Metabolism of Magnolol from Magnoliae Cortex. II.\textsuperscript{1)}
Absorption, Metabolism and Excretion of [ring-\textsuperscript{14}C]Magnolol in Rats

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After single oral administration of [ring-\textsuperscript{14}C]magnolol, a central nervous system-depressive, muscle-relaxant and bactericidal principle of Magnoliae Cortex to rats, the blood levels of radioactivity showed two peaks at 15 min and 8 h, suggesting an enterohepatic circulation of magnolol and its metabolites. The radioactivity was distributed mostly in the gastrointestinal tract and liver, and next in the kidney, pancreas and lung. A major metabolite excreted in the bile was [ring-\textsuperscript{14}C]magnolol-2-O-glucuronide.

After oral and intraperitoneal administration of [ring-\textsuperscript{14}C]magnolol, most of the radioactivity was eliminated into the feces and urine within the first 12 h in either case. The oral dose was recovered to a greater extent from the feces (72\% of the administered radioactivity) than from the urine (7.4\%) in 144 h, and the intraperitoneal dose was similarly recovered from the feces (67\%) and from the urine (12\%).

On repeated oral administration of cold and [ring-\textsuperscript{14}C]magnolol, the composition of the fecal metabolites significantly changed, and tetrahydromagnolol, 5-((E)-1-propenyl)-5'-propyl-2,2'-dihydroxybiphenyl, 5-allyl-5'-propyl-2,2'-dihydroxybiphenyl, isomagnolol and 5-allyl-5'-(2E)-1-propenyl)-2,2'-dihydroxybiphenyl were detected.

**Keywords**—Magnolia obovata; Magnolia officinalis; magnolol; metabolism

In contrast to the situation with modern medicines, only a few studies have been conducted on the absorption, metabolism and excretion of crude drugs or their components in animals. During the course of our studies on the metabolism of crude drug components by intestinal flora, we have previously reported the metabolism of magnolol in rats from Magnoliae Cortex,\textsuperscript{2)} which is one of the most important constituents of prescriptions for the therapy of anxiety, nervous disturbance or gastrointestinal disorder in traditional Chinese medicine. By analyzing benzene-soluble fractions of feces and urine after oral administration of magnolol to rats, we have shown that magnolol is transformed into a series of metabolites including tetrahydromagnolol, 5-((E)-1-propenyl)-5'-propyl-2,2'-dihydroxybiphenyl, 5-allyl-5'-propyl-2,2'-dihydroxybiphenyl, isomagnolol and 5-allyl-5'-(2E)-1-propenyl)-2,2'-dihydroxybiphenyl, and that isomerization of magnolol could be caused, in part, by intestinal bacteria.

In the present paper, we report the fate of [ring-\textsuperscript{14}C]magnolol orally or intraperitoneally administered to rats in single or repeated doses.

**Materials and Methods**

**Instruments**—Ultraviolet (UV) spectra were measured with a Shimadzu UV-210A digital double-beam
spectrophotometer. Radioactivity was measured with a Packard A-300 C liquid scintillation counter (United Technologies Packard). Radioactive metabolites were analyzed with a Hitachi 655 high-performance liquid chromatography (HPLC) system equipped with a radioanalyzer (Aloka RLC-551), a data processor (Hitachi 655-60) and a variable-wavelength UV monitor (Hitachi 638-41). Chemicals—Magnolol was isolated from Magnoliae Cortex according to the reported method. Isomagnolol and tetrahydro magnolol were synthesized as reported previously. Protosol (tissue and gel solubilizer, 0.5 m solution) was purchased from New England Nuclear. A liquid scintillation medium, ACS-II, was obtained from Amersham Corp. Glucuronidase from bovine liver was purchased from P-L Biochemicals Inc. This enzymatic activity was determined to be 16 units/g according to the method of Talalay et al. Pentobarbital sodium (Nembutal) was purchased from Abbott. B-Saccharic acid-1,4-lactone was purchased from Sigma Chem. Co. (St. Louis).

