Mutagenicity of Anthraquinone and Hydroxylated Anthraquinones in the Ames/Salmonella Microsome System

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The mutagenicity of anthracene, anthraquinone, and four structurally similar compounds of each was evaluated in the Ames/Salmonella microsome assay. Anthraquinone was shown to be mutagenic for strains TA1537, TA1538, and TA98 in the absence of rat liver homogenate. The four anthraquinone derivatives tested were mutagenic for TA1537 exclusively. None of the anthracenes exhibited mutagenic activity.

Many natural and synthetic hydroxy-, amino-, nitro-, and benz-anthraquinones are mutagenic in the Ames/Salmonella assay (2–4, 7, 9). The majority of these are classified as frameshift mutagens due to their reactivity for Ames strain TA1537, TA1538, or TA98.

We have reported recently that chrysophanol (1,8-dihydroxy-3-methylanthraquinone) and islandicin (1,4,8-trihydroxy-3-methyl-anthraquinone) mycotoxins produced by Penicillium islandicum are frameshift mutagens which require metabolic activation (S9) (6). The mutagenic activity associated with these two mycotoxins is quantitatively different from that observed with emodin (1,4,6-tri-hydroxy-3-methylanthraquinone), a known mutagenic mycotoxin for TA1537 (6, 9).

A comparison of the structure-mutagenic activity relationships for these three mycotoxins suggested that chrysophanol might represent the basic mutagenic structure (6). We suggested that the oxygen moiety at position 10, the methyl group at position 3, or both may form part of the mutagenic site, and that the quantitative differences in mutagenic activity between chrysophanol, islandicin, and emodin were due to the position of the additional OH group found in emodin and islandicin. Placement of this OH group at position 6 (emodin) facilitated, or at position 4 (islandicin) restricted, either the interaction with DNA or the activation to the mutagenic species by rat liver homogenate (6).

The present study was initiated to assess further the structural relationships and mutagenic potentials of hydroxylated anthraquinones.

FIG. 1. Structures of compounds tested.

MATERIALS AND METHODS

Test compounds. The anthracenes (AC) and anthraquinones (AQ) (Fig. 1) were obtained from Aldrich Chemical Co., Milwaukee, Wis. All stock dilutions were made in dimethyl sulfoxide (MCB, Norwood, Ohio) immediately before use.
Mutagen assay. The five *Salmonella* tester strains, TA98, TA100, TA1535, TA1537, and TA1538 were obtained from B. Ames, Department of Biology, University of California, Berkeley, and G. Walker, Department of Biology, Massachusetts Institute of Technology, Cambridge. The liver homogenate (Aroclor-induced S9) was obtained from Litton Bionetics, Inc., Kensington, Md. The quantitative mutagen assay procedure was performed as described by Ames et al. (1). Each determination was performed in duplicate, and all compounds were tested at least three times. 2-Amino AC (Sigma Chemical Co., St. Louis, Mo.) was used as the positive control mutagen for the metabolic activation with S9. 9-Aminoacridine (TA1537), 2-nitrofluorene (TA98 and TA1538) (both from Aldrich), and sodium azide (TA100 and TA1535) (Sigma) were control mutagens not requiring S9 activation.

A positive result was defined as a reproducible, dose-related increase in the number of histidine-independent colonies, whereas a negative result was defined as the absence of a reproducible increase in the number of histidine-independent colonies (5). In addition, the criterion that at least two of the points on the dose-response curve be equal to or greater than twice the spontaneous number of mutant colonies was used.

Solubility determination. The solubility of each test compound was determined as follows. Preweighed material was dissolved and diluted in dimethylsulfoxide to yield a series of stock concentrations. From these solutions, 0.2 ml was added to 4 ml of test mixture (all of the nonagar components of the top agar of Ames et al. [1]). These solutions were kept at 37°C for 48 h. The concentrations of the various compounds in solution were determined spectrophotometrically (Spectronic 21; Bausch & Lomb, Inc., Rochester, N.Y.). The appropriate wavelengths selected for this determination were obtained from reference 8.

