Biochemical Pharmacology, Vol. 26, pp. 1535–1538. Pergamon Press, 1977. Printed in Great Britain

PRELIMINARY COMMUNICATIONS

METABOLISM IN VIVO OF DIOXANE: IDENTIFICATION OF p-DIOXANE-2-ONE AS THE MAJOR URINARY METABOLITE*

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(Received 5 March 1977; accepted 4 May 1977)

Previous investigations (1-3) from our laboratory have established p-dioxane (I), a commonly used industrial and laboratory solvent, as a hepatic carcinogen. This finding was confirmed by Kociba et al. (4). The literature on the metabolism of I is very scanty. Based on results obtained from the oxidation of I by nitric acid, Fairley et al. (5) postulated oxalic acid and diglycolic acid to be the metabolites of I. However, this hypothesis could not be substantiated by animal studies (6). In order to gain an insight into the mechanism of carcinogenic action of I, we have undertaken studies of its metabolism in vivo. In analyzing by gas chromatography (g. c.) the urine samples obtained from rats treated with I, we have identified a major metabolite of I. The present communication describes these studies.

Male Sprague-Dawley rats (95-130 g) were given dioxane (50-400 mg/100 g body weight) intraperitoneally. Urine samples were collected in 8- or 12-hr periods for 2 days, using 0.2 ml of glacial acetic acid as a preservative; the samples were then treated with kaolin, filtered through Whatman No. 42 paper and the filtrate pH was checked and adjusted to between 4.0 and 4.5. Gas chromatographic (Microtek GC DSS-162 equipped with a flame ionization detector) separation of the volatile compounds present in the kaolin-treated urine on Porapak Q or QS (mesh 50-80, 6 ft. x $\frac{1}{4}$ in., inlet 280°, column 180°, detector 200°) revealed two major peaks: one with a retention time of 7 min corresponding to I and the other with a retention time of 32 min hereafter called "the metabolite." The appearance of the metabolite was dependent on the pH of the urine. At high pH (in excess of pH 12), no metabolite can be detected; reacidification of the solution brought about the re-appearance of the metabolite peak, suggesting the presence of an acidic group in the metabolite. The excretion of the metabolite was dose dependent and time dependent,

*Presented in part at the 173rd Meeting of the American Chemical Society in New Orleans, March 1977, Abstr. No. 79 MEDI.

reaching a maximum between 20 to 28 hr after administration of I. Administration of diethylene glycol (II) also yielded the same metabolite; however, it was almost entirely excreted prior to 20 hr, suggesting that II may represent an intermediate stage in the metabolism in vivo of I. Under similar conditions, diglycolic acid, ethylene glycol and oxalic acid did not give rise to excretion of the metabolite.

Isolation of the metabolite was achieved by preparative g.c. (Varian model 90-P equipped with a thermal conductivity detector). Urine samples were first concentrated 30- to 40-fold by lyophilization. The metabolite was then isolated from the lyophilized urine by trapping the effluent gas in a U-shaped collecting tubule immersed in ice. The freshly isolated sample was in the form of a yellowish liquid which would gradually solidify to a white amorphous material after several days, on-standing at ambient temperature. Generally, after isolation the metabolite was immediately diluted with deuterated chloroform or with methylene chloride for spectral analyses. The purified metabolite showed an intense carbonyl band at 1750 cm^{-1} in the infrared (IR) spectrum (Perkin Elmer 257 grating IR spectrometer). Nuclear magnetic resonance (NMR) spectra (Varian A-60 or HA-100) showed two triplets and one singlet with equal intensity at δ 3.85, 4.48 and 4.37 respectively. Gas chromatography-mass spectrometric studies were performed on a Hewlett-Packard 5982A quadrupole mass spectrometer equipped with a model 5710A gas chromatograph using OV-101 as support. Figure 1 shows the g.c.-mass spectrum of the

