Studies on Choleretic Constituents in *Artemisia capillaris* THUNB.

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Twelve constituents (1, capillarin; 2, scoparone; 3, scopoletin; 4, isoscpoletin; 5, capillaratemisin-7-methyl ether; 6, cirsimaritin; 7, capillarin; 8, arteminin A; 9, capillaratemisin B1; 10, arteminin C; 11, capillarin; 12, capielle) were isolated after testing the choleretic activity of various fractions derived from the water extract of *Artemisia capillaris*. In tests of the choleretic activity in Wistar rats, four constituents (2, 8, 9, 10) were found to make an overwhelming contribution to the activity. Five constituents (3, 4, 8, 10, 12) were found for the first time in this plant. All of the compounds caused bile secretion to increase without increasing biliary bile acid, cholesterol or phospholipid excretion.

**Keywords**—choleretic constituent; bile flow; biliary lipid; *Artemisia capillaris*; arteminin A, C; capillaratemisin B1; cipelle; capillarisin; scoparone; scopoletin

The extract of *Artemisia capillaris* has been reported to show a choleretic effect in dogs, rabbits or rats,1-4 and 6,7-dimethyleculetin (scoparone)2-3 and capillarisin5 were isolated as effective constituents. Recently, Kitagawa et al.6 isolated capillaratemisin B1, as a new effective constituent from this plant. However, it remains uncertain whether the choleretic activity detected in the water extract of this plant is entirely due to these compounds. To identify clearly the active components, we repeatedly fractioned the water extract and examined the effects of these fractions and the 12 compounds isolated from them on bile flow and biliary lipid secretion in rats. We also studied the effects of dehydrocholic acid, p-hydroxyacetophenone7 and chlorogenic acid8; the last two compounds have also been reported to be choleretic principles of this plant.

**Materials and Methods**

Dehydrocholic acid and chlorogenic acid were purchased from Nakarai Chemicals (Tokyo, Japan), and p-hydroxyacetophenone from Wako Pure Chemicals Ind. (Tokyo, Japan). Twelve compounds, capillarin9 (mp 125-127°C, scoparone10 (mp 143-145°C), scopoletin11 (mp 210°C), isoscpoletin12 (mp 186-187°C), capillarisin 7-methyl ether13 (mp 206-207°C), cirsimaritin14 (mp ca. 260°C, diacetate 197-199°C), capillarisin15 (mp 226°C), arteminin A (mp 127-129°C), capillaratemisin B1,6 (mp 146-148°C), arteminin C (mp 150-152°C), capillarisin15 (mp 81°C) and capielle16) (yellowsish oil) were isolated from the commercially available material "inchinko",17 which is composed of flower heads of *Artemisia capillaris* THUNB. The procedures used to isolate these compounds are summarized in Fig. 1. The ten compounds other than arteminin A and C were identified on the basis of spectral and other physical data. Arterinin A and C gave very similar spectral data to those of capillaratemisin B1, and their structures were assumed to be as shown in Fig. 2 (the absolute stereochemistry at C-8 and C-9 was not assigned). Scopoletin and isoscpoletin were prepared by demethylation of scoparone because only small amounts could be isolated from the plant.

Arteminin A (8), C_{19}H_{15}O_{4} (Calcd: C 72.12, H, 7.65. Found: C 71.85, H, 7.51). [α]_{D}^{25} + 25.3 ± 0.6 (c = 1.045, CHCl_{3}). IR ν_{max}: 3350-2600 (br), 1683, 1624, 1596cm⁻¹. UV λ_{max}(ε): 324 (23500), 240.5 (13500), 220.5 (14300) nm. 1H-NMR (CDCl_{3}) δ ppm: 7.67 (1H, d, J = 17 Hz, H-17), 7.18 (1H, br s, H-2 or H-6), 7.13 (1H, br s H-6 or H-2), 6.23 (1H, d, J = 17 Hz, H-18), 5.25 (1H, br t, H-13), 4.73 (1H, q, J = 8 Hz, H-8), 3.73 (2H, d, J = 6.5 Hz, H-10), 3.23 (2H, m, H-12), 3.05 (2H, m, H-7), 2.08 (1H, m, H-9), 1.72 (6H, s, H-15, H-16), 0.97 (3H, d, J = 6.5 Hz, H-11). 13C-NMR
Fig. 1. Isolation of Twelve Compounds from the Cude Drug "Inchinko"
(Artemisia capillaris THUNB.)

