tanilide may account for an even greater percentage of the total metabolism.

Laboratory of Chemical Pharmacology, National Heart and Lung Institute, National Institutes of Health, Bethesda, Md. 20014, U.S.A.

JACK A. HINSON*
JERRY R. MITCHELL
DAVID J. JOLLOW

* Recipient of National Institutes of Health Postdoctoral Fellowship 5 FO2 HL54291-02.

REFERENCES

1. J. Miller and E. Miller, *Prog. exp. Tumor Res.* **11**, 273 (1969).
2. J. H. Weisburger and E. K. Weisburger, *Pharmac. Rev.* **25**, 1 (1973).
3. L. A. Poirier, J. A. Miller and E. C. Miller, *Cancer Res.* **23**, 790 (1963).
4. M. Kiese and W. Lenk, *Biochem. Pharmac.* **20**, 379 (1971).
5. P. H. Grantham, T. Matsushima, J. H. Weisburger, R. C. Yamamoto and E. K. Weisburger, *Toxic. appl. Pharmac.* **19**, 380 (1971).
6. W. Troll and S. Belman, in *Bladder Cancer, a Symposium*, pp. 35–44. Aesculapius, Birmingham (1967).
7. S. Belman, W. Troll, G. Teebor and F. Mukai, *Cancer Res.* **28**, 535 (1968).
8. J. R. Mitchell, D. J. Jollow, W. Z. Potter, D. C. Davis, J. R. Gillette and B. B. Brodie, *J. Pharmac. exp. Ther.* **187**, 185 (1973).
9. D. J. Jollow, J. R. Mitchell, W. Z. Potter, D. C. Davis, J. R. Gillette and B. B. Brodie, *J. Pharmac. exp. Ther.* **187**, 195 (1973).
10. W. Z. Potter, D. C. Davis, J. R. Mitchell, D. J. Jollow, J. R. Gillette and B. B. Brodie, *J. Pharmac. exp. Ther.* **187**, 203 (1973).
11. J. R. Mitchell, D. J. Jollow, W. Z. Potter, J. R. Gillette and B. B. Brodie, *J. Pharmac. exp. Ther.* **187**, 211 (1973).
12. J. R. Mitchell, W. Z. Potter, J. A. Hinson, W. R. Snodgrass, J. A. Timbrell and J. R. Gillette, in *Handbook of Experimental Pharmacology* (Eds. O. Eichler, A. Farah, H. Herken and A. Welch), pp. 383–419. Springer, Berlin (1975).
13. J. A. Hinson, J. R. Mitchell and D. J. Jollow, *Molec. Pharmac.* **11**, 462 (1975).
14. U. Priyadarshini and S. Tandon, *J. chem. eng. Data* **12**, 143 (1967).
15. J. Booth and E. Boyland, *Biochem. J.* **91**, 362 (1964).
16. J. Daly, *Biochem. Pharmac.* **19**, 2979 (1970).
17. G. Zweig and J. Sherma (Eds.), in *Handbook of Chromatography*, Vol. II, p. 161. CRC Press, Cleveland (1972).

Biochemical Pharmacology, Vol. 25, pp. 601–602. Pergamon Press, 1976. Printed in Great Britain.

Metabolism of the carcinogenic bifunctional olefin oxide, 4-vinyl-1-cyclohexene dioxide, by hepatic microsomes

(*Received* 3 *September* 1975; *accepted* 2 *October* 1975)

Alkene oxides are known to produce malignant tumors; the bifunctional epoxides such as 4-vinyl-1-cyclohexene dioxide (1) and 1,3-butadiene dioxide have been reported to show notable carcinogenic activity on mouse skin [1]. These findings are of importance in relation to a strongly suggested role of *K*-region epoxides as metabolically formed proximate carcinogens in carcinogenicity exerted by polycyclic aromatic hydrocarbons, as they have been shown to produce malignant transformations of cells in culture [2–5]. The suggested carcinogenic mechanism involving the *K*-region epoxides, however, is an open question since previous work indicates that they have no carcinogenic activity *in vivo* [1].

