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Studies on Choleretic Constituents in *Artemisia capillaris* THUNB.

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Twelve constituents (**1**, capillarin; **2**, scoparone; **3**, scopoletin; **4**, isoscopoletin; **5**, capillartemisin-7-methyl ether; **6**, cirsimaritin; **7**, capillarisin; **8**, artepillin A; **9**, capillartemisin B_1; **10**, artepillin C; **11**, capillin; **12**, capillene) 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*; artepillin A, C; capillartemisin B_1; capillene; 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-dimethylesculetin (scoparone)[2,3] and capillarisin[5] were isolated as effective constituents. Recently, Kitagawa *et al.*[6] isolated capillartemisin B_1, 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*-hydroxyacetophenone[7] and chlorogenic acid[8]; 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, capillarin[9] (mp 125—127 °C), scoparone[10] (mp 143—145 °C), scopoletin[11] (mp 210 °C), isoscopoletin[12] (mp 186—187 °C), capillarisin 7-methyl ether[13] (mp 206—207 °C), cirsimaritin[14] (mp *ca.* 260 °C, diacetate 197—199 °C), capillarisin[5] (mp 226 °C), artepillin A (mp 127—129 °C), capillartemisin B_1[6] (mp 146—148 °C), artepillin C (mp 150—152 °C), capillin[15] (mp 81 °C) and capillene[16] (yellowish 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 artepillins A and C were identified on the basis of spectral and other physical data. Artepillins A and C gave very similar spectral data to those of capillartemisin B_1 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 isoscopoletin were prepared by demethylation of scoparone because only small amounts could be isolated from the plant.

Artepillin A (**8**), $C_{19}H_{24}O_4$ (Calcd: C 72.12, H, 7.65. Found: C 71.85, H, 7.51). $[\alpha]_D^{25}$ $+25.3\pm0.6°$ ($c=1.045$, $CHCl_3$). IR ν_{max}^{KBr}: 3350—2600 (br), 1683, 1624, 1596 cm^{-1}. UV λ_{max}^{EtOH}: 324 (23500), 240.5 (13500), 220.5 (14300) nm. ^{1}H-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), ^{13}C-NMR

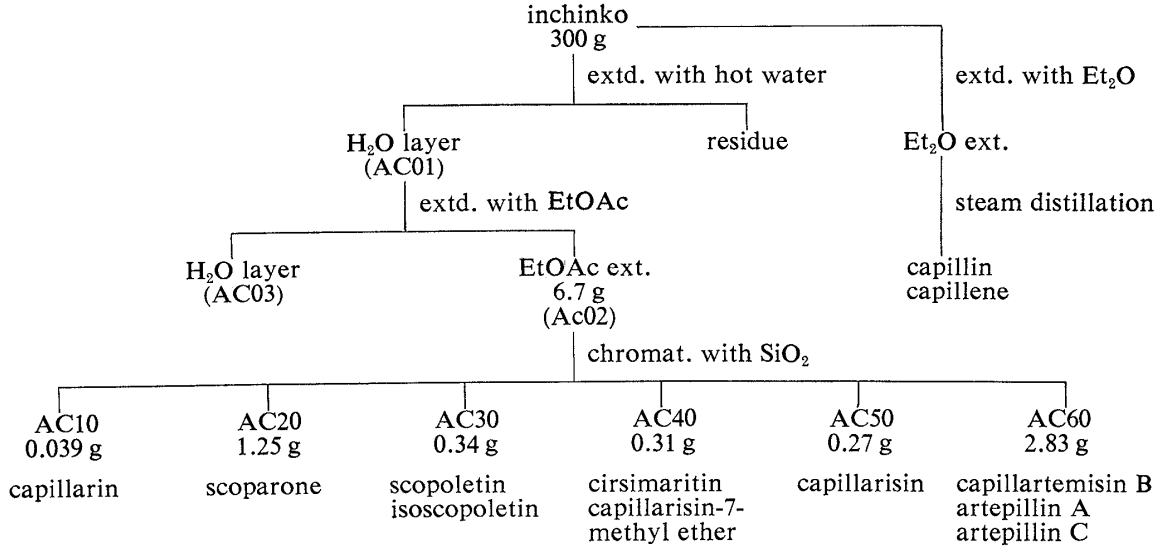

Fig. 1. Isolation of Twelve Compounds from the Cude Drug "Inchinko" (*Artemisia capillaris* THUNB.)

