CLXXVII. THE BIOLOGICAL INERTNESS OF IRRADIATED MYCOSTEROLS OTHER THAN ERGOSTEROL.

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(Received October 29th, 1928.)

THE evidence so far available indicates that of the three groups of "protoplasmic" sterols, the zoo-, phyto- and myco-sterols, only one member of the last group, *i.e.* ergosterol, can be antirachitically activated by irradiation. Besides ergosterol there is present in ergot a second mycosterol, to which Tanret [1908] has given the name fungisterol. In view of the apparently unique property of ergosterol, it became essential to study the behaviour of fungisterol in this direction. Our previous attempt to settle this question gave indecisive results [Rosenheim and Webster, 1927], partly owing to the difficulty of separating the substance from crude ergosterol, containing only about 10 % fungisterol, but mainly owing to the small total amount of sterols obtainable from ergot. We have since been able, through the kindness of Mr F. H. Carr, of the British Drug Houses, Ltd., to work up the mother-liquors resulting from the recrystallisation of large amounts of ergosterol from ergot. The biological examination of the sterol isolated by us led to the conclusion that the slight antirachitic activity acquired by it on irradiation was due to contamination with traces of ergosterol. This result is in agreement with the spectroscopic analysis which indicated the presence of less than 5 % of ergosterol in this specimen of fungisterol. Another specimen of an apparently identical sterol, prepared from ergot, was kindly put at our disposal by Messrs Burroughs, Wellcome and Co. and gave identical results. The physical constants of the two sterols agree with each other, but not with those of fungisterol, as described by Tanret. Apparently the latter product was still a mixture of several sterols, of which two further constituents were isolated and are being investigated chemically in Messrs Burroughs, Wellcome and Co.'s laboratories. Their biological examination, which we carried out, also gave negative results.

These facts lend further confirmation to the view expressed previously [Rosenheim and Webster, 1928] that ergosterol is the only substance which can be converted into vitamin D by irradiation.

EXPERIMENTAL.

Chemical. The residue of the mother-liquors after removal of crystallisable ergosterol, representing the yield from about 250 kg. ergot, was a yellowish powder (5.5 g.) which melted at 107–110° and had $[\alpha]_{5461}^{19^\circ} - 63^\circ$. The product was acetylated and the acetate recrystallised from acetic anhydride. The apparently uniform product (M.P. 117–118°, $[\alpha]_{5461}^{19^\circ} - 50^\circ$) was fractionated from methyl alcohol at various temperatures. The fractions obtained at 36° and 18°, containing presumably the bulk of the less soluble ergosterol, were discarded, and the colourless crystals deposited at – 5° from the filtrates were again recrystallised from methyl alcohol. The final product weighed 0.195 g., $[\alpha]_{5461}^{20^\circ} - 43^\circ$ (*c* in chloroform = 1), M.P. 117–118°, solidified at 102° (a mixed M.P. with cholesterol acetate, 107–108°).

The sterol isolated in Messrs Burroughs, Wellcome and Co.'s experimental laboratories had a constant rotation of $[\alpha]_{5461} - 34^\circ$ (*c* in chloroform = 1.5). It had no definite melting point, but the acetate melted at 117–120°. A mixed M.P. of this and our own preparation showed no depression. The two specimens appear to be identical, but both were still contaminated with traces of ergosterol, as shown by a specific colour reaction (to be published shortly) and by their spectroscopic examination.

Tanret's fungisterol had M.P. 144° and $[\alpha]_D - 22^\circ$; the acetate M.P. 158.5° and $[\alpha]_D - 15.9^\circ$. There is no criterion by which Tanret's success in freeing his product from ergosterol may be judged, and, although its physical constants differ from those of the presumably purer preparations above described, it seems advisable to retain the name "fungisterol" for the sterol which constitutes the largest amount in the sterol mixture accompanying ergosterol in ergot. It is interesting to note that zymosterol which occurs together with ergosterol in yeast [Smedley-MacLean, 1928] is evidently not identical with fungisterol.

The following information was kindly supplied to us by Messrs Burroughs, Wellcome and Co. with regard to the two other sterols isolated by them from ergot.

Sterol (1) had $[\alpha]_{5461} + 60^\circ$ (*c* in chloroform = 0.9). It melted at 172° and gave a benzoate M.P. 173° (mixed M.P. of sterol with its benzoate 150°) and $[\alpha]_{5461} + 72^\circ$ (*c* in chloroform = 0.8).

Sterol (2). A substance giving the colour reaction of a sterol. It had M.P. ca. 260°, $[\alpha]_{5461} - 90^\circ$ (*c* in alcohol = 0.2).

Spectroscopic examination. An alcoholic solution of fungisterol (1 : 20,000) showed no absorption when compared on the same plate with a solution of ergosterol of the same strength, in which the characteristic bands were conspicuous. An approximate match with the latter was obtained only when the strength of the fungisterol solution was increased to 1 : 1000. The ergosterol content of this specimen was therefore probably less than 5 %, and that of the second specimen was found to be of the same order.

Sterol (1) showed no absorption even in the highest strength tested (1 : 1000), and gave an entirely negative test with the above-mentioned specific colour reaction for ergosterol. It may therefore be considered as practically free from ergosterol.

Sterol (2). Spectroscopic examination indicated about 8 % ergosterol as impurity. The colour reaction for ergosterol was strongly positive.

Biological examination. This was carried out by our usual technique. The sterols were irradiated in alcoholic solution and the modified Sherman-Pappenheimer diet was used in the feeding experiments. Continued experience with this technique in a large number of tests on hundreds of rats has shown us that the minimum effective dose of irradiated ergosterol lies between 1/10,000 and 1/20,000 mg. *per diem*. On the assumption that the ergosterol percentage of the specimens of fungisterol, as shown by spectroscopic analysis, was of the order of 5 %, the doses were so adjusted that the minimum dose of ergosterol, which would be effective after irradiation, was present in the largest dose (1/500 mg.) of fungisterol, whilst the smallest dose (1/5000 mg.) of fungisterol, although still double the amount of the effective dose of ergosterol, would only contain about 1/100,000 mg. of the latter. The results were decisive and in general agreement with the spectroscopic test, showing that doses of 1/5000 and 1/2500 mg. of irradiated fungisterol did not prevent severe rickets, whilst the largest dose induced healing, though not as complete as in the control experiments receiving 1/20,000 mg. of irradiated ergosterol. This result justifies the conclusion that the effect produced by these specimens of irradiated fungisterol is due to the ergosterol still contained in them as impurity. A similar series of experiments was found to be conclusive in demonstrating the biological inactivity of irradiated zymosterol, one of the mycosterols of yeast [Hume, Smith and Smedley-MacLean, 1928].

Sterol (2) gave practically identical results, whilst sterol (1) proved to be entirely inactive, even in the largest doses administered, thus again confirming the evidence of the spectroscope and the colour reaction.

SUMMARY.

A mycosterol, accompanying ergosterol in ergot, was isolated, for which Tanret's name "fungisterol" is retained. This, as well as two other sterols from the same source, was found to be biologically inactive after irradiation. The results confirm the evidence previously adduced for the unique function of ergosterol as the mother-substance of vitamin D.

REFERENCES.

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