Inhibition of Chlorination in *Streptomyces aureofaciens* by Nitriles and Related Compounds

JOSEPH J. GOODMAN and MARY MATRISHIN

Lederle Laboratories, Division of American Cyanamid Co., Pearl River, New York 19065

Received for publication 10 August 1972

A number of nitriles and cyano compounds inhibited chlorination by *Streptomyces aureofaciens*. In most cases, this inhibition was enhanced by bromide. Methylene blue and p-amino-propiophenone reversed the inhibition to some extent.

The antithyroid agent 1,1,3-tricyano-2-amino-1-propene (TCAP) prevents organification of iodine (5). It also serves as a halogen acceptor in the reaction catalyzed by the chloroperoxidase of *Caldariomyces fumago* (7). We therefore tested it as a chlorination inhibitor in our previously described *Streptomyces aureofaciens* BC-41 system where inhibition leads to the production of a high concentration of tetracycline rather than chlorotetracycline (2). Since bromide ion, itself a chlorination inhibitor (4), has been shown to potentiate the effect of some organic (3; M. Arishima and Y. Sekizawa, U.S. Patent 2,949,407, 1960) and inorganic inhibitors (6), these experiments were run without and with 200 μg of bromide ion/ml (as KBr) included in the medium.

TCAP was indeed found to be active, and this led to the testing of a total of 36 other nitriles and cyano compounds, of which half were found to be active. These are shown in Table 1. Included in Table 1 is the toxicity index (2), which represents the ratio of total potency in the control to total potency with inhibitor. Total potencies in the controls ranged from 5,500 to 8,600 μg/ml over the course of these experiments.

As can be seen in Table 1, all of the compounds except benzonitrile, phthalonitrile, and dicyanobenzene were potentiated by bromide. Thiocyanate, itself a chlorination inhibitor (1), as is bromide, did not potentiate. Iodide and fluoride were also inactive in this respect.

Using cyanide and tetracyanoethylene as model compounds, we found that excess chloride ion did not reverse the inhibition. Slight reversal was found with Fe³⁺ and somewhat more with Cu²⁺ ions. p-Amino-propiophenone reversed the inhibition by about half and methylene blue by over half (Table 2). The methylene blue was added after the fermentation had progressed for 48 hr; otherwise no antibiotic was synthesized.

