Communications to the Editor

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CAPILLARTEMISIN A AND B, TWO NEW CHOLERETIC PRINCIPLES FROM ARTEMISIAE CAPILLARIS HERBA

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By monitoring with a choleretic activity test for rat-duodenum, two new choleretic principles named capillartemisin A (1) and B (2) were isolated from Artemisiae Capillaris Herba ("Inchinko" in Japanese, the aerial part of *Artemisia capillaris* Thunb.), and their structures were determined on the basis of spectral evidence and by a synthesis of the methyl ester of 1 (1a). Capillartemisin A (1) and B (2) showed more potent and lasting choleretic activity than sodium dehydrocholate and the other hitherto identified choleretic principles of "Inchinko". A new flavonoid isoarcapillin (6) was also chemically elucidated.

KEYWORDS —— isopentenylated *p*-coumaric acid derivative; capillartemisin; isoarcapillin; choleretic activity; *Artemisia capillaris*; ^{1}H NMR; ^{13}C NMR

Artemisiae Capillaris Herba ("Inchinko" in Japanese, the aerial part of *Artemisia capillaris* Thunb., Compositae) is known as an important ingredient of Chinese traditional medicines which are specified as remedies for jaundice, hepatitis, and cholecystitis. In regard to the bioactive principles of this crude drug, esculetin 6,7-dimethyl ether[1] and capillarisin[2] were identified as the choleretic principles, and eupatolitin and arcapillin[3] were shown to be the antihepatotoxic constituents. During the course of chemical studies on the bioactive constituents of naturally occurring drug materials,[4] we isolated two new choleretic principles named capillartemisin A (1) and B (2) from "Inchinko". This paper communicates evidence which is consistent with their structures. In addition, a new flavonoid, isoarcapillin (6), was chemically elucidated.

By monitoring with a choleretic activity test for rat-duodenum,[5] two major active principles, capillartemisin A (1, 0.04% from the crude drug) and B (2, 0.13%) together with isoarcapillin (6, 0.11%), were isolated from "Inchinko" by use of various chromatographic separations.

Capillartemisin A (1), mp 127-128°C, $C_{19}H_{24}O_4$,[6] IR ν_{max}^{KBr} cm^{-1}: 3460, 3000-2500 (br), 1675, 1618, 1590, MS *m/z* (%): 316 (M^+, 4), 298 (M^+-H_2O, 100), was a disubstituted *p*-coumaric acid derivative as shown by its UV λ_{max}^{MeOH} nm (ε): 303 (21000),

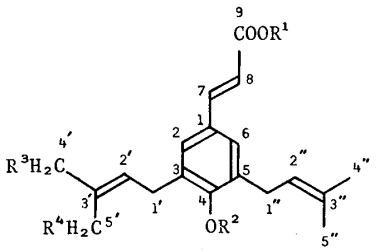
234 (20500), 219 (20000)[7] and the ^{1}H NMR spectrum (Table I). The ^{1}H NMR spectrum of 1 also indicated the presence of one isopentenyl and one hydroxyisopentenyl residue located at both *ortho* of the phenolic hydroxyl group.[7,8] By comparison of the ^{13}C NMR data for 1 (Table II) with those for *p*-coumaric acid,[9,10] the substitution pattern in the *p*-coumaric acid moiety of 1 and the presence of isopentenyl and *E*-hydroxyisopentenyl[9] residues were confirmed. CH_2N_2 methylation of 1 furnished the monomethyl ester (1a), colorless oil,[11] $C_{20}H_{26}O_4$, MS: 330 (M^+, 19), IR ($CHCl_3$): 3600-3300 (br), 1704, which was acetylated (Ac_2O-pyridine) to give the diacetyl-monomethyl ester (1b), colorless oil, $C_{24}H_{30}O_6$, UV (MeOH): 281 (17800), 223 (sh, 17200), 200! (22800), IR (CCl_4): 1766, 1740, 1726. The spectral properties (including ^{1}H NMR) of 1a and 1b evidenced the structure of 1, which was finally substantiated by the following synthesis of 1a. Thus, isopentenylation of *p*-coumaric acid with 1-bromo-3-methyl-2-butene in the presence of 6.7% aq. NaOH (at 22°C, 4 h)[12] followed by CH_2N_2 methylation yielded 3[7a,b] (16%) and 4[7c] (31%). SeO_2 oxidation[13] of 4 in EtOH by heating under reflux for 3 h furnished 1a (62%).

