is convenient to run these extractions simultaneously with other work.

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C. Newton Blakesley*
J. Loots

Division of Food Chemistry
National Food Research Institute
Council for Scientific and Industrial Research
Pretoria, 0001, South Africa

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Identification of Benzene as a Volatile Metabolite of *p*-Toluic Acid Phenylhydrazide (TAPH)

Benzene was characterized as a volatile metabolite of *p*-toluic acid phenylhydrazide (TAPH) in the rat. The relationship of benzene as the volatile metabolite of TAPH and the phenyl groups bound to heme and globin from the treatment of sheep with *p*-toluoyl chloride phenylhydrazone (Jaglan et al., *J. Agric. Food Chem.* **24**, 659, 1976) is discussed.

In a recent article (Jaglan et al., 1976), we described the disposition of anthelmintic *p*-toluoyl chloride [^{14}C]-phenylhydrazone (TCPH) in sheep. We found that only the phenylhydrazine part of the molecule was responsible for the retention of radioactivity in blood. The radioactivity was selectively localized in erythrocytes and specifically and covalently bound to hemoglobin.

Under aqueous conditions, TCPH hydrolyzes to form *p*-toluic acid phenylhydrazide (TAPH). When sheep were treated with an equivalent dose of TAPH (uniformly ring-labeled phenylhydrazine), seven to ten times higher blood residues as compared with TCPH were observed (Jaglan et al., 1973). The blood radioactivity was again selectively localized in erythrocytes and covalently bound to hemoglobin, but the accountability of the dose in feces and urine was only about 60% as compared with 90% from TCPH. This indicated that some volatile metabolite had been lost. Further studies were done in rats in order to conserve [^{14}C]TAPH as well as to reduce handling problems.

Blood residue patterns in rats were found to be similar to that observed in sheep from TCPH or TAPH treatments. We report here the characterization of benzene as a volatile metabolite of TAPH in the rat. The probable relationship of benzene identified in this study to the phenyl group bound to hemoglobin from TCPH is discussed. This is the first recorded example of the formation of benzene from a hydrazide in vivo.

EXPERIMENTAL SECTION

A Sprague-Dawley male rat weighing 241 g was given a single oral dose of [^{14}C]TAPH (20.9 mg of uniformly ring-labeled phenylhydrazine containing 10.4×10^6 DPM, 51.8 μCi/mmol) and secured in a metabolism cage (Aerospace Ind., Garnerville, N.Y.). The cage was attached to a series of traps containing, in order, 50 mL of sulfuric acid (3 N), 50 mL of methanol, 50 mL of methanol cooled in dry ice–ethanol, and 500 mL of phenethylamine (2 N in methanol). Polyethylene tubing was used for all the connections. The system was connected to a vacuum line and the air flow adjusted to 60 mL/min. The traps were removed 24 h after treatment and aliquots from each trap were counted. Feces, urine, and cage wash were counted as described before (Jaglan et al., 1976).

The rat was killed by cervical dislocation and the radioactivity in the whole skinned animal containing all the tissues, blood, and gastrointestinal tract was determined. The carcass was homogenized in four volumes of water with a Polytron homogenizer for 10 min, then aliquots of this homogenate combusted and counted as described for feces.

The contents of the methanol traps, which contained most of the volatile radioactivity, were combined, and 5 mL of 1 N sodium hydroxide was added to convert any phenols and acids present to their respective sodium salts. The solution was then distilled at 50 °C under water aspirator vacuum; the distillate was collected in a cooled (ice bath) receiving flask. About 85% of radioactivity distilled into the receiver. Half of the distillate (6×10^5 DPM) was diluted with 20 mL of benzene and 300 mL of pentane, then shaken with 700 mL of water for 30 s in a separatory funnel, and the aqueous phase was discarded. The organic phase was washed with 200 mL of saturated sodium chloride solution, then dried over anhydrous sodium sulfate. The dried solution was distilled through a glass column, 280 mm long and 18 mm wide packed with 4-mm glass beads, until all the pentane was removed. The residual benzene solution was derivatized to benzophenone by the classical Friedel-Crafts acylation. The benzophenone was recrystallized several times from ethanol–water to a constant specific activity. Three aliquots of the benzophenone were weighed (23.8, 28.5, 23.7 mg) into

