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# SYNTHESIS AND CHARACTERIZATION OF m- AND p-METHYLBENZYL-MERCAPTURIC ACIDS DERIVED FROM m- AND p-XYLENES

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## ABSTRACT

Chemical synthesis and physical properties of two mercapturic acids suggested as urinary metabolites of m- and p-xylenes are described. These compounds may be used for the identification and quantivative determination by high-performance liquid chromatography of the corresponding mercapturic acids in urine. © 1997 Elsevier Science Ltd

Key Words, m-methylbenzyl mercapturic acid, p-methylbenzyl mercapturic acid, Synthesis

## **INTRODUCTION**

Toluene and isomeric xylenes are widely used as solvents (1). In urine of rat or humans exposed by these aromatic compounds, the excretion of the corresponding hippuric acids has been published (2,3). Since these hippuric acids were synthesized chemically before the 1960s', the high-performance liquid chromatography (HPLC) has been introduced for the quantitative determination after the latter half of 1970s' (4-7). In 1980, R von Doorn et al. have proposed the exretion of mercapturic acids ( thioethers ) in the urine of rats treated with toluene and isomeric xylenes (8). The urinary benzyl mercapturic acid ( N-acetyl-S-benzyl-L-cysteine; BMA ) was identified by using the Rf value of the standard sample, synthesized chemically, by thin layer chromatography. The identification of o-methylbenzyl mercapturic acid ( N-acetyl-S-(o-xylyl)-L-cysteine; o-MBM ) in urine was carried out by the comparison of nuclear

magnetic resonance and mass spectra data of the isolated sample with those of the standard sample synthesized by chemical reaction. On the other hand, urinary m-methylbenzyl mercapturic acid (m-MBM) and p-methylbenzyl mercapturic acid (p-MBM) were not sufficiently identified, but were suggested through the increase of SH concentration according to the Ellman method (9), which may be due to the failure of chemical synthesis of these standard samples by using the same procedure used for BMA or o-MBM.

In this report, we report the chemical syntheses of m-MBM and p-MBM and describe the physical properties.

### EXPERIMENTAL

#### Marerials

α -Bromo-m-xylene, α -bromo-p-xylene and N-acetyl-L-cysteine were obtained from Aldrich Chemical Company. 2-Methoxyethanol and other chemicals were purchased from Tokyo Kasei.

Chemical Syntheses of m-methylbenzyl and p-methylbenzyl mercapturic acids (m-MBM and p-MBM)

A solution of ten m mole (1.85g) of  $\alpha$  -bromo-m-xylene or  $\alpha$ -bromo-p-xylene in 10 ml of 2-methoxyethanol was shaken for three hours at room temperature with ten m mole (1.64g) of N-acetyl-L-cysteine dissolved in 10 ml of 10% (W/V) NaOH in water. The mixture was made acid to Congo Red by the addition of conc. hydrochloric acid and was extracted several times with ethyl acetate. The ethyl acetate layers were combined, dried with sodium sulfate and evaporated to afford a residue which was crystallized. Crude yields were 83.5% (m-MBM) and 85.2% (p-MBM) respectively. These white crystals were recrystallized several times from the solution of hexane/ethanol(1:1) to be used for the determination of melting point, elemental analysis and mass and nuclear magnetic resonance spectra are described in Results and Discussion.

#### **Results and Discussion**

The m.p. of m-MBM and p-MBM were 129 - 130 °C and 140 - 141 °C respectively. Elemental analysis; Found (m-MBM): C, 58.43; H, 6.36; N, 5.25, S 11.92 (%); Found (p-MBM): C, 58.53; H, 6.38; N, 5.28, S 11.95 (%); Calcd for C13H17SNO3; C, 58.41; H, 6.41; N, 5.24, S, 11.99 (%).

In the infrared spectra of m-MBM and p-MBM were recorded on Perkin Elmer 983G, two carbony groups were observed clearly at 1655 cm-1 (NH-CO) and 1760 cm-1 (COOH).

The purified m-MBM and p-MBM were dissolved in deuteromethanol and NMR spectra were determined by using JEOL EX 270 MHz, which are shown in Table 1. These NMR spectra are in good agreement with those published for o-MBM (8).

Assignment	Integral	m-MBM (ppm <sup>b</sup> )	р-МВМ (ррт <sup>ь</sup> )
-CO-CH3	3	2.02 (s)	2.00 (s)
-C6H4-CH3	3	2.38 (s)	2.36 (s)
-S-CH2-	2	2.85 - 2.95 (d)	2.84 - 2.95 (d)
−C₅H₄−CH₂−	2	3.65 (s)	3.70 (s)
-CH2-CH-	1	4.76 - 4.86 (q)	4.58 - 4.62 (q)
CH-NH-	1 ۴	No signal	No signal
СН-СООН	1 °	No signal	No signal
-C 5 H 4 -	4	7.10 - 7.20 (m)	7.15 - 7.22 (q)

Table 1 'H NMR spectral data of the synthesized m-methylbenzy! and p-methylbenzy! mercaputric acids ( m-MBM and p-MBM ) in CD<sub>3</sub>OD\*

\* CD<sub>3</sub>OD; solvent signals at 3.32 and 4.85 ppm

<sup>b</sup> NMR spectra were made with TMS at 0.00 ppm

<sup>5</sup> No signals in CD<sub>3</sub>OD (Reference 8)

Abbreviations: (s) singlet, (d) doublet, (q) quartet, and (m) multiplet

The mass spectra of m-MBM and p-MBM with JEOL JMS DX 303 are shown in Table 2. The chemical ionization method ( CI method; methane ) was used at an ionization energy of 70 ev. The molecular ion peak showed at  $m/e \approx 268$  (  $M^{+} + 1$  ).

R von Doorn et al synthesized BMA and o-MBM chemically and identifed them by the following procedures (8): The identification of urinary BMA was carried out by the comparison of the thin-layer chromatographic Rf values of the synthesized standard. The structure of isolated o-MBM from urine of rat exposured by o-xylene was determined by the agreement with the nuclear magnetic resonance (NMR) and mass spectra, and melting point of the standard synthesized chemically. On the other hand, the urinary m-MBM and p-MBM were not identified sufficiently and suggested through the increase of SH

concentration ( Eliman method ) after sodium borohydride reduction of urine sample of rat administrated by m- and p-xylenes.

p-methylbenzyl mercaputric acids ( m-MBM and p-MBM )			
compound	m/e ( relative intensity, % )		
m≁MBM	268 (100), 250 (9), 226 (4), 208 (6), 163 (4), 130 (7), 105 (12)		
p- <b>MBM</b>	268 (100), 250 (8), 208 (7), 130 (7), 105 (34)		

Table 2 Mass spectral data of the synthesized m-methylbenzyl and

The HPLC determination of urinary BMA and o-MBM was successful, but that of urinary m-MBM and p-MBM was not possible as described in the previous report (10), for the standards of the corresponding mercapturic acids were not obtainable by the same chemical synthesis used for BMA or o-MBM

In the chemical synthesis of o-MBM, triethylamine and catalytic amount of sodium iodide were used for hydrogen halide elimination (8). The elimination reaction of hydrogen halide between m- or p-methylbenzyl halides and N-acetyl-L-cysteine did not proceed by triethylamine, and from the reaction mixture the corresponding methylbenzyl alchols and N-acetyl-L- cystine were obtained after long time instead of m-MBM and p-MBM respectively. In using sodium hydroxide as elimination reagent, m- or p-MBM was obtained in a high yield without catalytic sodium iodide.

At the next stage of this study, these standards will be used for the identification by HPLC determination of urinary m-MBM and p-MBM.

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