Evaluation of Alternariol and Alternariol Methyl Ether for Mutagenic Activity in *Salmonella typhimurium*

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Alternariol and alternariol methyl ether were tested in the Ames *Salmonella typhimurium* assay, and both were shown, with and without metabolic activation, to be nonmutagenic to strains TA98 and TA100. The finding of other investigators that alternariol methyl ether is weakly mutagenic to TA98 without metabolic activation could have resulted from the presence of a small amount of one of the highly mutagenic altertoxins in the alternariol methyl ether originally tested.

Alternaria species are widely distributed plant pathogens and decay organisms of mature fruits and vegetables. In a survey carried out by Bruce et al. (2), *Alternaria* propagules were found in 184 of 230 test samples of grains from the United States. These molds also produce a large variety of toxic and nontoxic secondary metabolites (5, 6), including the dibenzo-pyrones alternariol (AOH) (3), alternariol methyl ether (AME) (3), and altenuene (1); the perylene derivatives alter-toxins I, II, and III (9) and stemphyltoxin III (4, 8); and a tetradic acid (2), tenuazoic acid (6). The structures of AME, AOH, and altertoxin III are shown in Fig. 1.

Our laboratories have previously shown that altertoxins I, II, and III and stemphyltoxin III are mutagenic in the Ames *Salmonella typhimurium* assay using strains TA98 and TA100 in both the absence and the presence of a liver microsomal fraction (S9) for metabolic activation (4, 9). It has also been reported that AME is weakly mutagenic to *S. typhimurium* TA98 without metabolic activation whereas tenuazoic acid is not mutagenic to the same strain (7). In the present study, highly purified AOH and AME were tested for mutagenic activity by using *S. typhimurium* TA98 and TA100 both with and without S9 to determine whether these dibenzopyrones are indeed mutagenic.

AOH and AME were obtained from F. S. Chu of the University of Wisconsin Food Research Institute, Madison, Wis. (3), and were repurified by liquid column chromatography on Silica Gel 60 (E. Merck AG, Darmstadt, Germany). AME was eluted from the column with methylene chloride, and

AOH was eluted with methylene chloride-acetone (4:1). AOH and AME were tested for purity by thin-layer chromatography on Silica Gel 60 plates. Separate 25-μg/ml solutions of AME, AOH, stemphyltoxin III, and altertoxins I, II, and III were prepared in methylene chloride. A 10-μl portion of each solution was spotted on the plate. After development with benzene-methanol-acetic acid (90:5:5) and drying at room temperature, AME was detected at its R_f of 0.94 by exposing the plate to NH_3 fumes, which also caused altertoxin III to become violet. The identities of AME, AOH, and altertoxin III were confirmed by mass spectrometry.

S. typhimurium TA98 and TA100 were used for identification of reverse mutations from histidine dependence by the plate incorporation method of Ames et al. (1). To 2 ml of molten top agar ¹were added 0.1 ml of bacteria from an overnight culture, 0.05 ml of the test substance dissolved in dimethyl sulfoxide, and 0.5 ml of the S9 mix, when used. The base agar contained 0.5% glucose instead of the 2% glucose used by Bruce et al. (2). The S9 was prepared from the livers of Aroclor 1254-induced male Sprague-Dawley rats according to the procedure described by Ames et al. (1). Compounds were dissolved in dimethyl sulfoxide immediately before plating, and the assay was completed as rapidly as possible because of the instability of the altertoxins. For each dose level, three plates were used with concurrent positive and solvent controls. The plates were incubated for 48 h at 37°C, and all colonies were counted manually. A revertant count equal to twice the background count was considered positive. Bacterial cultures
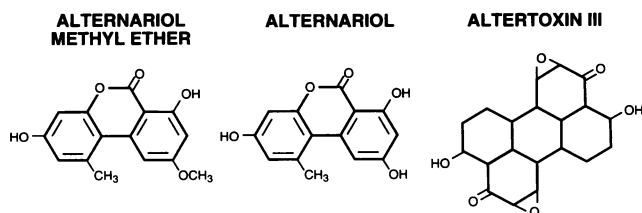

FIG. 1. Structures of AME, AOH, and altertoxin III.

