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IDENTIFICATION OF URINARY METABOLITES OF THE NEPHROTOXIC HYDROCARBON 2,2,4-TRIMETHYLPENTANE IN MALE RATS

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SUMMARY. The compound 2,2,4-trimethylpentane (2,2,4 TMP) is reported to be especially potent in inducing kidney lesions in male rats (1,2). Although the pathology produced by 2,2,4 TMP has been examined (1), there are no reports concerning the metabolism of 2,2,4 TMP by the male rat. The eight principal urinary metabolites of 2,2,4 TMP found in the urine of Fischer 344 male rats are: 2,2,4-trimethyl-1-pentanol, 2,4,4-trimethyl-1-pentanol, 2,4,4-trimethyl-2-pentanol, 2,2,4-trimethyl-1-pentanoic acid, 2,4,4-trimethyl-1-pentanoic acid, 2,4,4-trimethyl-2-hydroxy-1-pentanoic acid, 2,2,4-trimethyl-5-hydroxy-1-pentanoic acid and 2,4,4-trimethyl-5-hydroxy-1-pentanoic acid.

INTRODUCTION. Male rats chronically exposed to gasoline vapors had nephropathy and a significant increase in the incidence of renal carcinomas. 2,2,4-Trimethylpentane (isooctane - a principal constituent of gasoline and the standard reference fuel for assigning "octane rating") produced kidney lesions in gavage studies identical to those seen in more acute fuel inhalation exposures. This is the first report on the urinary metabolites of a branched chain, nephrotoxic alkane.

Many compounds have been shown to produce kidney lesions, including hyaline deposition, proximal tubular cell degeneration, intratubular casts, and/or renal carcinomas in male rats but not in female rats or in either sex of other rodents tested. Among those compounds shown to produce kidney damage are Stoddard solvent (a mixture of aliphatic hydrocarbons with some naphthenes and benzene derivatives), decalin, methylisobutylketone, gasoline, 2,2,4-trimethylpentane (isooctane), petroleum-based aviation fuels such as JP-4 and JP-5, and the synthetic Cruise Missile fuel, JP-10. The relevance of these pathologic findings in male rats to the possible production of similar lesions in man becomes of great importance.

The male rat is unique in that a low molecular weight protein, alpha 2u globulin (MW 26,400), is produced by the liver at puberty, predominantly under the influence of testoterone. This globulin is readily filtered

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through kidney glomeruli and is the major urinary protein in young adult, male rats, but is not synthesized to any extent in the female rat or in other rodents. There is also evidence of differences in metabolism of xenobiotics between the sexes. Metabolite identification after dosing with compounds known to produce renal damage in male rats may yield valuable information for explaining mechanisms of toxicity.

MATERIALS AND METHODS. MATERIALS: The hydrocarbon 2,2,4-trimethylpentane was purchased from Aldrich Chemical Company (Milwaukee, WI). 2,2,4-Trimethyl-1-pentanol was purchased from Wiley Organics (Columbus, OH). 2,4,4-Trimethyl-1-pentanol was synthesized via the hydroboration of 2,4,4-trimethyl-1-pentene (Wiley Organics) (3). 2,4,4-Trimethyl-2-pentanol was prepared by the oxymercuration - demercuration of 2,4,4-trimethyl-1-pentene (4). 2,2,4-Trimethyl-1-pentanoic acid and 2,4,4-trimethyl-1-pentanoic acid were synthesized by the chromium trioxide-acetic acid oxidation of 2,2,4-trimethyl-1-pentanol and 2,4,4-trimethyl-1-pentanol, respectively (5). α,α,γ-Trimethyl-δ-valerolactone and α,γ,γ,-trimethyl-δ-valerolactone were prepared by the Baeyer-Villiger oxidation of 2,2,4-trimethylcyclopentanone and 2,4,4-trimethylcyclopentanone (Wiley Organics), respectively (6). 2,2,4-Trimethyl-5-hydroxy-1-pentanoic acid and 2,4,4-trimethyl-5-hydroxy-1-pentanoic acid were isolated by the basic hydrolysis of α,α,γ-trimethyl-δ-valerolactone and α,γ,γ,-trimethyl-δ-valerolactone respectively (7). 2,4,4-Trimethyl-2-hydroxy-1-pentanoic acid was prepared by the literature procedure (8). 2,4,4-Trimethyl-1,2-pentanediol was prepared by the osmium tetroxide oxidation of 2,4,4-trimethyl-1-pentene (9). 2,2,4-Trimethyl-1,5-pentanediol was prepared using the literature procedure (10).

GAVAGE STUDY: Eight male Fischer 344 rats (254 $\pm$ 7 g) were dosed by gavage with 1.0 ml (0.692 g) 2,2,4-trimethylpentane every other day for 14 days. Rats were placed in metabolism cages for the first 48 hours following initial dosing for urine collection. Urine was also collected from water-treated control rats. Food and water were provided ad libitum.