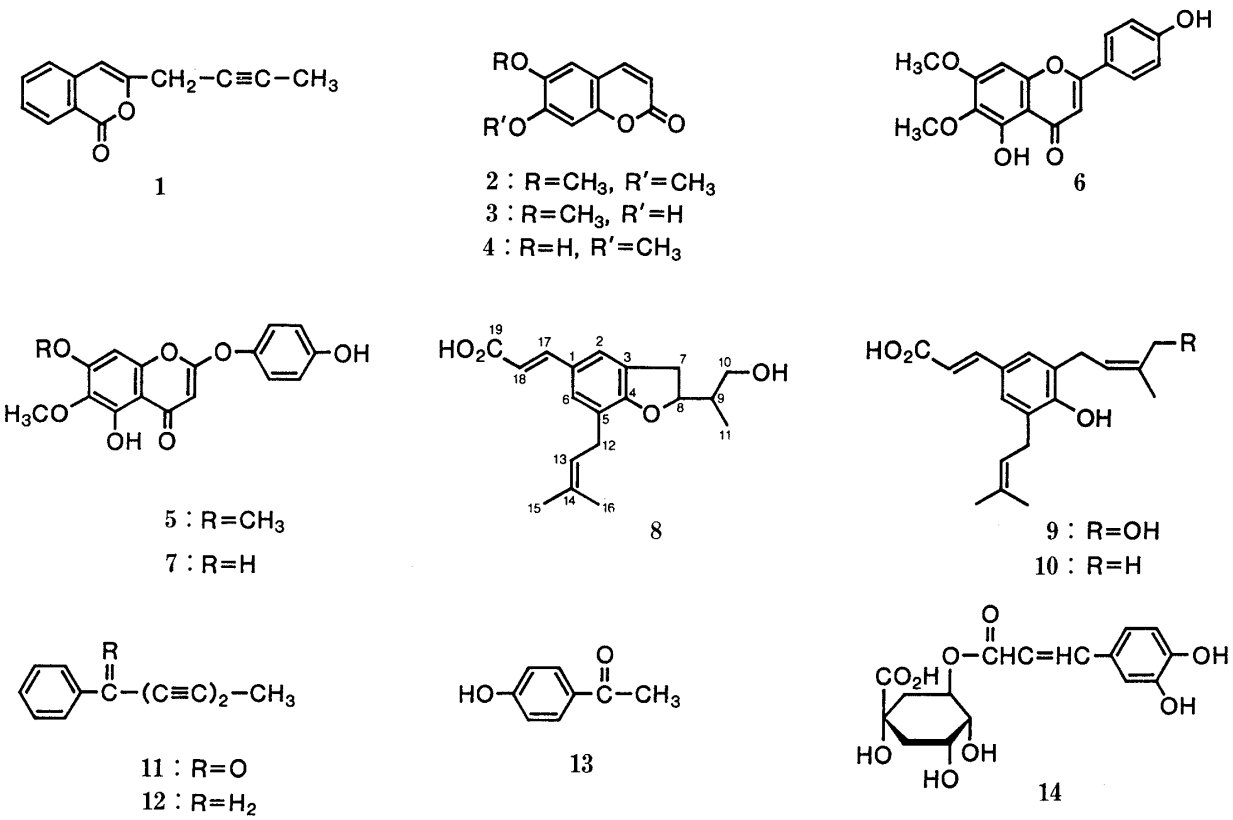

Fig. 2. Structures of Test Compounds

($CDCl_3$) 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 × 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**), $C_{19}H_{24}O_3$ (Calcd: C, 75,97; H, 8.05. Found: C, 76.01; H, 8.02). IR ν_{max}^{Nujol}: 3400, 3200-2500 (br), 1685, 1625, 1595 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ ppm: 7.67 (1 H, d, J=17 Hz, H-17), 7.15 (2H, br s, H-2, H-6), 6.23 (1H, d, J=17 Hz, H-18), 5.28 (2H, br t, 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). ^{13}C-NMR (acetone-d_6) δ ppm: 169.7 (s, C-19), 155.7 (s. C-4), 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 I. Effects of *Artemisia capillaris* Extract and Its Fractions on Bile Flow in Rats