Synthesis of [ring-14C]Magnolol—[ring-14C]Magnolol (specific activity: 2.48 mCi/mmol) was synthesized from [14C]aniline via [14C]5,5'-dibromo-2,2'-dimethoxybiphenyl according to the method of Runenberg. The radiochemical purity, 98.1% was determined by thin layer chromatography (TLC) on Silica gel 60 F254 plate (0.25 mm in thickness, Merck) with CHCl3–MeOH (9:1).

Animals—Male Wistar rats (4 or 8 weeks of age) were used. Prior to the experiments, the rats were fasted for 17–18 h but drinking water was allowed ad libitum.

Excretion of Radioactivity into Feces and Urine—A suspension (1 ml) of [ring-14C]magnolol (2.0 μCi) in 5% arabic gum was orally or intraperitoneally administered to each of 5 rats (4 weeks of age, weighing 109–126 g). The animals were housed in individual metabolism cages which permitted the separate collection of feces and urine. The latter was collected in bottles, to which toluene had been added as a preservative. Food and water were allowed ad libitum 4 h after the administration and the excrement was collected at intervals. The feces were suspended in 10 volumes of water. The urine was diluted with water to give an appropriate volume. Aliquots (100 μl) of these suspensions and dilutions were each mixed with an emulsion-scintillation medium (10 ml), and counted for radioactivity in a liquid scintillation counter. As a control experiment, feces and urine were collected from five rats which had not received radioactive magnolol and were processed as described above.

Excretion of Radioactivity into Bile—Six rats (8 weeks of age, weighing 220–280 g) were anesthetized with pentobarbital sodium (1 mg/kg) and cannulae (single lumen polyethylene tubing, 1.5 mm i.d., 2.7 mm o.d., Natsume, and intramedic polyethylene tubing, PE-10, 1.5 mm i.d., 2.7 mm o.d., Clay Adams) were placed in the trachea and bile duct of each animal. After oral or intraperitoneal administration of a suspension (1 ml) of [ring-14C]magnolol (2 μCi) in 5% arabic gum to the rats, bile was collected at intervals in tubes chilled with ice-water and then diluted with 5 volumes of water. Aliquots (100 μl) of the dilutions were transferred to an emulsion-scintillation medium (10 ml) and counted for radioactivity as usual. A control experiment was carried out with animals that received no magnolol. Similarly, bile was collected from bile-duct-cannulated rats (8 weeks of age, weighing ca. 245 g) whose gastric pylorus was tied with a thread, after oral administration of the same dose.

Distribution of Radioactivity in Organs and Biological Fluids—After oral administration of [ring-14C]magnolol (1.6 μCi) as a 5% arabic-gum suspension (0.6 ml) to 27 rats (4 weeks of age, weighing 75–110 g), three of them were decapitated at 15 and 30 min, and then at 1, 2, 4, 8, 12, 24, and 48 h. In each case, the organs or tissues were immediately removed, washed in isotonic saline (0.9% NaCl), and weighed. Each organ was homogenized in 100 volumes (v/w) of isotonic saline in a glass Potter-Elvehjem type homogenizer with a Teflon pestle. Aliquots (100 μl) were then mixed with EtOH (200 μl) and Protosol (200 μl), kept for 12–15 h at room temperature and incubated for 2 h at 45°C. Hydrogen peroxide (30% H2O2, 200 μl) was then added, and the mixtures were incubated for 60 min at 45°C, then cooled. An emulsion scintillation medium (10 ml) and 1 n HCl (100 μl) were added with stirring. The resulting emulsions were kept overnight at room temperature and measured for radioactivity in a liquid scintillation counter. Similarly, aliquots (100 μl) of the blood collected at intervals were mixed with Protosol–EtOH (1:2, 300 μl) for 2 h at 45°C, then 30% H2O2 (300 μl) was added. The mixtures were incubated for 60 min at 45°C, mixed with an emulsion-liquid scintillation medium (10 ml) after cooling, and counted for radioactivity as usual.