(CDC13) δ ppm: 172.6 (s, C-19), 159.9 (s, C-4), 147.1 (d, C-17), 133.4 (s, C-14), 129.7 (d, C-2 or C-6), 127.3 (s x 2) and
123.7 (s) (C-1, C-3, C-5), 122.5 (d, C-2 or C-6), 121.3 (d, C-13), 114.2 (d, C-18), 87.3 (d, C-8), 65.9 (t, C-10), 41.0 (d, C-9),
33.8 (t, C-7), 28.2 (t, C-12), 25.7, 17.8, 12.7 (each q, C-11, C-15, C-16).

Artepillin C (10), C18H22O5 (Calcd: C, 75.97; H, 8.05. Found: C, 76.01; H, 8.02). IR v max cm⁻¹: 3400, 3200-2500 (br),
1685, 1625, 1595. 1H-NMR (CDCl3) δ ppm: 7.67 (1 H, d, J=17 Hz, H-17), 7.15 (2H, brs, H-2, H-6), 6.23 (1H, d, J=17 Hz, H-18), 5.28 (2H, brt, H-8, H-13), 3.33 (4H, m, H-7, H-12), 1.78 (12H, s, H-10, H-11, H-15 and H-16).

13C-NMR (acetone-d6) δ ppm: 169.7 (s, C-19), 155.7 (s, C-14), 146.6 (d, C-17), 133.4 (s, C-9, C-14), 129.2 (s, C-3,
C-5), 128.4 (d, C-2, C-6), 127.0 (s, C-1), 122.8 (d, C-8, C-13), 115.3 (d, C-18), 29.2 (t, C-7, C-12), 25.8, 17.8 (each q, C-
10, C-11, C-15, C-16). Each compound was suspended in 5% gum arabic water solution and administered intraduodenally.

Wistar strain male rats (9 to 11 weeks of age, bred at Shionogi Aburahi Laboratories, Shiga, Japan) were anesthetized with sodium pentobarbital (60 mg/kg, i.p.) and the bile duct was cannulated with polyethylene tubing (PE-10). Bile was collected every 30 min for 3 h and weighed. The test compounds were injected intraduodenally after the first bile collection. Biliary bile acids were determined enzymatically by the method reported by Mashige et al.,18) cholesterol by GLC,19) and phospholipids by the method of Gomori.20) The significance of differences in values was determined by using Student's t-test.

Results

The procedures for isolating the twelve compounds are shown in Fig. 1. Ethyl acetate was added to the water extract (AC01) of the crude material and two fractions were obtained, the ethyl acetate extract (AC02) and the remaining water layer (AC03). AC02 was subjected to column chromatography on silicic acid and divided into six fractions (AC02-10-AC02-60). The effects of the fractions AC01, AC02 and AC03 on bile flow were examined (Table I). The dose for injection was adjusted to contain 2 or 4 g of the crude material per rat. The bile flow in the control rats was about 0.9 ml/30 min and remained almost constant during the period of the experiment. The AC01 fraction showed a tendency to increase the bile flow in the first and second periods but not thereafter. The AC02 fraction was ineffective at a dose of 2 g of crude material but increased the bile flow at a dose of 4 g of crude material. The AC03 fraction was ineffective even at the higher dose.

The effects of subdivisions of fraction AC02 were also examined; each dose corresponded to 4 g of crude material. The amount of the first fraction (AC02-10) was so small that the assay could not be performed. As shown in Table I, the fractions AC02-20 and -60 showed

