Bull. Environ. Contam. Toxicol. (1985) 35:745–749


Environmental Contamination and Toxicology

# Biliary Metabolites of 2,6-Diisopropylnaphthalene in Rats

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Isopropylnaphthalenes are now used in duplicating papers and heat-transfer media as a substitute for polychlorinated biphenyls. In view of the increase in the use of isopropylnaphthalenes, it is desirable to study the toxicity and biological fate in animals of isopropylnaphthalenes, since their release into the environment appears inevitable. Sumino (1977) found diisopropylnaphthalenes in sludge and fish from bays in Japan. Recently, we reported the absorption, distribution, and excretion of 2,6-diisopropylnaphthalene (2,6-DIPN) after single and repeated oral administration of the compound to rats (Kojima et al. 1978; Kojima et al. 1979). In addition, we reported that upon oral administration of 2,6-DIPN to rats, five urinary metabolites were identified, and that these metabolites were the major urinary metabolites of 2,6-DIPN (Kojima et al. 1982).

The present study was carried out in rats after oral administration of 2,6-DIPN to examine the biliary metabolites of the compound.

## MATERIALS AND METHODS

Pure 2,6-DIPN (mp 69.5°C) was a gift from Kureha Chemical Co. (Tokyo). Hexamethyldisilazane and trimethylchlorosilane in anhydrous pyridine were purchased from Tokyo Kasei Chemical Co. (Tokyo). β-Glucuronidase (Type IX) was obtained from Sigma Chemical Co. (St. Louis, MO). 5-Dimethylaminonaphthalene-1-(2-hydroxyethyl)sulfone was supplied by the Department of Pharmaceutical Analytical Chemistry in this Faculty. Authentic samples used for the identification of metabolites of 2,6-DIPN in this study, 2,6-naphthalenedi(2-propan)-2-ol (metabolite B, mp 156-157°C), 2-[6-(1-hydroxy-1-methyl)ethylnaphthalene-2-yl]-2-propionic acid (metabolite C, mp 140-143°C), 2,6-naphthalenedi-2-propionic acid (metabolite D, mp 190-193°C), 2-[6-(1-hydroxy-1-methyl)ethylnaphthalene-2-yl]-2-hydroxypropionic acid (metabolite E,

dec p 115-118°C), and 2-[6-(1-hydroxy-1-methyl)ethyl-naphthalene-2-yl]-1,2-propanediol (metabolite F, mp 139-141°C), were obtained from the urine of rats after oral administration of 2,6-DIPN, according to the procedure reported in our previous paper (Kojima et al. 1982). All other chemicals and solvents were of reagent grade.

Male Wistar rats, weighing 200-220 g, were anesthetized with ether and the bile duct was cannulated with polyethylene tubing (PE-10) as described previously (Kojima and Maruyama 1979). After oral administration of 2,6-DIPN (100 mg/kg) as an olive oil solution, each rat was housed in a Bollman cage with diet and water ad libitum, and the bile was collected for 24 h after administration. The bile was adjusted to pH 1.0-2.0 with 1N HCl and centrifuged at 3000 rpm for 10 min. The supernatant was extracted three times with 20-ml portions of $CHCl_3$. The extract was evaporated to dryness in vacuo at 40°C (Extract 1).

The aqueous layer that remained after extraction at pH 1.0-2.0 with $CHCl_3$ was adjusted to pH 6.8 with 1N NaOH. The solution was incubated with 10 mg of β-glucuronidase (438 Fishman units/mg) for 24 h at 37°C after the addition of 2 drops of $CHCl_3$. The mixture was adjusted to pH 1.0-2.0 with 1N HCl and extracted three times with 20-ml portions of $CHCl_3$. The extract was evaporated to dryness in vacuo at 40°C (Extract 2).

Gas-liquid chromatography was as previously described (Kojima et al. 1982).

