Aristolochic acid (AA) (4), a naturally occurring nephrotoxin (1) and Carcinogen (4), is implicated in a unique type of renal fibrosis (5), designated Chinese herbs nephropathy (5) (CHN), which can develop to urothelial cancer (5). Understanding which enzymes are involved in AA activation and/or detoxication is important in the assessment of an individual susceptibility to this natural carcinogen. We examined the ability of prostaglandin H synthase (PHS)(3) to activate AA to metabolites forming DNA adducts with the nuclease P1 (3) and 1-butanol extraction enrichment procedure (10) of the (32)P-postlabeling assay (10). PHS is a prominent enzyme in the kidney (1) and urothelial tissues (1). Ram (7) seminal vesicle (1) (RSV) microsomes (1), which contain high levels of PHS, generated AA-DNA adduct patterns reproducing those found in renal tissues (1) in CHN patients. 7-(Deoxyadenosin-N(6)-yl)aristolactam I (4), 7-(deoxyguanosin-N(2)-yl)aristolactam I (4) and 7-(deoxyadenosin-N(6)-yl)aristolactam II (4) were identified as AA-DNA adducts formed by AAI (4). Two adducts, 7-(deoxyguanosin-N(2)-yl)aristolactam II (4) and 7-(deoxyadenosin-N(6)-yl)aristolactam II (4), were generated from AAII (4). According to the structures of the DNA adducts identified, nitroreduction (6) is the crucial pathway in the metabolic activation of AA. The identity of PHS as the activating enzyme in RSV microsomes was proven with different cofactors and inhibitors. Only indomethacin (4), a selective inhibitor of PHS, significantly decreased the amount of adducts formed by RSV microsomes. The inhibitor of NADPH:CYP reductase (3) (alpha-lipoic acid (4)) and some selective inhibitors of cytochromes P450 (CYP) (3) were not effective. Likewise, only cofactors of PHS, arachidonic acid (4) and hydrogen peroxide (4), supported the DNA adduct formation of AAI and AAII, while NADPH (4) and NADH (4) were ineffective. These results demonstrate a key role of PHS in the activation pathway of AAI and AAII in the RSV microsomal system and were corroborated with the purified enzyme, namely ovine PHS-1 (3). The results presented here are the first report demonstrating a reductive activation of nitroaromatic compounds (4) by PHS-1.