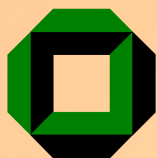


# Oxidative metabolism of alternariol and alternariol monomethylether by rat, porcine, and human liver microsomes

Nils Helge Schebb[1], Erika Pfeiffer[1], Ronald Maul[2], Timo Gehring[3], Joachim Podlech[3], and Manfred Metzler[1]

[1]Institute of Applied Biosciences, Section of Food Chemistry and Toxicology, University of Karlsruhe
[2]Institute of Biochemistry and Food Chemistry, University of Hamburg [3]Institute of Organic Chemistry, University of Karlsruhe

## Introduction

Alternariol (AOH) and alternariol monomethylether (AME) (Fig. 1) 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. Alternaria toxins have been found in many kinds of foodstuff, e.g. grains and vegetables. Only few data are available about the toxicity of AME and AOH. In particular, information about their biotransformation is scarce and inconsistent. Therefore, we have studied the oxidation of AME and AOH in human (HLM), porcine (PLM), and rat liver microsomes (RLM).

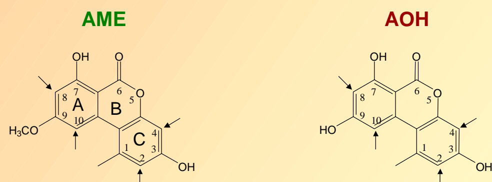



**Fig. 1:** Chemical structures of AME and AOH. Arrows indicate possible sites for aromatic hydroxylation.

## Materials and Methods

For oxidative metabolism, AME and AOH (50 µM) were incubated with microsomes (0.7-1 mg protein per ml) and a NADPH-generating system in 0.1 M phosphate buffer pH 7.4. Incubations without cosubstrate and with heat-inactivated microsomes served as control. AME, AOH 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 by GC-MS after derivatisation with BSTFA and deuterated BSA.

## Results of oxidative metabolism

Oxidative metabolism of AME and AOH led to several products with similar UV/VIS spectra (Insert in Fig. 2) as the parent compounds for all tested microsomes. Metabolic conversion of AME (8-20%) was generally higher than that of AOH (3-15%). In the case of AME, the HPLC chromatogram (Fig. 2) exhibited the formation of 5 oxidative metabolites (M1-M5) after incubation with RLM. Furthermore, AME was demethylated to AOH. M1 was not detected with HLM and PLM. HLM and PLM gave rise to similar patterns of metabolites, but M5 was formed preferentially by HLM instead of M2 (Fig.3).

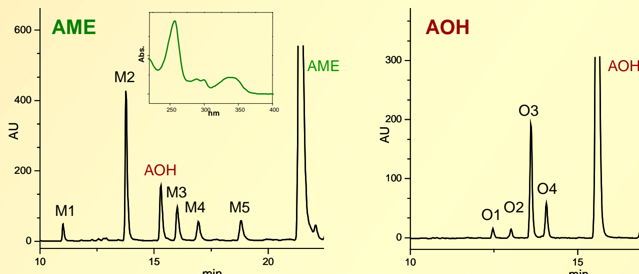



**Fig. 2:** HPLC chromatogram of AME (left) and AOH (right) at 254 nm after incubation with rat liver microsomes. Insert: UV/VIS spectrum of AME

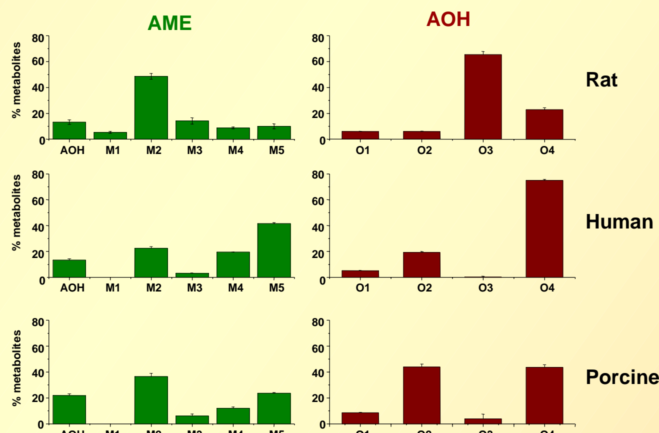


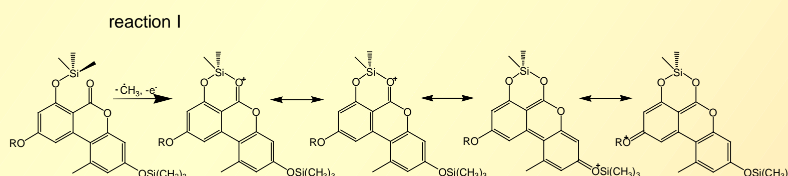

**Fig. 3:** Pattern of oxidative metabolites of AME and AOH after incubation with porcine (bottom), human (center) and rat (top) liver microsomes.

