[Chem. Pharm. Bull. 36(6)2267—2269(1988)]

An Unstable Epoxy Intermediate Formed by the Microsomal Oxidation of 4-Nitrophenyl Vinyl Ether

MASAKAZU ISOBE, TOMOMICHI SONE,* and EIGO TAKABATAKE

Department of Toxicology, Faculty of Pharmaceutical Sciences, Setsunan University, 45–1 Nagaotoge-cho, Hirakata, Osaka 573–01, Japan

(Received November 5, 1987)

An epoxy intermediate, 2-(4-nitrophenoxy)oxirane (NPO), previously proposed to be a short-lived intermediate in the microsomal metabolism of 4-nitrophenyl vinyl ether (NPVE), was isolated by high-performance liquid chromatography and identified by mass spectrometric comparison with an authentic specimen. NPO decomposed to glycolaldehyde and 4-nitrophenol with a half life of 4.6 min in 0.1 M phosphate buffer, pH 7.4, at 37 °C. The hydrolytic decomposition was accelerated by the addition of rat hepatic microsomes, but the accelerating effect was reduced by the addition of 3,3,3-trichloropropene oxide, a microsomal epoxide hydrolase inhibitor.

These results indicate that NPO is an obligatory intermediate in the microsomal conversion of NPVE to glycolaldehyde and 4-nitrophenol.

Keywords——2-(4-nitrophenoxy)oxirane; epoxide; vinyl ether; microsomal metabolism; stability; hydrolysis; epoxide hydrolase

It has been presumed that vinyl ethers are metabolized by monooxygenase systems to glycolaldehyde and an alcohol or a phenol *via* unstable epoxy intermediates.[1–5] We reported that 4-nitrophenyl vinyl ether (NPVE) was a useful substrate for the assay of microsomal olefinic epoxidation activity, because the formation of 4-nitrophenol was easily monitored by colorimetry.[3] The present study provides evidence for the formation of an unstable epoxy intermediate, 2-(4-nitrophenoxy)oxirane (NPO), from NPVE incubated with rat hepatic microsomes.

Experimental

Chemicals——Nicotineamide adenine dinucleotide phosphate (NADP), glucose 6-phosphate, and glucose 6-phosphate dehydrogenase were purchased from Oriental Yeast Co., Tokyo, and 3,3,3-trichloropropene oxide (TCPO) from Aldrich Chemical Co., Milwaukee, Wis., U.S.A. NPVE was synthesized according to the method of Dombroski and Hallensleben.[6] All other reagents used were of reagent grade.

Synthesis of NPO——NPVE (50 mg) dissolved in absolute $CHCl_3$ (0.4 ml) was added to a $CHCl_3$ solution (0.2 ml) of perbenzoic acid[7] (100 mg), and the mixture was kept at room temperature for 2 h. The reaction mixture was diluted with benzene (2 ml) and passed through a silica gel column (packed with C_6H_6, 7 × 30 mm) within 2 min. The residue obtained by evaporation of solvents from the effluent was crystallized from *n*-hexane/ether (4/1). The epoxide of NPVE, NPO, was isolated as an amorphous precipitate in 30% yield (mp 93.5—94.5 °C). The purity was confirmed to be over 95% by nuclear magnetic resonance (NMR) spectrometry (JEOL FX-90Q spectrometer). ^{1}H-NMR (in $CDCl_3$) δ: 3.00 (1H, dd, J=2.5 and 4.2 Hz, 3-H), 3.12 (1H, dd, J=1.2 and 4.2 Hz, 3-H), 5.18 (1H, dd, J=1.2 and 2.5 Hz, 2-H), 7.1—8.3 (4H, aromatic H). Electron impact-mass spectrum (EI-MS) was recorded on a JEOL model DX-300 mass spectrometer under the following conditions: ionization chamber temperature, 200 °C; ionization energy, 30 eV. MS *m/z*: 181 (M^+), 152 ($M^+ - CHO$).

