Metabolism of 4,4'-Dihalogenobiphenyls

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4,4'-Dichlorobiphenyl in rabbits gave three major urinary metabolites: 4,4'-dichlorobiphenyl-3-ol, 3,4'-dichlorobiphenyl-4-ol, and 4'-chlorobiphenyl-4-ol. 4,4'-Dibromobiphenyl gave the bromo analogues of the three 4,4'-dichlorobiphenyl metabolites. These results are consistent with 3,4-epoxidation of the biphenyl nucleus followed by epoxide ring opening accompanied by a 1,2-halogen shift (NIH shift). 4,4'-Di-iodobiphenyl gave only a single metabolite, 4,4'-di-iodobiphenyl-3-ol, and rearrangement products were not observed.

POLYCHLORINATED biphenyls (PCB) are among the most widespread and persistent pollutants in the global ecosystem.[1-3] Laboratory studies with micro-organisms and animals have shown that PCB are degraded to hydroxylated metabolites [4-10] and recent work has confirmed the presence of hydroxylated PCB in seal tissue.[11]

The metabolisation of 2,2',5,5'-tetrachlorobiphenyl by the rabbit [12] gives two phenolic metabolites, 2,2',5,5'-tetrachlorobiphenyl-4-ol and 2,2',5,5'-tetrachlorobiphenyl-3-ol, and a third more polar metabolite, *trans*-3,4-dihydro-2,2',5,5'-tetrachlorobiphenyl-3,4-diol. This result suggests metabolisation of the tetrachlorobiphenyl *via* an arene oxide intermediate. Hydrolysis of 2,2',5,5'-tetrachloro-3,4-epoxy-3,4-dihydrobiphenyl would yield the *trans*-dihydro-diol, and rearrangement of the epoxide or dehydration of the dihydro-diol could give the two phenolic metabolites. This study is concerned with the comparative metabolism of 4,4'-dihalogenobiphenyls in the rabbit.

The dihalogenobiphenyls were administered by intraperitoneal injection to rabbits housed in metabolic cages. The urine from the 4,4'-dichlorobiphenyl experiment was extracted, and the mass spectrum of the crude ethereal extract showed molecular ions at *m/e* 238 and 204, corresponding to dichlorobiphenylol ($C_{12}H_8Cl_2O$) and chlorobiphenylol ($C_{12}H_9ClO$), respectively. T.l.c. gave three major metabolite fractions (I)—(III); their n.m.r. and mass spectral properties are given in the Table. The spectra of the least polar metabolite (I) were identical with that of 4,4'-dichlorobiphenyl-3-ol, the sole goat and rat urinary metabolite of 4,4'-dichlorobiphenyl.[5,10] The n.m.r. spectrum of the metabolite of intermediate polarity (II) showed a typical AA'BB' quarter for the 4-protons on the unsubstituted chlorophenyl ring at τ 2.61 and 2.55 and the signals for the three protons on the hydroxylated ring appeared at τ 2.91 (d, J 8.5 Hz), 2.63 (q, J 8.5 and 2.2 Hz), and 2.48 (d, J 8.2 Hz). The high-field doublet was assigned to the proton *ortho* to the hydroxy-group and coupled with the proton resonating at τ 2.63; the latter is also *meta*-coupled to the proton resonating at τ 2.48. The chemical shift data are consistent with the 3,4'-dichlorobiphenyl-4-ol structure and the assignment was confirmed by unambiguous synthesis. The n.m.r. and mass spectra of the metabolite (III) were identical with the spectra of 4'-chlorobiphenyl-4-ol. Since biological hydroxylation occurs at the 3- and 4-positions of the biphenyl nucleus this suggests the formation of a 4,4'-dichloro-3,4-epoxy-3,4-dihydrobiphenyl intermediate which can rearrange *via* a 1,2-chlorine shift (NIH shift) [13,14] as indicated in the Scheme. Presumably loss of Cl from the rearranged carbocation intermediate (see

Relative concentrations and ^1H n.m.r. data for the rabbit urinary metabolites of the 4,4'-dihalogenobiphenyls