2,6-DIPN (100 mg/kg) was administered orally to rats in which the bile duct was cannulated with polyethylene tubing. Each animal was housed in Bollman cage with diet and water ad libitum and the 24-h bile was collected. The bile (about 10 ml) was adjusted to pH 1.0-2.0 with 1N HCl and centrifuged at 3000 rpm for 10 min. The supernatant was extracted three times with 20-ml portions of $CHCl_3$. The extract was evaporated to dryness in vacuo and the residue was dissolved in 10 ml of $CHCl_3$ (sample solution 1 for the determination of unconjugated metabolites). The aqueous layer that remained after extraction at pH 1.0-2.0 with $CHCl_3$ was adjusted to pH 6.8 with 1N NaOH and incubated with 10 mg of β-glucuronidase for 24 h at 37°C after the addition of 2 drops of $CHCl_3$. The mixture was adjusted to pH 1.0-2.0 with 1N HCl and extracted three times 20-ml portions of $CHCl_3$. After removal of the $CHCl_3$ in vacuo, the residue was dissolved in 10 ml of $CHCl_3$ (sample solution 2 for the determination of conjugated metabolites). To 1 ml of sample solution 1 or 4 ml of sample solution 2 was added 0.2 ml of 5-dimethylaminonaphtha-

lene-1-(2-hydroxyethyl)sulfone solution (500 μg/ml of $CHCl_3$) as an internal standard and evaporated to dryness. After addition of 0.1 ml of hexamethyldisilazane and trimethylchlorosilane in anhydrous pyridine to the residue, the mixture was heated for 5 min in a boiling water-bath and then subjected to GLC. The calibration curves for the metabolites of 2,6-DIPN were the same as described in a previous paper (Kojima et al. 1982).

## RESULTS AND DISCUSSION

To examine the biliary unconjugated metabolites of 2,6-DIPN, Extract 1 was trimethylsilylated and analyzed by GLC. As shown in Figure 1, five peaks, which were not identical with those of the control, were detected and the retention times showed good correspondence with those of trimethylsilyl derivatives of 2,6-naphthalenedi(2-propan)-2-ol (metabolite B), 2-[6-(1-hydroxy-1-methyl)ethylnaphthalene-2-yl]-2-propionic acid (metabolite C), 2,6-naphthalenedi-2-propionic acid (metabolite D), 2-[6-(1-hydroxy-1-methyl)ethylnaphthalene-2-yl]-2-hydroxypropionic acid (metabolite E), and 2-[6-(1-hydroxy-1-methyl)ethylnaphthalene-2-yl]-1,2-propanediol (metabolite F). In addition, unchanged 2,6-DIPN (metabolite A) in Extract 1 was identified by GLC (data not shown). These results showed that five unconjugated metabolites (B, C, D, E, and F) of 2,6-DIPN together with the unchanged compound were excreted in the bile of rats given 2,6-DIPN.

Next, in order to examine the presence of possible conjugated metabolites of 2,6-DIPN, the conjugated metabolites in Extract 2 were hydrolyzed with β-glucuronidase. The retention times for the trimethylsilyl derivatives of aglycones, which were extracted from the above hydrolysate with $CHCl_3$, showed good correspondence with those of the trimethylsilylated metabolites (B, C, D, E, and F) (Figure 1). Thus, these aglycones were identified as metabolites B, C, D, E, and F. These results showed that the kinds of metabolites of 2,6-DIPN found in the bile were similar to those in the urine.

Furthermore, we determined the unconjugated and conjugated metabolites in the 24-h bile of rats given a single oral dose of 2,6-DIPN (100 mg/kg) (Table 1). The results showed that the metabolites of 2,6-DIPN excreted in the bile amounted to about 17% of the dose, and that the total excretion of conjugated metabolites was much smaller than those of unconjugated metabolites. The biliary excretions of metabolites C and E were relatively small. The major metabolites in the bile were metabolites B and D. In our previous paper (Kojima et al. 1982), we reported that upon urinary metabolites of 2,6-DIPN, metabolite E was the major metabolite and