RESULTS

Table 1 presents the solubility values of each test compound and a summary of their mutagenic activities. No mutagenic activity was observed when AC and four AC derivatives were tested (Tables 1 and 2). AQ and the various dihydroxyAQs tested in a similar fashion were mutagenic (Tables 1 and 2).

The mutagenic activity of AQ against strains TA98, TA1538, and TA1537 was observed in the absence of liver homogenate (Fig. 2). The four dihydroxyAQs were mutagenic for strain TA1537 exclusively. The 1,2- and 1,8-dihydroxyAQs were more mutagenic when tested in the presence of S9 (Fig. 3A,B). The mutagenic activity of 2,6-dihydroxyAQ was observed only in the presence of S9 (Fig. 3C). The activity of 1,4-dihydroxyAQ was approximately the same in the presence or absence of S9 (Fig. 3D).

DISCUSSION

Our results confirm the previous report (3) that 1,2-, 1,4-, and 1,8-dihydroxyAQ are frameshift mutagens in the Ames/Salmonella assay. In addition, we have shown that AQ and anthraflavic acid (2,6-dihydroxyAQ) are also frameshift mutagens.

The observation that danthon (1,8-dihydroxyAQ) was active in the presence as well as the absence of S9 is of interest because of our recent comments concerning the mutagenicity of 3-methylAQs (6). We have suggested that the methyl group at the 3 position and the oxygen moiety at the 10 position may form part of the mutagenic site. The mutagenic activity of danthon (chrysophanol minus the methyl group) is comparable to that of emodin (6, 9). This would suggest that the methyl group at position 3 on emodin, isodachyrrhizin, and chrysophanol is modified (or eliminated) by the activation mixture or has no involvement in the mutagenic activities of these mycotoxins.

AQ is mutagenic for the three Ames strains that respond to frameshift mutagens (Tables 1 and 2). The four other AQs tested were mutagenic for TA1537 exclusively. The loss of TA1538 and TA98 directed mutagenic activity after treatment of AQ with S9 is consistent with the results of Brown and Dietrich (4) for other substituted AQs.

In contrast to reports indicating that the number or placement of OH groups on AQ cannot be correlated with mutagenic activity (3, 4), we feel that the placement of OH groups at positions 1,
TABLE 2. Mutagenicity of AC and AQ compounds

<table>
<thead>
<tr>
<th>Chemical</th>
<th>µg/plate</th>
<th>Revertants/plate* for strain:</th>
<th>TA98</th>
<th>TA100</th>
<th>TA1535</th>
<th>TA1537</th>
<th>TA1538</th>
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<tbody>
<tr>
<td></td>
<td>nmol/plate</td>
<td>-S9</td>
<td>+S9</td>
<td>-S9</td>
<td>+S9</td>
<td>-S9</td>
<td>+S9</td>
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<td>13.8</td>
<td>11.5</td>
<td>10.5</td>
<td>4.8</td>
<td>6.1</td>
<td>9.5</td>
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<tr>
<td>2-Nitrofluorene(^b)</td>
<td>5</td>
<td>23.7</td>
<td>140.0</td>
<td>70.0</td>
<td>30.0</td>
<td>40.0</td>
<td>40.0</td>
</tr>
<tr>
<td>Sodium azide(^b)</td>
<td>10</td>
<td>153.8</td>
<td>140.0</td>
<td>70.0</td>
<td>30.0</td>
<td>40.0</td>
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<td>2-AminoAC(^b)</td>
<td>25</td>
<td>128.7</td>
<td>140.0</td>
<td>70.0</td>
<td>30.0</td>
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<td>AC</td>
<td>20</td>
<td>112.2</td>
<td>140.0</td>
<td>70.0</td>
<td>30.0</td>
<td>40.0</td>
<td>40.0</td>
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<tr>
<td>9-Anthraldehyde</td>
<td>2</td>
<td>11.2</td>
<td>7.0</td>
<td>13.5</td>
<td>24.5</td>
<td>18.0</td>
<td>8.0</td>
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<td>9-ACmethanol</td>
<td>20</td>
<td>97.0</td>
<td>9.5</td>
<td>19.0</td>
<td>73.8</td>
<td>71.8</td>
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<td>AC-9-carboxylic acid</td>
<td>2</td>
<td>9.6</td>
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<td>15.5</td>
<td>38.5</td>
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<td>Anthrone</td>
<td>20</td>
<td>103.0</td>
<td>13.5</td>
<td>14.8</td>
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<td>58.0</td>
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<tr>
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<td>Danthon</td>
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<td>Alizarin</td>
<td>20</td>
<td>83.5</td>
<td>14.0</td>
<td>16.5</td>
<td>57.3</td>
<td>65.0</td>
<td>12.5</td>
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<tr>
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<td>8.35</td>
<td>13.5</td>
<td>7.0</td>
<td>35.5</td>
<td>53.0</td>
<td>7.0</td>
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<tr>
<td>Anthraflavic acid</td>
<td>40</td>
<td>167.0</td>
<td></td>
<td>23.5</td>
<td>52.5</td>
<td>11.0</td>
<td>20.0</td>
</tr>
</tbody>
</table>