URINARY METABOLITE IDENTIFICATION: Urine was acidified to a pH of 4.0, and treated with beta-glucuronidase-sulfatase (Calbiochem, LaJolla, CA) for 18 hours at 37°C. It was then cooled to room temperature and filtered through a Clin-Elut column (Analytichem International, Harbor City, CA) using neat methylene chloride as eluent. The methylene chloride extract was analyzed using gas-liquid partition chromatography (GLC), gas-liquid partition chromatography/mass spectrometry (GC/MS), and thin layer chromatography (TLC). For GLC, a carbowax 20M column (25 m x 0.25 mm I.D.) and a temperature program of 60° to 170°C at 5°/min were used. The GC/MS analyses were accomplished using a quadrupole instrument. A DX-4 column (15 m x 0.25 mm I.D.) was used with the same temperature programming; ionization was obtained by electron impact at a voltage of 70 eV and an ion source temperature of 200°C. The TLC was performed on silica gel plates Polygram Sil G (0.25 mm Brinkmann) and developed in $CHCl_3$:CH_3CN (60:40). The metabolites were identified by a comparison of their retention times (GLC), fragmentation patterns (MS), and R_f values (TLC) with those of independently synthesized compounds. Reduction of a urine sample was accomplished by treating with lithium aluminum hydride (0.25 g, 0.007 mole) in 30 ml of ether for 2 hours. After hydrolysis, the ether solution was analyzed by GLC, GC/MS, and TLC.

RESULTS. Fischer 344 male rats treated with 2,2,4-trimethylpentane yielded the urinary metabolites listed in Table 1. These metabolites were

TABLE 1. URINARY METABOLITES OF 2,2,4 TMP IN FISCHER 344 MALE RATS

URINARY METABOLITES
2,2,4-Trimethyl-1-Pentanol
2,4,4-Trimethyl-2-Pentanol
2,4,4-Trimethyl-1-Pentanol
2,4,4-Trimethyl-1-Pentanoic Acid
2,2,4-Trimethyl-1-Pentanoic Acid
2,4,4-Trimethyl-5-Hydroxy-1-Pentanoic Acid
2,2,4-Trimethyl-5-Hydroxy-1-Pentanoic Acid
2,4,4-Trimethyl-2-Hydroxy-1-Pentanoic Acid

all compared with purchased or synthesized compounds to insure no ambiguity with regard to metabolite identification.

DISCUSSION. Trimethylhydroxypentanoic acids were not directly detected on gas chromatographic or gas chromatographic/mass spectrometric analyses. Trimethyl-5-hydroxy-1-pentanoic acids form cyclic compounds under the conditions of gas chromatography. α,α,γ-Trimethyl-δ-valerolactone and α,γ,γ-trimethyl-δ-valerolactone peaks were tentatively identified by mass spectrometry and confirmed by comparing fragmentation patterns and GLC retention times with those of synthesized valerolactones. By hydrolyzing aliquots of these synthesized lactones to form the trimethyl-5-hydroxy-1-pentanoic acids and performing thin layer chromatography with these acids, with the valerolactones, and with processed urine, an excellent match was found between R_f values of the urine and acid chromatograms but not with that of the valerolactones.

Processed rat urine was reduced with lithium aluminum hydride to form alcohols from any acid, aldehyde, or ketone functionalities in order to identify positions of carbon atoms of the TMP molecule which underwent oxidation. Large amounts of 2,4,4-trimethyl-1,2-pentanediol were found and identified by GC/MS. Synthesized 2,4,4-trimethyl-2-hydroxy-1-pentanoic acid matched a major urine component on TLC but was not detected on GLC analyses of the urine. Figure 1 is a representative GLC tracing of processed urine from a male rat treated with 2,2,4-TMP by gavage.

The Fischer 344 male rat tended to excrete 2,2,4-trimethylpentane primarily as carboxylic acid derivatives, with a smaller portion of the hydrocarbon appearing in the urine as the monosubstituted alcohol. n-Hexane and n-heptane, on the other hand, are metabolized to yield alcohols and hydroxyketones as primary products (11, 12). Various hydroxy substituted carboxylic acids were the principal disubstituted urinary metabolites of 2,2,4-TMP, there being no trace of any hydroxyketone or diol detected in the urine by GLC. The structure of the carboxylic acid metabolites indicated that the carboxylic acid group can form at either end of the molecule.

Reduction of a urine sample with lithium aluminum hydride produced only the three mono-alcohols listed in table 1 as well as 2,4,4- trimethyl-pentane-1,2-diol and the trimethylpentane-1,5-diols which are believed to be formed from the various hydroxy carboxylic acids listed in table 1.

Since carboxylic acids are normally formed from carboxaldehydes, it is possible that various carboxaldehydes or carboxylic acids themselves are actively involved in the mechanism of nephropathy found in the male Fischer 344 rat following gavage with 2,2,4-trimethylpentane. Chemical interactions in the kidney between metabolites and alpha 2u globulin may form compounds that cannot be adequately degraded and result in abnormal accumulation and necrosis.

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