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# Oxidation of styrene in liver microsomes

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DIHYDRODIOLS are metabolic products of oxidation of aromatic hydrocarbons such as naphthalene in mammalian liver microsomes.[1, 2] Oxidation of the nonaromatic double bond of some cyclic compounds to glycols has also been demonstrated. Thus, dihydronaphthalene[3] and indene[4] give rise to glycols in mixed-function oxidations in liver microsomes.

El Masri *et al.*[5] showed that rabbits fed styrene (phenylethylene) excrete the glucuronide of phenylethylene glycol in the urine. We have found that the dihydroxylation of this noncyclic olefin occurs in the microsomes of mammalian liver.[6] This communication describes some of the characteristics of glycol formation from styrene.

The enzyme preparation was the reconsituted lyophilized 9000 *g* supernatant fraction, prepared as previously described,[4, 7] from homogenates of livers of male Holtzman rats or male New Zealand White rabbits that had been injected i.p. with sodium phenobarbital, 75 mg/kg/day (rats) or 15 mg/kg/day (rabbits), for 4 days. The last injection occurred 20 hr before the animals were killed. Incubation, deproteinization and extraction of glycol with ethyl acetate were carried out by methods similar to those employed for the study of indene metabolism.[4] The substrate solution containined 50 μmole styrene in 0·5 ml dimethylformamide. The recovery of added phenylethylene glycol in the extraction procedure was essentially complete.

Gas chromatograms of the extracts in two different systems are shown in Fig. 1. In each case, peaks were observed in the chromatograms of experimental extracts which were identical in retention time to those given by authentic phenylethylene glycol. All other peaks in the experimental chromatograms were matched by peaks in those of blank samples prepared as above, but with the styrene and dimethylformamide added at the end of the incubation period, immediately before deproteinization. In each system, two peaks were observed for phenylethylene glycol reference samples and corresponding peaks were obtained from experimental extracts. This phenomenon was investigated with the OV-1/OV-17 column. One peak varied in height over a 10-fold range proportionally to the amount of glycol injected, whereas the other was not proportional to sample size, but was relatively constant in height. This is also shown in tracings E and F of Fig. 1, in which one peak was higher in the chromatogram of an extract made after 60 min of incubation than in that after 10 min, whereas

the other had about the same height in both chromatograms. It was concluded that the relatively invariant peak was due to a degradation product of phenylethylene glycol formed at a constant rate on the gas chromatographic column. The other peak increased linearly with incubation time, at least during the first 30 min, and continued to rise as incubation time was extended to 2 hr.

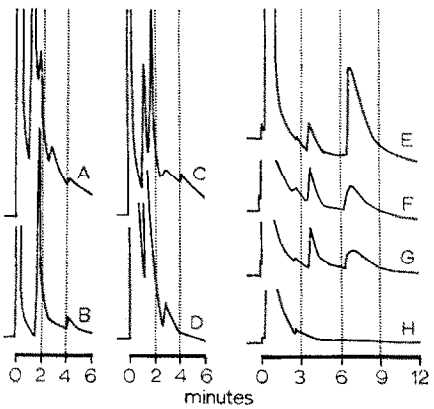


FIG. 1. Gas chromatograms of extracts of incubation mixtures with rat (A-D) and rabbit (E-H) liver 9000 *g* supernatant fraction. Chromatographic conditions: A-D, 3·8% SE-30 on Diatoport S, 80–100 mesh; column 120°, flash heater 255°, detector 185°. E-F, 2% OV-1/3% OV-17 on Chromosorb W (AW-DMCS), 60–80 mesh; column 125°, flash heater 175°, detector 185°. Glass 4-ft U-columns, hydrogen flame ionization detection and a carrier helium flow rate of 100 ml/min were used throughout. A, complete reaction mixture; B, reference phenylethylene glycol; C, extract A "spiked" with reference phenylethylene glycol; D, blank (prepared as described in text); E and F, complete reaction mixtures after 60 and 10 min of incubation respectively; G and H, blank (prepared as described in text) with and without added reference phenylethylene glycol respectively.

Table 1 shows the results of TLC of the metabolite and of two derivatives. The trimethylsilyl derivative was also subjected to gas chromatography as recently described.[8] The retention time of the derivative prepared from the metabolite (3·6 min) exactly matched that of the trimethylsilyl ether of phenylethylene glycol.

TABLE 1. THIN-LAYER CHROMATOGRAPHY OF METABOLITE AND DERIVATIVES

| Compound chromatographed | Solvent system* | $R_f$ | $R_f$ of phenylethylene glycol or of corresponding derivative |
|---|---|---|---|
| Metabolite† | A | 0·51 | 0·52 |
| Acetate derivative‡ | B | 0·36 | 0·35 |
| Trimethylsilyl ether§ | C | 0·32 | 0·32 |

* Eastman chromagram sheets no. 6060 (silica gel + fluorescent indicator) were activated for 15 min at 110° prior to applying samples. Solvent systems: A, organic phase from benzene:water:ethyl acetate:glacial acetic acid (7:8:3:2, v/v/v/v); B, hexane:ether (9:1, v/v); C, heptane. Spots were visualised under u.v., and, in the case of the metabolite itself, with ammoniacal $AgNO_3$[4].

† Metabolite was extracted from the reaction mixture without prior deproteinization when direct TLC was performed.

‡ Prepared analogously to the acetate derivative of inanediol[4].

§ Prepared by the procedure recently described.[8]

The amount of phenylethylene glycol formed from styrene was estimated by a recently described colorimetric method.[9] A preparation of rat liver 9000 *g* supernatant fraction catalyzed the production of 78 mμmole phenylethylene glycol per hr, while in the presence of a rabbit liver preparation, 95

mμmole glycol was formed. The spectrum of the chromophore was identical to that obtained when phenylethylene glycol was used; the maximum occurred in each case at 375 mμ.

Thus, another class of unsaturated hydrocarbons, that of the phenyl-substituted ethylenes, may now be added to the two classes (aromatic polycyclic and nonaromatic cyclic hydrocarbons) previously known to be oxidized to glycols by mammalian liver microsomes. Studies on the specificity and mechanism of oxidative reactions of this class are currently in progress in our laboratory.

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