Compound	Relative yield	τ (J in Hz)
Band (I) (X = Cl, M^+ 238) (4,4'-dichlorobiphenyl-3-ol)	4	2.95 (1 H, q, J 8.5 and 2.2), 2.79 (1 H, d, J 2.2), 2.64 (1 H, d, J 8.5), 2.62 (2 H, d, J 8.5), 2.53 (2 H, d, J 8.5)
Band (II) (X = Cl, M^+ 238) (3,4'-dichlorobiphenyl-4-ol)	1	2.91 (1 H, d, J 8.5), 2.63 (1 H, q, J 8.5 and 2.2), 2.61 (2 H, d, J 8.5), 2.55 (2 H, d, J 8.5), 2.48 (1 H, d, J 2.2)
Band (III) (X = Cl, M^+ 204) (4'-chlorobiphenyl-4-ol)	3	3.09 (2 H, d, J 8.5), 2.62 (2 H, d, J 8.5), 2.55 (2 H, d, J 8.5), 2.53 (2 H, d, J 8.5)
Band (I) (X = Br, M^+ 326) (4,4'-dibromobiphenyl-3-ol)	10	2.98 (1 H, q, J 8.5 and 2.2), 2.79 (1 H, d, J 2.2), 2.59 (2 H, d, J 8.5), 2.50 (1 H, d, J 8.5), 2.45 (2 H, d, J 8.5)
Band (II) (X = Br, M^+ 326) (3,4'-dibromobiphenyl-4-ol)	1	2.91 (1 H, d, J 8.5), 2.65 (2 H, d, J 8.5), 2.61 (1 H, q, J 8.5 and 2.2), 2.47 (2 H, d, J 8.5), 2.36 (1 H, d, J 2.2)
Band (III) (X = Br, M^+ 248) (4'-bromobiphenyl-4-ol)	2	3.19 (2 H, d, J 8.5), 2.60 (2 H, d, J 8.5) ,2.57 (2 H, d, J 8.5), 2.47 (2 H, d, J 8.5)
Band (I) (X = I, M^+ 422) (4,4'-di-iodobiphenyl-3-ol)		2.83 (1 H, q, J 8.2 and 2.2), 2.73 (1 H, d, J 2.2), 2.72 (2 H, d, J 8.5), 2.26 (2 H, d, J 8.5), 2.15 (1 H, d, J 8.5)

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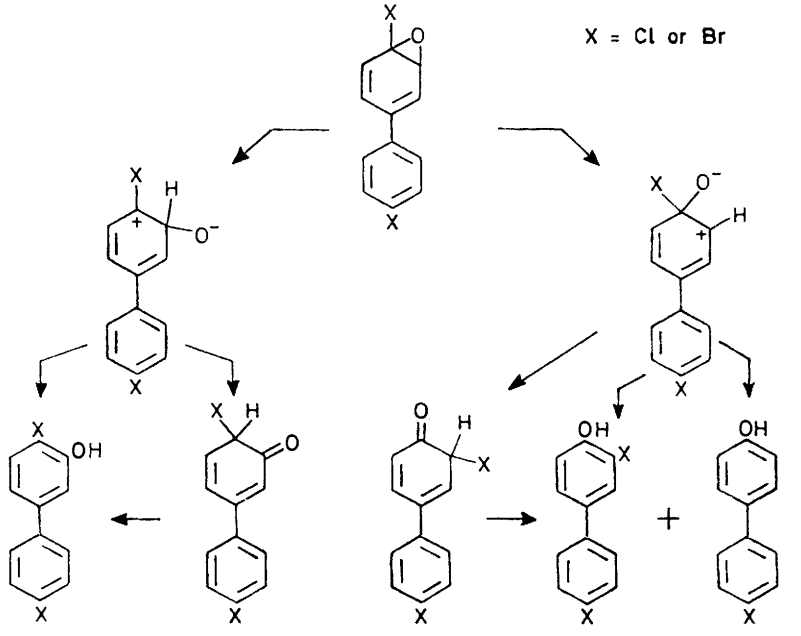
Scheme) would then give the dechlorination–hydroxylation metabolite 4′-chlorobiphenyl-4-ol. Previous work has shown that biological hydroxylation of other chlorinated aromatic substrates in which a 1,2-chlorine shift is observed is often accompanied by a dechlorination–hydroxylation product.[15-19]