Incubation Conditions——Hepatic microsomes prepared from untreated male Wistar rats (180 g) were used in the experiment. For the isolation of the epoxide, the incubation mixture consisted of the microsomal suspension (2 mg protein/ml), NPVE (0.2 mM, added as an MeOH solution, 100 μl), TCPO (1 mM, added as an MeOH solution, 10 μl), NADP (0.5 mM), glucose 6-posphate (5 mM), $MgCl_2$ (5 mM), and glucose 6-phosphate dehydrogenase (1 U/ml) in 0.1 M phosphate buffer, pH 7.4, to make a final volume of 5.0 ml. Incubations were carried out at 37 °C for 15 min under

aerobic conditions. 4-Nitrophenol and glycolaldehyde were assayed according to the methods described in a previous paper.[3]

High-Performance Liquid Chromatography (HPLC)——HPLC was carried out on a Shimadzu model LC-5A apparatus equipped with a silica gel column (LiChrosorb SI-60, 5μ, 4 × 250 mm) and a JASCO model UVIDEC-100-IV UV spectrophotometer (280 nm). The column was eluted at a flow rate of 2 ml/min with *n*-hexane/tetrahydrofuran (THF) (20/1).

Results and Discussion

Isolation and Identification of NPO

Semiempirical molecular orbital calculations have shown that the epoxides of alkyl vinyl ethers are generally too unstable to isolate.[4] On the other hand, NPO was synthesized and isolated as a stable material and its structure was confirmed by EI-mass spectrometry and NMR spectrometry, as presented in Experimental.

After incubation of NPVE with rat hepatic microsomes in the presence of a reduced nicotinamide adenine dinucleotide phasphate (NADPH)-generating system and TCPO, an *n*-hexane extract of the incubation mixture was examined directly by HPLC. The chromatogram showed a weak peak at 9.8 min, the retention time of authentic NPO, in addition to the major peak of NPVE at 3.0 min. The solvents in the NPO fraction eluted from the column were evaporated under reduced pressure and the residue was examined by EI-mass spectrometry. The EI-MS was superimposable on that of authentic NPO (Fig. 1). The molecular ion peak was seen at *m/z* 181 and the base peak at 152 was assignable as the $M^+ - CHO$ ion. When the NADPH-generating system was omitted from the incubation mixture, the formation of NPO was not observed.

Hydrolysis of NPO

The hydrolytic decomposition of NPO in an aqueous solution was examined. NPO (100 nmol) dissolved in 50 μl of THF was added to 0.1 M phosphate buffer, pH 7.4 (3.0 ml), at 37 °C and the release of 4-nitrophenol was monitored continuously at 420 nm in a cuvette. It was also shown that the hydrolysis of NPO proceeded stoichiometrically to form 4-nitrophenol and glycolaldehyde. The amount of NPO unhydrolyzed in the mixture was

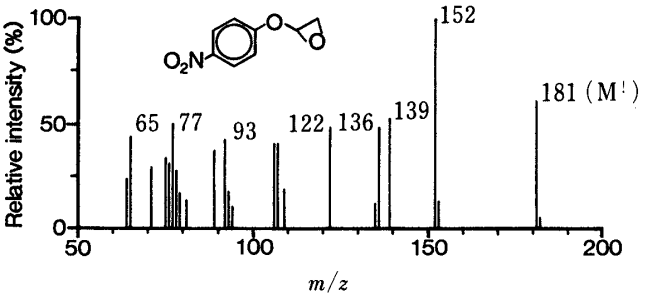

Fig. 1. Mass Spectral Identification of the Epoxy Intermediate

The spectrum shown is that of NPO formed from NPVE by microsomal incubation and it is the same as that of authentic NPO.

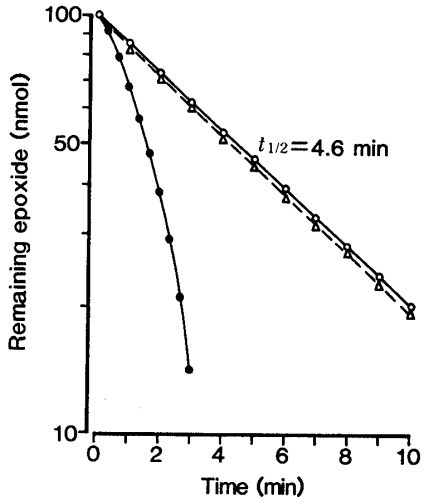

Fig. 2. Hydrolytic Degradation of NPO in an Aqueous Medium and the Microsomal Acceleration of the Hydrolysis

Additions: ○—○, none; ●—●, microsomes (430 μg of protein/ml); △---△, microsomes (430 μg of protein/ml) + TCPO (1 mM).

