Identification and Determination of Urinary and Biliary Metabolites of 2-Isopropynaphthalene in Rats

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Four unconjugated metabolites of 2-isopropynaphthalene (2-IPN) were identified by thin-layer chromatography and gas-liquid chromatography (GLC) from the bile of rats receiving 2-IPN orally; they were 2-(2-naphthyl) propionic acid, 2-(2-naphthyl)-2-propanol, 2-(2-naphthyl)-2-hydroxypropionic acid, and 2-(2-naphthyl)-1,2-propanediol, together with the unchanged compound. The presence of glucuronides of these four metabolites was also suggested by GLC of the extract obtained after hydrolysis by β-glucuronidase. In addition, quantitative determination of the metabolites indicated that the total urinary and biliary excretions of these metabolites in 24 h after administration were about 23% and about 18% of the dose, respectively, and that the urinary and biliary metabolite excretion patterns were the same.

Keywords — alkynaphthalene; 2-isopropynaphthalene; metabolism; urinary metabolite; biliary metabolite

Isopropynaphthalenes have recently been used in duplicating paper and heat transfer media as a substitute for polychlorinated biphenyls. In view of the increase in the use of isopropynaphthalenes, it is desirable to study the toxicity and biological fate in animals of isopropynaphthalenes, since their release into the environment appears inevitable. Sumino et al. found diisopropynaphthalenes in sludge and fish from bays in Japan. Recently, we reported the absorption, distribution, excretion, and metabolism of 2,6-diisopropynaphthalene after single and continuous oral administration of the compound to rats. In addition, we studied the absorption, tissue distribution, and excretion of 2-isopropynaphthalene (2-IPN) in rats after single and continuous oral administration of 2-IPN and also reported the identification of urinary metabolites of 2-IPN in rats.

The present study deals with the identification of biliary metabolites of 2-IPN and the quantitative determination of urinary and biliary metabolites of 2-IPN in rats after oral administration.

Experimental

Materials — Pure 2-IPN (bp 287°C) was a gift from Kureha Chemical Co. (Tokyo). β-Glucuronidase (type IX) was obtained from Sigma Chemical Co. (St. Louis, Mo.). N,O-Bis (trimethylsilyl) acetamide was purchased from Tokyo Kasei Chemical Co. (Tokyo). Anthrone and pentobarbital were purchased from Nakarai Chemical Co. (Tokyo). Authentic samples used for the identification of metabolites of 2-IPN in this study, 2-(2-naphthyl) propionic acid (metabolite B, mp 138
-140°C), 2-(2-naphthyl)-2-propanol (metabolite C, mp 66–67°C), 2-(2-naphthyl)-2-hydroxypropionic acid (metabolite D, mp 163–165°C), and 2-(2-naphthyl)-1,2-propanediol (metabolite F, mp 95–96°C), were obtained from the urine of rats after oral administration of 2-IPN, according to the procedure reported in our previous paper. All other chemicals and solvents were of reagent grade.

**Extraction of Biliary Metabolites** — Male Wistar rats, weighing 190–220 g, were anesthetized with ethyl ether and the bile duct was cannulated with polyethylene tubing (PE-10) as described previously. After oral administration of 2-IPN (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 1 n HCl and centrifuged at 3000 rpm for 10 min. The supernatant was extracted three times with 10 ml portions of CHCl₃. The extract was concentrated *in vacuo* at about 40°C (Extract 1).

**Enzymatic Hydrolysis of Biliary Conjugated Metabolites** — The aqueous layer that remained after extraction at pH 1.0–2.0 with CHCl₃ was adjusted to pH 6.8 with 1 n 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 CHCl₃. The mixture was adjusted to pH 1.0–2.0 with 1 n HCl and extracted three times with 10 ml portions of CHCl₃. The extract was concentrated *in vacuo* at 40°C (Extract 2).

**Chromatography** — The apparatus and experimental conditions for thin-layer chromatography (TLC) and gas-liquid chromatography (GLC) were the same as described in a previous paper.

**Quantitative Determination of Urinary and Biliary Metabolites** —

1) Sample solutions from urine: 2-IPN (100 mg/kg) was administered orally to rats. The animals were housed in Bollman cages with diet and water *ad libitum* and the 24-h urine was collected. The urine was treated according to the procedure described in a previous paper to obtain sample solution 1 for the determination of unconjugated metabolites and sample solution 2 for the determination of conjugated metabolites.

2) Sample solutions from bile: 2-IPN (100 mg/kg) was administered orally to rats in which the bile duct was cannulated with polyethylene tubing. The 24-h bile was collected, adjusted to pH 1.0–2.0 with 1 n HCl and centrifuged at 3000 rpm for 10 min. The supernatant was extracted three times with 20-ml portions of CHCl₃. The extract was evaporated to dryness *in vacuo* and the residue was dissolved in 10 ml of CHCl₃ (sample solution 3 for the determination of unconjugated metabolites). The aqueous layer that remained after extraction at pH 1.0–2.0 with CHCl₃ was adjusted to pH 6.8 with 1 n NaOH and incubated with 10 mg of β-glucuronidase for 24 h at 37°C after the addition of 2 drops of CHCl₃. The mixture was adjusted to pH 1.0–2.0 with 1 n HCl and extracted three times with 20-ml portions of CHCl₃. After removal of the CHCl₃ *in vacuo*, the residue was dissolved in 10 ml of CHCl₃ (sample solution 4 for the determination of conjugated metabolites).

