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Metabolism of Bromobenzenes in the Rabbit

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A series of brominated (Br_1–Br_5) benzenes was administered to rabbits by intraperitoneal injection and mass spectrometric analysis revealed the presence of oxygenated bromobenzene metabolites in the urine and feces. The metabolites were isolated and their structures determined. At least two major metabolites were identified from each bromobenzene (Br_1–Br_3) fed. Tetrabromo- and pentabromobenzene yielded only trace amounts of metabolite in the urine and none was detected in the feces. The compounds obtained from 1,4-dibromo- and 1,3,5-tribromobenzene showed migration of bromine atoms, suggesting the possibility of arene oxide intermediates.

Halogenated aromatic compounds are among the most persistent and ubiquitous environmental pollutants. The metabolic degradations of DDT (Feil et al., 1973, 1975), DDD (Reif et al., 1974), DDE (Sundstrom et al., 1975), 2,4-D (Feung et al., 1975), and polychlorinated biphenyls (Safe et al., 1975) have recently been reported and in all cases hydroxylated metabolites have been identified. Mechanistic studies suggest that many halogenated aromatics are metabolized via arene oxide intermediates which can rearrange into the phenolic products. The rearrangement is often accompanied by a 1,2-H (or ^2H) or substituent (e.g., Cl, Br, CH_3) shift from the site of hydroxylation to the adjacent carbon atom and is referred to as the NIH shift (Daly et al., 1972).

Brominated benzenes are industrial compounds produced in North America by several companies (Great Lakes Chemical Co., Dow Chemical, and Mallinckrodt Chemical Works) and are used as fuel additives and top cylinder compounds. In addition to the potential environmental hazard resulting from their entrance into the ecosystem they are interesting as analogues of other compounds already identified as pollutants such as the brominated biphenyls (Ruzo and Zabik, 1975). The metabolites of bromobenzene have previously been reported and dihydrodiols (Azouz et al., 1953), mercapturic acids (Knight and Young, 1958), and bromophenols (Tomaszewski et al., 1975) have been identified. This paper describes the isolation and structure of several brominated benzene metabolites in rabbit urine.

MATERIALS AND METHODS

Bromobenzenes. Bromobenzene, 1,2-, 1,3-, and 1,4-dibromobenzene, 1,2,4- and 1,3,5-tribromobenzene, and 1,2,4,5-tetrabromobenzene were obtained from ICN-K&K Pharmaceuticals, Plainview, N.Y. Pentabromobenzene was kindly supplied by Dr. Sundstrom, University of Amsterdam, Netherlands.

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

Bromophenols. 2-, 3-, and 4-bromophenol, 2,4- and 2,6-dibromophenol, 2,4,6-tribromophenol, and pentabromophenol were obtained from ICN as well.

Preparation of Bromophenol Standards. Nitration of 1,2-dibromobenzene, followed by reduction with Zn–acetic acid, yielded a mixture of two dibromoanilines. Diazotization of the mixture followed by treatment with 50% copper(II) sulfate (aqueous) (Goto et al., 1974) gave two dibromophenols. The mixture was separated by thin-layer chromatography (1:3, benzene–petroleum ether). The mass spectra of the two major products (R_f 0.16 and 0.34) exhibited molecular ions at m/e 250. A summary of their nuclear magnetic resonance spectra is given in Table I and the structures are assigned as 3,4-dibromophenol (R_f 0.16) and 2,3-dibromophenol (R_f 0.34).

Nitration, reduction, and diazotization of 1,2,4-tribromobenzene followed by treatment with aqueous copper(II) sulfate gave a single major product isolated by TLC. Its mass spectrum showed a molecular ion at m/e 328 and the spectroscopic data were consistent with the structure of 2,4,5-tribromophenol (Table I). Diazotization of 2,5-dibromoaniline, after treatment with aqueous copper(II) sulfate, yielded 2,5-dibromophenol (m/e 250).

