Metabolism of Chlorinated Naphthalenes

Luis Ruzo, Dan Jones, Stephen Safe,* and Otto Hutzinger

1-Chloro-4-[^{2}H]naphthalene was administered to a pig and the 4-chloronaphthol metabolite retained 18% of the deuterium. 1,4-Dichloro- and 1,4-dibromonaphthalene gave 2,4-dihalonaphthols as the major products and the results are consistent with the intermediacy of arene oxides; decomposition of these intermediates is accompanied by a 1,2 migration of deuterium and halogen, respectively. 1,2-Dichloronaphthalene and 1,2,3,4-tetrachloronaphthalene also give phenolic metabolites; however, the higher chlorinated isomer, 1,2,3,4,5,6-hexachloronaphthalene, was not metabolized.

In recent years it has been shown that chlorinated aromatic compounds are among the most widespread and persistent environmental pollutants (Risebrough et al., 1968; Fishbein, 1973). Among this class of compounds are polychlorinated biphenyls (PCB), polychlorinated terphenyls, polychlorinated benzenes, DDT, and DDE. The metabolism of PCB (Hutzinger et al., 1972; Safe et al., 1974, 1975), chlorinated benzenes (Azouz et al., 1955; Parke and Williams, 1955, 1960; Jondorf et al., 1955, 1958), DDT and DDE (Feil et al., 1975; Menzie, 1974) gave a range of hydroxylated products and related compounds. In addition recent results also suggest that these halogenated aromatic substrates are hydroxylated via arene oxide intermediates (Sundstrom et al., 1975; Selander et al., 1975; Safe et al., 1976).

Polychlorinated naphthalenes (PCN) are also industrial chlorinated aromatic compounds (commercial Halowaxes) with properties and uses similar to PCB. PCN have been identified in commercial PCB preparations and can interfere in the gas chromatographic analysis of PCB and chlorinated pesticides (De Vos et al., 1970; Stalling and Huckins, 1973). The biological properties of specific chlorinated naphthalenes have been reviewed (Kimbrough, 1972) and some specific isomers are associated with X disease in cattle (Sikes et al., 1952; Bell, 1953).

In a recent communication we have identified 4-chloronaphthol and 3-chloro-2-naphthol as the major pig urinary metabolites of 1- and 2-chloronaphthalene, respectively (Ruzo et al., 1975). This report deals with the mechanism of the metabolism of 1-chloronaphthalene, and some 1,4-dihalonaphthalenes and the metabolites of related chlorinated naphthalenes are also outlined.

MATERIALS AND METHODS

Synthesis of 1-Chloro-4-[^{2}H]naphthalene. 4-Chloronaphthylamine was converted into 1-chloro-4-iodonaphthalene (76% yield) by diazotization and decomposition of the diazonium salt with potassium iodide. 1-Chloro-4-iodonaphthalene (800 mg) was dissolved in dry tetrahydrofuran (20 ml) and lithium aluminum deuteride (100 mg, Merck Sharp and Dohme) was added with care. The mixture was refluxed for 20 h and then quenched by the addition of deuterium oxide (5 ml). The mixture was extracted with ether and the residual product (340 mg) was purified by thin-layer chromatography (TLC) in hexane. The mass spectrum exhibited a molecular ion at *m/e* 163 and showed >90% incorporation of a single ^{2}H atom.

Other Halonaphthalene Substrates. 1,4-Dichloronaphthalene was prepared by conventional diazotization of commercial 4-chloronaphthylamine (Aldrich) and decomposition of the diazonium salt in cuprous chloride hydrochloric acid solution. The crude product was purified by TLC in hexane and recrystallized from methanol and gas chromatographic analysis indicated a purity of >99%. The mass spectrum gave a molecular ion at *m/e* 196. 1,2-Dichloronaphthalene and 1,2,3,4-tetrachloronaphthalene were obtained from commercial sources (Chemical Procurement Laboratories and Aldrich, respectively); 1,2,3,4,5,6-hexachloronaphthalene was prepared by zinc–acetic acid reduction of 1,2,3,4,5,6-hexachloro-7-nitronaphthalene (Aldrich) to give the corresponding amine; diazotization and deamination with hypophosphorus acid gave the hexachloro substrate (M^+ 332). 1,4-Dibromonaphthalene was commercially available (Eastman).