In contrast, the chromatogram of AOH (Fig.2) showed 4 oxidative metabolites (O1-O4), with remarkable differences in the pattern between the three species. HLM und PLM gave rise only to a trace of O3 whereas O3 was formed as dominating product by RLM. HLM showed a strong formation of O4, and after incubations with PLM, O2 and O4 were found in similar amounts (Fig.3).

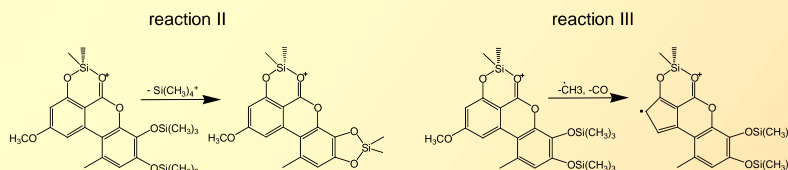
According to LC-ESI-MS these products are monohydroxylated compounds, only M1 is dihydroxylated. Considering the possible sites for hydroxylation (Fig.1), all of them are catechols, except 10-HO-AME.

## Structure determination by GC-MS fragmentation

Trimethylsilylated AME, AOH and metabolites did not exhibit molecular ions in GC-EI-MS. The base peaks in the mass spectra were $[M-15]^+$. It is suggested that silylation of the hydroxy group in position 7 led to the loss of a methyl radical of the TMS-group under formation of a 5-membered ring with mesomeric stabilisation and no further fragmentation (reaction I).

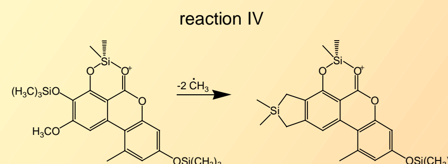



The metabolites M4, M5 and O1-O4 gave rise to a characteristic ion at $[M-88]^+$ due to the loss of $Si(CH_3)_4$ and formation of a bridging $Si(CH_3)_2$ group between two vicinal hydroxy groups (reaction II). It is therefore concluded that these metabolites are catechols.




The spectra of AME, M4 and M5, but not AOH, showed the formation of a $[M-43]^+$ ion. This is attributed to the loss of $CH_3$ and CO from the aromatic methoxy group (reaction III). Hydroxylation of ring A prevents this fragmentation, and it is deduced that M4 and M5 are hydroxylated in ring C. After structure determination of M4 as 4-HO-AME by comparison with a chemically synthesized reference substance, M5 was identified as 2-HO-AME by reaction I-III.

M2 and M3 gave rise to intense $[M-30]^+$ ions, thus implying the formation of a bridging $Si(CH_3)_2$ group from a hydroxy and a neighboring methoxy group (reaction IV), similar to reaction II. It is concluded that M2 and M3 are hydroxylated at ring A.




Of the two possible products of ring A hydroxylation, 8-HO-AME is a catechol whereas 10-HO-AME is not. Because the hydroxy group in position 7 of 8-HO-AME is already bridged (reaction I), no $[M-88]^+$ ions were detectable. However, M2 was identified as 8-HO-AME because it was a substrate of catechol-O-methyltransferase in contrast to M3, which is therefore 10-HO-AME.

The most polar AME metabolite M1 had the mass of dihydroxylated AME in its ESI mass spectrum, but did not give a mass spectrum in GC-EI-MS.

## Conclusions

Our studies have shown that both AME and AOH undergo extensive oxidative metabolism *in vitro*. Human, pig and rat microsomes formed the same hydroxylated metabolites with a remarkably different quantitative pattern. The majority of the oxidative metabolites are catechols which may cause cell damage by redox cycling or reaction with critical nucleophiles.

## Acknowledgement

Supported by the Landesschwerpunkt Baden-Württemberg “Lebensmittel und Gesundheit”, Schwerpunkt “Mykotoxine” at the University of Karlsruhe.