Administration of Substrates. The bromobenzene (600 mg) was dissolved in corn oil and equally administered to two albino rabbits (~50 mg/kg) by intraperitoneal injection. Urine and feces were collected for 10 days after administration of the substrate and stored at −25°C prior to use.

Extraction and Analysis. The urine samples were acidified to pH 5 with acetic acid, cooled, and extracted with an equal volume of ether. The aqueous layer was then diluted with sufficient concentrated sulfuric acid to make a 6 *N* acid solution and then heated for 2 hr at 100°C. The solution was then diluted with an equal volume of distilled water and extracted with ether. Both ether extracts were kept separate and were dried over anhydrous sodium sulfate, concentrated, and purified by preparative thin-layer chromatography (TLC) on silica gel HF_{254} (Merck) using benzene as the eluting solvent. Bands with R_f values similar to corresponding bromophenol standards were removed from the plates and extracted with ether to give

Table I. Chromatographic and Spectroscopic Properties of Synthetic Bromophenols

Compound (M^+)	R_f[a]	t_R	NMR data, ppm
2,3-Dibromophenol (250)	0.34	11.6	6.69 (d of d, J = 8.2, 2.1 Hz), 6.97 (d, J = 8.2 Hz), 7.02 (d of d, J = 8.2, 2.1 Hz)
3,4-Dibromophenol (250)	0.16	16.8	6.49 (d of d, J = 8.2, 2.1 Hz), 6.97 (d, J = 2.1 Hz), 7.33 (d, J = 8.2 Hz)
2,4,5-Tribromophenol (328)	0.21	13.2	7.29 (s), 7.49 (s)

[a] R_f values obtained with benzene–petroleum ether (1:3) as eluent. [b] Column temperatures: dibromophenols at 130°C, tribromophenol at 140°C.

Table II. Summary of Rabbit Urinary Metabolites of Some Isomeric Brominated Benzenes

Substrate[a]	Metabolite	R_f[b]	t_R[c]	Yield,[d] %
Bromobenzene	4-Bromophenol	0.04	12.4	1.2
	3-Bromophenol	0.07	12.1	1.0
1,2-Dibromobenzene	2,3-Dibromophenol	0.34	11.6	0.9
	3,4-Dibromophenol	0.16	16.8	0.9
1,4-Dibromobenzene	2,4-Dibromophenol	0.18	9.3	1.5
	2,5-Dibromophenol	0.25	11.0	1.0
1,3,5-Tribromobenzene	2,4,6-Tribromophenol	0.37	12.4	1.3
	2,3,5-Tribromophenol[e]	0.18	13.0	1.1
1,2,4-Tribromobenzene	2,4,5-Tribromophenol	0.21	13.2	1.0
	2,4,6-Tribromophenol	0.37	12.4	0.4

[a] Three-hundred milligrams was administered to each of two rabbits. [b] R_f values calculated in benzene for the monobromophenols and in 1:3 benzene-petroleum ether for the higher brominated phenols. [c] Column temperatures: monobromophenols at 100°C, dibromophenols at 130°C, and tribromophenols at 140°C. [d] The figure shown is the percent yield of metabolite obtained by extraction and purification of the ether extracts of the hydrolyzed rabbit urine. [e] Not conclusively identified due to the unavailability of an authentic standard.

a hydroxy fraction. The remainder of the plate was also extracted with ether–methanol. The extracts were further purified by TLC using 1:3 benzene–petroleum ether (bp 40–60°C) as the eluting solvent. Feces samples (100 g) were extracted with ether (200 ml) and filtered. Thin-layer chromatography as described above yielded hydroxy fractions identical with those obtained from urine.

Gas Chromatography and Spectroscopic Procedures. Samples were analyzed by gas–liquid chromatography on a Hewlett-Packard Model 5710A apparatus equipped with a 1/8 in. × 6 ft glass column packed with 3% SE-30 on Chromosorb W (80–100 mesh). The column was operated isothermally at temperatures of 100–140°C using helium (30 ml/min) as carrier gas. Detection of products was by flame ionization, with injector and detector temperatures of 300°C.