Administration of the Substrates to Pigs. The substrates were dissolved in domestic corn oil (3 ml) and to this solution was added an aqueous solution (19 ml) which contained sodium chloride (1.8 g), Pluronic F_{68} (0.5 g, BASF Wyandotte), and Tween 80 (0.5 g, Atlas Chemicals). The mixture was sonicated (Biosonic 4, Bronwill VWR Scientific) for 3 min and the resulting emulsion was used for administration of the chlorinated naphthalene substrate. A female, Yorkshire pig (average weight, 7.5 kg) was anaesthetized by intraperitoneal administration of sodium pentobarbital (25 mg/kg, Dibutal, Diamond Laboratories). The oil emulsion of the chloronaphthalene substrate was administered retrocarotidly over a period of 2 min followed by injection of a 0.9% saline flush solution (2 ml). Prior to injection of the emulsion the carotid artery was cannulated with intramedic polyethylene tubing (PE240, Clay Adams). After 6 h the pig was sacrificed and the total urine and bile samples were obtained and stored at −25 °C until used.

Extraction and Isolation of Metabolites. Preliminary urine analysis was carried out by hydrolysis of small samples (2 ml) with excess β-glucuronidase enzyme (5 mg, Sigma Chemical, 380000 Fishman units per g) in 0.1 M acetate buffer (pH 5.0) at 38 °C or by treatment with concentrated sulfuric acid (1.0 ml) followed by refluxing for 2 h at 100 °C. The hydrolyzed solutions were diluted with water (5 ml) and extracted with ether (2 × 5 ml), the ether extracts were dried and concentrated, and the residue was purified by thin-layer chromatography (TLC) on silica gel HF_{254} (Merck) using chloroform as the eluting solvent. Bands which corresponded to standard chloronaphthol metabolites were removed from the plate, extracted with ether, and identified by gas–liquid chromatography (GLC) and mass spectrometry. The bulk of the urine was worked up using the acid treatment since the yields of metabolites were comparable to those obtained using enzyme hydrolysis. The bile workup was also carried out using the acid treatment. The only exception to these procedures

Guelph-Waterloo Centre for Graduate Work in Chemistry, University of Guelph, Guelph, Ontario, Canada (L.R., D.J., S.S.) and Millieuchemie, University of Amsterdam, Amsterdam, The Netherlands (O.H.).

Table I. Spectroscopic and Chromatographic Properties of Chloronaphthalene Metabolites

Substrate	Metabolite (M^+)	Yield, %	t_R	NMR data, ppm
1-Chloro-4-[^{2}H]naphthalene	4-Chloronaphthol (178, 179)	5.5		
1,2-Dichloronaphthalene	3,4-Dichloronaphthol (212)	0.8	3.8 (180 °C)	7.94 (m, 1 H), 7.52 (m, 1 H), 7.35 (m, 2 H), 7.00 (m, 1 H), 6.96 (s, 1 H)
1,4-Dichloronaphthalene	2,4-Dichloronaphthol (212)	3.5		8.27 (m, 2 H), 7.61 (m, 2 H)
1,4-Dibromonaphthalene	2,4-Dibromonaphthol (300)	1.5	7.4 (170 °C)	8.25 (m, 1 H), 8.14 (m, 1 H), 7.79 (s, 1 H), 7.60 (m, 2 H)
	5,8-Dibromonaphthol (300)	0.5	8.6 (170 °C)	7.94 (q, 1 H, J = 8.2, 2.2 Hz), 7.59 (d, 1 H, J = 8.2 Hz), 7.55 (t, 1 H, J = 8.2 Hz), 7.49 (d, 1 H, J = 8.2 Hz), 7.16 (q, 1 H, J = 8.2, 2.2 Hz)
1,2,3,4-Tetrachloronaphthalene	5,6,7,8-Tetrachloronaphthol (280)	0.7	4.0 (250 °C)	7.93 (q, 1 H, J = 8.2 Hz), 7.57 (t, 1 H, J = 8.2 Hz), 7.16 (q, 1 H, J = 8.2, 2.2 Hz)
	5,6,7,8-Tetrachloro-2-naphthol (280)	0.3	4.8 (250 °C)	Insufficient material
1,2,3,4,5,6-Hexachloronaphthalene				

