Metabolism of Chloronaphthalenes

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Representative isomers of polychloronaphthalenes (PCN) were synthesized and administered orally to rats. Urinary and fecal metabolites were isolated and identified. Hydroxylation and/or hydroxylation–dechlorination were found to be common metabolic pathways for these compounds. Thus, 1,2-dichloronaphthalene was biotransformed to the glucuronide conjugate of 5,6-dichloro-1,2-dihydroxy-1,2-dihydronaphthalene. 2,7-Dichloronaphthalene was metabolized to free and conjugated 7-chloro-2-naphthol. 2,6-Dichloronaphthalene gave rise to free and conjugated 6-chloro-2-naphthol and 2,6-dichloronaphthol.

This work is part of our studies on the metabolic dispositions of polychloronaphthalenes (PCN) in mammals (Chu et al., 1976a,b). PCN are known causative agents of X-disease of cattle (Sikes and Bridge, 1952) and chloracne in humans (Kleinfeld et al., 1972). Recently PCN were detected in polychlorobiphenyl-contaminated environmental samples (Crump-Weisner et al., 1973; Goerlitz and Law, 1974). There has been considerable interest in the metabolism of PCN in mammals (Ruzo et al., 1975, 1976), and the published reports indicate that hydroxylation via arene oxide intermediate is the sole metabolic pathway for these compounds. However, we have found that in addition to hydroxylation, PCN can also undergo hydroxylation–dechlorination. The present investigations were designed to study metabolism of 1,2-, 2,7-, and 2,6-dichloronaphthalene in rats.

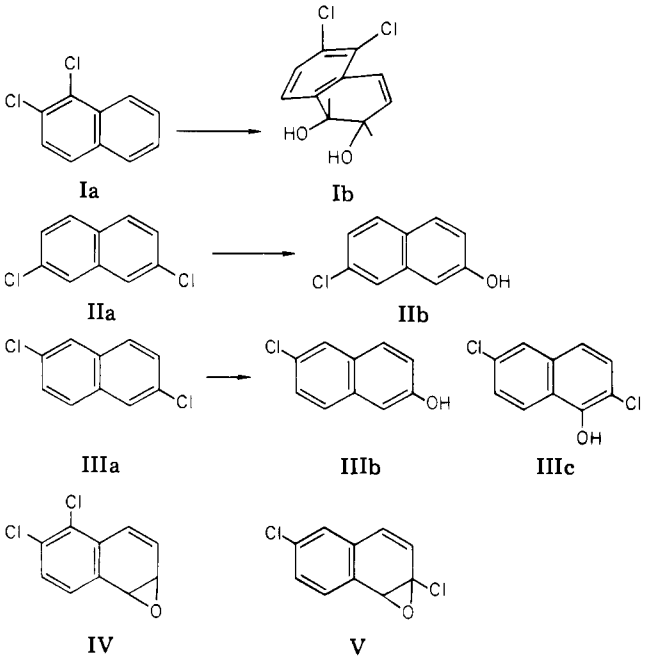
MATERIALS AND METHODS

Gas chromatography–mass spectrometry (GC–MS) analysis was performed at 70 eV on a Varian MAT 311A with a 12 × $^1/_8$ in. i.d. glass column packed with 3% OV-17 on 80–100 mesh Chromosorb W-HP. Nuclear magnetic resonance (NMR) spectra were recorded on a Varian HA 100 spectrometer located in the Department of Chemistry, University of Alberta, Edmonton or on a 220-MHz NMR spectrometer in the Department of Medical Genetics, University of Toronto, Toronto. Tetramethylsilane was used as internal standard, deuterated dichloromethane or acetone was used as solvent.

Chemical Synthesis. Synthesis of 1,2-dichloronaphthalene, 1,2-dichloronaphthalene-*1,4,5,8*-^{14}C (Chu et al., 1976b), 2,7-dichloronaphthalene, 2,6-dichloronaphthalene, and 7-chloro-2-naphthol have been described previously (Chu et al., 1976a).