Extract or fraction dose	No. of rats	Time after administration (min)					
		−30—0	0—30	30—60	60—90	90—120	120—150
Control (gum arabic)	6	0.90±0.05[b]	0.86±0.04 (96)[c]	0.92±0.04 (103)	0.92±0.04 (103)	0.86±0.04 (97)	0.84±0.04 (95)
AC01 3 ml of extract/rat (=2 g of "inchinko"/rat)[a]	3	0.84±0.06	1.04±0.09 (126)	1.04±0.08 (124)	0.90±0.08 (108)	0.92±0.08 (110)	0.86±0.01 (104)
AC02 48 mg/rat (=2 g of "inchinko"/rat)	2	0.85	0.79 (94)[c]	0.85 (100)	0.81 (97)	0.82 (98)	0.82 (98)
AC02 96 mg/rat (=4 g of "inchinko"/rat)	4	0.85±0.06	1.45±0.12[d] (170)[e]	1.38±0.08[d] (163)[e]	1.12±0.03[d] (133)[e]	1.02±0.04 (120)[e]	0.90±0.06 (107)
AC03 410 mg/rat (=2 g of "inchinko"/rat)	2	0.77	0.73 (96)	0.75 (98)	0.77 (100)	0.81 (106)	0.75 (98)
AC03 820 mg/rat (=4 g of 'inchinko"/rat)	2	0.77	0.77 (100)	0.82 (107)	0.84 (108)	0.87 (113)	0.83 (107)
AC02-20 22 mg/rat	3	0.79±0.07	1.07±0.07[d] (135)	0.97±0.08 (123)	0.88±0.07 (111)	0.82±0.05 (104)	0.79±0.04 (100)
AC02-30 5.7 mg/rat	3	0.83±0.08	1.01±0.07 (122)	0.92±0.10 (111)	0.86±0.09 (104)	0.82±0.10 (99)	0.85±0.08 (102)
AC02-40 5.4 mg/rat	3	0.93±0.13	1.05±0.08 (113)	0.94±0.06 (101)	0.84±0.03 (90)	0.83±0.05 (89)	0.83±0.06 (89)
AC02-50 4.7 mg/rat	3	0.76±0.03	0.88±0.01[d] (116)	0.86±0.02 (113)	0.83±0.04 (109)	0.90±0.07 (118)	0.90±0.09 (118)
AC02-60 49 mg/rat	3	0.81±0.07	1.19±0.07[d] (147)[e]	1.07±0.07[d] (132)[e]	1.05±0.06 (130)	1.02±0.06 (126)	0.96±0.09 (119)