The metabolisation of 4,4′-dibromobiphenyl by rabbits also gave three urinary metabolites, whose mass and n.m.r. spectra are given in the Table. Mass spectrometric analysis indicated that two of the metabolites



were dibromobiphenylols ($C_{12}H_8Br_2O$, M^+ 326) and the third was a bromobiphenylol ($C_{12}H_9BrO$, M^+ 248). The n.m.r. spectra for band (I) gave chemical shift data which were consistent with the 4,4′-dibromobiphenyl-3-ol structure. The high-field proton signal at τ 2.98 showed coupling to both *ortho*- and *meta*-protons (J 8.5 and 2.2 Hz) whereas the remaining two protons on the hydroxylated ring resonated as doublets at τ 2.79 (J 2.2 Hz) and 2.50 (J 8.5 Hz). The AA′BB′ bromophenyl ring gave two doublets at τ 2.59 and 2.45 (J 8.5 Hz). The chemical shift data for metabolite (II; X = Br) could be analysed like the spectrum of metabolite (II; X = Cl) and the results are consistent with the 3,4′-dibromobiphenyl-4-ol structure, in which a 1,2-bromine shift has occurred. The spectral results for metabolite (III; X = Br) supported the 4′-bromobiphenyl-4-ol structure, showing that debromination–hydroxylation of the parent substrate had occurred. The metabolites formed from both 4,4′-dibromo- and 4,4′-dichlorobiphenyl are consistent with the Scheme, and previous work has shown that both Br and Cl migrations can occur in the biological hydroxylation of the halogenoacetanilides [19] and halogenotyrosines.[19]

The metabolisation of 4,4′-di-iodobiphenyl gave only a single product, whose mass spectrum (M^+ 422) indicated it to be a di-iodobiphenylol ($C_{12}H_8I_2O$). Previous work has shown [14] that a major metabolite of *para*-substituted aromatic compounds is the deiodination–hydroxylation product; however the corresponding 4′-iodobiphenyl-4-ol was not detected in our extracts. The 220 MHz n.m.r. spectrum (see Table) gave chemical shift data and coupling constants which confirmed that the metabolite was 4,4′-di-iodobiphenyl-3-ol. Rat liver microsomal oxidation of *p*-iodoanisole [20] also gave a metabolite hydroxylated *ortho* to the iodo-group. It would appear that the NIH shift of an iodo-group is an unfavourable metabolic pathway since this rearrangement does not accompany the metabolisation of any iodo-aromatic compounds which have been investigated.[14]

PCB and polybrominated biphenyls (PBB) are both widely used industrial compounds whose biological properties are not fully understood. The metabolism of the model 4,4′-dichloro- and 4,4′-dibromo-biphenyls suggests that the hydroxylated metabolites are formed from arene oxide intermediates which undergo 1,2-Cl (or Br) NIH shifts.

EXPERIMENTAL

Mass spectra were obtained on a Varian CH7 mass spectrometer and n.m.r. spectra with a Varian HR220 spectrometer. G.l.c. was performed with a Hewlett–Packard 700 chromatograph (6 ft glass column packed with 3% OV 17 at 200 °C).

Administration of 4,4′-Dichlorobiphenyl.—The 4,4′-dihalogenobiphenyl (0.3 g) in vegetable oil (10 ml) was administered intraperitoneally to a male rabbit (4 kg), and urine and faeces were collected for 7 days after injection.

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Extraction and Purification of the Metabolites.—The rabbit urine was acidified to pH 5 with acetic acid and extracted with ether to give the free metabolite extract. The dried extract was concentrated and purified by preparative t.l.c. in chloroform. The fluorescent zones on the t.l.c. plate were examined by mass spectrometry and three bands (I)—(III) were shown to contain chlorinated or brominated metabolites. Analysis of the extracts from the metabolisation of 4,4′-di-iodobiphenyl gave only one metabolite [band (I)]. The mass and n.m.r. spectral properties of the metabolites are given in the Table.

A small portion (5%) of the urine was acidified to pH 5 with acetic acid, diluted with an equal volume of acetate buffer (5 ml; 0.2M; pH 5.2) and treated with an excess of β-glucuronidase (10 mg; Sigma Chemical Co., 380 000 Fishman units per g) for 48 h at 38 °C. The metabolites were isolated by extraction with ether and the relative concentrations of these compounds in both the enzyme-treated and untreated urine were compared by g.l.c. The results showed that less than 10% of the metabolites were present in the urine in a bound form (*e.g.* as glucuronides).

Synthesis of 4,4′-Dichlorobiphenyl-3-ol, 3,4′-Dichlorobiphenyl-4-ol, and 4′-Chlorobiphenyl-4-ol.—Authentic samples of the three compounds were obtained by synthesis as described.[21]

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