Mass spectra were obtained using a Varian MAT CH7 apparatus at 70 eV. The samples were inserted into the ion source using a temperature controlled probe at temperatures between 80 and 140°C. Nuclear magnetic resonance spectra (NMR) were obtained using a Varian HR220 spectrometer using deuteriochloroform as solvent and tetramethylsilane (Me_4Si) as internal standard. Product identification was done by comparison of R_f values, retention times, and NMR spectra with authentic standards.

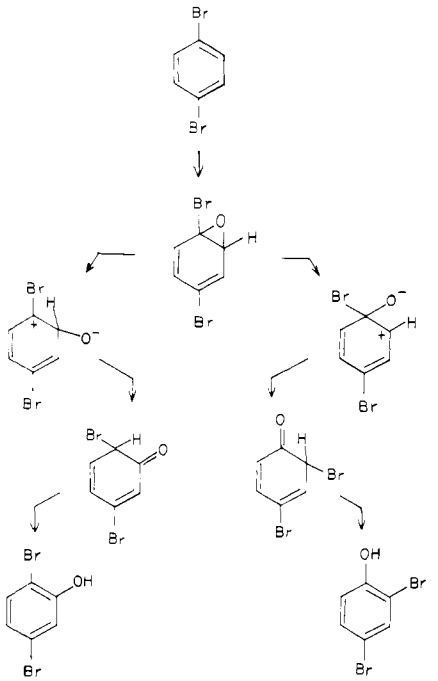
RESULTS AND DISCUSSION

Nitration of 1,2-dibromobenzene gave two mononitration products which were converted to their corresponding dibromophenols and separated by TLC. The NMR spectra of the two compounds are shown in Table I. The spectrum of the least polar component exhibited a typical ABC pattern; the resonances at 6.69 and 7.02 ppm appeared as quartets with ortho and meta coupling (J = 8.2 and 2.1 Hz). The triplet at 6.97 ppm (J = 8.2 Hz) consisted of a pair of overlapping doublets which is typical of the B proton of the ABC pattern. These data are consistent with the 2,3-dibromophenol structure. Assuming that the vicinal dibromo substituents have not been changed during the reaction then the second and more polar phenolic product must be 3,4-dibromophenol. The NMR spectrum exhibited the predicted ABX pattern (see Table I) and confirms the structure assignment. The synthesis of 2,5-dibromophenol was accomplished unambiguously from the corresponding aniline. Nitration of 1,2,4-tribromobenzene gave a single product which was converted into a tribromophenol. The NMR spectrum exhibited two singlets at 7.29 and 7.49 ppm which is consistent with a 1,2,4,5-substituted compound. The only possible structure for this product was, therefore, 2,4,5-tribromophenol.

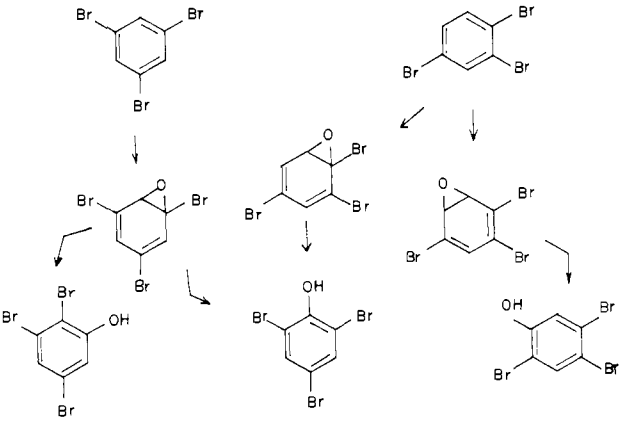
TLC purification of the extracts gave several fluorescent bands for each mono-, di-, and tribromobenzene fed to the rabbits. Using mass spectrometric analysis the bromine containing fractions could easily be detected by the characteristic isotope distribution pattern of bromine. Each compound gave two major metabolites (Table II). Comparison of the yields obtained by direct extraction of the urine with ether with that obtained after acid hydrolysis showed approximately 70–80% of the metabolite to be unbound. Total yields were calculated by combining the bromophenols obtained by ether extraction prior and after acid treatment.

