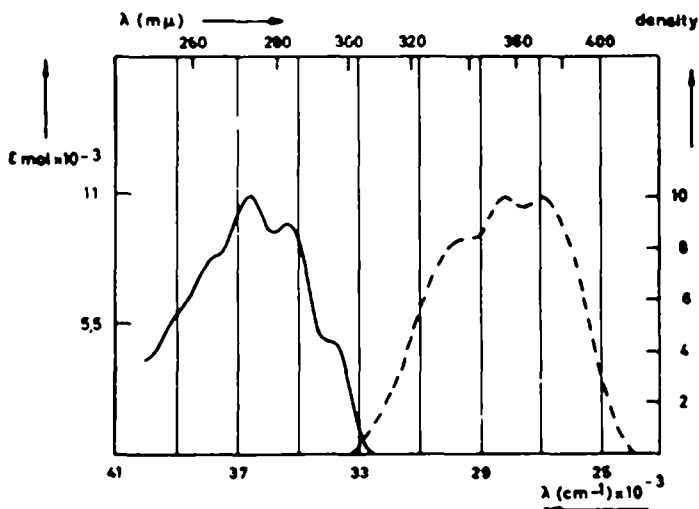


FIG. 6. – — Ultra-violet absorption spectrum of lumisterol₂ at 80°K. – – – – Fluorescence spectrum of lumisterol₂ in EPA at 80°K.

ergosterol or lumisterol₂ can move away from each other smoothly without passing through a highly unfavourable state at any time.    The molecule will thus transform into the open cis-hexatriene derivative (pre-ergocalciferol).

This ring opening constitutes one of the main photochemical reactions occurring with 1,3-cyclohexadiene systems and also occurs with α-phellandrene and 1,3-cyclohexadiene.[11]  The reverse reaction, i.e. the ring closure of excited hexatriene derivatives,

[11] R. J. de Kock, N. G. Minnaard and E. Havinga, Rec. Trav. Chim. 79, 922 (1960); cf. R. J. de Kock, Thesis Leiden (1959); D. H. R. Barton, Helv. Chim. Acta 42, 2604 (1959); R. Srinivasan. Personal communication.
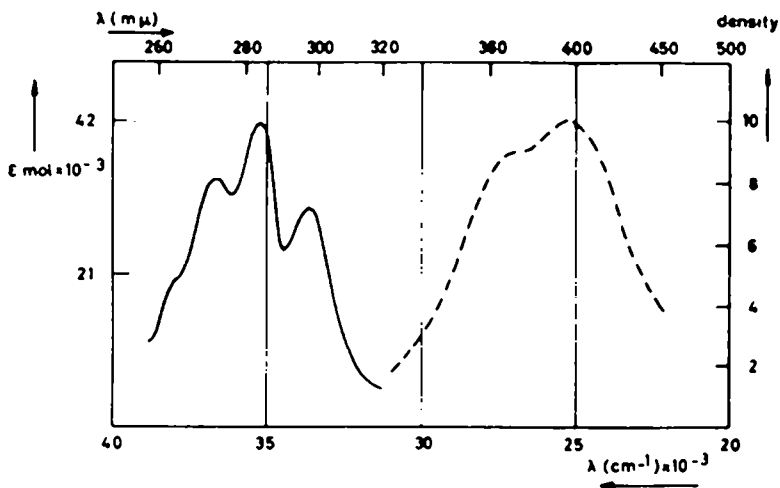
FIG. 7. --- Ultra-violet absorption spectrum of tachysterol₂ at 80°K. ---- Fluorescence spectrum of tachysterol₂ in EPA at 80°K.
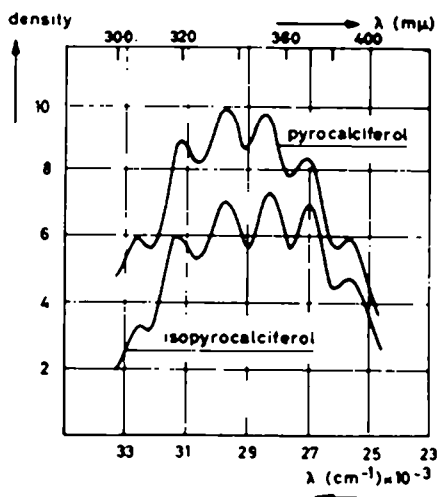