Gross Chemical Fractionation of Metabolites—A fecal, urinary or biliary suspension/solution was adjusted to pH 7 with 1 n HCl and extracted three times with equal volumes of benzene. The separated benzene phases were combined and designated as the benzene-soluble fraction. The aqueous layer was acidified to pH 1 with 1 n HCl and extracted three times with equal volumes of ethyl acetate (AcOEt). The combined AcOEt phases and the aqueous residual phase were designated as the AcOEt-soluble and water-soluble fractions, respectively. An aliquot (100 μl) of each fraction was mixed with an emulsion-scintillation medium (10 ml), and counted for radioactivity in a liquid scintillation counter. The above three fractions were next subjected to enzymic digestion and HPLC for analysis of their radioactive components.

Enzymic Digestion of Conjugates—A portion (ca. 1 × 106 dpm) of the AcOEt-soluble or water-soluble fraction which had been adjusted to pH 7 was passed through a filter paper and a membrane filter (25 mm in diameter, 0.45 μm pore size). The filtrate was evaporated in vacuo to yield a residue. The residue was dissolved in sodium acetate buffer (pH 4.5, 100 μl) and used for the following experiments.

i) Digestion by β-Glucuronidase–Arylsulfatase: Aliquots (20 μl each) of the above solution were mixed with β-glucuronidase–arylsulfatase solutions (20 μl each, concentration ranging from 0.4 to 50 mg protein/ml). The mixtures
were incubated for 4 h at 37 °C and applied to a polyamide TLC plate (Polygram, Macherey-Nagel, Co., Dueren), which was developed with benzene–EtOH (9:1). The radioactive spots were detected by autoradiography (Fuji X-ray film, RX Medical), scraped into vials containing an emulsion-scintillator (10 ml), and counted in a liquid scintillation counter.

ii) Digestion by Arylsulfatase: Aliquots (20 μl each) of the above solutions were mixed with the β-glucuronidase-arylsulfatase solutions (20 μl each, 0.4—50 mg/ml) and 1.0 mg of a β-glucuronidase inhibitor (t-saccharic acid-1,4-lactone). The mixtures were incubated for 4 h at 37 °C. Under these conditions, the β-glucuronidase activity was completely inhibited but the arylsulfatase activity remained intact. The reaction mixtures were then applied to polyamide TLC plates and analyzed as described above.

Analysis of Fecal and Urinary Metabolites after Repeated Administration of [ring-14C]Magnolol—A suspension (1 ml) of magnolol (3 mg) and [ring-14C]magnolol (2 μCi) in 5% arabic gum was orally administered to each of 6 rats (8 weeks of age, weighing 224—246 g) at 24-h intervals for 6 d. The rats were housed in individual metabolism cages and allowed food and water ad libitum. Feces and urine were collected every 24 h after administration. The feces were extracted three times with MeOH (200 ml). The MeOH solution was evaporated in vacuo to yield a residue. The residue was suspended in water (50 ml), adjusted to pH 7 and then extracted three times with benzene (50 ml each). The aqueous layer was acidified to pH 1, and extracted three times with AcOEt (50 ml each). Aliquots of the benzene-soluble, AcOEt-soluble and water-soluble fractions were separately mixed with an emulsion scintillation medium (10 ml) and counted for radioactivity in a liquid scintillation counter. Furthermore, aliquots (2 μl each) of the above fractions were analyzed with an HPLC-radioanalyzer.

Analysis of Metabolites with an HPLC-Radioanalyzer—Aliquots containing radioactive metabolites were injected into a column (30 cm × 4.6 mm i.d.) of TSK gel-ODS-120A (particle size, 10 μm; Toyo Soda Kogyo Co., Tokyo) attached to the HPLC-radioanalyzer system. HPLC was carried out under the following conditions: mobile phases, CH3CN-H2O-AcOH (50:50:0.5) for the benzene-soluble fraction, CH3CN-H2O-AcOH (40:60:0.5 or 30:70:0.5) for the AcOEt-soluble fraction, CH3CN-H2O-AcOH (40:60:0.5) for the water-soluble fraction; flow rate 0.4 ml/min; UV trace at 250 nm; radioanalyzer sensitivity, 300 counts/30 s.

