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The separation of the photochemical isomers of ergosterol by thin-layer chromatography

The separation of the photochemical isomers of ergosterol presents a difficult experimental problem. The investigations of WINDAUS[1], VELLUZ[2] and HAVINGA[3] have shown that UV irradiation of ergosterol at a temperature below 20° gives rise to five isomers: prévitamin D, tachysterol$_2$, lumisterol$_2$, toxisterol$_I$ and toxisterol$_{II}$. If the irradiation temperature is higher than 20° three additional isomers are formed: vitamin D$_2$, suprasterol$_I$ and suprasterol$_{II}$.

Many known chromatographic methods have been used in the study of the ergosterol isomers[4–7] and recently we have separated vitamin D$_2$ and the suprasterols[8].

Experimental

Preparation of solutions. Solutions of ergosterol in ethyl ether (5 g/l) were irradiated in quartz cells by two Mazda TG16 germicide lamps at a wavelength of 2537 Å and at a temperature below 20°. Two ergosterol solutions containing different

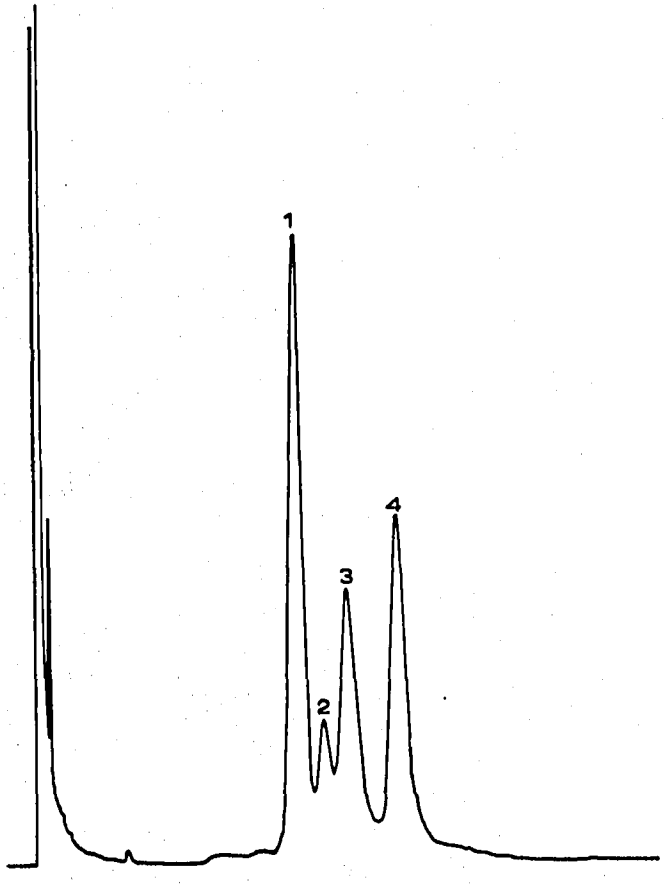

Fig. 1. Gas phase chromatography of the photochemical isomers of ergosterol. 1 = Pyrocalciferol$_2$ + lumisterol$_2$; 2 = isopyrocalciferol$_2$; 3 = ergosterol; 4 = tachysterol$_2$. Column: 3% JXR.

isomer concentrations are obtained after irradiations of 2.30 h and 15 h duration. Since the lumisterol$_2$ concentration is very small for the wavelength used, a 1 g/l solution of lumisterol$_2$ in ethyl ether is added to the two solutions of irradiated ergosterol. The purity of the solution is checked by gas phase chromatography on an apolar stationary phase[11] (Fig. 1).

Preparation of the plates. Three types of plates were used. Type E aluminium oxide plates (Merck ref. 5713), thickness 250 μm. Type T aluminium oxide plates (Merck ref. 1065), thickness 1 mm. Kieselgel plates (Merck ref. 5715), thickness 250 μm.

The plates are activated for 3 h at 115° and a 15 mm wide band is scraped off the layer at each side in order to reduce the edge effect.