General Procedure for Isolation of Metabolites. Male Wistar rats weighing approximately 250 g were given a single oral dose of a dichloronaphthalene (400 mg/kg) in 1% tragacanth suspension or corn oil. The rats were kept in metabolism cages and provided with food and water ad libitum for 2 days. Urine was collected and the combined urine (200 mL) was passed through an Amberlite XAD-2 column (100 g). Water (50 mL) was passed through the column, followed by ethanol (300 mL). The ethanol fraction was evaporated to dryness and the residue was incubated with β-glucuronidase (Glucurase, 20 mL, 5000 Sigma units/mL) at 37 °C for 24 h. The mixture was extracted with chloroform (3 × 20 mL), and the combined extracts were dried. The residue was chromatographed on silica gel plate (HF 254 1000 μ, Merck Ag Darmstadt) using (a) ether–petroleum ether (30–70 °C, 3:7) or (b) benzene–dioxane–acetic acid (80:25:7) as developing solvents. The band containing chloronaphthalenes as detected by UV lamp or a radiochromatograph scanner (Actigraph III, Nuclear Chicago) was removed from the plate and extracted with chloroform (20 mL). Evaporating the solvent gave crude metabolites which were recrystallized from hexane or vacuum sublimed before performing GC–MS or NMR analysis.

Scheme I



RESULTS AND DISCUSSION

Analysis showed that metabolies I_b, II_b, III_b, and III_c were found in the urine of rats dosed with dichloronaphthalenes I_a, II_a, and III_a, respectively (Scheme I). The structural elucidation of metabolites was aided by NMR and MS and is presented below.

1,2-Dichloronaphthalene (Ia). The metabolite showed a molecular ion at *m/e* 230, 232 (M/M + 2 = 3/2) and a fragment at *m/e* 212 attributed to the loss of H_2O. The accurate mass of the molecular ion at 229.9899 conformed to a dihydrodiol ($C_{10}H_8O_2Cl_2$, 229.9901). The position of hydroxylation was unequivocally established by NMR (Figure 1a).

The "doublet of triplets" centered at 4.41 ppm was assigned to H-2 because of its chemical shift and coupling pattern. H-1 (4.66 ppm) would be expected to resonate at lower field than H-2 because of its benzylic nature. H-2 was split into a doublet by H-1 with a J_{12} of 11.5 Hz,

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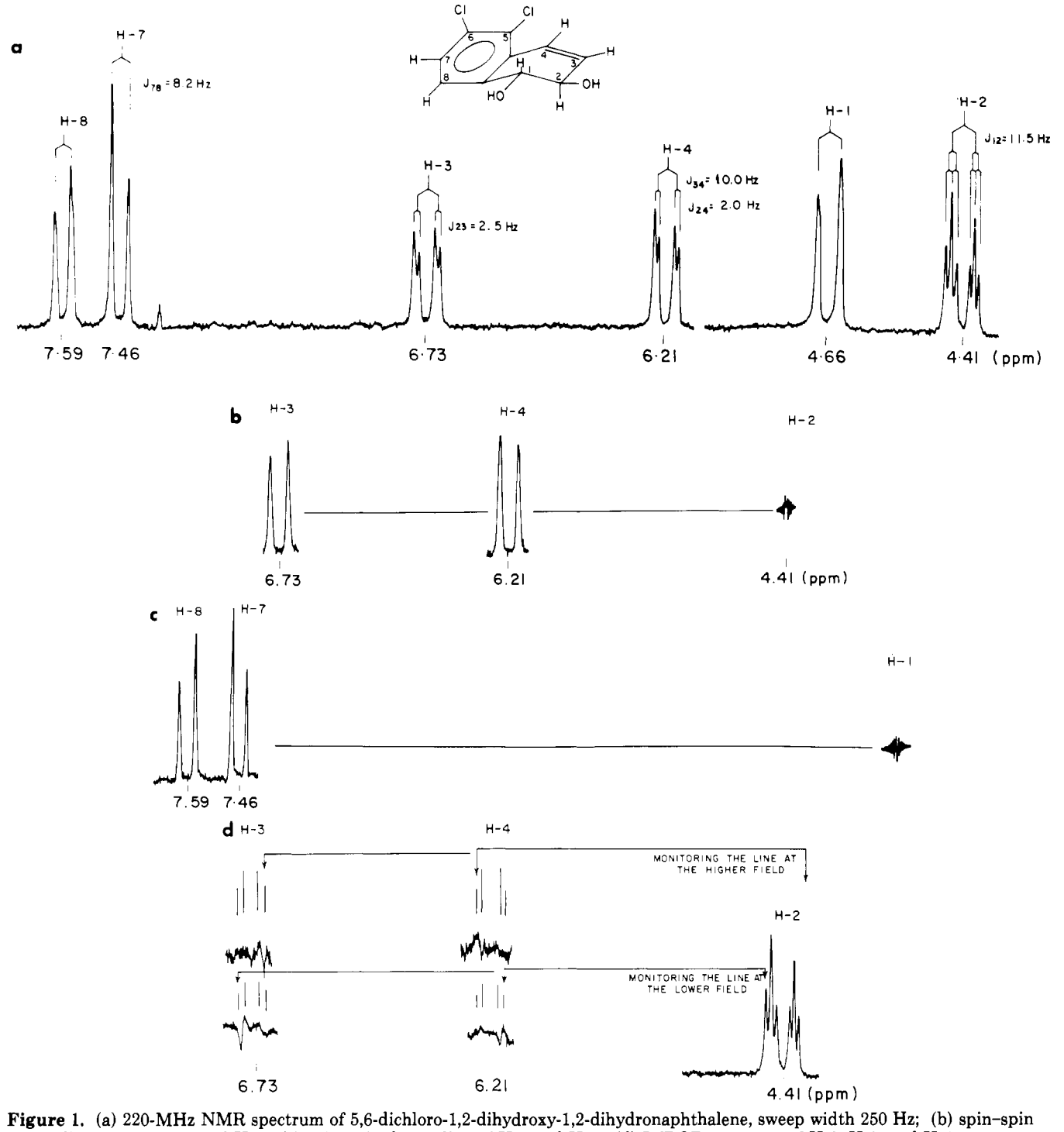