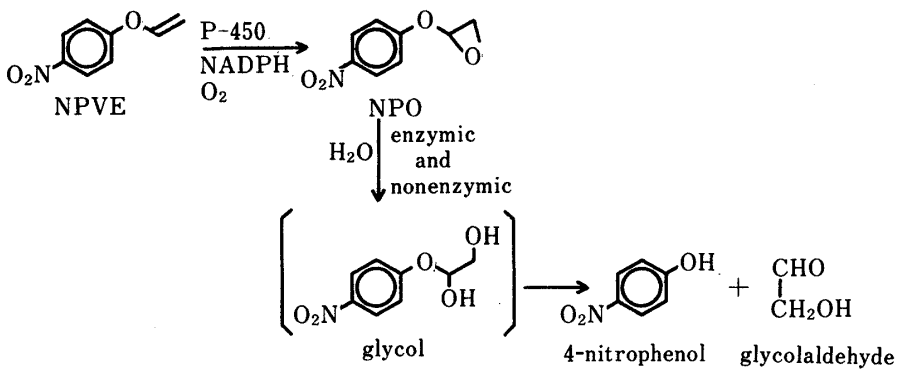

Fig. 3. The Metabolic Pathway of NPVE *via* an Epoxy Intermediate

calculated from 4-nitrophenol formed. NPO decomposed according to first-order kinetics with a half life of 4.6 min (Fig. 2). The activation energy for the nonenzymic hydrolysis of NPO was calculated to be 15.3 kcal/mol from the Arrhenius plot of the rate constants measured at various temperatures. The initial rate of hydrolysis of NPO increased with the amount of microsomal fraction added to the reaction mixture. When 430 μg of protein/ml (final concentration) of microsomes was added, the initial rate was about twice that of the spontaneous hydrolysis. The acceleration of hydrolysis by microsomes was accounted for by the epoxide hydrolase, because this effect was strongly inhibited by the addition of TCPO (1.0 mM), a microsomal epoxide hydrolase inhibitor.[8,9]

These results indicate that NPO is an obligatory intermediate in the metabolism of NPVE to 4-nitrophenol and glycolaldehyde and that NPO formed from NPVE is hydrolyzed both enzymically and nonenzymically (Fig. 3).

References

1) D. A. Blake, R. S. Rozman, H. F. Cascorbi, and J. C. Krantz, Jr., *Biochem. Pharmacol.*, **16**, 1237 (1967).
2) J. A. Marsh, J. J. Bradshaw, G. A. Sapeika, S. A. Lucas, L. S. Kaminsky, and K. M. Ivanetich, *Biochem. Pharmacol.*, **26**, 1601 (1977).
3) M. Isobe, T. Sone, and E. Takabatake, *J. Pharmacobio-Dyn.*, **8**, 614 (1985).
4) S. M. Adams, M. J. Murphy, and L. S. Kaminsky, *Mol. Pharmacol.*, **20**, 423 (1981).
5) M. J. Murphy, D. A. Dunbar, and L. S. Kaminśky, *Toxicol. Appl. Pharmacol.*, **71**, 84 (1983).
6) J. R. Dombroski and M. L. Hallensleben, *Synthesis*, **1972**, 693.
7) G. Braun. "Organic Syntheses," Coll. Vol. I, ed. by A. H. Blatt, John Wiley and Sons, Inc., New York, 1941, p. 431.
8) T. Watabe, M. Isobe, K. Yoshikawa, and E. Takabatake, *J. Pharmacobio-Dyn.*, **1**, 98 (1978).
9) F. Oesch, N. Kaubisch, D. M. Jerina, and J. W. Daly, *Biochemistry*, **10**, 4858 (1971).