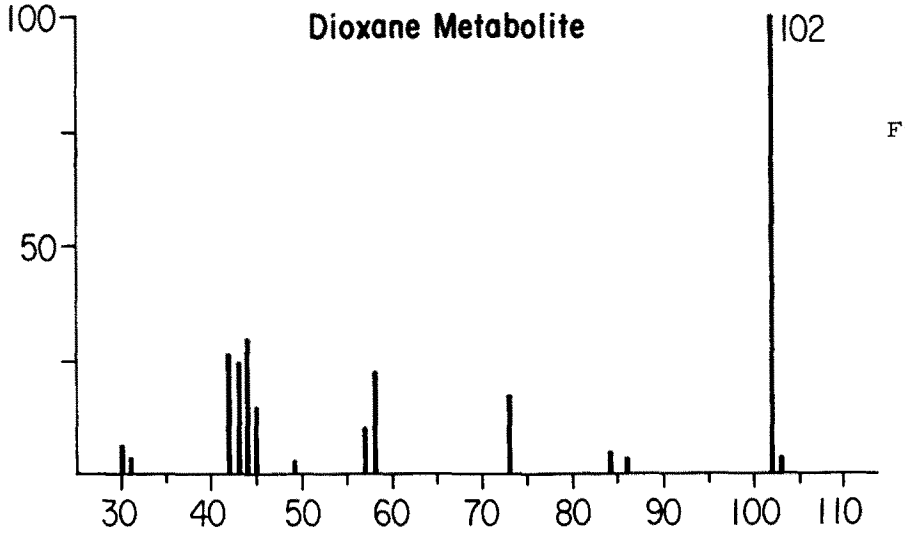

Fig. 1. Gas chromatography-mass spectrum of the dioxane metabolite.

metabolite isolated from lyophilized urine by preparative g.c. and diluted with methylene chloride. A parent peak at m/e 102 was observed. Molecular weight determinations by cryoscopy and by the Rast method (Galbraith Labs., Knoxville, Tenn., and Huffman Labs., Wheatridge, Colo.) yielded, however, inconsistent values ranging from 147 to 565, which attests the instability of the isolated compound. Based on the information obtained, the structure of the metabolite has been deduced and identified as p-dioxane-2-one (also known as 2-hydroxyethoxyacetic acid δ-lactone) (IV). This conclusion was supported by the facts that IV is a lactone with a six-membered ring, which has the peculiar characteristic of spontaneously polymerizing to linear polymers (V) on standing (7), and that IV can be reversibly converted to 2-hydroxyethoxyacetate (III) (which is non-volatile) by adjusting

the acidity or alkalinity of the solution (8). Synthetic p-dioxane-2-one was obtained from Aldrich Chemical Co. and purified by distillation. The authentic compound possesses the same chemical properties and exhibits identical IR, NMR and g.c.-mass spectra as the dioxane metabolite. Evidence that IV is an actual metabolite of I was provided by our studies using ^{14}C-labeled I. Forty to sixty per cent of the radioactivity may be recovered from the urine of rats given ^{14}C-labeled I. Using preparative g.c., ^{14}C-labeled IV was isolated from the urine by bubbling the column effluent directly through dioxane-based liquid scintillation solution (Bray's solution). The amount of radioactivity collected correlated well with the amount of IV determined by the flame ionization detector.

To establish the absolute amounts of IV excreted by rats dosed with I, the purified authentic compound was diluted with kaolin-treated urine from untreated rats to a final concentration of 10 mg/ml and the solution was used to standardize the g.c. calibration. The average excretion of IV in 48 hr was found to be 227.8±15.8 mg (mean ± S. E. of eight determinations)/200 g body weight from rats given I at a dose of 300 mg/100 g body weight. Thus, 33 per cent of I given was recovered as IV in the urine. An average of 10.8 per cent of I was excreted unchanged. Comparison of these data with those obtained by using ^{14}C-labeled I indicated that IV and I accounted for most, if not all, of the urinary excretion.

Our data clearly establish that p-dioxane-2-one is the major urinary metabolite of dioxane. Other experiments (9) revealed that the metabolism of I to IV can be significantly increased by pretreatment of rats with inducers of microsomal mixed-function oxidases (MFO) and decreased by inhibitors of MFO, suggesting the involvement of MFO in the metabolism *in vivo* of I. That the metabolism of I may be related to its toxicity and/or carcinogenicity is implicated from acute toxicity studies which indicate that certain agents that modify the metabolism of I also modify its toxicity in the same manner. Preliminary data on the acute toxicity of IV indicate that IV is considerably more toxic than I. The possibility that IV may be a proximate carcinogen of I is currently under study in view of the fact that a number of lactones with similar structure are known to be carcinogenic (10, 11). It is of interest that IV and derivatives of IV have been used commercially and industrially as preservatives for animal specimens and other purposes. The potential health hazard of the compound remains to be assessed.