was the workup and extraction of the 1-chloro-4-[^{2}H]-naphthalene metabolite: for this sample the urine was adjusted to pH 5 with acetic acid and extracted with ether. The hydroxylated metabolite was isolated from the crude ether extract by preparative TLC.

Gas–Liquid Chromatographic and Spectroscopic Analyses. GLC analyses were performed on a Hewlett Packard 5710 instrument on a glass column (0.3 cm × 2 m) packed with 3% SE-30 on 80–100 mesh Chromosorb W. Operating conditions were: flame ionization detector and injection port temperatures, 300 °C; helium carrier gas flow, 30 ml/min; hydrogen gas flow, 60 ml/min; air gas flow, 200 ml/min; oven temperature (as indicated in Table I).

Mass spectra were recorded on a low-resolution Varian CH7 mass spectrometer at 70 eV. Nuclear magnetic resonance (NMR) spectra were obtained on a Varian HR220 spectrometer.

RESULTS

Metabolite Identification. The major pig urinary metabolite of 1-chloronaphthalene was 4-chloronaphthol (Ruzo et al., 1975) and this naphthol was isolated as the metabolite of 1-chloro-4-[^{2}H]naphthalene. Mass spectrometric analysis of this product indicated two molecular ion species at m/e 178 and 179 and the relative ratios of these peaks showed that 18% of the ^{2}H was retained in the 4-chloronaphthol. The 18% figure was corrected for the percent purity of the deuterated substrate.

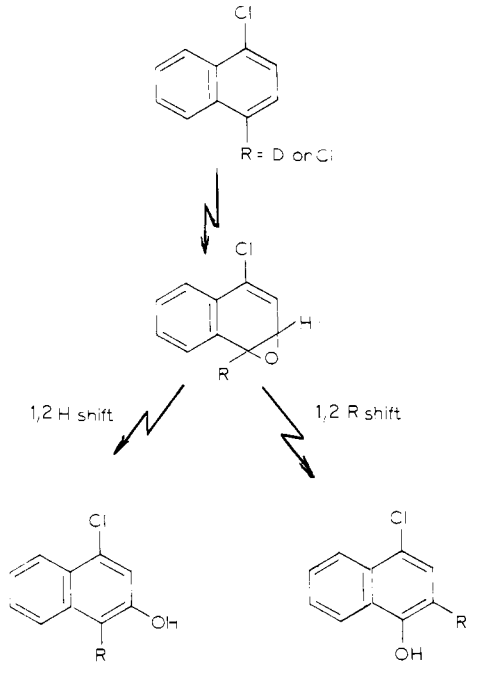
The 1,4-dichloronaphthalene substrate gave one major metabolite which exhibited a molecular ion in its mass spectrum at m/e 212. The NMR spectrum and GLC retention time data (see Table I) were identical with the results obtained with an authentic sample of 2,4-dichloronaphthol (Eastman). A second dichloronaphthol metabolite (M^+ 212) was also detected but due to insufficient material its structure could not be determined. For comparative purposes the metabolism of 1,4-dibromonaphthalene was also investigated. Analysis of the pig urine extracts by TLC and mass spectrometry showed that two dibromonaphthols were formed (M^+ 300). The GLC and NMR properties of the major product (see Table I) were identical with the data obtained from an authentic sample of 2,4-dibromonaphthol (Eastman). The NMR spectrum of the minor product was consistent with hydroxylation of the naphthalene nucleus on the 1 position of the unsubstituted ring to give 5,8-dibromonaphthol. The two vicinal protons on the bromine-substituted ring appeared as a doublet (J = 8.2 Hz) at 7.49 and 7.59 ppm. The high-field proton ortho to the hydroxyl group appeared as a quartet at 7.16 ppm (J = 8.2 and 2.2 Hz) whereas the H_4 proton gave a quartet at 7.94 ppm (J = 8.2 and 2.2 Hz). H_3 which is ortho to two protons appeared as a triplet at 7.55 (J = 8.2 Hz). These data confirmed the structure of the metabolite as 5,8-dibromonaphthol.