Results

Blood Levels of Radioactivity

After oral administration of 2 μCi of [ring-14C]magnolol to rats, the blood level of radioactivity reached the first maximum (1.3 × 104 dpm/ml) at 15 min, showing rapid absorption of magnolol from the gastrointestinal tract (Fig. 1). This level then declined progressively during the period between 30 min and 4 h, again increased to give the second peak (3.0 × 103 dpm/ml) at 8 h, and decreased gradually until 48 h.

Organ Distribution

Table I shows the distribution of radioactivity in the organs at intervals after oral administration of [ring-14C]magnolol to rats. High radioactivity was observed in the gastrointestinal tract and liver (11% of the administered dose at 15 min), and relatively high activity in the kidney and pancreas. The radioactivity of the liver, lung and kidney also

![Fig. 1. Blood Levels of Radioactivity After Oral Administration of [ring-14C]Magnolol to Rats (n = 3)](image-url)
<table>
<thead>
<tr>
<th>Organs</th>
<th>0.25</th>
<th>0.5</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>8</th>
<th>12</th>
<th>24</th>
<th>48</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testis</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.27 ± 0.21</td>
<td>0.10 ± 0.10</td>
<td>0.13 ± 0.13</td>
<td>0.05 ± 0.02</td>
<td>0.05 ± 0.03</td>
<td>0.04 ± 0.03</td>
<td>0.02 ± 0.01</td>
</tr>
<tr>
<td>Adrenal</td>
<td>0.00 ± 0.00</td>
<td>0.03 ± 0.02</td>
<td>0.05 ± 0.04</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.05 ± 0.02</td>
<td>0.05 ± 0.00</td>
<td>0.05 ± 0.01</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.00 ± 0.00</td>
<td>0.01 ± 0.01</td>
<td>0.36 ± 0.19</td>
<td>0.10 ± 0.07</td>
<td>0.14 ± 0.11</td>
<td>0.21 ± 0.12</td>
<td>0.20 ± 0.08</td>
<td>0.06 ± 0.01</td>
<td>0.02 ± 0.02</td>
</tr>
<tr>
<td>Pancreas</td>
<td>0.22 ± 0.14</td>
<td>0.39 ± 0.09</td>
<td>0.67 ± 0.31</td>
<td>0.75 ± 0.12</td>
<td>0.13 ± 0.11</td>
<td>0.67 ± 0.28</td>
<td>0.30 ± 0.13</td>
<td>0.15 ± 0.05</td>
<td>0.03 ± 0.01</td>
</tr>
<tr>
<td>Liver</td>
<td>11.11 ± 2.55</td>
<td>7.62 ± 2.03</td>
<td>4.18 ± 0.30</td>
<td>4.27 ± 0.63</td>
<td>2.35 ± 0.22</td>
<td>3.71 ± 0.87</td>
<td>2.47 ± 0.48</td>
<td>1.72 ± 0.26</td>
<td>0.77 ± 0.04</td>
</tr>
<tr>
<td>Thymus</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.04 ± 0.02</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.14 ± 0.06</td>
<td>0.09 ± 0.04</td>
<td>0.05 ± 0.01</td>
<td>0.02 ± 0.01</td>
</tr>
<tr>
<td>Heart</td>
<td>0.13 ± 0.03</td>
<td>0.02 ± 0.02</td>