Figure 1. (a) 220-MHz NMR spectrum of 5,6-dichloro-1,2-dihydroxy-1,2-dihydronaphthalene, sweep width 250 Hz; (b) spin–spin decoupling of H-2, H-3, and H-4; (c) spin–spin decoupling of H-1 and H-8; (d) INDOR spectrum of H-2, H-3, and H-4.

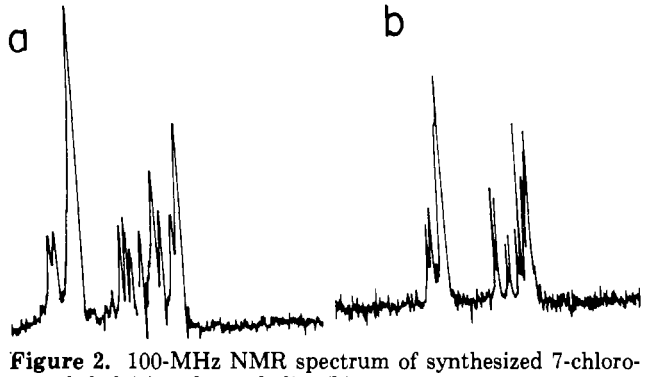

Figure 2. 100-MHz NMR spectrum of synthesized 7-chloro-2-naphthol (a) and metabolite (b).

characteristic of a trans diaxial coupling, and this was further split by H-3 (J_{23} = 2.5 Hz) and H-4 (J_{24} = 2.0 Hz) into two apparent triplets. Irradiation of H-2 should result in the simplification of the signals H-3 (6.73 ppm) and H-4 (6.21 ppm). Indeed, these two doublets of doublets collapsed to an AB doublet of doublets upon irradiation of the signal at 4.41 ppm (Figure 1b). H-3 and H-4 are coupled with J_{34} = 10 Hz. The line broadening of H-1 and H-8 (7.59 ppm) arose from the virtual coupling as was proved by double resonance (Figure 1c).

In order to ascertain which of the two signals (6.21 and 6.73 ppm) was from H-3 and H-4, INDOR technique was applied (Johanneson and Coyle, 1972). If the higher field line of H-2 is irradiated at low power, only the higher field