3) Determination of the metabolites: For the determination of metabolites B, D and F, 0.1 ml of anthrone (250 µg/ml of CHCl₃) was added as internal standard to 1 ml of sample solution 1, 2, 3, or 4 and the mixture was evaporated to dryness. After addition of 0.1 ml of N,O-bis(trimethylsilyl) acetamide to the residue, the mixture was heated for 10 min at 92°C in a water-bath and then subjected to GLC. For the determination of metabolite C, 1 ml of sample solution was evaporated to dryness, 0.1 ml of pentobarbital (5 mg/ml of CHCl₃) was added as an internal standard, and the mixture was subjected to GLC. The calibration curve for each metabolite was obtained by plotting the concentration of the metabolites against the peak height ratio of the metabolite to the internal standard. When the metabolites (B, 100 µg; C, 150 µg; D
and F, 200 μg) of 2-IPN were added to the control urine and bile, and the compounds were analyzed by the method described above, mean recoveries of these compounds were 95% for B, 93% for F, 87% for C, and 77% for D.

**Results and Discussion**

To examine the biliary unconjugated metabolites of 2-IPN Extract 1, which was obtained by the procedure described in the experimental section, was analyzed by TLC. As shown in Fig. 1, five spots, which were not found in the control, were detected and the Rf values showed good correspondence with those of unchanged 2-IPN (metabolite A), 2-(2-naphthyl) propionic acid (metabolite B), 2-(2-naphthyl)-2-propanol (metabolite C), 2-(2-naphthyl)-2-hydroxypropionic acid (metabolite D) and 2-(2-naphthyl)-1,2-propanediol (metabolite F). In addition, Extract 1 was trimethylsilylated and analyzed by GLC. Four peaks, which were not identical with those of the control, were detected and the retention times showed good correspondence with those of trimethylsilylated metabolites B, C, D, and F (Fig. 2). These results showed that four unconjugated metabolites (B, C, D, and F) of 2-IPN together with the unchanged compound (metabolite A) were excreted in the bile of rats given 2-IPN.

In addition, in order to examine the presence of possible conjugated metabolites of 2-IPN, 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 CHCl₃, showed good correspondence with those of the trimethylsilylated metabolites (B, C, D, and F) (Fig. 2). Thus, these aglycones were identified as metabolites B, C, D, and F. These results showed that the kinds of metabolites of 2-IPN found in the bile were similar to those in the urine.

Furthermore, we determined the unconjugated and conjugated metabolites in 24-h urine and bile of rats given a single oral dose of 2-IPN (100 mg/kg) (Table 1). The results showed that the metabolites of 2-IPN excreted in the urine and bile amounted to about 23% and about 18% of the dose, respectively. The urinary and biliary excretions of unchanged 2-IPN and unconjugated metabolites B, D, and F were relatively small. The major unconjugated metabolite excreted in the urine and bile was metabolite C. In
Table I. Metabolites of 2-IPN in 24-h Urine and Bile after a Single Oral Administration

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Formula</th>
<th>Urine Unconjugated</th>
<th>Urine Conjugated</th>
<th>Bile Unconjugated</th>
<th>Bile Conjugated</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>CH₃</td>
<td>CH₃</td>
<td>CH₃</td>
<td>CH₃</td>
<td>CH₃</td>
</tr>
<tr>
<td>B</td>
<td>COOH</td>
<td>CH₃</td>
<td>CH₃</td>
<td>CH₃</td>
<td>CH₃</td>
</tr>
<tr>
<td>C</td>
<td>COH</td>
<td>CH₃</td>
<td>CH₃</td>
<td>CH₃</td>
<td>CH₃</td>
</tr>
<tr>
<td>D</td>
<td>COOH</td>
<td>CH₃</td>
<td>CH₃</td>
<td>CH₃</td>
<td>CH₃</td>
</tr>
<tr>
<td>E</td>
<td>CHO</td>
<td>CH₃</td>
<td>CH₃</td>
<td>CH₃</td>
<td>CH₃</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>17.12±1.23</td>
<td>5.49±2.19</td>
<td>15.06±2.02</td>
<td>3.36±0.15</td>
</tr>
</tbody>
</table>

a) The values represent means ± standard deviation for 3 to 7 animals.

In addition, the major glucuronide conjugate in the urine and bile was metabolite F. The total urinary and biliary excretions of conjugated metabolites were much smaller than those of unconjugated metabolites, although the excretion of metabolite F in the conjugated form was greater than that in the unconjugated form. Thus, the excretion pattern of urinary metabolites of 2-IPN was almost the same as that of biliary metabolites.

In our previous paper, we reported that during 24 h after oral administration of monoisopropyl [¹⁴C] naphthalene (MIPN-¹⁴C), about 50% of the dose was excreted in the urine and about 2% of the dose was excreted in the feces, and that the biliary excretion of radioactivity in rats orally given MIPN-¹⁴C represented about 60% of the dose during the first 24 h. These findings suggest that enterohepatic circulation plays an important role in the reabsorption of the unconjugated and conjugated metabolites of 2-IPN excreted in the intestine via the bile, and that the major route of excretion of metabolites of 2-IPN is via the urine. Judging from the finding mentioned above that the total excretion of urinary metabolites of 2-IPN was about 23%, it seems likely that some further metabolite(s) other than the metabolites which have been identified by us are present in the urine and bile of 2-IPN-treated rats.

References and Notes

4) S. Kojima, M. Nakagawa, R. Suzuki, M. Horio, Y. Taniguchi and Y. Tanaka, Eisei Kagaku, 25,