GAS CHROMATOGRAPHIC STUDY OF STEROIDS IN THE PRODUCTION OF VITAMIN D_2

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The determination of ergosterol in yeast or in the irradiated solutions obtained during the production of vitamin D_2 by precipitation with digitonin is a lengthy operation and in a number of cases does not give accurate results since besides ergosterol digitonin precipitates many other steroids [1]. There are reports in the literature on the use of gas liquid chromatograph (GLC) for the separation of synthetic mixtures of ergosterol, cholesterol, and certain other steroids [2] and also for the analysis of vitamin D. The vitamin appears on the chromatogram as 2 peaks which correspond with pyro- and isopyrocalciferol, which are formed by thermal isomerization on the chromatographic column [3]. The GLC method has been used for the determination of vitamin D in blood and liver [4].

We have studied the possible use of the GLC method for the determination of ergosterol and other steroids in such substances as yeast and fungi and in the irradiation products of ergosterol.

The chromatographic behavior of ergosterol, lumisterol, vitamin D_2, and their acetates, and also the acetates of tachysterol, precalciferol, and ergosterol peroxide were studied in order to identify the substances which could be present in the irradiation products. Furthermore the retention volume of cholesterol and its acetate was determined. The results of the determination of retention times are given in Table 1.

During the analysis of the free alcohols, lumisterol, which is one of the main irradiation products of ergosterol, gives a peak which coincides with the 2nd peak of vitamin D_2. A more complete separation of these substances is obtained by GLC of their acetates (q.v. Table 1). Moreover, the acetate of precalciferol gives rise to 2 peaks which are similar to the peaks of vitamin D_2 acetate, this enables a simultaneous determination of vitamin and previtamin to be carried out. The acetate of tachysterol is evidently decomposed on the column and does not give a peak. The acetate of ergosterol peroxide which is practically always formed under industrial conditions does not interfere with the determination of the main components of the mixture.

In Fig. 1 is represented the chromatogram of the mixture of steroids separated from bakers' yeast. It is evident that the separation of the components on the chromatogram permits their quantitative determination to be carried out. Similar results are obtained by the analysis of the nonsaponifiable fraction of the lipids of Blakeslea trispora.*

EXPERIMENTAL

Substances Studied. Ergosterol, vitamin D_2, precalciferol, tachysterol, and lumisterol were obtained as described earlier [5]; photosensitized oxidation of ergosterol in alcohol gave ergosterol peroxide, mp 170-173° [6]; cholesterol had mp 149°.

Acetylation of the above substances with acetic anhydride in pyridine gave the acetates. The substances were purified by recrystallization, the acetates of precalciferol and tachysterol were purified chromatographically on an alumina column (activity II, eluting with a mixture of petroleum ether and benzene, 2:1). All the acetates were homogeneous on chromatographing on an alumina plate in a petroleum ether–benzene system (2:1 by volume). Given below for the substances are the melting point and R_S relative

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TABLE 1. Retention Time of Steroids and Their Acetates (Carrier Gas Flow 75 ml/min, Temperature 245°C)

Substances	Retention time (in min)	
	free alcohol	acetate
Ergocalciferol	87 and 97	82 and 115
Lumisterol	97	93
Cholesterol	–	106
Ergosterol	122	130
Ergosterol peroxide	–	160
Precalciferol	–	82 and 115
Tachysterol	–	Retained on column

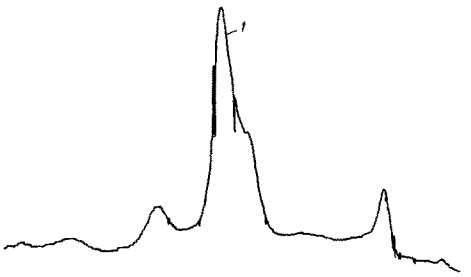

Fig. 1. Chromatogram of the mixture of steroids isolated from bakers' yeast. First peak is ergosterol.

to precalciferol acetate; ergosterol acetate: 167-170°, 0.49; lumisterol acetate: 108-110°, 0.42; vitamin D_2 acetate: 83-86°, 0.89; cholesterol acetate: 114-116°, 0.75; ergosterol peroxide acetate: 195-197°; 0.40; tachysterol acetate: – 0.64; precalciferol acetate: – 1.0.

The nonsaponifiable fraction of the lipids of bakers' yeast and the fungus Blakeslea trispora were obtained in the usual manner [1].

Chromatography. A "Pye" argon chromatograph was used, stationary phase 10% SE-30 on Celite-545 (100-120 mesh), length of glass column (borosilicate glass) 1.2 m, carrier gas flow 75 ml/min, pressure 1.4 atm. The substance was applied to the column from a micro pipette as a 5-20% solution in benzene.

CONCLUSIONS

The feasibility of determining steroids in yeast, fungi, and the products of irradiation of ergosterol by a gas liquid chromatographic method is demonstrated. The optimum conditions found for the separation of the main substances formed during the irradiation of ergosterol and their retention volumes are given.

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