Capillartemisin B (2), mp 146-148°C, $C_{19}H_{24}O_4$, was also a *p*-coumaric acid derivative having one isopentenyl group at the C-5 and one hydroxyisopentenyl group at the C-3 as shown by its spectral properties: UV (MeOH): 303 (21200), 234 (20700), 218 (22400), IR (KBr): 3400, 3000-2500 (br), 1675, 1618, 1585, ^{1}H and ^{13}C NMR (Table I, II). The ^{13}C NMR data for the hydroxyisopentenyl residue in 2 clearly indicated the *Z* geometry. CH_2N_2 methylation of 2 gave the monomethyl ester (2a), colorless oil, $C_{20}H_{26}O_4$, MS: 330 (M^+, 48), IR (CCl_4): 3600-3100 (br), 1695, which was acetylated to furnish 2b, mp 108-109°C, $C_{24}H_{30}O_6$, UV (MeOH): 282 (17000), 223 (sh, 17000), 200! (22200), IR (CCl_4): 1764, 1736, 1723. Comparison of the spectral properties (including ^{1}H and ^{13}C NMR) of 2a and 2b with those of 1a and 1b further confirmed the geometries of respective hydroxyisopentenyl residues in capillartemisin A (1) and B (2). Acetylation of 2 furnished the diacetate (2c), colorless oil, $C_{23}H_{28}O_6$, UV (MeOH): 280 (18200), 222 (sh, 27200), 200! (30500), IR ($CHCl_3$): 3000-2500 (br), 1767, 1740, 1693, which, on treatment with conc. HCl-dioxane (1:1) at 100°C for 1 h and subsequent CH_2N_2 methylation, was converted to the pyrane derivative (2d), colorless oil, $C_{20}H_{26}O_4$, MS: 330 (M^+, 2), 257 (100), UV (MeOH): 319 (22000), 240 (15700), 218 (17600), IR ($CHCl_3$): 3580, 1699, 1632, 1602. The ^{1}H NMR spectrum of 2d indicated the formation of one pyrane ring by the signals attributable to the dimethyl pyrane ring.[14] Based on the this evidence, the structure of 2, a geometric isomer of 1 at the hydroxyisopentenyl residue, was determined.

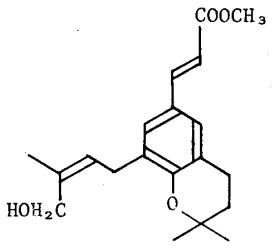
One of two flavonoids isolated together with 1 and 2 was found to be arcapillin (5)[3] by comparing its spectral data with those reported. The spectral data (UV, IR, ^{1}H and ^{13}C NMR) for another flavonoid isoarcapillin (6), mp 288°C, $C_{18}H_{16}O_8$, led to an assumption that 6 was an isomer of 5 differing only in the location of one methoxyl group either at C-2' (in 6) or at C-6 (in 5). The assumption was verified by the examination of the UV spectrum of 6 taken under various conditions (with addition of 1% $AlCl_3$, 1% $AlCl_3$-5% HCl, or sat. aq. AcONa) and by the preparation of a common pentamethyl ether (7) from both 5 and 6 by CH_2N_2 methylation.

As given in Table III, capillartemisin A (1) and B (2) showed more potent and

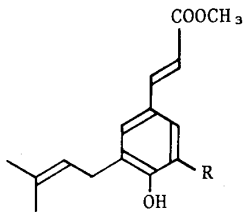
lasting choleretic activities than sodium dehydrocholate, esculetin 6,7-dimethyl ether, and capillarisin. One of the two flavonoids, arcapillin (5), was found to exhibit a weak choleretic activity.[5)]



1 $R^1=R^2=$ H, $R^3=$ OH, $R^4=$ H (capillartemisin A)
1a $R^1=CH_3$, $R^2=$ H, $R^3=$ OH, $R^4=$ H
1b $R^1=CH_3$, $R^2=$ Ac, $R^3=$ OAc, $R^4=$ H

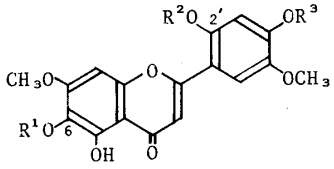


2d



3 R= H
4 R=

2 $R^1=R^2=R^3=$ H, $R^4=$ OH (capillartemisin B)
2a $R^1=CH_3$, $R^2=R^3=$ H, $R^4=$ OH
2b $R^1=CH_3$, $R^2=$ Ac, $R^3=$ H, $R^4=$ OAc
2c $R^1=$ H, $R^2=$ Ac, $R^3=$ H, $R^4=$ OAc



5 $R^1=CH_3$, $R^2=R^3=$ H (arcapillin)
6 $R^1=$ H, $R^2=CH_3$, $R^3=$ H (isoarcapillin)
7 $R^1=R^2=R^3=CH_3$

