Mutagenicity of the Mycotoxin Emodin in the Salmonella/ Microsome System

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The mycotoxin emodin was found to be a frameshift mutagen for Salmonella typhimurium strain TA 1537 after metabolic activation in a mammalian microsome system.

Emodin (2-methyl-4,5,7-trihydroxyanthraquinone, Fig. 1, structure II) is a diarrheagenic toxin (10) produced by various fungi, including such ubiquitous species as Cladosporium fulvum Cooke (1), Penicillium rugulosum Thom (8), Aspergillus ochraceus Wilhelm (12) and Aspergillus wentii Wehmer (10). Since the compound has an anthraquinoid nature, it is regarded as a precursor of many of the naturally occurring fungal anthraquinones (4).

Considering that the anthraquinone moiety is a key structure of the potent carcinogenic mycotoxin, luteoskyrin (9), as well as of the mutagenic fungal metabolite, versicolorin A (11), it is surprising that the mutagenicity and carcinogenicity of emodin has not been investigated more extensively. Ueno and Kubota (8) reported that emodin does not possess any deoxyribonucleic acid-attacking abilities in the rec assay using the recombination-deficient mutant of Bacillus subtilis M45 (rec-) and the parent strain H17 (rec+). Recently Brown and Brown (5) and Brown et al. (6) included emodin in a list of 9,10-anthraquinone derivatives assayed for mutagenicity to histidine-requiring strains of Salmonella typhimurium and established that this compound acts as a frameshift mutagen after metabolic activation.

This paper presents a detailed account of the mutagenic activity of emodin for S. typhimurium, and discusses the relationship between the chemical structure and mutagenicity of the compound.

Emodin (K & K Laboratories, Inc., Plainview, N.J.) was purified by preparative thin-layer chromatography (Merck PLC plates) using chlo-roform-acetone (93:7) as developing solvent and eluting the emodin ($R_f \approx 0.5$) with acetone. The identity of the emodin peak was confirmed by its ultraviolet absorption spectrum (3). Contaminating substances were eluted in two fractions from the preparative thin-layer chromatography plates. The two fractions consisted of all compounds with higher $R_f$ values than emodin and all with lower values than emodin. Mutagenicity testing (2) revealed that both fractions of contaminating substances possessed weak mutagenic activity for S. typhimurium after metabolic activation. A serial dilution (0, 0.5, 5 and 500 μg per plate) of purified emodin was made in dimethylsulfoxide and assayed by the plate incorporation test described by Ames et al. (2). S. typhimurium TA 98, TA 100, TA 1535, TA 1537, and TA 1538 (kindly supplied by B. N. Ames, University of California, Berkeley, Calif.) were used as bacterial tester strains. The strains were routinely checked for efficiency as recommended (2). All assays were carried out in the presence and absence of Aroclor 1254 (Monsanto) -induced rat liver microsomes, containing a reduced nicotinamide adenine dinucleotide phosphate-generating system (S-9 mix) at a rate of 40 μl per plate. As a positive control, 9-aminoacridine (Sigma) was incorporated in the test at the same concentrations as emodin.

As shown in Table 1, mutagenic activity by emodin was exhibited only for strain TA 1537, after metabolic activation. This indicates a frameshift mutagenic action by the mycotoxin and supports the results of Brown and Brown (5) and Brown et al. (6). To verify these findings, emodin was retested for mutagenicity to strain TA 1537 at a wider concentration range (Fig. 2). The dose response presented in Fig. 2 confirms the mutagenicity of the compound.

It is difficult to equate the results of this mutagenicity test with in vivo carcinogenicity tests because, to the best of our knowledge, no detailed information is available on the chronic toxicity of either pure emodin or culture material of the fungi producing the toxin.

Insight into the relationship between structure and biological action of emodin can be gained by a comparison of the nature of its mutagenicity to different S. typhimurium strains with that of the mutagenic fungal metabolite versicolorin A and the compound 3a,8a-dihydrofuro [2,3-b]benzofuran (Fig. 1). The chemical
structure of emodin occurs in versicolorin A whereas 3a,8a-dihydrofuro[2,3-b]benzofuran resembles that part of the versicolorin A molecule not containing the emodin structure. Versicolorin A is known to be mutagenic for S. typhimurium strains sensitive to both base-pair substitution and frameshift mutagens and displays this ability both with and without activation by microsomal enzymes (F. C. Wehner, P. G. Thiel, S. J. van Rensburg, and I. P. C. Demasius, Mutat. Res., in press). The base-pair substitution mutagenic activity of versicolorin A seems to originate from the bisfuran ring structure of the molecule because 3a,8a-dihydrofuro[2,3-b]benzofuran only mutates strain TA 100 and not TA 98 (7). Our observations indicate that the emodin structure in versicolorin A may

be responsible for the frameshift mutagenic effect of versicolorin A because only strain TA 1537, which is specific for frameshift mutagens (2), was sensitive to emodin. Frameshift mutations can be brought about by intercalation of emodin in a deoxyribonucleic acid base-pair stack which seems to be facilitated by the metabolism of emodin to a reactive entity by microsomal enzymes. In the case of versicolorin A, frameshift mutation could be effected without metabolic activation, suggesting that the occurrence of the bisfuran moiety in the molecule

![Chemical structures](image)

**Fig. 1. Chemical structures of (I) 3a,8a-dihydrofuro[2,3-b]benzofuran, (II) emodin, and (III) versicolorin A.**

**Table 1. Reversion of S. typhimurium tester strains with emodin**

<table>
<thead>
<tr>
<th>Strain</th>
<th>S-9</th>
<th>Optimal concn of emodin (µg/plate)*</th>
<th>Spontaneous mutants per plate</th>
<th>Histidine revertants per plate at optimal concentration*</th>
<th>Mutagenicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>TA 98</td>
<td>+</td>
<td>5</td>
<td>43</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td></td>
<td>−</td>
<td>0.5</td>
<td>7</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>TA 100</td>
<td>+</td>
<td>0.5</td>
<td>152</td>
<td>157</td>
<td></td>
</tr>
<tr>
<td></td>
<td>−</td>
<td>5</td>
<td>92</td>
<td>110</td>
<td></td>
</tr>
<tr>
<td>TA 1535</td>
<td>+</td>
<td>0.5</td>
<td>37</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td></td>
<td>−</td>
<td>5</td>
<td>23</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>TA 1537c</td>
<td>+</td>
<td>500</td>
<td>22</td>
<td>210</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>−</td>
<td>500</td>
<td>9</td>
<td>15</td>
<td></td>
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<tr>
<td>TA 1538</td>
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<td>0.5</td>
<td>31</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td></td>
<td>−</td>
<td>5</td>
<td>13</td>
<td>8</td>
<td></td>
</tr>
</tbody>
</table>

*a Concentration at which the highest reversion of S. typhimurium occurred. Concentration range: 0, 0.5, 5, 50, and 500 µg of emodin per plate.

*b Mean revertant value of triplicate plates per concentration. Spontaneous mutants subtracted.

*c Revertant colonies of TA 1537 per plate after incubation with the positive control 9-aminoacridine at concentrations of 0, 0.5, 5, 50 and 500 µg/plate were 20, 20, 53, 76 and 140 with metabolic activation and 12, 16, 15, 17, and 377 without metabolic activation, respectively.
facilitates intercalation in the deoxyribonucleic acid molecule, obviating the need for metabolic activation as in the case of emodin.

LITERATURE CITED