a Mean of six or more determinations. Spontaneous revertant colonies were not subtracted. -S9, No S9 added; +S9, S9 added.

b 2-Nitrofluorene was the positive unactivated control for TA98 and TA1538; sodium azide was the positive unactivated control for TA100 and TA1535; 9-aminoacridine was the positive unactivated control for TA1537; and 2-aminoanthracene was the positive activated control for all strains.

c Lawn was weakened.
Mutagenicity of Anthraquinone to TA1537, TA1538 & TA98

FIG. 2. Comparison of the mutagenic activity of AQ to the Ames strains TA1537, TA1538, and TA98 in the presence (●) and absence (○) of S9. The mean induced number of mutants ± 1 standard deviation was plotted.

8, or both gives rise to more active mutagens in the presence of S9. Our results suggest that hydroxylation at positions other than 1 and 8 reduces mutagenicity for TA1537 in the presence of S9.

We do not believe that these mutagenicity differences relate to the solubilities of these compounds. Danthron is both less soluble and more mutagenic than alizarin, and AQ, which is less soluble than danthron, exhibits the broadest mutagenic profile (Table 1). We do feel, however, that the solubility of the compound is a parameter which should be considered in any in vitro bioassay for mutagens. For example, the decrease in the number of danthron-induced mutants (in the presence of S9) (Fig. 3) and the "plateau" observed for alizarin were observed at mutagen concentrations which essentially exceed the solubility limit for these compounds.

In addition, the Ames test requires a seed layer containing the test compound, indicator strain, and, depending on the test, liver homoge-
FIG. 3. Response of TA1537 to four AQs in the presence of S9 (●) and in its absence (○). The mean induced number of mutants ± 1 standard deviation was plotted.
nate. This mixture is then poured over a base layer which contains Vogel-Bonner salts, 2% glucose, and 1.5% agar (1). Danthron and alizarin diffuse poorly from the seed layer into the base layer. In solution danthron is yellow to brown (depending on the concentration), whereas alizarin is orange. At concentrations which exceed the respective relative solubilities, there is a distinct separation such that the color is confined to the seed layer. At lower concentrations there is some slight color penetration into the base layer (data not presented). These observations indicate that one should not assume that the test substance in dimethylsulfoxide in the top agar will diffuse into the base layer.

In conclusion, we offer a note of caution. Negative mutagenic data in the absence of solubility information under the conditions of the test may be misleading. AQ and anthraflavic acid, both weak mutagens in this report, have been tested previously at concentrations which exceed those reported here and classified as nonmutagenic (2–4). It is important that the solubility of test compounds be determined and that the concentration range tested be adjusted accordingly.

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LITERATURE CITED