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TABLE 1. Number of His⁺ revertants in positive and solvent controls in the *S. typhimurium* mutagenicity assay

| Strain	Chemical	Dose of S9 (μg/plate)	No. of His⁺ revertants/plate Positive control	Solvent control
TA98	2-Fluorenylacetamide	10	+692	38
TA100	4-Nitro-*o*-phenylenediamine	5.0	−703	30
TA100	2-Aminoanthracene	0.5	+841	143
TA100	Nitrofurantoin	0.5	−812	134
TA1535	2-Aminoanthracene	2.5	+72	7
TA1535	Sodium azide	1.25	−84	6
TA1537	2-Aminoanthracene	2.5	+88	10
TA1537	4-Nitro-*o*-phenylenediamine	10	−96	8

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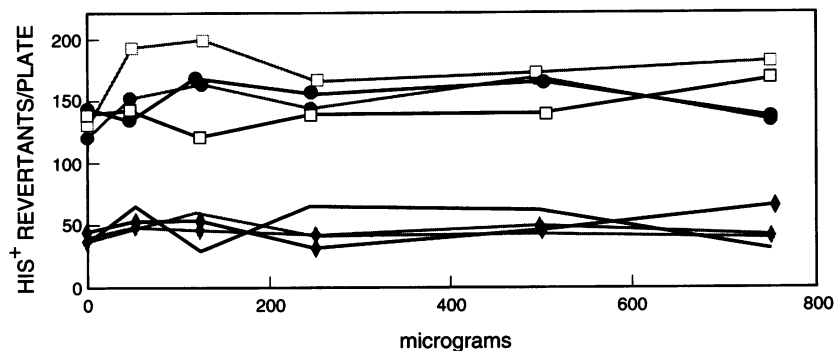
FIG. 2. Results of mutagenicity assays with AOH (unbroken symbols) and AME (hatched symbols). Lines, TA98 without S9; diamonds, TA98 with S9; squares, TA100 without S9; circles, TA100 with S9.
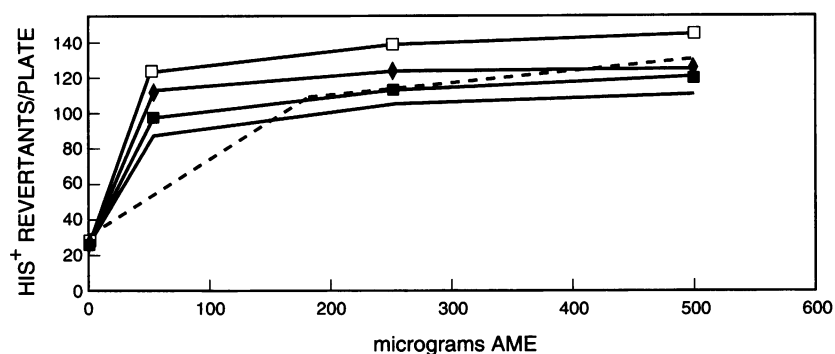

FIG. 3. Mutagenic responses of strain TA98 to AME and altertoxin III. ———, AME plus 0.6 μg of altertoxin plus S9; ◆, AME plus 1.8 μg of altertoxin III plus S9; ■, AME plus 0.6 μg of altertoxin III without S9; □, AME plus 1.8 μg of altertoxin III without S9; — — —, altertoxin III.

were routinely tested for viability as well as sensitivity to ampicillin and crystal violet.

Both AME and AOH were shown to be free from contamination with the altertoxins, as determined by thin-layer chromatography. The results of the mutagenicity assays of AME and AOH are shown in Fig. 2. Responses for the concurrent positive controls are shown in Table 1. AME and AOH were tested at doses of 50 to 750 μg per plate with *S. typhimurium* TA98 and TA100, both with and without metabolic activation. No positive increases in revertants over the spontaneous levels were observed. AME and AOH were also tested with strains TA1537 and TA1538 at the same doses, and no positive responses were induced (data not shown). As expected, altertoxin III, at doses of 0.6 and 1.8 μg per plate, induced a mutagenic response with TA98 (Fig. 3). A mutagenic response was also obtained when AME (Fig. 3) and AOH were tested in the presence of very small amounts of altertoxin III, 0.6 and 1.8 μg.

We conclude from these experiments that AME and AOH are not mutagenic. The previous report by Scott and Stoltz (7) of marginal mutagenicity of AME in the *S. typhimurium* test could have resulted from the presence of a very small amount of one of the altertoxins in the AME that they tested.

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