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

	Dose	No. of rats	Time after administration (min)					
			−30—0	0—30	30—60	60—90	90—120	120—150
Control		11	0.98±0.03[a]	1.03±0.03	1.06±0.03	1.01±0.03	0.99±0.03	0.92±0.02
				(106±2)[b]	(109±3)	(104±3)	(102±4)	(95±4)
Capillarin	50 mg/kg	4	0.96±0.05	1.10±0.05	1.09±0.07	1.01±0.03	0.96±0.03	0.95±0.05
				(115±5)	(115±8)	(106±7)	(102±8)	(101±11)
Scoparone	50 mg/kg	5	0.94±0.09	1.19±0.10	1.14±0.09	1.06±0.07	1.02±0.08	0.96±0.06
				(128±7)[d]	(123±7)	(117±13)	(113±14)	(106±13)
Scopoletin	50 mg/kg	4	0.91±0.08	1.35±0.11[c]	1.02±0.08	0.99±0.06	0.96±0.05	0.88±0.03
				(149±6)[d]	(112±4)	(110±4)	(107±7)	(99±10)
Isoscopoletin	50 mg/kg	4	0.88±0.10	1.06±0.09	0.95±0.10	0.87±0.06	0.86±0.05	0.84±0.03
				(122±3)[d]	(109±1)	(101±6)	(101±8)	(99±10)
Capillarisin-7-methylether	50 mg/kg	5	0.89±0.06	0.92±0.05	0.94±0.05	0.90±0.06	0.92±0.06	0.91±0.05
				(103±2)	(106±2)	(101±4)	(104±6)	(104±8)
Cirsimaritin	50 mg/kg	5	0.95±0.06	0.96±0.07	0.99±0.07	0.93±0.05	0.94±0.05	0.92±0.04
				(101±2)	(104±2)	(99±4)	(100±3)	(100±4)
Capillarisin	50 mg/kg	5	0.92±0.03	1.13±0.02[c]	0.99±0.04	0.98±0.03	0.95±0.02	0.93±0.06
				(123±4)[d]	(108±4)	(107±5)	(103±3)	(101±8)
Artepillin A	50 mg/kg	5	0.98±0.03	1.12±0.02[c]	1.17±0.03[c]	1.06±0.05	1.06±0.04	1.06±0.02
				(114±2)[d]	(120±2)[d]	(108±2)	(108±2)	(108±4)
Capillartemisin B_1	50 mg/kg	5	0.96±0.01	1.34±0.03[c]	1.17±0.06[c]	1.11±0.07	1.03±0.07	0.96±0.07
				(139±3)[d]	(122±7)	(116±8)	(107±8)	(100±8)
Artepillin C	50 mg/kg	5	1.01±0.06	1.39±0.06[c]	1.20±0.03[c]	1.11±0.02	0.99±0.06	0.89±0.06
				(138±7)[d]	(119±7)	(111±6)	(99±7)	(90±9)
Capillin	50 mg/kg	4	0.92±0.07	1.06±0.04	1.03±0.05	0.92±0.04	0.89±0.06	0.82±0.04
				(117±7)	(115±10)	(103±12)	(99±10)	(92±10)
Capillene	50 mg/kg	5	0.97±0.04	1.13±0.05[c]	1.09±0.07	1.00±0.04	0.95±0.05	0.93±0.04
				(116±2)[d]	(111±4)	(103±3)	(98±2)	(96±5)
p-Hydroxyacetophenone	50 mg/kg	4	0.84±0.03	1.43±0.03[c]	1.02±0.08	0.96±0.07	0.93±0.07	0.88±0.03
				(169±4)[d]	(121±6)	(114±6)	(110±5)	(104±2)
Chlorogenic acid	50 mg/kg	5	0.90±0.06	0.93±0.07	0.92±0.07	0.91±0.07	0.89±0.04	0.85±0.04
				(103±3)	(102±4)	(101±5)	(99±5)	(96±8)
Dehydrocholic acid	50 mg/kg	5	0.95±0.06	1.31±0.05[c]	1.18±0.04[c]	1.08±0.04	1.02±0.03	0.95±0.03
				(139±4)[d]	(126±6)[d]	(116±8)	(109±7)	(102±7)

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, capillartemisin B_1 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, scopoletin, isoscopoletin, capillarisin-7-methyl ether, cirsimaritin, capillarisin, artepillin A, capillartemisin B_1, artepillin C, capillin 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, scopoletin, isoscopoletin, capillarisin, artepillin A, capillartemisin B_1, 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 scopoletin, capillartemisin B_1 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