Bromobenzene gave 3-bromo- and 4-bromophenol (5:6). 1,2-Dibromobenzene gave three different metabolites (m/e 250); the two major products (~90%) were identified as 3,4- and 2,3-dibromophenols by comparison with synthetic standards. The third compound could not be identified beyond its molecular weight; however, since there are only two possible substitution positions that do not involve rearrangement of the bromine atoms it can be surmised that this minor metabolite arises via an arene oxide which isomerizes. 1,4-Dibromobenzene gave only two metabolites, 2,5- and 2,4-dibromophenol, the latter being the result of 1,2-migration of bromine. The structures were confirmed by comparison with authentic standards. 1,3-Dibromobenzene was also metabolized to give three different phenolic products. Owing to the small amounts of metabolite and the unavailability of authentic standards these metabolites could not be identified. Since three positions are available for direct hydroxylation it was not possible to ascertain if bromine rearrangement had taken

Scheme I



Scheme II



place. Two of the metabolites gave R_f values and GLC retention times that corresponded with those of authentic 2,4- and 2,6-dibromophenol standards.

1,2,4-Tribromobenzene was metabolized to give three different bromophenols (m/e 328). Comparative spectroscopic and chromatographic data indicated that two of the metabolites were 2,4,6- and 2,4,5-tribromophenol. The former product can only form by a 1,2-migration of Br from the site of hydroxylation to the adjacent carbon atom. The third metabolite could not be identified. 1,3,5-Tribromobenzene gave 2,4,6-tribromophenol, the direct hydroxylation product, and another tribromophenol (m/e 328) which could not be conclusively identified. However, since there is only one position available for direct hydroxylation of 1,3,5-tribromobenzene therefore the unidentified tribromophenol must be formed with bromine migration to give 2,3,5-tribromophenol. Attempts to synthesize this phenol unfortunately were not successful. The tetra- and pentabromobenzenes did not yield appreciable quantities of metabolites. 1,2,4,5-Tetrabromobenzene gave trace amounts of a compound with m/e 188 containing one bromine atom and pentabromobenzene yielded only a trace (<0.01 mg) of pentabromophenol (m/e 484). Mass spectrometric examination of the ethereal feces extracts from the mono-, di-, and tribromobenzene experiments showed the presence of phenolic metabolites in the feces. The expected phenolic metabolites of the higher brominated benzene isomers (1,2,3,4-tetrabromobenzene and pentabromobenzene) could not be detected in the feces. In addition the crude urine and fecal extracts were analyzed by mass spectrometry to determine the presence of any further metabolic breakdown products. Only the ions characteristic of the phenolic metabolites were observed; however, it is possible that lower levels of more highly degraded bromobenzenes were present but were not detected by mass spectrometric analysis.

Thus, the lower brominated benzenes are metabolized to give phenols and the hydroxylation of some of these substrates is accompanied by a 1,2-migration of a bromine atom. This observation is consistent with an arene oxide intermediate. Several mechanisms for the decomposition of arene oxides have been demonstrated (Bruice et al., 1973; Kasperek et al., 1972; Kasperek and Bruice, 1972) and Scheme I illustrates a possible pathway for the metabolism of 1,4-dibromobenzene. Fission of the 1,2-arene oxide at C_1–O and C_2–O gives two carbonium ion intermediates which rearrange via 1,2-H or 1,2-Br shifts to the dienones. Enolization of the two dienones gives the observed metabolites, 2,5- and 2,4-dibromophenol. A schematic summary of the metabolism of 1,3,5- and 1,2,4-tribromobenzene via arene oxides is also shown (Scheme II).

It is clear from the above experiments that polybrominated benzenes are susceptible to metabolic degradation in the rabbit and are excreted in the urine and, to a lesser extent, in the feces. Since the toxicology and biological properties of brominated phenols are not known this is, therefore, an area of potential environmental hazard and concern.

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