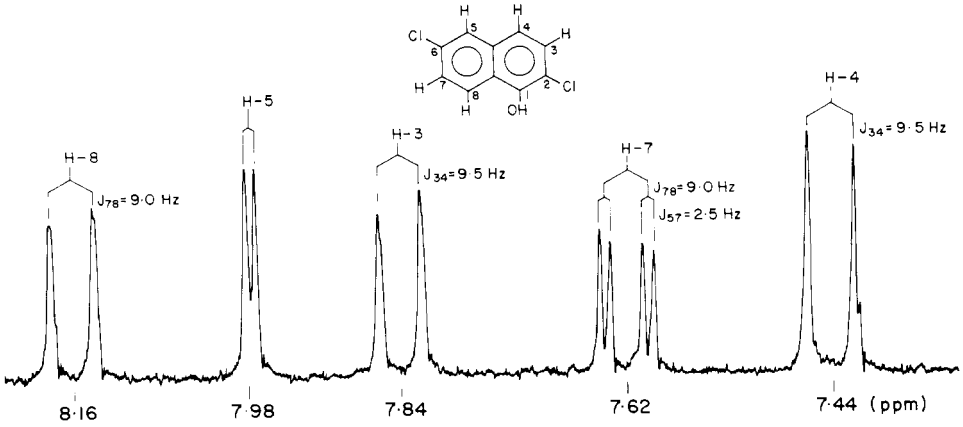

Figure 3. 220-MHz NMR spectrum of 2,6-dichloronaphthol, sweep width 250 Hz.

line of H-3 and lower field line of H-4 are effected. Similarly, it can be seen that the lower field line of H-2 is connected to the lower field line of H-3 and higher field line of H-4. This result showed that J_{23} and J_{24} had opposite signs with former being positive and the latter being negative. Since it is known that vicinal coupling usually is positive, it can be deduced that the signal at 6.73 ppm was due to H-3 and the signal at 6.21 ppm due to H-4.

2,7-Dichloronaphthalene (IIa). A monochloronaphthol was isolated from the urine of rats dosed with 2,7-dichloronaphthalene. The MS of the metabolite gave a molecular ion at m/e 178 and 180 (M/M + 2 = 3), indicative of a monochloro compound. An NMR spectrum of the compound purified by TLC was obtained using Fourier transform techniques (Figure 2). Since the spectrum was similar to that of synthetic 7-chloro-2-naphthol (IIb), and since the GC retention times were identical, the metabolite was tentatively identified as 7-chloro-2-naphthol. Further supports on the structural identification were that the NMR spectra and GC retention times of certain other possible monochloronaphthols were different from the metabolite IIb (Chu et al., 1976a). Formation of IIb from IIa is significant and it supports our previous result that dechlorination–hydroxylation is one of the metabolic pathways for PCN (Chu et al., 1976a).

2,6-Dichloronaphthalene (IIIa). A previous report (Chu et al., 1976a) showed that compound IIIa was metabolized to 6-chloro-2-naphthol (IIIb) and a dichloronaphthol ($C_{10}H_6OCl_2$) of unestablished structure. A 220-MHz NMR spectrum now indicates that the latter metabolite is 2,6-dichloronaphthol (IIIc). The spectrum (Figure 3) is interpreted as 2,6-dichloronaphthol as follows: All protons fall in the aromatic proton region. H-4 (7.44 ppm) and H-3 (7.84 ppm) were ortho coupled (J_{34} = 9.5 Hz. H-8 (8.16 ppm) and H-7 (7.62 ppm) were also ortho coupled with J_{78} = 9 Hz. H-7 was further coupled to H-5 (J_{57} = 2.5 Hz) forming a doublet of doublets. H-8 was broadened because of the para coupling to H-5. It was logical to expect H-8 to be most deshielded by the oxygen atom at the peri position, and hence this proton is assigned to the signal at lowest field. It was possible that 1,6-dichloro-2-naphthol could also result a similar NMR spectrum assigned as IIIc. Therefore this possibility should not be ruled out.

When several representative isomers of PCN are examined for their metabolites, it becomes feasible to establish general metabolic pathways for these compounds. This is the purpose of the present studies. It has been suggested that arene oxide is the intermediate metabolite of aromatic compounds (Daly et al., 1972). The present results are consonant with arene oxides as intermediates in PCN metabolism.

Dihydrodiol Ib can be considered as a hydration product of the arene oxide IV. Formation of hydroxylated compound IIIc from IIIa may also be mediated by an arene oxide (V) which undergoes a spontaneous isomerization to give IIIc. In addition to hydroxylation, dechlorination–hydroxylation has also been established as an alternative pathway for these compounds. The correlation between the pattern of substitution and metabolic pathway (dechlorination–hydroxylation vs. simple hydroxylation) was not established in the present study, but further study is in progress to clarify this point.

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