<td>0.11 ± 0.02</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.11 ± 0.03</td>
<td>0.24 ± 0.11</td>
<td>0.07 ± 0.02</td>
<td>0.02 ± 0.01</td>
</tr>
<tr>
<td>Lung</td>
<td>0.22 ± 0.03</td>
<td>0.22 ± 0.11</td>
<td>0.27 ± 0.11</td>
<td>0.00 ± 0.00</td>
<td>0.01 ± 0.01</td>
<td>0.47 ± 0.22</td>
<td>0.22 ± 0.10</td>
<td>0.11 ± 0.04</td>
<td>0.06 ± 0.01</td>
</tr>
<tr>
<td>Bladder</td>
<td>0.00 ± 0.00</td>
<td>0.01 ± 0.01</td>
<td>0.04 ± 0.02</td>
<td>0.38 ± 0.18</td>
<td>0.07 ± 0.02</td>
<td>0.15 ± 0.04</td>
<td>0.09 ± 0.01</td>
<td>0.04 ± 0.01</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.83 ± 0.05</td>
<td>0.60 ± 0.34</td>
<td>0.61 ± 0.07</td>
<td>0.40 ± 0.10</td>
<td>0.68 ± 0.11</td>
<td>0.74 ± 0.07</td>
<td>0.65 ± 0.15</td>
<td>0.38 ± 0.19</td>
<td>0.09 ± 0.04</td>
</tr>
<tr>
<td>Brain</td>
<td>0.07 ± 0.07</td>
<td>0.04 ± 0.03</td>
<td>0.12 ± 0.04</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.08 ± 0.02</td>
<td>0.24 ± 0.04</td>
<td>0.13 ± 0.03</td>
<td>0.07 ± 0.02</td>
</tr>
<tr>
<td>Stomach</td>
<td>26.37 ± 6.99</td>
<td>14.70 ± 2.03</td>
<td>7.12 ± 3.25</td>
<td>1.95 ± 0.28</td>
<td>0.98 ± 0.17</td>
<td>0.44 ± 0.11</td>
<td>0.42 ± 0.10</td>
<td>0.40 ± 0.20</td>
<td>0.11 ± 0.03</td>
</tr>
<tr>
<td>Content/stomach</td>
<td>13.63 ± 1.84</td>
<td>27.39 ± 6.01</td>
<td>13.80 ± 9.54</td>
<td>1.44 ± 0.24</td>
<td>0.67 ± 0.05</td>
<td>1.48 ± 0.17</td>
<td>0.94 ± 0.38</td>
<td>0.36 ± 0.19</td>
<td>0.04 ± 0.03</td>
</tr>
<tr>
<td>Small intestine</td>
<td>8.90 ± 2.83</td>
<td>20.42 ± 2.74</td>
<td>23.19 ± 4.61</td>
<td>29.17 ± 1.47</td>
<td>15.64 ± 1.90</td>
<td>4.45 ± 0.20</td>
<td>2.25 ± 0.06</td>
<td>0.91 ± 0.44</td>
<td>0.28 ± 0.13</td>
</tr>
<tr>
<td>Content/s. intestine</td>
<td>21.50 ± 9.35</td>
<td>27.57 ± 2.84</td>
<td>35.07 ± 4.56</td>
<td>28.83 ± 8.53</td>
<td>15.09 ± 3.01</td>
<td>6.91 ± 0.94</td>
<td>3.80 ± 0.93</td>
<td>1.56 ± 0.77</td>
<td>0.06 ± 0.05</td>
</tr>
<tr>
<td>Large intestine</td>
<td>0.48 ± 0.06</td>
<td>1.84 ± 0.42</td>
<td>6.15 ± 1.34</td>
<td>9.38 ± 1.98</td>
<td>18.63 ± 1.66</td>
<td>7.08 ± 1.10</td>
<td>4.05 ± 0.74</td>
<td>0.84 ± 0.31</td>
<td>0.14 ± 0.05</td>
</tr>
<tr>
<td>Content/l. intestine</td>
<td>0.17 ± 0.08</td>
<td>1.70 ± 0.14</td>
<td>6.74 ± 3.76</td>
<td>7.15 ± 1.47</td>
<td>33.48 ± 10.34</td>
<td>24.20 ± 6.46</td>
<td>11.61 ± 3.41</td>
<td>3.16 ± 1.45</td>
<td>0.21 ± 0.03</td>
</tr>
</tbody>
</table>

**Table I.** Distribution of Radioactivity in the Organs After Oral Administration of [ring-¹⁴C]Magnolol to Rats (n = 3)
showed two peaks at 15 min and 8 h after administration (Fig. 2).

