Triparanol Inhibition of Sterol Biosynthesis in Chlorella ellipsoidea¹,²

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Abstract
The sterol composition of C. ellipsoidea was markedly changed when this alga was grown in the presence of 1 µg/g triparanol. Triparanol appears to inhibit the removal of 14α-methyl group, the second alkylation at C-24, Δ⁵-reductase, and Δ⁷ → Δ⁵-isomerase. The effect of triparanol in Chlorella is much more diversified than the specific effect originally assigned to it in animals.

Materials and Methods
Chlorella ellipsoidea was grown in a glucose-inorganic salts medium in carboys as described elsewhere (15). Triparanol-treated cells were grown under identical conditions, except for the addition of 1 µg/g triparanol to the culture medium. This level of triparanol gave a 50% inhibition of growth. Cells obtained were freeze-dried, and extracted with CHCl₃-CH₂OH (2:1, v/v). After saponification, sterols were precipitated from the nonsaponifiable fraction with digitonin and sterols were recovered by the method of Issidorides et al. (10). Total free sterols were separated into dimethyl-, methyl-, and desmethyl sterols on a Woelm grade II neutral alumina column (8). After acetylation, sterol acetates were further separated by AgNO₃-silica gel column chromatography (9). The separations on the alumina and the silica gel columns were monitored by gas-liquid chromatography on an SE-30 column (14). Gas-liquid chromatography analyses were made on Glowall Chromalab, Model A-110 and Model A-310 gas chromatographs, with operating conditions as described previously (14). Sterols were identified using gas-liquid chromatography data from four columns and by gas chromatography-mass spectroscopy as previously described by Doyle et al. (8). Mass spectroscopy was recorded on an LKB Model 9000 gas chromatograph-mass spectrometer. The compounds were introduced into the ion chamber through a 0.75% SE-30 column.

Results and Discussion
A large qualitative and quantitative difference between the control and inhibited cultures were revealed when their sterol mixtures were separated by AgNO₃-silica gel column chromatography and analyzed on an SE-30 column. Relative retention times of the peaks obtained from control cultures indicated that the major sterols are identical to those described previously (7,15).

Sterols observed in inhibited cultures are listed in Table I. They were identified by their movement on the Al₂O₃ column and AgNO₃-silica gel column, their relative retention times on four gas-liquid chromatography columns (14) and by gas chromatography-mass spectroscopy. The chromatographic characteristics of all these sterols were identical to those of authentic compounds. A quantitative comparison of sterols from control versus inhibited cultures was performed by gas chromatography. There was a reduction in total sterol concentration of approximately 68% in the inhibited culture. This indicates that triparanol blocks certain steps of sterol biosynthesis before the cyclization of squalene, as previously postulated in rats (9) and in Ochromonas (1).

Production of the three major sterols of the control culture was reduced from near 100% of the total sterol to 23% of the total sterol inhibited cultures. Porriferasterol was diminished by the greatest amount (89%). Significant accumulations of ergosta-5,7,14-dien-3β-ol, Δ⁷-chondrillasterolen, 5α-(24S-stigmasta-8,14-dien-3β-ol, obtusifoliol, 5α-ergost-7-en-3β-ol, and 5α-ergosta-8,14-dien-3β-ol were found. These sterols were not detected in control cultures. Ten of the sterols identified in inhibited cultures were also present in the AY-9944-treated cultures of the same alga (7). The effect of triparanol in C. ellipsoidea seems to be similar in some respects to that postulated for C. emersonii (8). The accumulation of 14α-methyl sterols (12% of total sterol) suggests that triparanol also prevents the removal of the 14α-methyl group in C. ellipsoidea.
Table I. A Quantitative Comparison of Sterols from Control and Triparanol-treated Cultures of Chlorella ellipsoidea