<table>
<thead>
<tr>
<th>Extract or fraction dose</th>
<th>No. of rats</th>
<th>0-30</th>
<th>30-60</th>
<th>60-90</th>
<th>90-120</th>
<th>120-150</th>
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<tbody>
<tr>
<td>Control (gum arabic)</td>
<td>6</td>
<td>0.90 ± 0.05</td>
<td>0.86 ± 0.04</td>
<td>0.92 ± 0.04</td>
<td>0.92 ± 0.04</td>
<td>0.86 ± 0.04</td>
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<tr>
<td>AC01 3 ml of extract/rat</td>
<td>3</td>
<td>0.84 ± 0.06</td>
<td>1.04 ± 0.09</td>
<td>1.04 ± 0.08</td>
<td>0.90 ± 0.08</td>
<td>0.92 ± 0.08</td>
</tr>
<tr>
<td>(=2 g of &quot;inchinko&quot;/rat)</td>
<td>(96)</td>
<td>(103)</td>
<td>(103)</td>
<td>(97)</td>
<td>(104)</td>
<td>(98)</td>
</tr>
<tr>
<td>AC02 46 mg/rat</td>
<td>2</td>
<td>0.85</td>
<td>0.79</td>
<td>0.85</td>
<td>0.81</td>
<td>0.82</td>
</tr>
<tr>
<td>(=2 g of &quot;inchinko&quot;/rat)</td>
<td>(94)</td>
<td>(100)</td>
<td>(97)</td>
<td>(98)</td>
<td>(98)</td>
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</tr>
<tr>
<td>AC02 96 mg/rat (=4 g of &quot;inchinko&quot;/rat)</td>
<td>4</td>
<td>0.85 ± 0.06</td>
<td>1.45 ± 0.12</td>
<td>1.38 ± 0.08</td>
<td>1.12 ± 0.03</td>
<td>1.02 ± 0.04</td>
</tr>
<tr>
<td>AC03 410 mg/rat (=2 g of &quot;inchinko&quot;/rat)</td>
<td>2</td>
<td>0.77</td>
<td>0.73</td>
<td>0.75</td>
<td>0.77</td>
<td>0.81</td>
</tr>
<tr>
<td>AC03 820 mg/rat (=4 g of &quot;inchinko&quot;/rat)</td>
<td>2</td>
<td>0.77</td>
<td>0.77</td>
<td>0.82</td>
<td>0.84</td>
<td>0.87</td>
</tr>
<tr>
<td>AC02-20 22 mg/rat</td>
<td>3</td>
<td>0.79 ± 0.07</td>
<td>1.07 ± 0.07</td>
<td>0.97 ± 0.08</td>
<td>0.88 ± 0.07</td>
<td>0.82 ± 0.05</td>
</tr>
<tr>
<td>AC02-30 5.7 mg/rat</td>
<td>3</td>
<td>0.83 ± 0.08</td>
<td>1.01 ± 0.07</td>
<td>0.92 ± 0.10</td>
<td>0.86 ± 0.09</td>
<td>0.82 ± 0.10</td>
</tr>
<tr>
<td>AC02-40 5.4 mg/rat</td>
<td>3</td>
<td>0.93 ± 0.13</td>
<td>1.05 ± 0.08</td>
<td>0.94 ± 0.06</td>
<td>0.84 ± 0.03</td>
<td>0.83 ± 0.05</td>
</tr>
<tr>
<td>AC02-50 4.7 mg/rat</td>
<td>3</td>
<td>0.76 ± 0.03</td>
<td>0.86 ± 0.01</td>
<td>0.86 ± 0.02</td>
<td>0.83 ± 0.04</td>
<td>0.90 ± 0.07</td>
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<tr>
<td>AC02-60 49 mg/rat</td>
<td>3</td>
<td>0.81 ± 0.07</td>
<td>1.19 ± 0.07</td>
<td>1.07 ± 0.07</td>
<td>1.05 ± 0.06</td>
<td>1.02 ± 0.06</td>
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</table>

a) Corresponding to extract (or fraction) contained in 2 g of "inchinko" (A. capillaris). b) Mean ± S.E. (g). c) Percentage of the initial level (Mean ± S.E.). d) Statistically significant compared with the initial level (p<0.05). e) Statistically significant compared with the control (p<0.05).
TABLE II. Effects of Fourteen Compounds Contained in *A. capillaris* and Dehydrocholic Acid on Bile Secretion in Wistar Rats

