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MUTAGENICITY AND IN VITRO METABOLISM OF ARISTOLOCHIC ACID

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INTRODUCTION

Currently a great interest in elucidating the basis of the biological properties of the nitroarenes is present. They are widely distributed environmental agents, some of which are extraordinarily mutagenic and cause cancer in rodents (1). Aristolochic acid (AA), a nitrophenanthrene derivative, was used as an anti-inflammatory agent in several pharmaceutical preparations up to 1982. In 1982 AA was reported to be highly carcinogenic (2) and mutagenic (3). Recent studies showed that the substance called Aristolochic acid contains at least 6 compounds (4). The main ingredients of the commercially available mixture are aristolochic acid I (AAI) and aristolochic acid II (AAII), which are different from each other by one methoxy group (Fig. 1).

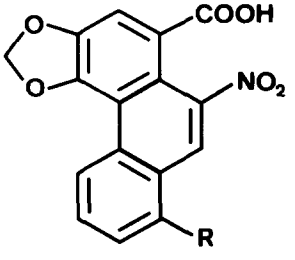

Figure 1

R = $-OCH_3$ = Aristolochic Acid I (AAI)
R = -H = Aristolochic Acid II (AAII)

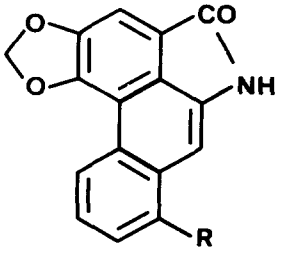

Figure 2

R = $-OCH_3$ = Aristolactam I
R = -H = Aristolactam II

MATERIALS AND METHODS

To determine the relative mutagenic activity of the components, three different AA samples (AAI 99% pure; AAI 65% + AAII 35%; AAI 32% + AAII 68%) were assayed with the method of Ames et al. (5) in Salmonella typhimurium TA 1537, TA 100 and TA 100 NR (6). The two main components (AAI) and AAII) were direct mutagens in Salmonella strains TA 1537 and TA 100 with almost equal mutagenic potency. In TA 100 NR the AA samples showed no or only a very low level of biological activity, indicating the necessity of nitroreduction for the bioactivation of the samples.

Isolation of AAI and AAII from the mixture is achieved by MPLC (middle pressure liquid chromatography) with a reversed phase 30 um ODS column (500 mm x 50 mm) (eluant methanol 60%, H_2O 39%, acetic acid 1%; flow 30 ml/min).

The in vitro metabolism of AAI and AAII by rat liver 9000 xg supernatant under aerobic and anaerobic conditions was investigated (6.25 ml incubations, 0.2 mM; 20 mg protein/ml). Metabolism was not detected in the absence of NADPH or heated S-9 mix for both aerobic and anaerobic incubations.

Under anaerobic conditions the rates of disappearence of AAI and AAII were clearly different. For AAI only 10% conversion was measured after 180 min whereas for AAII approximately 60% had disappeared after 180 min (Fig. 3). The ethylacetate-soluble metabolites were separated by HPLC and identified by their spectral properties and by comparison to standards. For both AAI and AAII the major metabolites under anaerobic conditions were the corresponding Aristolactams (Fig. 2).

In contrast to the results described above aerobic incubations of AAI and AAII with rat liver S-9 mix resulted in 90% conversion of AAI after 180 min but no measurable conversion of AAII (Fig. 4). This metabolite derived from AAI is still not identified. These findings suggest that in vitro the acids are metabolized by two totally different pathways, namely an oxidative pathway for AAI to yield an unidentified metabolite and a reductive pathway for AAII to yield the corresponding amino compound as was observed for most nitroarenes (7).

To detect covalent in vitro binding to DNA the enzymatic ^{32}P-postlabeling method of Randerath et al. (8) was used. Until now the ^{32}P-postlabeling assay was applied only for an anaerobic incubation with AAII in the presence of S-9 mix and calf thymus DNA. Two distinct spots were found after removal of ^{32}P-labeled normal DNA nucleotides and autoradiography indicating two aromatic carcinogen-DNA adducts for which the structures are not yet established.

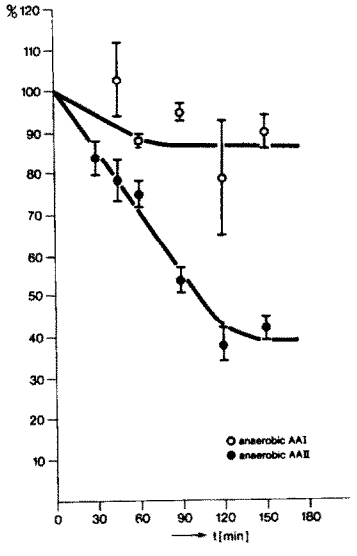

Figure 3

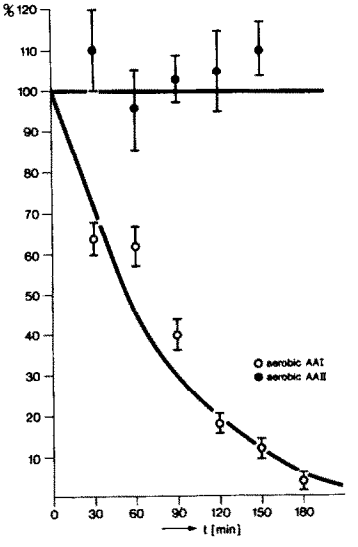

Figure 4

Rates of metabolism of AAI and AAII under unaerobic and aerobic conditions.

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