<table>
<thead>
<tr>
<th>Sterols</th>
<th>Control</th>
<th>Triparanol-Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% sample</td>
<td>µg/µl dry wt</td>
</tr>
<tr>
<td>4α, 14α-Dimethyl-5α-(24S)-stigmast-8-en-3β-ol</td>
<td>— 1</td>
<td>1.2</td>
</tr>
<tr>
<td>Cycloartenol</td>
<td>0.1</td>
<td>1</td>
</tr>
<tr>
<td>24-Methylene cycloartenol</td>
<td>0.7</td>
<td>7</td>
</tr>
<tr>
<td>Obtusifoliol</td>
<td>7.5</td>
<td>76</td>
</tr>
<tr>
<td>24-Dihydroobtusifoli</td>
<td>1.3</td>
<td>13</td>
</tr>
<tr>
<td>14α-Methyl-5α-(24S)-stigmast-8-en-3β-ol</td>
<td>0.1</td>
<td>1</td>
</tr>
<tr>
<td>14α-Methyl-5α-ergost-8-en-3β-ol</td>
<td>0.9</td>
<td>9</td>
</tr>
<tr>
<td>5α-Ergosta-8, 14-dien-3β-ol</td>
<td>7.8</td>
<td>79</td>
</tr>
<tr>
<td>5α-Ergost-8(9)-en-3β-ol</td>
<td>2.7</td>
<td>28</td>
</tr>
<tr>
<td>5α-Ergosta-7(8)-en-3β-ol</td>
<td>7.1</td>
<td>72</td>
</tr>
<tr>
<td>Ergosta-5, 7-dien-3β-ol</td>
<td>29.3</td>
<td>297</td>
</tr>
<tr>
<td>5α-(24S)-Stigmasta-8, 14-dien-3β-ol</td>
<td>7.5</td>
<td>76</td>
</tr>
<tr>
<td>5α-(24S)-Stigmasta-8(9)-en-3β-ol</td>
<td>1.7</td>
<td>17</td>
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<tr>
<td>24R-Stigmasta-5, 7, 22-trien-3β-ol</td>
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<tr>
<td>Δ1-Chondrillastanol</td>
<td>6.8</td>
<td>69</td>
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<tr>
<td>Ergost-5-en-3β-ol</td>
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<td>1062</td>
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<tr>
<td>Poriferasterol</td>
<td>62.3</td>
<td>1993</td>
</tr>
<tr>
<td>Clionasterol</td>
<td>4.5</td>
<td>144</td>
</tr>
<tr>
<td>Total</td>
<td>100.0</td>
<td>3199</td>
</tr>
</tbody>
</table>

1 No sterols detected where data are not given.

The ratio of sterols with 10-carbon side chains to those with 9-carbon side chains is 2:1 in the control but 2:5 in inhibited cultures. This indicates that triparanol is inhibiting alkylation that leads to sterols with 10-carbon side chains. The accumulation of 24-methylene cycloartenol and obtusifoliol provide further evidence for the postulated inhibition, since 24-methylene sterols are usually considered as precursors for the second alkylation (3). However, this study did not detect 24-methylene polinastanol or 14α-methyl-5α-ergosta-8(9), 24(28)-dien-3β-ol which accumulated to a great extent in triparanol-treated C. emersonii (8). The increase of Δ5 sterols from unnoticeable amounts in the control (less than 0.1% of total sterol) to 31% of the total sterol in inhibited cultures, reveals the inhibitory action of triparanol on Δ5-reductase (6). This was not found in C. emersonii, since that organism apparently does not contain a Δ5-reductase (13). There is also an increase in ratio of Δ5/Δ7 sterols in the inhibited cultures. Similar results were obtained with triparanol-treated C. emersonii and with AY-9944-treated cultures of C. ellipsoidea in this laboratory (7, 8). This effect was reported earlier with sterols in rat skin (4). These data suggest an inhibitory effect of triparanol on Δ5 → Δ7-isomerase.

From preceding observations, triparanol seems to have a much more diversified effect than has been anticipated. Some of these effects could possibly account for its adverse effects on human beings as a hypocholesterolemic drug (11, 12).

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**LITERATURE CITED**