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BROMO(METHYLTHIO)BENZENES AND RELATED SULFUR-CONTAINING COMPOUNDS: MINOR URINARY METABOLITES OF BROMOBENZENE IN RATS

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Summary: Urinary metabolites of bromobenzene in rats were examined. Isomeric bromo(methylthio)benzenes were identified by gas chromatographic and mass spectral comparison with authentic samples. The presence of solvent-unextractable precursors which produce the methylthio metabolites upon alkali-treatment was also revealed. The amounts of bromo(methylthio)benzenes and their precursors were not increased when rats were given a higher amount of bromobenzene or pretreated with diethyl maleate. These results suggest that the known covalent binding of bromobenzene to liver tissue is not responsible for the formation of these metabolites.

INTRODUCTION

Recent studies (1,2) on the metabolism of chlorobiphenyls have shown that some isomers are metabolized to the chloro-(methylthio)biphenyls and chloro(methylsulfonyl)biphenyls. In order to determine whether these new metabolic reactions can be extended to other halogenated aromatic compounds, bromobenzene was chosen as a model with more simple structure and its metabolic fate was studied in rats.

The present paper reports evidence for the occurrence of the three isomeric bromo(methylthio)benzenes, their solvent-unextractable precursors, and four other structurally related sulfur-containing metabolites.

METHODS

Administration of materials: Male Wistar rats weighing approximately 250 g were dosed i.p. with either 40 or 120 mg of bromo-

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benzene dissolved in soybean oil. The lower dosage of bromobenzene is approximately two-third of the dosages which were reported to cause minimal necrosis in rat liver (3,4). In other experiment, rats were pretreated with diethyl maleate (0.5 ml/kg, i.p.) and then dosed with 40 mg of bromobenzene 45 min after the pretreatment. Urine was collected during the 48 hr after administration.

Isolation of metabolite fractions: The urine was adjusted to pH 3.0 with acetic acid and extracted with ether. The organic phase was then washed with aqueous sodium hydroxide to remove known acidic metabolites such as bromophenols (5) and mercapturic acid (6) (extract A). The extract was dried, evaporated to near dryness, taken up in hexane, and placed on a silica gel dry column (Merck Kieselgel 60 containing 5% of water, 8.5 cm x 8 mm id). Successive elution with hexane and benzene-ethanol (95:5 v/v) gave fractions 1 and 2, respectively. The extracted aqueous layer of urine was adjusted to pH 10 with aqueous sodium hydroxide and, after standing for 18 hr at room temperature, extracted with ether (extract B). The remaining procedures were the same as described above for extract A.

The chromatographic fractions obtained from both extracts A and B were examined by gas chromatography and by gas chromatography-mass spectrometry.

Instrumental analysis: Gas chromatography was performed on a Shimadzu GC-3E gas chromatograph equipped with an electron capture detector. The analysis of fraction 1 was conducted on a 2 m x 3 mm id glass column packed with Chromosorb W containing 5% Bentone 34. The column had an inlet pressure of 0.8 kg/cm^2 N_2 and was operated at 165°. The analysis of fraction 2 was conducted on a 1 m x 3 mm id glass column packed with Chromosorb W containing 2% OV-1. The column had an inlet pressure of 0.6 kg/cm^2 N_2 and was operated at 122°.

The mass spectra were obtained by using a JEOL JMS-D100 mass spectrometer coupled to a JEOL JGC-20K gas chromatograph by means of a single-stage jet separator. The ionizing energy was 25 eV. The columns and operating conditions were the same as described above for gas chromatography.

RESULTS AND DISCUSSION

Three peaks (a, b, and c) occurred in the gas chromatogram of fraction 1 of extract A (Fig. 1). The mass spectra of all the three peaks showed identical molecular ions at m/e 202 and qualitatively similar fragments at m/e 187 (M^+ - Me), 169 (M^+ - SH), 156 (M^+ - SCH_2), 122 (M^+ - HBr), and 108 (M^+ - Me - Br), suggesting the presence of bromo(methylthio)benzene isomers. By gas chromatographic and mass spectral comparison with authentic samples (7,8) peaks a, b, and c were identified as *p*-, *m*-, and *o*-

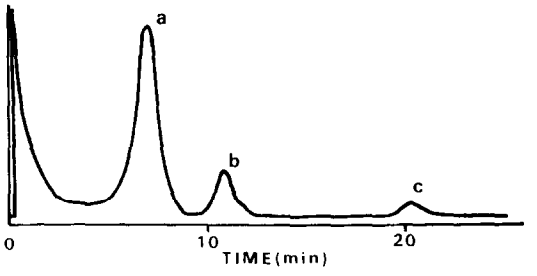

Fig. 1. Gas chromatogram of fraction 1 of urinary extract A on a Bentone 34 column.

bromo(methylthio)benzenes, respectively.