<table>
<thead>
<tr>
<th>Dose</th>
<th>No. of rats</th>
<th>Time after administration (min)</th>
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<td>30-60</td>
<td>60-90</td>
<td>90-120</td>
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<tr>
<td>Control</td>
<td>11</td>
<td>0.98 ± 0.03 ^a</td>
<td>1.03 ± 0.03</td>
<td>1.06 ± 0.03</td>
<td>1.01 ± 0.03</td>
<td>0.99 ± 0.03</td>
<td>0.92 ± 0.02</td>
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<tr>
<td>Capillarin 50 mg/kg</td>
<td>4</td>
<td>0.96 ± 0.05</td>
<td>1.10 ± 0.05</td>
<td>1.09 ± 0.07</td>
<td>1.01 ± 0.03</td>
<td>0.96 ± 0.03</td>
<td>0.95 ± 0.05</td>
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<tr>
<td>Scoparone 50 mg/kg</td>
<td>5</td>
<td>0.94 ± 0.09</td>
<td>1.19 ± 0.10</td>
<td>1.14 ± 0.09</td>
<td>1.06 ± 0.07</td>
<td>1.02 ± 0.08</td>
<td>0.96 ± 0.06</td>
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<tr>
<td>Scoleptin 50 mg/kg</td>
<td>4</td>
<td>0.91 ± 0.08</td>
<td>1.35 ± 0.11 ^a</td>
<td>1.02 ± 0.08</td>
<td>0.99 ± 0.06</td>
<td>0.96 ± 0.05</td>
<td>0.88 ± 0.03</td>
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<tr>
<td>Isoscopoletin 50 mg/kg</td>
<td>4</td>
<td>0.88 ± 0.10</td>
<td>1.06 ± 0.09</td>
<td>0.95 ± 0.10</td>
<td>0.87 ± 0.06</td>
<td>0.86 ± 0.05</td>
<td>0.84 ± 0.03</td>
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<tr>
<td>Capillarasin-7- methylether 50 mg/kg</td>
<td>5</td>
<td>0.89 ± 0.06</td>
<td>0.92 ± 0.05</td>
<td>0.94 ± 0.05</td>
<td>0.90 ± 0.06</td>
<td>0.92 ± 0.06</td>
<td>0.91 ± 0.05</td>
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<tr>
<td>Cirsimaritin 50 mg/kg</td>
<td>5</td>
<td>0.95 ± 0.06</td>
<td>0.96 ± 0.07</td>
<td>0.99 ± 0.07</td>
<td>0.93 ± 0.05</td>
<td>0.94 ± 0.05</td>
<td>0.92 ± 0.04</td>
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<tr>
<td>Capillarin 50 mg/kg</td>
<td>5</td>
<td>0.92 ± 0.03</td>
<td>1.13 ± 0.02 ^a</td>
<td>0.99 ± 0.04</td>
<td>0.98 ± 0.03</td>
<td>0.95 ± 0.02</td>
<td>0.93 ± 0.06</td>
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<tr>
<td>Artepillin A 50 mg/kg</td>
<td>5</td>
<td>0.98 ± 0.03</td>
<td>1.12 ± 0.02 ^a</td>
<td>1.17 ± 0.03 ^a</td>
<td>1.06 ± 0.05</td>
<td>1.06 ± 0.04</td>
<td>1.06 ± 0.02</td>
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<tr>
<td>Capillartemin B1 50 mg/kg</td>
<td>5</td>
<td>0.96 ± 0.01</td>
<td>1.34 ± 0.03 ^a</td>
<td>1.17 ± 0.06 ^a</td>
<td>1.11 ± 0.07</td>
<td>1.03 ± 0.07</td>
<td>0.96 ± 0.07</td>
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<tr>
<td>Artepillin C 50 mg/kg</td>
<td>5</td>
<td>1.01 ± 0.06</td>
<td>1.39 ± 0.06 ^a</td>
<td>1.20 ± 0.03 ^a</td>
<td>1.11 ± 0.02</td>
<td>0.99 ± 0.06</td>
<td>0.89 ± 0.06</td>
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<tr>
<td>Capillan 50 mg/kg</td>
<td>4</td>
<td>0.92 ± 0.07</td>
<td>1.06 ± 0.04</td>
<td>1.03 ± 0.05</td>
<td>0.92 ± 0.04</td>
<td>0.89 ± 0.06</td>
<td>0.82 ± 0.04</td>
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<tr>
<td>Capillene 50 mg/kg</td>
<td>5</td>
<td>0.97 ± 0.04</td>
<td>1.13 ± 0.05 ^a</td>
<td>1.09 ± 0.07</td>
<td>1.00 ± 0.04</td>
<td>0.95 ± 0.05</td>
<td>0.93 ± 0.04</td>
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<tr>
<td>p-Hydroxyacetophenone 50 mg/kg</td>
<td>4</td>
<td>0.84 ± 0.03</td>
<td>1.43 ± 0.03 ^a</td>
<td>1.02 ± 0.08</td>
<td>0.96 ± 0.07</td>
<td>0.93 ± 0.07</td>
<td>0.88 ± 0.03</td>
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<tr>
<td>Chlorogenic acid 50 mg/kg</td>
<td>5</td>
<td>0.90 ± 0.06</td>
<td>0.93 ± 0.07</td>
<td>0.92 ± 0.07</td>
<td>0.91 ± 0.07</td>
<td>0.89 ± 0.04</td>
<td>0.85 ± 0.04</td>
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<tr>
<td>Dehydrocholic acid 50 mg/kg</td>
<td>5</td>
<td>0.95 ± 0.06</td>
<td>1.31 ± 0.05 ^a</td>
<td>1.18 ± 0.04 ^a</td>
<td>1.08 ± 0.04</td>
<td>1.02 ± 0.03</td>
<td>0.95 ± 0.03</td>
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</table>