	Dose	No. of rats	Time after administration (min)					
			−30—0	0—30	30—60	60—90	90—120	120—150
Control		7	10.05±1.63[a]	8.90±1.38	10.69±1.16	10.16±0.86	9.96±1.18	8.29±0.97
				(89±3)[b]	(114±13)	(114±17)	(119±26)	(101±22)
Scoparone	50 mg/kg	4	8.05±1.18	8.27±1.60	8.18±1.02	9.65±2.25	8.28±2.78	6.87±2.81
				(103±12)	(109±18)	(144±54)	(131±66)	(113±66)
Scopoletin	50 mg/kg	4	8.65±1.61	8.25±2.43	8.51±1.84	9.77±1.37	9.56±1.08	8.22±1.89
				(91±8)	(98±5)	(118±17)	(120±23)	(112±34)
Isoscopoletin	50 mg/kg	4	6.87±2.03	5.51±1.83	5.71±1.74	5.03±0.61	5.73±0.43	6.32±0.73
				(77±7)	(81±7)	(86±15)	(110±33)	(129±51)
Capillarisin	50 mg/kg	5	9.67±1.39	9.29±1.39	8.17±1.27	7.80±1.37	8.17±1.57	8.37±1.97
				(96±7)	(86±11)	(82±11)	(84±8)	(86±15)
Artepillin A	50 mg/kg	4	8.46±0.75	6.35±0.44	7.57±1.03	8.55±1.77	10.41±1.98	10.95±0.78
				(76±4)[d]	(90±10)	(100±18)	(122±18)	(135±21)
Capillartemisin B_1	50 mg/kg	5	9.97±1.12	8.64±0.71	10.97±1.17	10.23±1.19	8.80±1.11	6.93±1.70
				(93±9)	(114±15)	(110±20)	(95±17)	(78±22)
Artepillin C	50 mg/kg	5	13.30±1.35	14.15±2.16	14.51±1.19	13.43±12.0	8.42±1.69	6.24±2.86
				(107±12)	(115±17)	(107±20)	(68±16)	(52±26)
Capillene	50 mg/kg	5	11.05±0.64	10.55±0.65	11.62±1.21	9.57±1.57	9.20±1.78	9.42±1.29
				(96±4)	(106±11)	(86±12)	(82±15)	(85±12)
Dehydrocholic acid	50 mg/kg	5	8.60±0.79	13.16±1.21[c]	11.77±1.85	10.77±2.04	10.59±1.48	8.99±0.71
				(153±7)[d]	(139±23)	(128±27)	(126±19)	(108±13)

a) Mean±S.E. (mg/30 min). *b*) Percentage of the initial level (Mean±S.E.). *c*) Statistically significant compared with the initial level. *d*) 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

Compounds	No. of rats	Time after administration (min)					
		−30—0	0—30	30—60	60—90	90—120	120—150
Control	7	73.9±6.2[a]	66.1±4.9	74.0±3.9	74.1±2.6	72.5±3.7	64.4±7.0
			(90±2)[b]	(103±6)	(105±9)	(103±11)	(92±13)
Scoparone	4	69.8±6.2	46.7±4.7[c]	45.8±3.1[c]	57.9±11.1	74.5±13.2	67.8±10.4
			(67±1)[d]	(68±10)[d]	(89±24)	(113±28)	(103±25)
Scopoletin	4	64.4±8.7	48.7±9.0	53.9±10.6	58.5±7.6	60.6±5.8	58.0±5.8
			(75±5)[d]	(84±7)	(92±7)	(98±11)	(99±21)
Isoscopoletin	4	58.7±13.7	43.5±12.4	46.5±13.5	47.8±7.5	48.0±3.2	48.6±3.8
			(72±6)	(76±8)	(87±9)	(94±18)	(100±28)
Capillarisin	5	62.0±6.2	50.3±5.4	47.7±5.4	48.6±5.9	50.0±5.4	48.3±8.0
			(81±6)	(78±9)	(80±11)	(81±8)	(79±13)
Artepillin A	4	49.6±3.0	42.8±1.3	45.8±4.4	47.1±4.9	52.6±5.9	52.1±2.8
			(87±5)	(95±13)	(97±14)	(108±16)	(107±9)
Capillartemisin B_1	5	76.2±8.0	51.5±2.5[c]	63.0±5.0	60.8±5.1	60.2±7.2	55.3±8.6
			(71±8)	(87±11)	(85±12)	(84±14)	(79±17)
Artepillin C	5	82.2±7.8	64.1±10.0	73.3±8.7	79.3±9.1	50.2±5.6[c]	37.2±7.6
			(77±6)	(89±9)	(97±9)	(66±12)[d]	(50±15)
Capillene	5	89.5±4.8	72.1±7.1	73.7±7.3	63.2±6.3[c]	60.2±8.4[c]	60.2±7.8[c]
			(81±7)	(84±10)	(71±9)[d]	(67±8)[d]	(68±9)
Dehydrocholic acid	5	64.2±7.1	58.4±5.3	61.1±7.1	57.5±7.2	60.6±7.09	53.8±4.9
			(92±5)	(97±11)	(93±14)	(99±16)	(88±13)