FIG. 8. Fluorescence spectra of pyro- and isopyro-calciferol.

seems to offer no special difficulties from a mechanistic point of view. It remains to be explained, however, why pre-ergocalciferol undergoes photo-induced ring closure into ergosterol whereas tachysterol₂ seems to be transformed preferentially into lumisterol₂. Further stereochemical specificities that offer problems with regard to mechanistic concepts, are found in the result of a special investigation that established the absence of the stereoisomers pyro- and isopyro-calciferol in the reaction mixture obtained by irradiation of an ethereal solution of ergosterol for a long time (see Experimental). Evidently, photochemical ring closure of pre-ergocalciferol and tachysterol] leads exclusively to ergosterol and lumisterol₂ (9, 10 anti-isomers) whereas the thermal ring closure of (pre)vitamin D₂ results specifically in the pyro- and isopyro-calciferols (9, 10 syn-isomers). It is remarkable that lumisterol₂ photo-isomerizes quantitatively into

pre-ergocalciferol while ergosterol forms some 15 per cent of another, still unidentified product. Finally, it may be remarked that pyro- and isopyro-calciferol show ultra-violet absorption and fluorescence spectra that are similar to those of ergosterol and lumisterol. The pyro-compounds undergo photo-isomerization, in which the cyclo-hexadiene B ring is transformed into a bicyclic system.[12] The photochemical *cis/trans* isomerization, pre-ergocalciferol ⇌ tachysterol$_2$, also shows some remarkable aspects.*

Extrapolation of the conclusions drawn from the energies calculated for the various conformations and configurations of the ground states and the first excited states in the case of *trans* and *cis* stilbene,[7] leads one to expect that the sterically hindered pre-ergocalciferol, upon being excited by the uptake of a light quantum, would be able to transform into the energetically more favourable *trans* conformation. This "down hill" movement would need no extra thermal activation. Thermal energy remains necessary for the conversion of the excited *trans* form into the *cis* conformation with probably a few kilocalories higher energy content. It was found, contrary to what happens at room temperature, that the *trans* compound tachysterol$_2$ is not isomerized into pre-ergo-calciferol by irradiation at 80 K, either in solid solution in EPA or in liquid solution in propane. The *cis* →*trans* isomerization, on the other hand, still occurs at 80 K even in solid solution. It thus appears that the pattern of the photo-interconversion of pre-ergocalciferol and tachysterol$_2$ is consistent with results obtained in the study of other *cis/trans* isomers, that of stilbenes in particular. With pre-ergocalciferol and tachy-sterol$_2$ the difference between the photo-excited *cis* and *trans* isomers is particularly evident from the difference of their chemical transformations.


### EXPERIMENTAL

*The photostationary state*

A solution of 1·0 g ergosterol in 2 l. ether was irradiated with ultra-violet light of wavelength 2537 Å. This radiation was obtained from a low-pressure mercury vapour lamp (Philips TUV 15 Watt), the light of which was filtered by 1 cm of gaseous chlorine at 2 atm press. The temp was kept between 10° and 20°. Samples taken at irradiation times of 3, 3½ and 4 hr contained equal amounts of pre-ergocalciferol and tachysterol$_2$. The irradiation technique and the analytical methods have been described elsewhere.[3] Similar experiments were carried out with lumisterol$_2$ and tachysterol$_2$ as starting materials. The values of the concentrations found at the irradiation times mentioned were averaged and expressed as percentages of the solute. The results are summarized in Table 1.