\(^{a)}\) Mean±S.E. \(^{b)}\) Percentage of the initial level. \(^{c)}\) Statistically significant compared with the initial level (\(p<0.05\)). \(^{d)}\) Statistically significant compared with the control (\(p<0.05\)).

prominent choleretic activity, and AC-50 showed slight activity. While AC02-20 was composed exclusively of scoparone, AC-60 contained at least three choleretic constituents, that is, capillartemin B1 and artepillins A and C, which were newly isolated in this study. Fractions AC02-30 and -40 increased the bile flow slightly but the changes were statistically insignificant (\(p > 0.05\)).

Next, we examined the effects on bile secretion of the twelve constituents isolated from *A. capillaris* (capillarin, scoparone, scoleptin, isoscopoletin, capitellarisin-7-methyl ether, cirsimaritin, capitellarisin, artepillin A, capitellarisin B1, artepillin C, capillan and capillene), two additional constituents of this plant reported to be choleretic (\(p\)-hydroxyacetophenone and chlorogenic acid), and dehydrocholic acid. The dose of each compound was 50 mg/kg (Table II). Eight isolated constituents (scoparone, scoleptin, isoscopoletin, capitellarisin, artepillin A, capitellarisin B1, artepillin C, capillene), \(p\)-hydroxyacetophenone and dehydrocholic acid increased the bile flow. The most pronounced effect was caused by \(p\)-hydroxyacetophenone, and the effects of scoleptin, capitellarisin B1 and artepillin C were similar to that of
### Table III. Effect of Twelve Compounds Isolated from *A. capillaris* and Dehydrocholic Acid on Biliary Bile Acid Secretion in Wistar Rats

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Dose (mg/kg)</th>
<th>No. of rats</th>
<th>Time after administration (min)</th>
<th>Control</th>
<th>Scoparone</th>
<th>Scopoletin</th>
<th>Isoscopoletin</th>
<th>Capillarin</th>
<th>Artepillin A</th>
<th>Capillartemin B&lt;sub&gt;1&lt;/sub&gt;</th>
<th>Artepillin C</th>
<th>Capillene</th>
<th>Dehydrocholic acid</th>
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<td>0–30 – 30–60 – 60–90 – 90–120 – 120–150</td>
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<tr>
<td>Control</td>
<td>7</td>
<td>7</td>
<td>10.05 ± 1.63&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.90 ± 1.38</td>
<td>10.69 ± 1.16</td>
<td>10.16 ± 0.86</td>
<td>9.96 ± 1.18</td>
<td>8.29 ± 0.97</td>
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<td>4</td>
<td>8.05 ± 1.18</td>
<td>8.27 ± 1.60</td>
<td>8.18 ± 1.02</td>
<td>9.65 ± 2.25</td>
<td>8.28 ± 2.78</td>
<td>6.87 ± 2.81</td>
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<td>4</td>
<td>8.65 ± 1.61</td>
<td>8.25 ± 2.43</td>
<td>8.51 ± 1.84</td>
<td>9.77 ± 1.37</td>
<td>9.56 ± 1.08</td>
<td>8.22 ± 1.89</td>
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<td>4</td>
<td>6.87 ± 2.03</td>
<td>5.51 ± 1.83</td>
<td>5.71 ± 1.74</td>
<td>5.03 ± 0.61</td>
<td>5.73 ± 0.43</td>
<td>6.32 ± 0.73</td>
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<td>Isoscopoletin</td>
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<td>8.97 ± 1.12</td>
<td>8.64 ± 0.71</td>
<td>10.97 ± 1.17</td>
<td>10.23 ± 1.19</td>
<td>8.80 ± 1.11</td>
<td>9.05 ± 0.78</td>
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<td></td>
<td></td>
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<td>9.97 ± 1.12</td>
<td>9.97 ± 1.12</td>
<td>10.17 ± 1.17</td>
<td>10.23 ± 1.19</td>
<td>8.80 ± 1.11</td>
<td>9.05 ± 0.78</td>
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<td>5</td>
<td>13.00 ± 1.35</td>
<td>14.15 ± 2.16</td>
<td>14.51 ± 1.19</td>
<td>13.43 ± 1.20</td>
<td>8.42 ± 1.69</td>
<td>6.24 ± 2.86</td>
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<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>11.05 ± 0.64</td>
<td>10.55 ± 0.65</td>
<td>11.62 ± 1.21</td>
<td>9.57 ± 1.57</td>
<td>9.20 ± 1.78</td>
<td>9.42 ± 1.29</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Dehydrocholic acid</td>
<td>5</td>
<td>5</td>
<td>8.40 ± 0.79</td>
<td>13.16 ± 1.21&lt;sup&gt;c&lt;/sup&gt;</td>
<td>11.77 ± 1.85</td>
<td>10.77 ± 2.04</td>
<td>10.59 ± 1.48</td>
<td>8.99 ± 0.71</td>
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</tr>
</tbody>
</table>