The metabolism of 1,2-dichloronaphthalene, 1,2,3,4-chloronaphthalene, and 1,2,3,4,5,6-hexachloronaphthalene was also investigated. 1,2-Dichloronaphthalene gave a dichloronaphthol product (M^+ 212) and the NMR spectrum is summarized in Table I. The familiar multiplets for the ABCD pattern of the unsubstituted naphthalene ring appeared at 7.35 (2 H), 7.52 (1 H), and 7.94 (1 H) ppm. In addition a one-proton singlet at 6.96 indicated that hydroxylation had occurred at C_1 or C_2 of the chloro-substituted naphthalene ring. The NMR spectra of chloronaphthalenes indicated that the protons at C_2 are at much higher field than the C_1 protons and the chemical shift at 6.96 ppm would suggest a C_2 proton ortho to a hydroxyl group. In addition previous work has shown that naphthalene is metabolized to yield naphthol and not 2-napthol. These facts suggest the structure of the 1,-2-dichloronaphthalene metabolite is 3,4-dichloronaphthol. The metabolism of 1,2,3,4-tetrachloronaphthalene gave two tetrachloronaphthol metabolites (M^+ 280). The NMR spectrum of the major metabolite (see Table I) exhibited signals at 7.16 (q, J = 8.2 and 2.2 Hz), 7.57 (t, J = 8.2 Hz), and 7.93 (q, J = 8.2 and 2.2 Hz) which were typical of an ABC pattern. These data thus were consistent with the 5,6,7,8-tetrachloronaphthol structure. The NMR spectrum of the unstable minor metabolite could not be obtained due to decomposition; however, since the four chloro groups were still retained the only other possible structure of this metabolite is 5,6,7,8-tetrachloro-2-naphthol. The metabolism studies with 1,2,3,4,5,6-hexachloronaphthalene did not yield any isolable metabolic transformation products.

DISCUSSION

The metabolic studies with the chloronaphthalene substrates give results which are comparable to recent work on the metabolism of other chlorinated aromatic substrates (Daly et al., 1972; Jerina and Daly, 1974). The major PCN metabolites were all identified as phenolic products and no dechlorination was observed with any of the substrates investigated. The mechanism of the metabolism of 1-chloro-4-[^{2}H]naphthalene and 1,4-dichloronaphthalene is summarized in Scheme I. The results are consistent with the formation of an arene oxide intermediate which on

Scheme I



decomposition is accompanied by a 1,2-^{2}H (or Cl) shift (Daly et al., 1972). 1,4-Dibromonaphthalene was also converted into 2,4-dibromonaphthol and the unrearranged product, 1,4-dibromo-2-naphthol, was not detected. Thus, it would appear that the decomposition and accompanying substituent shifts with 1,2-arene oxide intermediates are dependent on the structure of the aromatic substrate and the substituent. The 1,4-dihalonaphthalenes (R = Br, Cl) give only the rearranged 2,4-dihalonaphthol product; 1,4-dichlorobenzene is converted exclusively into the unrearranged 2,5-dichlorophenol whereas 1,4-dibromobenzene gave 2,4-dibromophenol and 2,5-dibromophenol (Ruzo et al., 1976); both 4,4'-dichloro- and dibromobiphenyl gave the unrearranged 4,4'-dihalo-3-biphenylol and the rearranged 3,4'-dihalo-4-biphenylol as well as the dechlorination–hydroxylation product 4'-halo-4-biphenylol (Safe et al., 1976a). Comparative studies with suitably labeled substrates are in progress since it is conceivable that alternative metabolic pathways may be a possibility (Tomaszewski et al., 1975) for some of these substrates.