Monoepoxides, including arene oxides, are detoxified by the catalytic action of either microsomal epoxide hydrolase [6, 7] (epoxide hydrase [8, 9]) or soluble epoxide-*S*-glutathione transferase [10], both of which are present in mammalian liver, to polar glycols or glutathione conjugates. However nothing is known of microsomal hydrolysis of the bifunctional epoxides, but only that no glutathione conjugation occurs with (1) *in vitro* [10] although monoepoxides such as cyclohexene oxide and styrene oxide, whose oxirane moieties are considered as its partial structures, are readily converted to the corresponding conjugates under the same conditions. This encouraged us to investigate the enzymatic hydrolysis of the carcinogen (1) by microsomal epoxide hydrolase not only from the view point of its detoxication mechanism, but also confirming whether enzymatic hydrolysis of the diepoxide follows evidence obtained by Kaubisch *et al.* [11] for structure–reactivity relationships in monoepoxides.

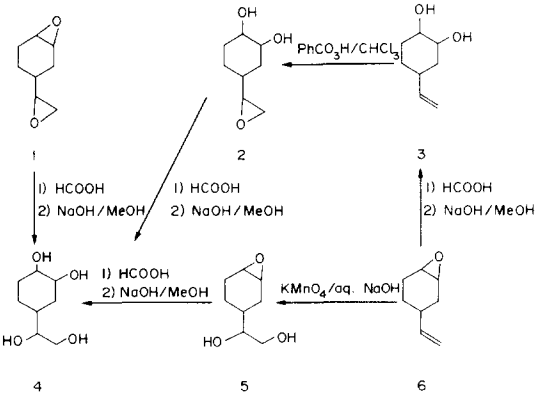

Fig. 1. Synthesis of 4-vinyl-1-cyclohexene dioxide metabolites.

The diepoxide (1) (2 mM) dissolved in acetone (0·2% v/v) was incubated at 37° with rabbit liver microsomes (1·2 mg protein/ml) in 0·1 M phosphate buffer, pH 7·4, and the

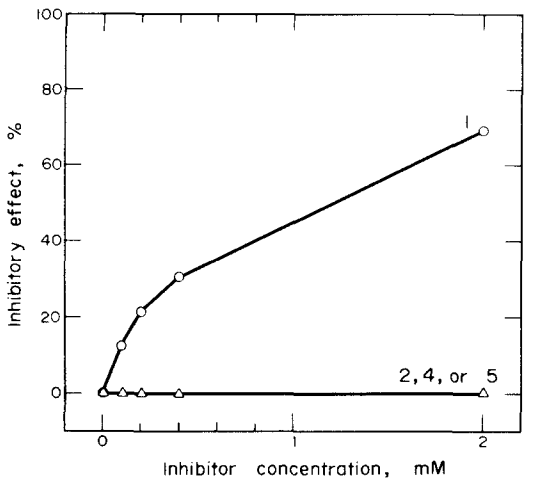

Fig. 2. Inhibition of microsomal hydrolysis of safrole oxide with 4-vinyl-1-cyclohexene dioxide (1) and its enzymatic hydrolysis products, 1,2-dihydroxy-4-vinylcyclohexane oxide (2), 4-(1',2'-dihydroxyethyl)-1,2-cyclohexanediol (4), and 4-(1',2'-dihydroxyethyl)-1-cyclohexene oxide (5). Concentration of the substrate, safrole oxide, was 0·2 mM and of microsomal protein 0·06 mg/ml. The reaction constant for the microsomal hydrolysis of safrole oxide in the absence of inhibitors was 50 nmoles/mg protein/min.

reaction terminated after 30 min by the addition of 5N NaOH so that the final concentration of the alkali was 1N. Following saturation of the mixture with sodium chloride, it was extracted with ethyl acetate. The residue obtained after evaporation of the solvent from the extract was trimethylsilylated in the standard manner, dissolved in *n*-hexane, and analyzed on a 1·5% SE-30 column at 140° by g.l.c. Chromatograms obtained indicated the formation of two possible types of monoepoxy-monoglycols, 1,2-dihydroxy-4-vinylcyclohexane oxide (2) and 4-(1',2'-dihydroxyethyl)-1-cyclohexane oxide (5); the former appeared as a single peak at a retention time of 4·1 min and the latter as a double peak with retention times of 4·9 and 5·4 min. G.l.c. mass spectra of the trimethylsilyl derivatives were superimposable on those of trimethylsilylated authentic specimens synthesized from commercially available (1) and 4-vinyl-1,2-epoxycyclohexane (6) as illustrated in Fig. 1; their molecular ion peaks appeared at *m/e* 302. A polar metabolite 4-(1',2'-dihydroxyethyl)-1,2-cyclohexane diol (4) was isolated from a residue obtained from the remaining aqueous layer by the addition of acetone and subsequent evaporation of the solvent from the aqueous acetone solution which was separated by centrifugation. Identification of the metabolite with authentic (4) was carried out by g.l.c. mass spectrometry after trimethylsilylation; the tetra-trimethylsilyl derivative appeared as a single peak at a retention time of 11·0 min at a column temperature of 140° and showed a molecular ion peak at *m/e* 464 in the spectrum.