Many but not all of the compounds in Table 1 feature a conjugated double bond. Malononitrile, one of the most active compounds, does not share this feature but is a monomer of TCAP, which does. Several compounds such as acrylonitrile, crotononitrile, and cinnamononitrile which do have a conjugated double bond were tested and found inactive. In view of the activity of cyanide at very low levels when added after medium sterilization, it is attractive to speculate that a low cyanogenicity of chemical or biological origin would account for activity. Structural consideration would indicate that this could be true pyruvonitrile and the cyanomethyl benzene and toluene sulfonylates. However, acetonitrile and butyronitrile, which might be expected to be cyanogenic, were tested and found inactive. Thus, there is no present common feature which would explain activity.
<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>Amt. added (ug/ml)</th>
<th>% TC</th>
<th>Toxicity index</th>
<th>% TC</th>
<th>Toxicity index</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0</td>
<td>4-8</td>
<td>1.0</td>
<td>12-20</td>
<td>1.0</td>
</tr>
<tr>
<td>Tricyano amino propene ( \text{NCCH}_2 \text{C} = \text{C} = \text{CN} )  ( \text{NH}_2 )</td>
<td>200</td>
<td>15</td>
<td>1.14</td>
<td>67</td>
<td>1.50</td>
</tr>
<tr>
<td>Tetracyano ethylene ( \text{NC} = \text{C} = \text{C} = \text{CN} )</td>
<td>200</td>
<td>8</td>
<td>1.58</td>
<td>64</td>
<td>1.36</td>
</tr>
<tr>
<td>Malononitrile ( \text{H}_2\text{C} = \text{C} = \text{CN} )</td>
<td>200</td>
<td>20</td>
<td>2.30</td>
<td>54</td>
<td>1.94</td>
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<tr>
<td>Ethoxy methylene malononitrile ( \text{C}_2\text{H}_5\text{OCH} = \text{C} = \text{C} = \text{CN} )</td>
<td>200</td>
<td>19</td>
<td>1.53</td>
<td>54</td>
<td>1.30</td>
</tr>
<tr>
<td>1-Cyclohexane malononitrile ( \text{C} = \text{C} = \text{CN} )</td>
<td>100</td>
<td>12</td>
<td>1.35</td>
<td>47</td>
<td>1.30</td>
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<tr>
<td>O-Chlorobenzylidene malononitrile ( \text{Cl} ) ( \text{CH} = \text{C} = \text{C} = \text{CN} )</td>
<td>200</td>
<td>10</td>
<td>1.32</td>
<td>43</td>
<td>1.66</td>
</tr>
<tr>
<td>Diphenyl methylene malononitrile ( (\text{C}_6\text{H}_5)_2 \text{C} = \text{C} = \text{CN} )</td>
<td>200</td>
<td>7</td>
<td>1.13</td>
<td>22</td>
<td>1.12</td>
</tr>
<tr>
<td>Cyanomethyl toluene sulfonate ( \text{CH}_3\text{S} - \text{OCH}_2\text{CN} )</td>
<td>100</td>
<td>12</td>
<td>1.21</td>
<td>50</td>
<td>1.00</td>
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<tr>
<td>Cyanomethyl benzene sulfonate ( \text{S} - \text{OCH}_2\text{CN} )</td>
<td>50</td>
<td>12</td>
<td>1.14</td>
<td>48</td>
<td>1.84</td>
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<tr>
<td>Pyruvonitrile ( \text{CH}_3\text{C} = \text{C} = \text{O} )</td>
<td>100</td>
<td>9</td>
<td>1.11</td>
<td>36</td>
<td>1.58</td>
</tr>
<tr>
<td>Fumaronitrile ( \text{NC} = \text{CH} = \text{CH} = \text{CN} )</td>
<td>50</td>
<td>13</td>
<td>2.49</td>
<td>31</td>
<td>2.77</td>
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<tr>
<td>Dicyano dichloro benzoquinone ( \text{Cl} - \text{O} - \text{O} - \text{CN} )</td>
<td>300</td>
<td>12</td>
<td>2.72</td>
<td>42</td>
<td>2.20</td>
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<tr>
<td>Dicyanobenzene ( \text{CN} )</td>
<td>50</td>
<td>27</td>
<td>3.58</td>
<td>22</td>
<td>3.24</td>
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<tr>
<td>Phthalonitrile ( \text{CN} - \text{CN} )</td>
<td>200</td>
<td>16</td>
<td>2.38</td>
<td>20</td>
<td>2.79</td>
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<tr>
<td>Benzonitrile ( \text{CN} )</td>
<td>300</td>
<td>24</td>
<td>3.50</td>
<td>20</td>
<td>2.62</td>
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<tr>
<td>KCN added before medium sterilization</td>
<td>100</td>
<td>11</td>
<td>2.57</td>
<td>52</td>
<td>1.36</td>
</tr>
<tr>
<td>KCN added after medium sterilization</td>
<td>5</td>
<td>10</td>
<td>1.23</td>
<td>31</td>
<td>1.21</td>
</tr>
<tr>
<td>Calcium Cyanamide ( \text{NC} - \text{N} \text{Ca} )</td>
<td>300</td>
<td>11</td>
<td>1.14</td>
<td>37</td>
<td>1.24</td>
</tr>
<tr>
<td>Hydrogen Cyanamide ( \text{NC} \text{NH}_2 )</td>
<td>100</td>
<td>10</td>
<td>1.00</td>
<td>34</td>
<td>1.00</td>
</tr>
</tbody>
</table>
TABLE 2. Reversal of chlorination inhibition activity of cyanide and tetracyanoethylene

<table>
<thead>
<tr>
<th>Reversing agent</th>
<th>Inhibitor</th>
<th>None</th>
<th>Bromide, 200 µg/ml</th>
<th>Cyanide, 100 µg/ml</th>
<th>Tetracyanoethylene, 200 µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total (µg/ml)</td>
<td>Percent TC</td>
<td>Total (µg/ml)</td>
<td>Percent TC</td>
</tr>
<tr>
<td>p-Amino-propiophenone</td>
<td>0 µg/ml</td>
<td>7,615</td>
<td>6</td>
<td>4,285</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>50 µg/ml</td>
<td>4,195</td>
<td>8</td>
<td>3,250</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>100 µg/ml</td>
<td>2,900</td>
<td>9</td>
<td>2,430</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>150 µg/ml</td>
<td>1,930</td>
<td>12</td>
<td>1,496</td>
<td>22</td>
</tr>
<tr>
<td>Methylene blue*</td>
<td>0 µg/ml</td>
<td>8,310</td>
<td>5</td>
<td>5,860</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>50 µg/ml</td>
<td>7,815</td>
<td>4</td>
<td>5,020</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>250 µg/ml</td>
<td>4,380</td>
<td>3</td>
<td>2,085</td>
<td>16</td>
</tr>
</tbody>
</table>

*a Tetracycline.  
* Added at 48 hr.

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