Table I. ^{1}H NMR Data for 1, 2 and 2d (δ at 90 MHz, J values in Hz)

Proton	1 (d_6-acetone)	2 (d_6-acetone)	2d ($CDCl_3$)
2, 6	7.22(2H,br s)	7.07(2H,br s)	7.03(2H,br s)
7	7.52(1H,d,J=15)	7.36(1H,d,J=15)	7.50(1H,d,J=15)
8	6.22(1H,d,J=15)	6.11(1H,d,J=15)	6.17(1H,d,J=15)
1',1"	3.42(1'),3.37(1") (both 2H,d,J=7)	3.53(1'),3.26(1") (both 2H,d,J=7)	3.30(1'-H_2,d,J=7.5) 2.73(1"-H_2,t,J=6.5)
2',2"	5.62(2'),5.34(2") (both 1H,br t,J=7)	5.22(2H,br t,J=7)	5.46(2'-H,br t,J=7.5) 1.77(2"-H_2,t,J=6.5)
4',4",5',5"	4.00(4'-H_2,s) 1.73(9H,s)	4.16(5'-H_2,s) 1.69(6H,s),1.73(3H,s)	4.14(5'-H_2,s) 1.33(6H,s),1.83(4'-H_3,s)

Table II. ^{13}C NMR Data for 1 and 2 (δc at 50 MHz, in d_6-acetone)[a)]

Carbon	1	2	Carbon	1	2	Carbon	1	2
1	129.3(s)	129.4(s)	1'	29.3(t)	29.3(t)	1"	28.7(t)	28.6(t)
2	129.1(d)[b)]	128.8(d)[c)]	2'	122.2(d)	126.0(d)	2"	122.8(d)	122.9(d)
3	128.3(s)	126.5(s)	3'	137.8(s)	135.6(s)	3"	133.4(s)	132.6(s)
4	155.5(s)	155.8(s)	4'	68.1(t)	22.5(q)	4"	25.9(q)	25.8(q)
5	127.2(s)	127.4(s)	5'	13.9(q)	62.1(t)	5"	17.9(q)	17.8(q)
6	128.3(d)[b)]	128.3(d)[c)]						
7	146.0(d)	145.7(d)						
8	115.5(d)	115.1(d)						
9	168.2(s)	168.0(s)						

a) The off-resonance patterns of the signals were given in parentheses.
b,c) The assignments are interexchangeable in the same column.

Table III. Facilitatory Effect of Capillartemisin A (1) and B (2), Esculetin-6,7-dimethyl ether, and Capillarisin on Biliary Secretion in Rats[a)]

Drugs	Dose mg/kg (i.d.)	0	Biliary Secretion (per cent) 0.5	1	1.5	2	3	4	5	6 (h)
Control	-	100	101.1 (±1.7)	102.9 (±3.3)	103.4 (±3.0)	96.4 (±2.3)	93.9 (±2.6)	88.1 (±2.5)	85.1 (±3.2)	80.7 (±4.4)
Sodium dehydrocholate	100	100	162.6*[b)] (±20.0)	138.8**[b)] (±9.9)	109.2 (±5.2)	98.1 (±5.7)	92.0 (±7.0)	84.6 (±7.6)	85.3 (±9.4)	82.1 (±10.1)
Capillartemisin A (1)	100	100	223.3** (±16.7)	219.0** (±22.0)	191.7** (±27.3)	158.6** (±25.1)	153.3** (±14.4)	139.6** (±10.8)	138.5** (±9.0)	131.9** (±11.5)
Capillartemisin B (2)	100	100	187.3** (±10.1)	164.3** (±6.9)	148.1** (±6.1)	144.5** (±6.7)	128.1** (±4.4)	111.1** (±3.4)	112.8** (±6.9)	108.3** (±5.3)
Esculetin 6,7-dimethyl ether	300	100	136.6** (±6.4)	133.0* (±8.8)	119.6 (±8.2)	113.5 (±10.2)	106.8 (±9.1)	102.8 (±11.8)	100.2 (±12.5)	90.9 (±9.5)
Capillarisin	300	100	110.6 (±3.9)	107.0 (±4.7)	116.2 (±7.2)	105.4 (±5.8)	111.6 (±8.6)	115.3 (±8.7)	109.1 (±14.8)	97.4 (±12.2)

a) Each drug was administered to the duodenum at 0 hour. Each value indicates: i) the per cent of the bile flow to the bile flow before administration of each drug and ii) mean (±S.E.) of 8 male rats. b) Significantly different from control, * $p < 0.05$, ** $p < 0.01$.

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