These three bromo(methylthio)benzene isomers were also present in fraction 1 of extract B in much higher amounts. This result indicates the presence of an alkali-labile precursor of each methylthio metabolite in the aqueous phase of urine. The possibility that these solvent-unextractable and alkali-labile precursors are some conjugated metabolites seems to be ruled out since no sulfur-containing metabolites were released after treatment of the aqueous phase of urine with β-glucuronidase and sulfatase. Although these precursors could not be structurally identified at present time, the insolubility in organic solvent and the facile release of the methylthio derivative by alkali suggest that each precursor is a sulfonium compound in which the sulfur atom of bromo(methylthio)benzene is bonded to the third unknown substituent.

Six peaks (d, e, f, g, h, and i) were observed in the chromatogram of fraction 2 of extract A (Fig. 2). Mass spectral evidence suggested peaks d and e to be known metabolites, trans-1,2-dihydro-1,2-dihydroxy-3- and 4-bromobenzenes (9,10), respectively or vice versa. The mass spectrum of peak f showed a molecular ion at m/e 220 with fragment ions at m/e 202 ($M^+ - H_2O$), 190 (M^+ -

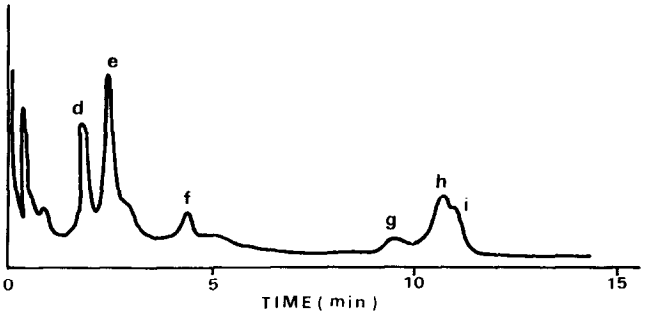

Fig. 2. Gas chromatogram of fraction 2 of urinary extract A on an OV-1 column.

HCHO), 172 (M^+ - MeSH), 144 (M^+ - MeSH - CO), 123 (M^+ - H_2O - Br), 93 (M^+ - MeSH - Br), and 65 (M^+ - MeSH - Br - CO), suggesting the presence of either 1,2-dihydro-1-hydroxy-2-(methylthio)-3, 4, 5, or 6-bromobenzene. The mass spectrum of peak g showed a molecular ion at m/e 218 with fragments at m/e 203 (M^+ - Me), 175 (M^+ -Me - CO), 155 (M^+ - MeSO), and 96 (M^+ - Me - CO - Br), indicating the presence of a bromo(methylsulfinyl)benzene. No attempt was made to further confirm these two metabolites, f and g. The mass spectra of both peaks h and i showed a common molecular ion at m/e 234 with very similar fragment ions at m/e 219 (M^+ - Me), 203 (M^+ - OMe, *via* rearrangement), 171 (M^+ - MeSO, *via* rearrangement), 155 (M^+ - $MeSO_2$), 143 (M^+ - MeSO - CO), 76 (M^+ - $MeSO_2$ - Br), which indicated the presence of bromo(methylsulfonyl)benzenes. The authentic samples (11-13) of the possible isomers were synthesized. By chromatographic and spectral comparison, peaks h and i were shown as to be *p*- and/or *m*-bromo(methylsulfonyl)benzene and *o*-bromo(methylsulfonyl)benzene, respectively. The mass spectra of the isomeric bromo(methylsulfonyl)benzenes were not substantially different from each other and the gas chromatographic retention times for *p*- and *m*-isomers were the same under the present condi-

tions. It was, therefore, not possible to distinguish between p- and m-bromo(methylsulfonyl)benzenes.

Any appreciable metabolite was absent in fraction 2 of extract B.

Among the above sulfur-containing metabolites bromo(methylthio)benzenes, especially in extract B, predominated. The total amounts (mean ± SD) of bromo(methylthio)benzenes found in both extracts A and B were: 80.0 ± 24.2, 62.5 ± 10.8, and 59.5 ± 22.2 μg/rat, respectively, for doses of 40, 40 (after treatment with diethyl maleate), and 120 mg of bromobenzene.

The liver necrosis produced by bromobenzene appears to be related to the degree of covalent binding of bromobenzene to tissue macromolecules (3,14). The necrosis by bromobenzene is also known to be enhanced by an increased dose of bromobenzene or by pretreatment with diethyl maleate (14), which depletes glutathione in liver. Therefore, if the formation of the methylthio metabolites from bromobenzene is mediated by its covalent binding to macromolecules as shown, e.g., in the metabolism of 2-acetylaminofluorene (15), an enhanced production of the methylthio metabolites would be caused in rats treated with the higher doses (120 mg) of bromobenzene and in those pretreated with diethyl maleate. However, this was not the case for the present results of the quantitative studies, suggesting that the covalent binding of bromobenzene is not responsible for the formation of the methyl thio metabolites identified in this work. Support is lent to this suggestion by the finding that no methylthio metabolites were released when the liver of rats recieved bromobenzene was treated with alkali.

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