<sup>a</sup> Mean ± S.E. (mg/30min). <sup>b</sup> Percentage of the initial level (Mean ± S.E.). <sup>c</sup> Statistically significant compared with the initial level. <sup>d</sup> Statistically significant compared with the control (p<0.05).

### Table IV. Effects of Twelve Compounds Isolated from *A. capillaris* and Dehydrocholic Acid on Biliary Cholesterol Secretion in Rats

<table>
<thead>
<tr>
<th>Compounds</th>
<th>No. of rats</th>
<th>Time after administration (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0–30 – 30–60 – 60–90 – 90–120 – 120–150</td>
</tr>
<tr>
<td>Control</td>
<td>7</td>
<td>66.1 ± 4.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>74.0 ± 3.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>74.1 ± 2.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>72.5 ± 3.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>64.4 ± 4.0</td>
</tr>
<tr>
<td>Scoparone</td>
<td>4</td>
<td>46.7 ± 4.7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>45.8 ± 3.1&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>57.9 ± 11.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>74.5 ± 13.2</td>
</tr>
<tr>
<td>Scopoletin</td>
<td>4</td>
<td>48.7 ± 9.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>53.9 ± 10.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>58.5 ± 7.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60.6 ± 5.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>58.0 ± 5.8</td>
</tr>
<tr>
<td>Isoscopoletin</td>
<td>4</td>
<td>43.5 ± 12.4</td>
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<tr>
<td></td>
<td></td>
<td>46.5 ± 13.5</td>
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<tr>
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<td>47.8 ± 7.5</td>
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<tr>
<td></td>
<td></td>
<td>48.0 ± 3.2</td>
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<tr>
<td>Capillarin</td>
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<td>47.7 ± 5.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>48.6 ± 5.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50.0 ± 5.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>48.3 ± 8.0</td>
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<tr>
<td>Artepillin A</td>
<td>4</td>
<td>42.8 ± 1.3</td>
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<tr>
<td></td>
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<td>45.8 ± 4.4</td>
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<tr>
<td></td>
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<td>47.1 ± 4.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>52.6 ± 5.9</td>
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<td></td>
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<td>52.1 ± 2.8</td>
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<tr>
<td>Capillartemin B&lt;sub&gt;1&lt;/sub&gt;</td>
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<td>51.5 ± 2.5&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>Artaepillin C</td>
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<td>64.1 ± 10.0</td>
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<tr>
<td></td>
<td></td>
<td>73.3 ± 8.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>79.3 ± 9.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50.2 ± 5.6&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Capillene</td>
<td>5</td>
<td>72.1 ± 7.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>73.7 ± 7.3</td>
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<tr>
<td></td>
<td></td>
<td>63.2 ± 6.3&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60.2 ± 8.4&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Dehydrocholic acid</td>
<td>5</td>
<td>58.4 ± 5.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>61.1 ± 7.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>57.5 ± 7.2</td>
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<tr>
<td></td>
<td></td>
<td>60.6 ± 7.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>53.8 ± 4.9</td>
</tr>
</tbody>
</table>

<sup>a</sup> Mean ± S.E. (µg/30min). <sup>b</sup> Percentage of the initial level. <sup>c</sup> Statistically significant compared with the initial level (p<0.05). <sup>d</sup> Statistically significant compared with the control (p<0.05).
**Table V.** Effects of Twelve Compounds Isolated from *A. capillaris* and Dehydrocholic Acid on Biliary Phospholipid Secretion in Wistar Rats