The possible metabolic pathways of I are shown in Fig. 2. The observation that IV is excreted from rats given II and at a rate faster than that of IV from I supports pathway (a); however, the absence of II in the urine from rats given I suggests either very rapid conversion of II to III (IV) or that other alternative mechanisms exist. One interesting alternative [pathway (b)] is the formation of a keto-peroxyl radical as an intermediate, similar to the reaction scheme proposed by Lorentzen *et al.* (12) for the oxidation of the carcinogen benzo(a)pyrene to benzo(a)pyrene diones. Another alternative [pathway (c)] that remains to be tested involves hydroxylation of I followed by oxidation of the hemiacetal (aldehyde) intermediate. The exact nature of the pathway awaits further studies.

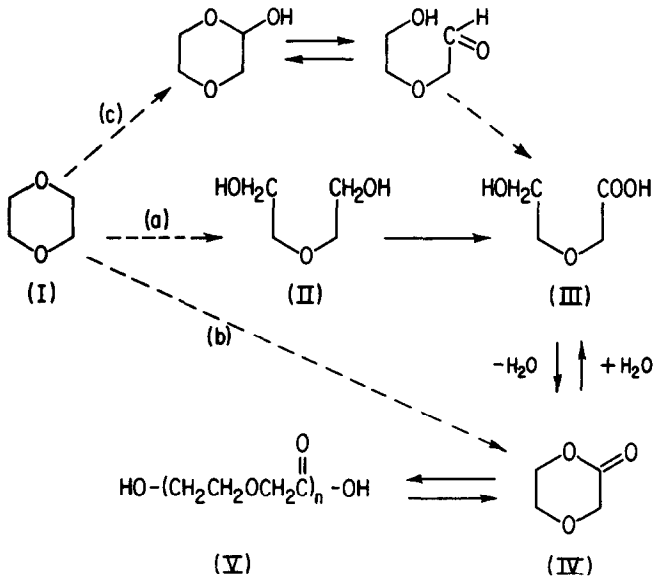

Fig. 2. Possible metabolic pathways of dioxane.

Acknowledgements — We are indebted to Dr. Kiyoyasu Ishikawa for helpful suggestions in some of these experiments and to Drs. John W. Russell and James F. Ryan from the Gulf South Research Institute for carrying out the g.c.-mass spectrography in a contractual arrangement. This investigation was supported by research grants CA-15111 and CA-18346 from the National Cancer Institute. Joseph C. Arcos was a recipient of a Faculty Research Award from the American Cancer Society.

REFERENCES

1. M. F. Argus, J. C. Arcos and C. Hoch-Ligeti. J. natnl. Cancer Inst. 35, 949 (1965).
2. C. Hoch-Ligeti, M. F. Argus and J. C. Arcos. Br. J. Cancer 24, 164 (1970).
3. M. F. Argus, R. S. Sohal, G. M. Bryant, C. Hoch-Ligeti and J. C. Arcos. Eur. J. Cancer 9, 237 (1973).
4. R. J. Kociba, S. B. McCollister, C. Park, T. R. Torkelson and P. J. Gehring. Toxic. appl. Pharmac. 30, 275 (1974).
5. A. Fairley, E. C. Linton and A. H. Ford-Moore. J. Hyg. 35, 341 (1936).
6. F. H. Wiley, W. C. Hueper, D. S. Bergen and F. R. Blood. J. ind. Hyg. Toxicol. 20, 269 (1938).
7. W. H. Carothers, G. L. Dorough and F. J. Van Natta. J. Am. chem. Soc. 54, 761 (1932).
8. E. Hollo. Ber. deut. chem. Ges. 61, 895 (1928).
9. Y.-T. Woo, M. F. Argus and J. C. Arcos. Biochem. Pharmac. 26, 1519 (1977).
10. F. Dickens. Br. med. Bull. 20, 96 (1964).
11. B. L. Van Duuren. Ann. N. Y. Acad. Sci. 163, 633 (1969).
12. R. J. Lorentzen, W. J. Caspary, S. A. Lesko and P. O. P. Ts'o. Biochemistry 14, 3970 (1975).