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

TABLE V. Effects of Twelve Compounds Isolated from *A. capillaris* and Dehydrocholic Acid on Biliary Phospholipid Secretion in Wistar Rats

	No. of rats	Time after administration (min)					
		−30—0	0—30	30—60	60—90	90—120	120—150
Control	7	2.97±0.35[a]	2.32±0.23 (82±7)[b]	2.50±0.22 (90±10)	2.48±0.13 (90±10)	2.28±0.19 (75±9)	2.03±0.23 (75±13)
Scoparone	4	2.14±0.20	1.52±0.21 (71±3)	1.45±0.13[c] (69±9)	2.03±0.38 (99±23)	2.21±0.48 (108±29)	2.17±0.51 (107±32)
Scopoletin	4	2.21±0.48	1.74±0.47 (75±4)	1.88±0.47 (84±3)	1.98±0.37 (92±6)	1.95±0.21 (96±14)	1.73±0.22 (93±24)
Isoscopoletin	4	1.98±0.56	1.30±0.42 (64±5)	1.59±0.48 (80±4)	1.47±0.26 (83±11)	1.59±0.15 (98±23)	1.53±0.11 (100±30)
Capillarisin	5	2.31±0.28	1.70±0.21 (73±2)	1.57±0.23 (73±4)	1.65±0.22 (73±8)	1.70±0.19 (76±7)	1.65±0.22 (75±11)
Artepillin A	4	2.40±0.15	1.64±0.07[c] (69±6)	2.03±0.17 (86±10)	2.17±0.29 (92±14)	2.43±0.28 (102±13)	2.41±0.12 (102±8)
Capillartemisin B_1	5	2.75±0.27	1.81±0.11[c] (68±8)	2.35±0.22 (88±10)	2.27±0.21 (87±13)	2.12±0.21 (82±13)	1.78±0.30 (72±19)
Artepillin C	5	3.08±0.32	2.23±0.32 (72±6)	2.45±0.18 (82±8)	2.43±0.20 (83±11)	1.75±0.22 (62±12)	1.28±0.41 (47±18)
Capillene	4	2.23±0.07	1.98±0.12 (89±4)	2.03±0.19 (92±9)	1.82±0.15 (83±8)	1.71±0.16 (77±7)	1.72±0.17 (78±8)
Dehydrocholic acid	4	2.26±0.22	1.98±0.21 (87±4)	2.15±0.28 (96±12)	2.06±0.30 (92±13)	2.15±0.32 (97±15)	1.94±0.17 (89±11)

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 isoscopletin) (Table III). Dehydrocholic acid, on the other hand, increased the biliary bile acid secretion.

Decrease of the biliary cholesterol secretion was caused by scoparone, scopoletin 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 capillarisin 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 choleretic activities were examined at doses corresponding to 2 or 4 g of the crude material (*A. capillaris*). Choleretic 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 choleretic activity of *A. capillaris*. The former fraction was mainly composed of scoparone and the latter contained two choleretic compounds, artepillins A and C, which were newly isolated, in addition to capillartemisin B_1. When the choleretic activities of these compounds were compared at a dose of 50 mg/kg, *p*-

hydroxyacetophenone was most effective, and artepillin C, scopoletin and capillartemisin B_1 had nearly the same activity as dehydrocholic acid. Scopoletin 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.*[3] 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.[21] 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 B_1 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 B_1 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.[22] According to this concept, the increase in bile secretion by these eight compounds seems to be attributable to the bile flow caused by orgainc anions of the compounds administered, as reported by Aburada *et al.*[23]

Identifying the components contributing most to the choleretic activity is difficult because the chemical composition of *A. capillaris* differs with the materials examined[24] and *p*-hydroxyacetophenone, which has not been found in Japanese *A. capillaris*, has been confirmed to be highly choleretic.[7] However, our present results suggest that four components, scoparone, capillartemisin B_1 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.

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