The calculated composition of the (quasi) photostationary state to be expected at various wavelengths started from the following equations:

$$E + P + T + L = 100$$

$$\frac{T}{P} = \frac{0.47}{0.077 - 0.033} \cdot \frac{\epsilon P}{\epsilon T}$$

$$\frac{T}{L} = \frac{0.41}{0.033} \cdot \frac{\epsilon L}{\epsilon T}$$

$$\frac{P}{E} = \frac{0.26 + 0.05}{0.002} \cdot \frac{\epsilon E}{\epsilon P}$$

$\epsilon P$, $\epsilon T$, $\epsilon L$, and $\epsilon E$ are the extinction coefficients. In Table 3 the $E_{1\,cm}^{1\%}$ of P, T, L and E is given at the wavelengths used.

[12] A. Windaus, K. Dimroth and W. Breywisch, *Liebigs Ann.* **543**, 240 (1940); W. G. Dauben and G. J. Fonken, *J. Amer. Chem. Soc.* **81**, 4060 (1959).

*The absence of pyro- and isopyro-calciferol in irradiation mixtures*

A solution of 9 g ergosterol in 4 l. ether was irradiated for 9 hr with the unfiltered light of a high-pressure mercury vapour lamp (Philips HOV 1500 watt). The temp of the solution was kept between 10° and 20°. After removal of the solvent, a resinous product with weak ultra-violet absorption was obtained. $\lambda_{max}$ 243 m$\mu$; $E_{1\,cm}^{1\%}$ 80. Heating of the resin for 1 hr at 200° or at 300° caused a slight increase of the extinction, but no maxima near 270 and 280 m$\mu$ developed. These maxima would be expected if an appreciable amount of photo-pyrocalciferol or photo-isopyrocalciferol had been present in the irradiation product. Therefore it may be concluded that pyro- and isopyro-calciferol are not formed by irradiation of ergosterol or of its photo-isomers. This conclusion is not justified in the hypothetical case that photo-pyro- and isopyro-calciferol are intermediates in the formation of "over-irradiation products".

TABLE 3. SPECIFIC EXTINCTIONS OF ERGOSTEROL AND ITS ISOMERS

| $\lambda$ in m$\mu$ | $E_{1\,cm}^{1\%}$ (ether) | | | |
|---|---|---|---|---|
| | E | P | T | L |
| 253·7 | 121 | 207 | 250 | 120 |
| 280 | 295 | 141 | 705 | 226 |
| 296·5 | 132 | 48 | 463 | 82 |
| 302 | 28 | 28 | 245 | 30 |
| 313 | 0·4 | 8·6 | 90 | 1·2 |

*Measurement of the ultra-violet absorption and luminescence spectra at 80 K*

(a) The ultra-violet absorption spectra were measured in a specially designed cell which was similar to the one described by Potts[13]. The solvent was a mixture of 3 parts isopentane and 1 part methylcyclohexane.

(b) The luminescence was achieved by means of a super-high-pressure mercury vapour lamp (Philips SP 500 watt). The light of this lamp passed successively through a 2 cm layer of a solution of 60 g $NiSO_4.7H_2O$ and 20 g $CoSO_4.7H_2O$ in 100 ml water, a 3 cm layer of gaseous chlorine and a Corning Filter No 9863. This filter combination removed all incident radiation above 300 m$\mu$.

The solutions to be studied were placed in a cylindrical quartz tube, immersed in an unsilvered Dewar vessel filled with liquid air. The solvents used were EPA (a mixture of 5 parts ether, 5 parts isopentane and 2 parts ethanol) and MIP (a mixture of 1 part methyl cyclohexane and 3 parts isopentane).

Phosphorescence was measured according to the method described by van Roggen and Vroom[14] using rotating shutters. In the fluorescence experiments the exciting light fell on the sample perpendicularly to the direction in which the emission was measured.

A quartz lens placed behind the sample condensed the luminescence light on the slit of a Hilger medium quartz spectrograph. The spectra were recorded by means of Ilford HPS plates or, when infra-red emission was investigated, with Kodak IL plates.

[13] W. J. Potts, *J. Chem. Phys.* **21**, 191 (1953).
[14] A. van Roggen and R. A. Vroom, *J. Sci. Instrum.* **32**, 180 (1955).