**Fecal and Urinary Excretion**

Figure 3 shows the cumulative fecal and urinary excretion of radioactivity after oral or intraperitoneal administration of [ring-\(^{14}\)C]magnolol (2 \(\mu\)Ci). *Circa* 65\% of the radioactivity was excreted in the feces and 11\% in the urine within 24 h following oral administration, while
52% and 24% were excreted in the feces and urine, respectively, within 24 h following intraperitoneal administration. Overall recoveries were as much as 80 and 81% of the dose in the former and the latter cases, respectively, in 6 d.

**Biliary Excretion**

Figure 4 shows the cumulative biliary excretion profiles of radioactivity after oral and intraperitoneal administration to bile-duct-cannulated rats. Following oral administration, the radioactivity was gradually excreted into the bile and reached as much as 47% of the dose in 10 h, while following intraperitoneal administration, it was more rapidly excreted into the bile (47% within 2 h), and then gradually excreted up to 80% in 10 h. On the other hand, the biliary excretion profile following oral administration to rats whose gastric pylorus was tied with a thread was similar to the former profile. This reveals that orally administered magnolol is absorbed well from the stomach and excreted into the bile.

**Composition of Fecal, Urinary and Biliary Metabolites**

Through a solvent extraction technique, fecal, urinary or biliary excrement was fractionated into benzene-soluble, AcOEt-soluble and water-soluble fractions as described in Materials and Methods. The latter two fractions were subjected to enzymic hydrolysis by using β-glucuronidase and arylsulfatase in combination with D-saccharic acid-1,4-lactone as a β-glucuronidase inhibitor. Table II summarizes the gross composition of metabolites excreted into the feces, urine and bile after oral administration of 2 μCi of [ring-14C]magnolol to rats. In the feces, most of the radioactivity was present as the free forms of magnolol and its metabolites (53%), which consisted of various reduced and isomerized products as reported previously,3) the conjugates (ca. 6%) and unidentified products (40%). In the urine, the radioactive ingredients consisted of the free forms (ca. 9%), the glucuronides (3%), the sulfates (3%) and a large amount of unidentified products (85—86%). In the bile excreted within 4 h, the glucuronides (48—49%) as well as unidentified products (42%) were the major metabolites, and the free forms (7%) and the sulfates (2%) were minor.

**Analysis of Biliary Metabolites by HPLC**

The AcOEt-soluble fraction of the biliary excrement, which accounted for 75% of the radioactivity excreted into the bile after single administration of [ring-14C]magnolol, was analyzed with an HPLC-radioanalyser (Fig. 5). One major and two minor peaks were observed on the radiochromatogram. These metabolites were tentatively designated as B1, B2 and B3 from the last peak. After treatment of this fraction with β-glucuronidase, all the peaks disappeared on the chromatogram. Furthermore, they gave blue and purple colors on reverse phase TLC plates when the plates were sprayed with a naphthoresorcinol reagent7) followed by heating for 10 min at 120°C. These findings indicated that the compounds were glucuronides of magnolol and its derivatives. The major component, B1, had UV $\lambda_{max}$ at 384 nm in
TABLE II. Composition of the Radioactive Fractions Obtained from the Urine, Feces and Bile After Oral Administration of [ring-14C]Magnolol to Rats

