35. On Ergosterin, Isolated from a Japanese Edible Mushroom, Cortinellus Shiitake.

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The author has recently isolated a kind of sterin from a Japanese edible mushroom Cortinellus Shiitake and proved it to be identical, both in its chemical properties and in its physiological significance, with the ergosterin first isolated from ergot by Tanret.1

A brief summary of this investigation is given in the following lines:

1. Isolation of Ergosterin:— 15 kg. of commercial air dried sample containing 13.8% moisture, were finely pulverized and extracted with ether several times. The residue therewith was then extracted with ethyl alcohol. The alcoholic extract was evaporated to a small volume, diluted with water and repeatedly shaken with ether. The ethereal solution thus obtained was united with the former ether extract and after dehydration with sodium sulphate, the ether was evaporated off. The resulted oily syrup was now boiled with 20% alcoholic potash for one hour for the purpose of saponifying the fatty matter present. After cooling, the saponified liquid was diluted with water and shaken with ether, the unsaponifiable substances being thus taken up in ether, while the soap remained in the aqueous solution. The ethereal solution gave on evaporation, a brown residue, consisting mostly of needle crystals, which were recrystallized from acetone and ethylalcohol. The yield 35 gm. m.p. 158-159°, [α]D = -128° in chloroform solution.

A few centigram of this crystals dissolved in pure chloroform gave following reactions:

(1) By adding a few drops of acetic anhydride and conc. H2SO4, a red colour was produced which became blue and finally bluish-green. (Liebermann and Burchard's reaction). (2) By shaking after the addition of conc. H2SO4, the acid layer became blood red while the chloroform layer remained colourless. (3) By adding a few drops of 30% chloroform solution of SbCl3, purple red colouration was first produced

1) Tanret: Compt. rend de l'Acad des Sci. 147, 75 (1908)
which turned sky blue on standing. (4) By the addition of dry acid clay, a deep blue colour developed gradually.

Moreover the alcoholic solution of this crystals was precipitated by digitonin.

The purified sample was dried at 100° and analysed with follow-
ing results:

<table>
<thead>
<tr>
<th>Subst. gm.</th>
<th>CO₂ gm.</th>
<th>H₂O gm.</th>
<th>C %</th>
<th>H %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1000</td>
<td>0.3101</td>
<td>0.0989</td>
<td>84.57</td>
<td>10.99</td>
</tr>
<tr>
<td>0.0793</td>
<td>0.2453</td>
<td>0.0773</td>
<td>84.35</td>
<td>10.83</td>
</tr>
<tr>
<td>Calculated for C₂₇H₄₂O₈</td>
<td>84.75</td>
<td>11.07</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

An acetyl derivative, melting at 169-170°, was also obtained.

From the above results we see that the sterin obtained by the author is quite identical with Tanret’s ergosterin.

Further, the absorption spectra of this substance, observed by ¹/₁₀₀₀₀ mol. solution in ethylalcohol (40 sec. exposure to H vapour arc lamp) exactly agreed with those of ergosterin prepared by the author from ergot; i.e. the absorption band was visible at 2920, 2820, 2700, 2618 and 2500 μ. μ respectively. (Plate I).

2. Irradiation of Ergosterin with ultraviolet rays:— For this purpose, the alcoholic solution of the purified crystals was sealed in a quartz tube and exposed to the ultraviolet rays (Silectra standard mercury vapor lamp. 90 Volts, 10 Amperes) for ½ hour, at a distance of 30 cm. from the light source. The solution was then evaporated and the light yellow residue was dried in vacuum descicator, dissolved in a little linseed oil and then tested for its antirachitic potency by feeding experiment.

3. Feeding experiment:— The albino rats weighing 35-40 gms. were previously fed on Steenbock’s ricket diet (No. 2965), consisting of 20% wheat gluten, 76% yellow corn meal, 3% CaCO₃ and 1% NaCl, and after 28 days, when the animals suffered from rickets (Plate II (1)), they were divided into three groups and treated as follows:—

Group (1) Three rats served as control and so they were kept on the same diet as above for three weeks more: All of them suffered from severe rickets.

Group (2) Four rats were fed on the above diet, supplemented with 0.5 mg. of the irradiated ergosterin per rat daily. After three weeks, three of them recovered completely. (Plate II (2)), while the fourth one recovered only partially.

Group (3) Five rats were fed for three weeks on the same diet, supplemented with 1 mg. irradiated ergosterin per rat daily. The healing was much more marked than that of the group (2).
From these observations the potency of the author's ergosterin seems to be somewhat weaker than those of A. Windaus,¹ A. Hess,² O. Rosenheim,³ F. Holtz⁴ and others,⁵ but this may be due to a certain different method in irradiation.

Further, the Shiitake powder was directly irradiated and tested on rats just in the same way as above. It was confirmed that 0.2 gm. of it was nearly equivalent to 0.5 mg. of irradiated ergosterin in its antirachitic potency. The author has also isolated ergosterin from the spores of *Aspergillus Oryzae* and made parallel experiment with the preparation obtained above. Also the spore itself, when irradiated directly, exhibited the same physiological function.

3) O. Rosenheim & A. Webster: Biochem. J. 21, 389, (1927)
4) F. Holtz: Klin. Wochensch. 6, 535, (1927)
5) A. Adam: "", "", 6, 1286, (1927)

Plate II.

(1) Fed on Steenbock's ricket diet No. 2965 for 28 days.
(2) Fed on the same ricket diet, supplemented with irradiated Shiitake Ergosterin dissolved in linseed oil, 0.5 mg. daily for three weeks.
Plate I.

Ultraspectroscopic plate
(1) Ergosterin from Shiitake.
(2) Ergosterin from Ergot.
(3) Ergosterin from Spores of Aspergillus Oryzae.
Comparison: Cu Arc Spectrum.