<table>
<thead>
<tr>
<th></th>
<th>No. of rats</th>
<th>-30-0</th>
<th>0-30</th>
<th>30-60</th>
<th>60-90</th>
<th>90-120</th>
<th>120-150</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>7</td>
<td>2.97±0.35(^a)</td>
<td>2.32±0.23</td>
<td>2.50±0.22</td>
<td>2.48±0.13</td>
<td>2.28±0.19</td>
<td>2.03±0.23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(82±7(^b))</td>
<td>(90±10)</td>
<td>(90±10)</td>
<td>(75±9)</td>
<td>(75±13)</td>
<td></td>
</tr>
<tr>
<td>Scoparone</td>
<td>4</td>
<td>2.14±0.20</td>
<td>1.52±0.21</td>
<td>1.45±0.13(^c)</td>
<td>2.03±0.38</td>
<td>2.21±0.48</td>
<td>2.17±0.51</td>
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<tr>
<td></td>
<td></td>
<td>(71±3)</td>
<td>(69±9)</td>
<td>(99±23)</td>
<td>(108±29)</td>
<td>(107±32)</td>
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<tr>
<td>Scoptoletin</td>
<td>4</td>
<td>2.21±0.48</td>
<td>1.74±0.47</td>
<td>1.88±0.47</td>
<td>1.98±0.37</td>
<td>1.95±0.21</td>
<td>1.73±0.22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(75±4)</td>
<td>(84±3)</td>
<td>(92±6)</td>
<td>(96±14)</td>
<td>(93±24)</td>
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<tr>
<td>Isoscoptoletin</td>
<td>4</td>
<td>1.98±0.56</td>
<td>1.30±0.42</td>
<td>1.59±0.48</td>
<td>1.47±0.26</td>
<td>1.59±0.15</td>
<td>1.53±0.11</td>
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<tr>
<td></td>
<td></td>
<td>(64±5)</td>
<td>(80±4)</td>
<td>(83±11)</td>
<td>(98±23)</td>
<td>(100±30)</td>
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<tr>
<td>Capillarasin</td>
<td>5</td>
<td>2.31±0.28</td>
<td>1.70±0.21</td>
<td>1.57±0.23</td>
<td>1.65±0.22</td>
<td>1.70±0.19</td>
<td>1.65±0.22</td>
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<tr>
<td></td>
<td></td>
<td>(73±2)</td>
<td>(73±4)</td>
<td>(73±8)</td>
<td>(76±7)</td>
<td>(75±11)</td>
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</tr>
<tr>
<td>Artepillin A</td>
<td>4</td>
<td>2.40±0.15</td>
<td>1.64±0.07(^d)</td>
<td>2.03±0.17</td>
<td>2.17±0.29</td>
<td>2.43±0.28</td>
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<td>(69±6)</td>
<td>(86±10)</td>
<td>(92±14)</td>
<td>(102±13)</td>
<td>(102±18)</td>
<td></td>
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<tr>
<td>Capillartemisin B(_1)</td>
<td>5</td>
<td>2.75±0.27</td>
<td>1.81±0.11(^e)</td>
<td>2.35±0.22</td>
<td>2.27±0.21</td>
<td>2.12±0.21</td>
<td>1.78±0.30</td>
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<td>(88±10)</td>
<td>(87±13)</td>
<td>(82±13)</td>
<td>(72±19)</td>
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<tr>
<td>Artepillin C</td>
<td>5</td>
<td>3.08±0.32</td>
<td>2.23±0.32</td>
<td>2.45±0.18</td>
<td>2.43±0.20</td>
<td>1.75±0.22</td>
<td>1.28±0.41</td>
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<tr>
<td></td>
<td></td>
<td>(72±6)</td>
<td>(82±8)</td>
<td>(83±11)</td>
<td>(62±12)</td>
<td>(47±18)</td>
<td></td>
</tr>
<tr>
<td>Capillene</td>
<td>4</td>
<td>2.23±0.07</td>
<td>1.98±0.12</td>
<td>2.03±0.19</td>
<td>1.82±0.15</td>
<td>1.71±0.16</td>
<td>1.72±0.17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(89±4)</td>
<td>(92±9)</td>
<td>(83±8)</td>
<td>(77±7)</td>
<td>(78±8)</td>
<td></td>
</tr>
<tr>
<td>Dehydrocholic acid</td>
<td>4</td>
<td>2.26±0.22</td>
<td>1.98±0.21</td>
<td>2.15±0.28</td>
<td>2.06±0.30</td>
<td>2.15±0.32</td>
<td>1.94±0.17</td>
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<tr>
<td></td>
<td></td>
<td>(87±4)</td>
<td>(96±12)</td>
<td>(92±13)</td>
<td>(97±15)</td>
<td>(89±11)</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Mean±S.E. (mg/30 min). \(^b\) Percentage of the initial level. \(^c\) Statistically significant compared with the initial level (p<0.05).

dehydrocholic acid.