<table>
<thead>
<tr>
<th>Sample</th>
<th>Time (h)</th>
<th>n</th>
<th>Composition</th>
<th>Radioactivity (%)</th>
<th>Benzene-soluble</th>
<th>AcOEt-soluble</th>
<th>Water-soluble</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td>0—72</td>
<td>5</td>
<td>Free forms</td>
<td>2.4 ± 0.4</td>
<td>3.6 ± 2.3</td>
<td>2.8 ± 0.5</td>
<td>8.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Glucuronides</td>
<td>2.3 ± 1.0</td>
<td></td>
<td>0.5 ± 0.3</td>
<td>2.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Sulfates</td>
<td>1.9 ± 1.3</td>
<td></td>
<td>1.0 ± 0.4</td>
<td>2.9</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Others</td>
<td>56.9 ± 3.4</td>
<td></td>
<td>28.6 ± 0.7</td>
<td>85.5</td>
<td></td>
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<tr>
<td>Feces</td>
<td>0—72</td>
<td>5</td>
<td>Free forms</td>
<td>45.0 ± 3.2</td>
<td>6.4 ± 1.1</td>
<td>2.0 ± 0.6</td>
<td>53.4</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Glucuronides</td>
<td>2.5 ± 1.0</td>
<td></td>
<td>1.7 ± 0.6</td>
<td>4.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Sulfates</td>
<td>0.1 ± 0.1</td>
<td></td>
<td>1.9 ± 0.6</td>
<td>2.0</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Others</td>
<td>22.3 ± 1.9</td>
<td></td>
<td>17.5 ± 1.1</td>
<td>39.8</td>
<td></td>
</tr>
<tr>
<td>Bile</td>
<td>0—4</td>
<td>6</td>
<td>Free forms</td>
<td>0.2 ± 0.0</td>
<td>5.2 ± 1.0</td>
<td>1.6 ± 0.4</td>
<td>7.0</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Glucuronides</td>
<td>47.2 ± 3.9</td>
<td></td>
<td>1.2 ± 0.4</td>
<td>48.4</td>
<td></td>
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<td>Sulfates</td>
<td>1.5 ± 1.3</td>
<td></td>
<td>0.8 ± 0.4</td>
<td>2.3</td>
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<td></td>
<td></td>
<td></td>
<td>Others</td>
<td>21.1 ± 3.9</td>
<td></td>
<td>21.2 ± 0.5</td>
<td>42.3</td>
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</table>

The data are presented as the mean ± standard error. n, number of animals.

methanolic solution, which was bathochromically shifted by 28 nm in alkaline methanolic solution (ca. pH 13). The aglycone obtained after treatment with β-glucuronidase was identified as magnolol by direct comparisons of the retention time on HPLC and the Rf values on TLC in various solvent systems with those of an authentic sample. Based on these findings, B1 was determined to be magnolol-2-O-glucuronide. The amounts of B1, B2 and B3 were 50, 2 and 3% of the excreted radioactivity, respectively, as measured with an HPLC-radioanalyzer. The total glucuronide content (55%) in the bile was in quite good agreement with the value obtained by the enzymic method as described above (Table II).

Analysis of Radioactivity in the Blood by HPLC

The benzene-soluble fraction (ca. 10%) of the blood which was collected during 15 min to
12 h after oral administration of \([\text{ring-}^{14}\text{C}]\)magnolol was analyzed with an HPLC-radioanalyzer (data not shown). The major radioactive peak was ascribed to unchanged magnolol on the basis of a comparison of the retention times (32—33.5 min). Similarly, the AcOEt-soluble (16%) and water-soluble (74%) fractions were also shown to consist mostly of magnolol-O-glucuronide and unidentified compounds, respectively.

**Analysis of Fecal Metabolites by HPLC**

As reported previously,\(^2\) repeated oral administration of magnolol resulted in increases of the amounts of isomagnolol, tetrahydromagnolol and other metabolites in the feces. However, due to the difficulty in determining the UV extinction coefficients of all the minor metabolites, the previous experiment was not fully quantitative. In the present experiment, more quantitative analysis of the fecal metabolites was carried out by using radioactive magnolol and an HPLC-radioanalyzer. Figure 6 shows the HPLC elution profiles of the benzene-soluble fraction of feces, monitored by measuring the UV absorbance at 250 nm and the radioactivity. The metabolites, tetrahydromagnolol, 5-((E)-1-propenyl)-5'-propyl-2,2'-dihydroxybiphenyl, 5-allyl-5'-propyl-2,2'-dihydroxybiphenyl, isomagnolol and 5-allyl-5'-(E)-1-propenyl)-5,5'-dihydroxybiphenyl, as well as unchanged magnolol, were identified by direct comparison of the retention times with those of authentic samples.\(^2\) The amounts of these metabolites were quantitatively measured with a radioanalyzer.