Phenolic metabolites of 1,2-dichloronaphthalene and 1,2,3,4-tetrachloronaphthalene were also identified whereas the higher chlorinated 1,2,3,4,5,6-hexachloronaphthalene did not yield any urinary metabolites. This observation is comparable with the resistance of higher chlorinated biphenyls (Safe et al., 1976b) to metabolic degradation. Higher chlorinated naphthalenes have been implicated in a number of farm animal diseases and the stability of these compounds may be a factor in their biological activity.

LITERATURE CITED

Azouz, W. M., Parke, D. V., Williams, R. T., *Biochem. J.* **59**, 410 (1955).
Bell, W. B., *Vet. Med.* **48**, 135 (1953).
Daly, J. W., Jerina, D. M., Witkop, W. T., *Experientia* **28**, 1129 (1972).
De Vos, J. G., Koeman, H. H., van der Maas, H. L., Ten Noever de Brauw, M. C., De Vos, R. H., *J. Food Cosmet. Toxicol.* **8**, 627 (1970).
Feil, V. J., Lamoureux, C. H., Zaylskie, R. G., *J. Agric. Food Chem.* **23**, 382 (1975).
Fishbein, L., *Sci. Total Environ.* 4, 304 (1973).
Hutzinger, O., Nash, D. M., Safe, S., DeFreitas, A. S. W., Norstrom, R. J., Wildish, D. J., Zitko, V., *Science* **178**, 312 (1972).
Jerina, D. M., Daly, J. W., *Science* **185**, 573 (1974).
Jondorf, W. R., Parke, D. V., Williams, R. T., *Biochem. J.* **55**, 512 (1955).
Jondorf, W. R., Parke, D. V., Williams, R. T., *Biochem. J.* **69**, 181 (1958).
Kimbrough, R. D., *Arch. Environ. Health* **25**, 125 (1972).
Menzie, C. M., "Metabolism of Pesticides and Update", United States Department of the Interior, Fish and Wildlife Service, Wildlife Report No. 184, Washington, D.C., 1974.
Parke, D. V., Williams, R. T., *Biochem. J.* **59**, 415 (1955).
Parke, D. V., Williams, R. T., *Biochem. J.* **74**, 5 (1960).
Risebrough, R. W., Rieche, P., Peakall, D. B., Herman, S. G., Kirven, M. N., *Nature (London)* **220**, 1098 (1968).
Ruzo, L. O., Safe, S., Hutzinger, O., *J. Agric. Food Chem.*, submitted for publication (1976).
Ruzo, L. O., Safe, S., Hutzinger, O., Platonow, N., Jones, D., *Chemosphere* **3**, 121 (1975).
Safe, S., Hutzinger, O., Ecobichon, D., *Experientia* **30**, 720 (1974).
Safe, S., Jones, D., Hutzinger, O., *J. Chem. Soc., Perkin Trans. 1*, in press (1976a).
Safe, S., Jones, D., Kohli, J., unpublished results (1976b).
Safe, S., Platonow, N., Hutzinger, O., *J. Agric. Food Chem.* **23**, 259 (1975).
Selander, H. G., Jerina, D. M., Piccolo, D. E., Berchtold, G. A., *J. Am. Chem. Soc.* **97**, 4428 (1975).
Sikes, D., Wise, J. I., Bridges, M. E., *J. Am. Vet. Med. Assoc.* **121**, 337 (1952).
Stalling, D., Huckins, J. N., *J. Assoc. Off. Anal. Chem.* **56**, 367 (1973).
Sundstrom, G., Jansson, B., Jensen, S., *Nature (London)* **255**, 627 (1975).
Ten Noever de Brauw, M. C., De Vos, R. H., *J. Food Cosmet. Toxicol.* 8, 627 (1970).
Tomaszewski, J. E., Jerina, D. M., Daly, J. W., *Biochemistry* **14**, 2024 (1975).

Received for review October 9, 1975. Accepted January 27, 1976. The assistance of the National Research Council of Canada and the Canadian 220-MHz NMR Centre is gratefully acknowledged.