Changes in biliary secretion of bile acids, cholesterol and phospholipids are shown in Tables III, IV and V, respectively. The biliary bile acid secretion showed no statistically significant change, but when expressed as a percentage of the value before administration to the same animals, the bile acid secretion decreased in the rats given artepillin A (and isoscoptoletin) (Table III). Dehydrocholic acid, on the other hand, increased the biliary bile acid secretion.

Decrease of the biliary cholesterol secretion was caused by scoparone, scoptoletin and capillartemisin B\(_1\). Artepillin C and capillene showed an initial tendency of decrease (0—60 min) which became significant later (60 min or later) (Table IV). Dehydrocholic acid caused no significant change in the cholesterol secretion.

The biliary phospholipid secretion showed a tendency to be decreased by most of the compounds during the first 30 min and gradually recovered thereafter, except for the groups given capillarasin and artepillin C (Table V). The levels in these two groups remained low during the experimental period.

**Discussion**

The water extract of *A. capillaris* was divided into several fractions and their choleric activities were examined at doses corresponding to 2 or 4 g of the crude material (*A. capillaris*). Choleric activity was found in the ethyl acetate fraction and its sub-fractions AC02-60, -20 and -50. Judging from their activities, fractions AC-20 and -60 were considered to contribute overwhelmingly to the choleric activity of *A. capillaris*. The former fraction was mainly composed of scoparone and the latter contained two choleric compounds, artepillins A and C, which were newly isolated, in addition to capillartemisin B\(_1\). When the choleric activities of these compounds were compared at a dose of 50 mg/kg, p-
hydroxyacetophenone was most effective, and artepillin C, scopoletin and capillartemisin B₄ had nearly the same activity as dehydrocholic acid. Scoopoletin and isoscopoletin were also isolated from fraction AC30, which caused no increase in bile secretion, presumably because their levels in the fraction were very low. Aburada et al.⁵ reported that the essential oil of this plant increased bile flow, and we also found that capillene, a major component of the essential oil, had choleretic activity.

Bile secretion was increased 30 min after administration of dehydrocholic acid, and biliary bile acid secretion increased at the same time, but bile acid secretion was not increased by the eight compounds which increased bile secretion. Biliary phospholipid secretion is generally considered to be dependent on bile secretion.⁶ Dehydrocholic acid increased biliary bile acid secretion, but did not change phospholipid secretion, although biliary phospholipid secretion tended to be decreased by most of the compounds examined as well as the control (5% gum arabic). Capillartemisin B₄ and artepillin A did not decrease biliary bile acid secretion, but the former significantly decreased the biliary phospholipid secretion.

Cholesterol secretion is thought to be less dependent on bile acid secretion than phospholipid secretion. Scoparone, scopoletin and capillartemisin B₄ scarcely changed biliary bile acid secretion (0—30 min), but significantly decreased cholesterol secretion. The mechanism of bile secretion is not fully understood, but the secretion is thought to be composed of a bile acid-dependent flow and a bile acid-independent flow.⁷ According to this concept, the increase in bile secretion by these eight compounds seems to be attributable to the bile flow caused by organic anions of the compounds administered, as reported by Aburada et al.⁸

Identifying the components contributing most to the choleretic activity is difficult because the chemical composition of A. capillaris differs with the materials examined⁹ and p-hydroxyacetophenone, which has not been found in Japanese A. capillaris, has been confirmed to be highly choleretic.¹⁰ However, our present results suggest that four components, scoparone, capillartemisin B₄ and artepillins A and C, are the most likely constituents, at least in Japanese A. capillaris, which show choleretic activity without relying on special mechanisms such as increased excretion of biliary lipids.

References and Notes

1) T. Yukawa, R. Takano and T. Miyoshi, Experimental Gastroenterology, 3, 1349 (1929).