Reaction constants for the enzymatic formation of (2), (5), and (4) from (1) were 4·7, 10·2, and 1·1 nmoles/mg protein/min, respectively. Polar monoepoxy-monoglycols, (2) and (5), also served as microsomal substrates and yielded the tetraol (4) at reaction rates of 10·0 and 7·6 nmoles/mg protein/min, respectively. Control experiments using boiled microsomal preparations indicated that the formation of (2), (4), and (5) in the above mentioned reactions was enzymatic. The extremely poor utilization of the nonpolar diepoxide (1) as a substrate for microsomal epoxide hydrolase compared with previously reported monoepoxyalkanes [12] was attributable in part to its inhibitory effect on the enzyme (Fig. 2) which was confirmed by the inhibition of enzymatic hydrolysis of safrole oxide according to the previously reported method [13]. Neither monoepoxy-monoglycols, (2) and (5) nor tetraol (4) inhibited enzymatic hydrolysis of safrole oxide to any extent. This is suggestive of the poorer yield of (4) from (1) than from (2) or (5) in the enzymatic reactions. It is of interest that in epoxides (1) and (5) the cyclohexene moiety was not necessarily resistant to microsomal hydrolysis compared with the vinylidene oxide moiety in epoxides (1) and (2) although in a series of monoepoxides a remarkable difference has been reported to exist in reactivity between simple alicyclic olefin oxides and vinylidene oxides, e.g. microsomal hydrolysis is known to occur over 15 times faster in styrene oxide and simple 1-olefin oxides than in cyclohexene oxide [11].

TADASHI WATABE
TADASHI SAWAHATA
Laboratory of Drug Metabolism and Toxicology,
Department of Hygienic Chemistry,
Tokyo College of Pharmacy,
Ueno-sakuragi, Taito-ku, Tokyo 110, Japan

REFERENCES

1. B. L. Van Duuren, L. Langseth, B. M. Goldschmidt and L. Orris, *J. nat. Cancer Inst.* **39**, 1217 (1967).
2. P. L. Grover, A. Hewer and P. Sims, *Biochem. Pharmac.* **21**, 2713 (1972).
3. G. R. Keysell, J. Booth, P. L. Grover, A. Hewer and P. Sims, *Biochem. Pharmac.* **22**, 2853 (1973).
4. H. Marquardt, T. Kuroki, E. Huberman, J. K. Selkirk, C. Heidelberger, P. L. Grover and P. Sims, *Cancer Res.* **32**, 716 (1972).
5. E. Huberman, T. Kuroki, H. Marquardt, J. K. Selkirk, C. Heidelberger, P. L. Grover and P. Sims, *Cancer Res.* **32**, 1391 (1972).
6. T. Watabe and E. W. Maynert, *Fedn Proc.* **27**, 302 (1968).
7. E. W. Maynert, R. L. Foreman and T. Watabe, *J. biol. Chem.* **245**, 5234 (1970).
8. D. M. Jerina, J. W. Daly, B. Witkop, P. Zaltzman-Nirenberg and S. Udenfriend, *J. Am. chem. Soc.* **90**, 6525 (1968).
9. D. M. Jerina, J. W. Daly, B. Witkop, P. Zaltzman-Nirenberg and S. Udenfriend, *Biochemistry* **9**, 141 (1970).
10. T. Hayakawa, R. A. LeMahieu and S. Udenfriend, *Archs Biochem. Biophys.* **162**, 223 (1974).
11. N. Kaubisch, J. W. Daly and D. M. Jerina, *Biochemistry* **11**, 3080 (1972).
12. F. Oesch, *Xenobiotica* **3**, 305 (1973).
13. T. Watabe and K. Akamatsu, *Biochem. Pharmac.* **23**, 2839 (1974).