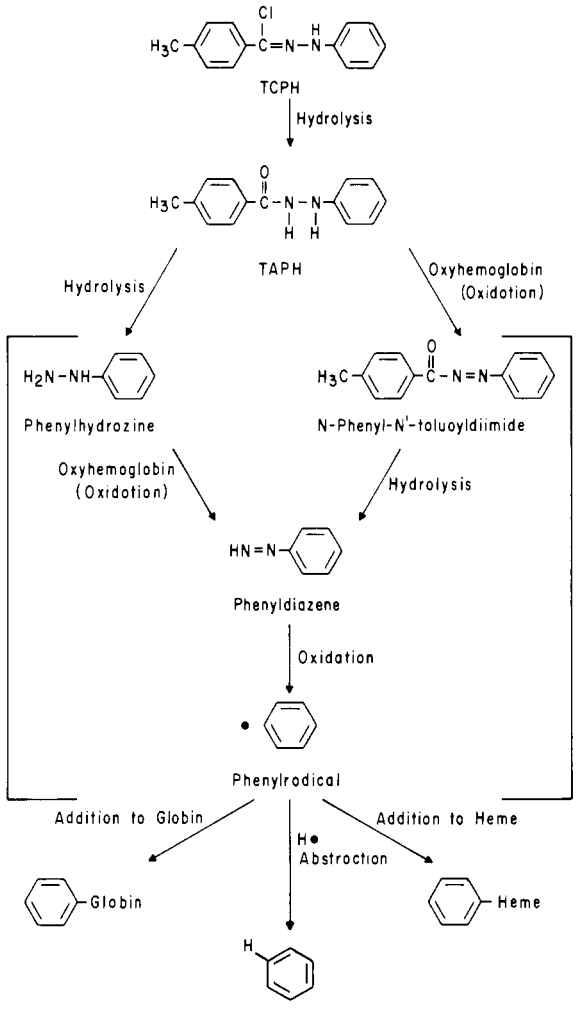

Figure 1. Postulated scheme for the generation of benzene from TAPH.

scintillation vials, 15 mL of diotol added, and each counted for 50 min. DPM was computed after adding internal standard.

RESULTS

From a rat treated with a single oral dose of [^{14}C]TAPH, the distribution of radioactivity was 19.3% in urine, 9.7% in feces, 25.6% in the carcass, and 13.4% in the expired air (Jaglan, 1973). The rest of the dose (32%) may also have been converted to volatiles, but was lost in greased joints, tubing, apparatus manipulation, and sample handling.

The absence of radioactivity in the phenethylamine trap indicated that carbon dioxide was not a major volatile metabolite. No radioactivity was found in the first sulfuric acid trap, which eliminated aniline, phenylhydrazine, or other basic volatile metabolites. Phenol was not present as the volatile metabolite because all of the radioactivity in the methanol traps was distilled from a sodium hydroxide solution. However, the ultraviolet absorption spectrum of the distillate strongly suggested the presence of benzene. Assuming that all of the radioactivity (6×10^5 DPM) in the distillate used for the derivatization was benzene (0.416 mg), the addition of 20 mL (17.58 g) of nonlabeled benzene to the distillate reduced the theoretical specific activity of benzene to 34.13 DPM/mg and of the derivative, benzophenone, to 14.61 DPM/mg. The observed specific activity of benzophenone was 14.72 DPM/mg. We therefore conclude that the volatile metabolite of TAPH is indeed benzene.

DISCUSSION

From these results, we postulate that the volatile metabolite, benzene, from TAPH and the bound hemoglobin residues from TCPH arise from a common intermediate (phenyl radical) as shown in Figure 1. In addition to reacting with heme and globin, the phenyl radical can abstract a hydrogen atom from some species in red cells, e.g., reduced pyridine nucleotide, to give rise to benzene (Kosower et al., 1965).

Although benzene has not been identified as the metabolite of phenylhydrazine (McIsaac et al., 1958) or its derivatives (Colvin, 1969; Juchau and Horita, 1972), Lawrason et al. (1949) observed symptoms typical of benzene poisoning from treatment of swine with phenylhydrazine. Beaven and White (1954) identified benzene as the end product of phenylhydrazine or acetylphenylhydrazine incubation with oxyhemoglobin. Traces of benzene were also observed when arylhydrazines were oxidized by atmospheric oxygen (Chattaway, 1907). Hardie and Tomson (1957) have indicated that air oxidation of arylhydrazines generated aryl radicals. Simultaneous to our work, Itano and Mannen (1976) demonstrated the binding of phenyl radicals to the aromatic amino acids of globin from in vitro incubation with phenylhydrazine. Nelson et al. (1976) demonstrated that reactive electrophiles were generated when acetylhydrazine was incubated with liver microsomes. Eyer and Kiese (1976) have also shown that covalent binding of 4-dimethylaminophenol to hemoglobin involved reactive intermediates.

The formation of benzene demonstrates that the nitrogen atoms have been metabolized away from TAPH. Indeed, when a rat was treated with [^{15}N]TAPH, no incorporation of ^{15}N in globin was observed (Jaglan et al., 1977).

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Prem S. Jaglan*
John L. Nappier
Rex E. Hornish
Alan R. Friedman

Agricultural Division
The Upjohn Company
Kalamazoo, Michigan 49001

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