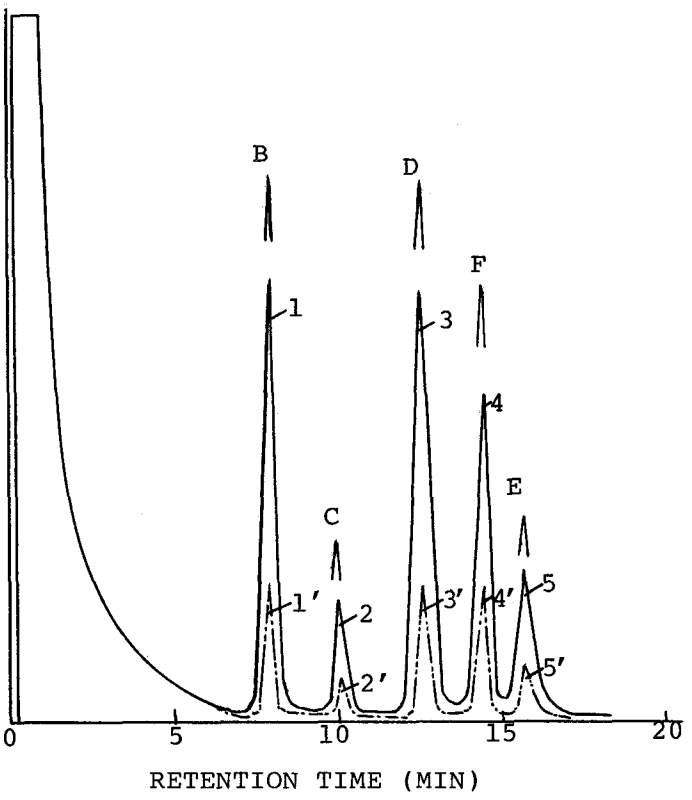
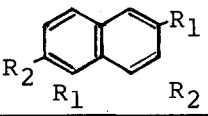



Figure 1. Gas-liquid chromatogram of biliary metabolites of 2,6-DIPN in rats. This chromatogram was obtained after the trimethylsilylation of the metabolites. Peaks 1-5 represent the unconjugated metabolites from Extract 1. Peaks 1'-5' represent the metabolites obtained by enzymatic hydrolysis of Extract 2.

metabolites B and D the minor metabolites. These findings suggest that metabolite B excreted in the bile is reabsorbed from the gut and metabolized to metabolite E, which is primarily excreted in the urine. In addition, the present results showed that the excretion pattern of biliary metabolites of 2,6-DIPN was different from that of urinary metabolites.

TABLE 1. Metabolites of 2,6-DIPN in 24-h bile after a single oral administration of the compound

| Metabolite | Formula | % of dose[a] | |
|---|---|---|---|
| |  | Unconjugated | Conjugated |
| A | $R_1$: $-CH(CH_3)_2$, $R_2$: $-CH(CH_3)_2$ | < 0.1 | |
| B | $R_1$: $-COH(CH_3)_2$, $R_2$: $-COH(CH_3)_2$ | 6.30 ± 0.60 | 0.20 ± 0.05 |
| C | $R_1$: $-CH(CH_3)_2$, $R_2$: $-COH(CH_3)_2$ | 0.52 ± 0.17 | < 0.1 |
| D | $R_1$: $-CH(CH_3)COOH$, $R_2$: $-CH(CH_3)COOH$ | 5.72 ± 1.10 | 0.77 ± 0.31 |
| E | $R_1$: $-COH(CH_3)COOH$, $R_2$: $-COH(CH_3)_2$ | 0.29 ± 0.03 | < 0.1 |
| F | $R_1$: $-COH(CH_3)CH_2OH$, $R_2$: $-COH(CH_3)_2$ | 2.45 ± 0.45 | 0.43 ± 0.05 |
| Total | | 15.30 ± 1.36 | 1.40 ± 0.16 |

a. The values represent means ± standard deviation for 3 to 6 animals.

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Received Jannuary 28, 1985; accepted February 11, 1985.