BENZIL, A SELECTIVE INDUCER AND A POTENT IN VITRO ACTIVATOR OF MICROSOMAL EPOXIDE HYDROLASE

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INTRODUCTION

It is now well known that many chemical carcinogens, including the polycyclic hydrocarbons, must be metabolized to reactive intermediates before they can exert their biological effects\(^1\). It is believed that both the carcinogenicity and mutagenicity of polycyclic hydrocarbons results from the covalent binding of such intermediates to protein, RNA, and/or DNA. In the case of benzo(a)pyrene the diol epoxide 7,8-dihydro-7,8-dihydroxybenzo(a)pyrene-9,10-epoxide may well be the ultimate carcinogen.

Formation of this diol epoxide in mammalian cells is a result of so-called recycling and requires both microsomal monooxygenase and epoxide hydrolase activities\(^2-4\). Thus, epoxide hydrolase may play a complicated role in the biotransformation of polycyclic hydrocarbons to carcinogenic intermediates. Many other xenobiotics containing aromatic rings or unsaturated alkyl chains are of such a size or chemical nature that they would not be expected to undergo significant recycling. In these cases epoxide hydrolase should protect the cell from toxic and mutagenic damage by metabolizing reactive epoxide intermediates to less dangerous diols.

While studying the induction of epoxide hydrolase by metabolites and structural analogues of trans-stilbene oxide, we found that benzil was an effective inducer of this enzyme\(^5\). Control experiments demonstrated that this compound is also a very potent activator of epoxide hydrolase IN VITRO. In the present investigation we have characterized the structural requirements for and the kinetics of this activation in more detail. We have also compared the
activating effect of benzil to that of other compounds identified earlier as activators of epoxide hydrolase, e.g., metyrapone\textsuperscript{6,7}, and chalcone epoxide\textsuperscript{8}.

MATERIALS AND METHODS

Chemicals

Dibenzyl (ICN Pharmaceuticals Inc., Life Sciences Group, Planview, N.Y., U.S.A.); \textit{cis}- and \textit{trans}-stilbene, \textit{trans}-stilbene oxide, desoxybenzoin, metyrapone, 2,2'-pyridil, dibenzoylethane, diacetyl and chalcone (EGA-Chemie, Steinheim/Albuch, Germany); benzoin and benzil (Fluka AG, Buchs S.G., Switzerland); \textit{meso}-1,2-diphenylethane-1,2-diol and phenanthrene quinone (Merck-Schuchardt, Munich, Germany); 3,3'-pyridil (Chem Service, West Chester, P.A., U.S.A.); dimethylsulfoxide (Sigma Chemical Co., St. Louis, Missouri, U.S.A.) and ethyl phenylglyoxalate (ABC, Aldrich Chemical Company Inc., U.S.A.) were all obtained from commercial sources. Benzylacetophenone was a kind gift from Dr. Ake Pilotti, Department of Organic Chemistry, University of Stockholm. \textit{cis}-Stilbene oxide and \textit{threo}-1,2-diphenylethane-1,2-diol were synthesized by Synthesis Service, Chemical Centre, Lund, Sweden. We synthesized chalcone epoxide according to Yang and Finnegan\textsuperscript{9}; the compound was recrystallized and its identity confirmed by NMR and melting point determination. [7-\textsuperscript{3H}]Styrene-7,8-oxide (10 Ci/mol) was purchased from the Radiochemical Centre, Amersham, U.K. All other chemicals used were of reagent grade and purchased from common commercial sources.

Animals and microsomes

Male Sprague-Dawley rats weighing 180–200g were used in all experiments. The animals were starved overnight before decapitation in order to reduce liver glycogen. Liver microsomes were then prepared according to Ernster \textit{et al}\textsuperscript{10}.

Induction

For all derivatives 1 mmol/kg body weight was administered once daily for 5 days. In one experiment (Table 3) twice as large dose of \textit{trans}-stilbene oxide and benzil was used. Routinely, the substance was injected intraperitoneally into rats dissolved in 1 ml sunflower oil.

Assay for microsomal epoxide hydrolase

Epoxide hydrolase was assayed using a modification\textsuperscript{11} of the method of Oesch \textit{et al}\textsuperscript{12}. To the 100 μl incubation volume containing buffer (pH 7.4) and 0.3–0.5 mg microsomal protein was added 2 μl of a solution containing the potential activator. After 5 min prein-