Figure 7 shows the changes in the composition of fecal metabolites after repeated administration of 2 µCi/24 h to rats. In accordance with the previous result, magnolol was recovered as a major radioactive component in the feces at the first 24 h, but tended to decrease in amount on repeated administration. Tetrahydromagnolol and other metabolites
increased in amount and reached a maximum after the fifth 24 h period.

Discussion

[ring-14C]Magnolol was rapidly absorbed from the gastrointestinal tract in rats after oral administration. A 14C peak in the blood was first attained within 15 min of oral administration and the second peak was attained at 8 h, suggesting an enterohepatic circulation of magnolol and its metabolites. Analysis of radioactive constituents of the blood with an HPLC-radioanalyzer showed the presence of unchanged magnolol (10%), magnolol-2-O-glucuronide (16%) and other unidentified compounds (74%). The latter products seem to be not the glucuronides and sulfates but other derivatives of magnolol, which may be bound with water-soluble carrier peptides on the basis of the gross chemical fractionation.

The radioactivity after oral administration was mostly distributed in the liver, stomach and intestine. The radioactivity of the liver, lung, kidney and pancreas also showed two appreciable peaks at 15 min and 8 h, coincident with the profile observed in the blood level.

Orally and intraperitoneally administered [ring-14C]magnolol was mostly excreted into the feces, accounting for 65 and 52%, respectively, of the administered dose within 24 h, while excretion into urine accounted for as much as 11 and 24%, respectively. These findings indicate that the main excretion route of the radioactivity following either oral or intraperitoneal administration is via the alimentary tract. This result is in contrast with the case of unsubstituted biphenyl, for which 58% was excreted into the urine.8) Magnolol, which possesses two hydroxyl groups in the biphenyl ring, was easily subject to conjugation, followed by excretion into the bile. In fact, the biliary radioactivity was present as glucuronides (48—49%), sulfates (2%) and unidentified water-soluble compounds (42%), while only 7% was present as the free forms of magnolol and its metabolites.

Analysis of the fecal and urinary metabolites by HPLC showed that magnolol was subject to isomerization, reduction and conjugation to yield a variety of metabolites, as reported previously.2) In the feces, the radioactivity was mostly present as the free forms (53%), tetrahydromagnolol, 5-((E)-1-propenyl)-5'-propyl-2,2'-dihydroxybiphenyl, 5-allyl-5'-propyl-2,2'-dihydroxybiphenyl, isomagnolol, 5-allyl-5'-(E)-1-propenyl)-2,2'-dihydroxybiphenyl and magnolol, and as unidentified water-soluble compounds (40%). Only ca. 6% was present as glucuronides and sulfates, in contrast to the case of the bile. This indicates that the conjugates which were excreted into the bile as the major radioactive components were hydrolyzed to the free forms by intestinal microflora. In addition, the amounts of tetrahydromagnolol and isomagnolol tended to increase on repeated oral administration to rats, suggesting that the formation of these metabolites is associated with the induction of metabolic enzymes of either animal tissues or intestinal bacteria. In the urine, major metabolites were unidentified water-soluble compounds (85—86%), but small amounts of the glucuronides (3%),9) sulfates (3%) and free forms (9%, magnolol and its reduced and isomerized compounds2) were also detected.

The bark of Magnolia officinalis REHD. et WILS. or M. obovata THUNB. contains magnolol and honokiol in various ratio as the major phenolic constituents.10) Magnolol and honokiol have been reported to produce central nervous system depressant effects and centrally-acting muscle relaxation11a-d) and to have potent antibacterial action against gram-positive bacteria including a cariogenic bacterium, Streptococcus mutans.4,12) Furthermore, the former compound was recently shown to have anti-gastric ulcer and antisecretery activities by Watanabe et al.11c) These pharmacological results suggest that both compounds are active principles of the crude drug, Magnoliaceae Cortex, which has been used for the therapy of neurosis and gastrointestinal complaints in traditional Chinese medicine. Since these compounds are similar in structure, honokiol may also be metabolized in a similar fashion to
magnolol. The metabolites, however, would be more complex because honokiol consists of
two heterogeneous phenylpropanoid units, unlike magnolol, which consists of two homo-
geneous units.

References and Notes

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