# *In vitro* metabolism of alternariol and alternariol monomethylether in rat liver microsomes

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## Introduction

Alternariol (AOH, Fig. 1) and alternariol monomethylether (AME) are the major mycotoxins produced by the molds of the genus *Alternaria*, in particular *A. alternata*. Both toxins are suspected to be mutagenic and are associated with the etiology of oesophageal cancer. AOH and AME have been found in many kinds of foodstuff, e.g. grains, sunflower seeds, oilseed rape and pecans as well as in various fruits including tomatoes, olives, mandarins, melons, peppers, apples and raspberries.

Only few data are available about the toxicity of AOH and AME. In particular, information about the biotransformation of AOH and AME is scarce and inconsistent. Therefore, we have studied the oxidation and glucuronidation of AOH and AME in rat liver microsomes from male Sprague-Dawley rats.

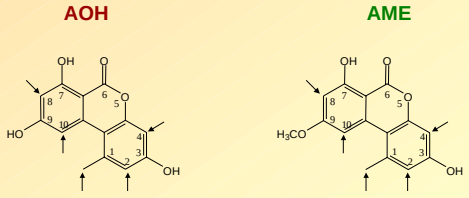


**Fig. 1** Chemical structures of AOH and AME. Arrows indicate possible sites for hydroxylation

## Materials and Methods

For oxidative metabolism, AOH and AME (50 µM) were incubated with microsomes (1 mg protein per ml) and a NADPH-generating system as cosubstrate in 0.1 M phosphate buffer pH 7.4. For glucuronidation, activated glucuronic acid (UDPGA) was used as cosubstrate instead of NADPH. Incubations without cosubstrate and with heat-inactivated microsomes served as control. AOH, AME and their metabolites were extracted from the incubations with ethylacetate and analyzed by HPLC-DAD. The metabolites were further characterized by HPLC-ESI-MS and after derivatisation with BSTFA by GC-MS.

## Results of oxidative metabolism (phase I)

Metabolic conversion of AOH was 25% whereas 40% of AME was transformed under the same conditions. In case of AME, the HPLC chromatogram (Fig. 2) exhibited the formation of 5 oxidative metabolites. In contrast, AOH showed 4 oxidative metabolites. Furthermore, AME was demethylated to AOH.

In addition, some products with a lower polarity and with similar UV/VIS spectra (Insert in Fig. 2) as the parent mycotoxins and the other metabolites were detected for both mycotoxins and are suggested to be polymers. The pattern of the metabolites is shown in Fig. 3.

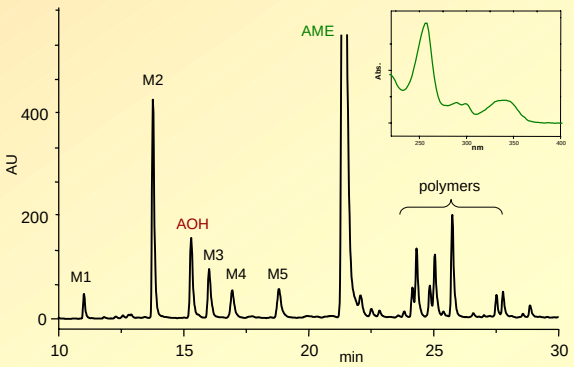


**Fig. 2** HPLC chromatogram of oxidative AME metabolites. Insert: UV/VIS spectrum of AME

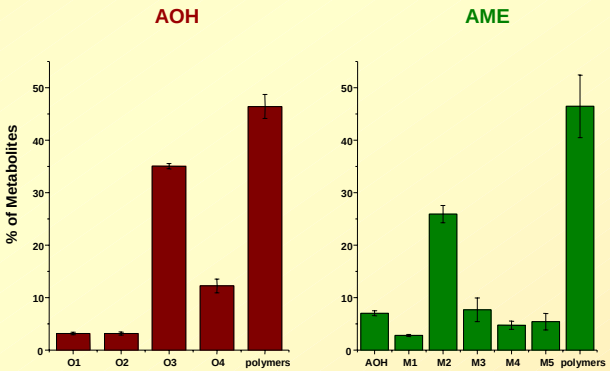


**Fig. 3** Pattern of oxidative metabolites of AOH and AME

According to their mass spectra, the oxidative metabolites O1-O4 of AOH and the oxidative metabolites M2-M5 of AME are monohydroxylated compounds. In ESI-MS the metabolite M1 showed the mass of dihydroxylated AME.

Considering the possible sites for oxidative metabolism (see arrows in Fig. 1) most of the metabolites O1-O4 and M2-M5 must be products of aromatic hydroxylation. In the case of AOH all these oxidation products are catechols. For AME the formations of catechols and one hydroquinone is expected.

First studies on the methylation of the AME metabolites showed that M2 is a substrate for catechol-O-methyltransferase, implying that the major metabolite of AME is a catechol.

The less polar products are most likely condensation products of the resulting polyphenols. The following findings led to this conclusion: (1) the substances show molecular ions in the ESI mass spectra at the mass of a dimer of the respective mycotoxin; (2) the addition of ascorbic acid decreased the formation of these products and increased the amount of the hydroxylated metabolites.

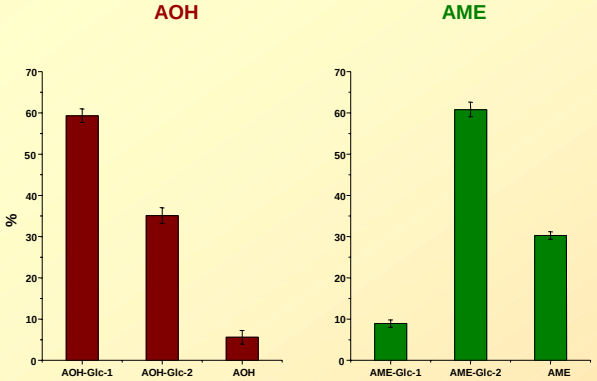


**Fig. 4** Pattern of glucuronides of AOH and AME

## Results of Glucuronidation (phase II)

Metabolic conversion of AOH to glucuronides was 95% whereas 65% of AME was conjugated under the same conditions. AOH gave rise to two glucuronides formed at a ratio of about 1:2. The corresponding ratio for AME was 1:7 (Fig. 4).

The proximity of the hydroxy group in position 7 to the keto group in position 6 (Fig. 1) of AME and AOH makes it less accessible for glucuronidation. It is therefore proposed that the two observed AOH glucuronides are conjugated at position 3 and 9. In the case of AME the major glucuronide is most likely conjugated at position 3.

## Conclusions

Our studies have shown that both AOH and AME undergo extensive oxidative metabolism and conjugation with glucuronic acid *in vitro*. AOH was markedly better glucuronidated than AME whereas more AME than AOH was hydroxylated. The majority of the oxidative metabolites are catechols which may be reactive and can cause cell damage by redox cycling or reaction with critical nucleophiles.

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