Solvent systems. Tests were carried out on some twenty solvents or pairs of solvents.

Four solvent pairs were chosen:

S1, methylene chloride–ethyl acetate (14:4) for the type T Al_2O_3 plates.

S2, carbon tetrachloride–acetone (10:1) for the type T Al_2O_3 plates and the Kieselgel plates.

S3, benzene–acetone (9:1) for the type E Al_2O_3 plates and the Kieselgel plates.

S4, benzene–ethyl acetate (10:2.5) for the type E Al_2O_3 plates.

Chromatography. The solutions were deposited 30 mm from the lower edge of the plates, in the form of spots containing between 10 and 20 μg of product. The chromatographic tanks did not contain any filter paper for saturation. The internal temperature of the development tanks was 20°. This factor plays a part, according to SINGH[12] and according to our own tests.

Detection. A 20% solution of antimony trichloride in chloroform is sprayed on to the plates after development. A 1-min heating with hot air is sufficient to show up the different isomers with their characteristic colours (Table I).

TABLE I

COLOURS OF THE ERGOSTEROL ISOMERS AFTER DETECTION ON THE THIN-LAYER PLATES

Isomer	*Colour*
Ergosterol	Blue
Lumisterol$_2$	Raspberry pink
Tachysterol$_2$	Dark brown
Prévitamin D	Light brown
Vitamin D_2	Light brown
Suprasterol$_I$	
Suprasterol$_{II}$	

Results

Table II shows the R_F values of the different isomers, in various solvent systems, of an ergosterol solution irradiated for 2.30 h with added lumisterol$_2$ and vitamin D_2 in ethyl ether.

The two irradiated solutions, with addition of the lumisterol$_2$ solution, were

TABLE II

R_F VALUES OF ERGOSTEROL STEREOISOMERS

	Type T Al_2O_3 plates		*Type E Al_2O_3 plates*		*Kieselgel plates*	
	S1	*S2*	*S3*	*S4*	*S2*	*S3*
Ergosterol	0.38	0.34	0.17	0.38	0.45	0.49
Vitamin D_2	0.51	0.47	0.27	0.51	0.59	0.60
Tachysterol$_2$	0.63	0.55	0.33	0.58	0.62	0.62
Lumisterol$_2$	0.68	0.62	0.52	0.67	0.70	0.68
Provitamin D	0.74	0.71	0.57	0.72	0.78	0.75

then applied in the form of lines rather than spots on the thin layer. A capillary pipette is used for this.

Kieselgel plates, 250 μm thick, were used. Using the solvent mixture carbon tetrachloride–acetone (10:1) it was possible, after development, to scrape the bands formed off the plates, elute them with ethyl ether and, after filtration on pre-washed Durieux paper, to obtain the UV spectra of the main isomers (see Fig. 2). These spectra are identical to those given by SHAW[13].

The reproducibility of the results was checked by running several tests. Using 2 mm thick layers it was possible to isolate enough product to use gas phase chromatography and obtain the retention times characteristic for each isomer.

Discussion

Using Kieselgel plates the separation of vitamin D_2 from tachysterol$_2$ is difficult

