THE FATTY ACIDS OF TRICHOPHYTON RUBRUM

by

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(with 2 figs.)

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Recent advances in gas and thin layer chromatographic techniques permit one to perform lipid analyses with great ease and rapidity, and detailed investigations of the fatty acids of bacteria (O'Leary, 1962; Scheuerbrandt & Bloch, 1962), protozoa (Erwin & Bloch, 1963; Korn, 1964) and saprophytic fungi (Bernhard, Abisch & Wagner, 1957; Coots, 1962; Van Etten & Gottlieb, 1964) have appeared. However, there are few reports dealing with the identification of lipids of medically important groups of organisms, such as the dermatophytic fungi. Equally lacking is knowledge of the fatty acid content during various stages of the growth cycle of the organisms, including the dermatophytes.

Prior to studies on the mechanism of fatty acid synthesis in the dermatophyte Trichophyton rubrum, it was essential that some information be available concerning the kinds and quantities of fatty acids which may be found at different growth periods.

This paper, therefore, reports the fatty acid content of Trichophyton rubrum during the logarithmic, early and late stationary phases of growth.

MATERIALS AND METHODS

Growth and Harvesting

Trichophyton rubrum (CDC strain) was grown in a liquid synthetic medium containing glucose, asparagine, tryptose, yeast extract, and trace metals (Zussman, Vicher & Lyon, 1961a). Five 2-liter Erlenmeyer flasks, each containing 1 liter of medium, were inoculated with 1 ml of T. rubrum homogenate (Vicher, Lyon &

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WHITE, 1959), incubated at room temperature (26±2°C) and agitated continuously on a rotary shaker at about 150 rpm.

Two cultures were harvested after 2 weeks of incubation, one consisting of white unpigmented balls, and the other of red-pigmented balls. A third culture was harvested after 3 weeks of growth, and two others after 4 weeks of growth; all these cultures were unpigmented. The mycelial balls (ZUSSMAN, VICHER & LYON, 1961b) were collected by filtration through 4 layers of cheese cloth, washed 3 times with phosphate buffer, pH 7, weighed and dried in an oven at 60°C overnight. The weight of the dried sample was determined and the material was ground to a fine powder in a poppy seed grinder. The yields obtained are listed in the table below.

<table>
<thead>
<tr>
<th>Age (weeks)</th>
<th>Dry Weight (g/flask)</th>
<th>Total Fatty Acid (mg)</th>
<th>% of Dry Mycelium</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 (Pigmented)</td>
<td>5.4</td>
<td>135.0</td>
<td>2.5</td>
</tr>
<tr>
<td>2 (Unpigmented)</td>
<td>12.8</td>
<td>469.3</td>
<td>3.6</td>
</tr>
<tr>
<td>3 (Unpigmented)</td>
<td>18.5</td>
<td>490.6</td>
<td>2.7</td>
</tr>
<tr>
<td>4 (Unpigmented)</td>
<td>14.9</td>
<td>1241.6</td>
<td>8.3</td>
</tr>
</tbody>
</table>

Extraction and methylation of the fatty acids

The fatty acids were extracted twice under nitrogen with dimethoxymethane (methylal)-methanol mixture1) (4:1, v/v, containing 0.1 mg of dl-alpha-tocopherol (General Biochemicals) per ml of mixture.

The extracts were pooled, concentrated, dissolved in petroleum ether and dried over anhydrous Na₂SO₄. The extract was filtered, evaporated to a small volume (1—2 ml) and transferred to a 25 ml round bottom methanolysis flask fitted with a reflux condenser equipped with a U-shaped drying tube (Bellco). The methyl esters of the fatty acids, prepared and separated from the reaction mixture by the method of STOFFEL, CHU & AHNRENS (1959), were collected in a tared vial and the yields were determined. For storage, the vial was filled with petroleum ether containing 0.1 mg/ml dl-alpha-tocopherol, flushed with nitrogen, stoppered and kept in the freezer. The petroleum ether was evaporated just prior to analysis by gas chromatography.

1) Unless otherwise stated, all chemicals were purchased from the Fisher Scientific Company, Chicago, Illinois.