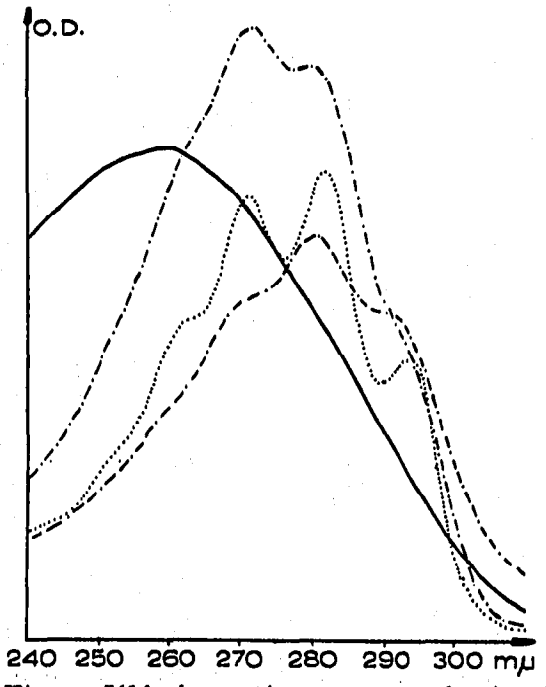

Fig. 2. UV absorption spectra obtained by elution of the bands formed on 250 μm plates. Ergosterol solution irradiated 2.30 h. (———) Prévitamin D; (—·—) lumisterol$_2$; (· · · · · ·) ergosterol; (· — — ·) tachysterol$_2$.

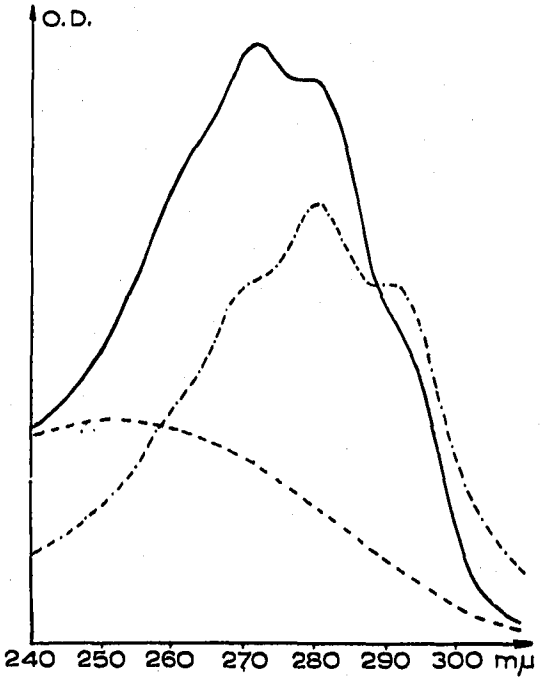

Fig. 3. UV absorption spectra obtained by elution of the bands formed on 250 μm plates. Ergosterol solution irradiated 15 h. (———) Lumisterol$_2$; (· — — ·) tachysterol$_2$; (— — —) toxisterol$_2$A or oxidation product.

whereas lumisterol$_2$ is completely separated from prévitamin D. With type E aluminium oxide, the results are the opposite of those obtained with Kieselgel. The best results for the separation of the five main isomers are in fact obtained with type T aluminium oxide. If the irradiations are carried out at temperatures below 20°, the transition from the prévitamin to vitamin D_2 is excluded and the four isomers formed may be studied on Kieselgel.

The UV spectra obtained on products separated by chromatography on a 250 μm layer, instead of 2 mm, are given for ergosterol solutions irradiated for 2.30 h (Fig. 2). The spectra obtained for the 15 h irradiation times, still with addition of a lumisterol$_2$ solution, are given in Fig. 3.

Two differences can be seen in the spectra of Figs. 2 and 3. Firstly, ergosterol does not appear in Fig. 3 since it now exists only in a very small quantity; this is confirmed by gas phase chromatography. Secondly, the prévitamin spectrum is different, the absorption maximum being displaced towards the short wavelengths.

The method giving the UV spectra by elution of the bands formed on thin 250 μm layers also proved suitable for the study of the photochemical isomers of 7-dehydrocholesterol.

The R_F values for 7-dehydrocholesterol, the prévitamin and tachysterol$_3$ are identical with those of the ergosterol isomers, using Kieselgel layers and the solvent mixtures benzene–acetone (9:1) and carbon tetrachloride–acetone (10:1).

Conclusions

In this work it was possible to use thin-layer chromatography and absorption spectra of four isomers were obtained by elution of the bands formed on 250 μm analytical plates.

This chromatographic technique, which has only occasionally been applied to the study of ergosterol isomers[5,6,9,10], seems to be very well suited for this problem.

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