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Studies on the Components of the Leaves of *Coptis japonica* Makino. I. The Structures of Coptiside I and II[1]

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Two new flavonoid glycosides, coptiside I, mp 190—191°, $C_{46}H_{58}O_{27}\cdot 4H_2O$ and coptiside II, mp 243—244°, $C_{21}H_{20}O_{11}\cdot 2\frac{1}{2}H_2O$, were isolated from the leaves of *Coptis japonica* Makino. The structure of coptiside I was established to be acacetin-7-O-β-D-glucopyranosyl-(1—2)-O-β-D-glucopyranosyl-(1—2)-O-[2,4-O-diacetyl-α-L-rhamnopyranosyl-(1—6)]-3-O-acetyl-β-D-glucopyranoside and of coptiside II was to be 7-O-β-D-galactopyranosylquercetin on the basis of chemical and spectral data.

There have been many studies done on the alkaloids of the rhizomes of *Coptis japonica* Makino, but few on the leaves of this plant.[3] Research on the leaves as a medicinal source has been carried out to give two new flavonoid glycosides, namely coptiside I and II, along with alkaloids. This paper deals with the structure determination of these new flavonoid glycosides.

The MeOH extract of the leaves of this plant was separated with water into soluble and insoluble portions, and the BuOH extract of the water soluble portion was repeatedly purified by polyamide and silica gel column chromatographies to give berberine, mp 213° (decomp.) (as hydrochloride), coptiside I (0.03%) and II (0.005%).

Coptiside I (I), a pale yellow amorphous powder, mp 190—191°, $[\alpha]_D^{25}$ −93° (pyridine), $C_{46}H_{58}O_{27}\cdot 4H_2O$, showed absorption maxima (270, 326 nm) characteristic of a flavonoid on the ultraviolet (UV) spectrum. The infrared (IR) spectrum of I exhibited absorption bands due to hydroxyl (broad, 3440 cm^{-1}), ester (1745 cm^{-1}) and conjugated carbonyl (1655 cm^{-1}) groups. I showed three singlets attributable to the acetyl groups (δ 1.96, 1.98, 2.00 ppm)

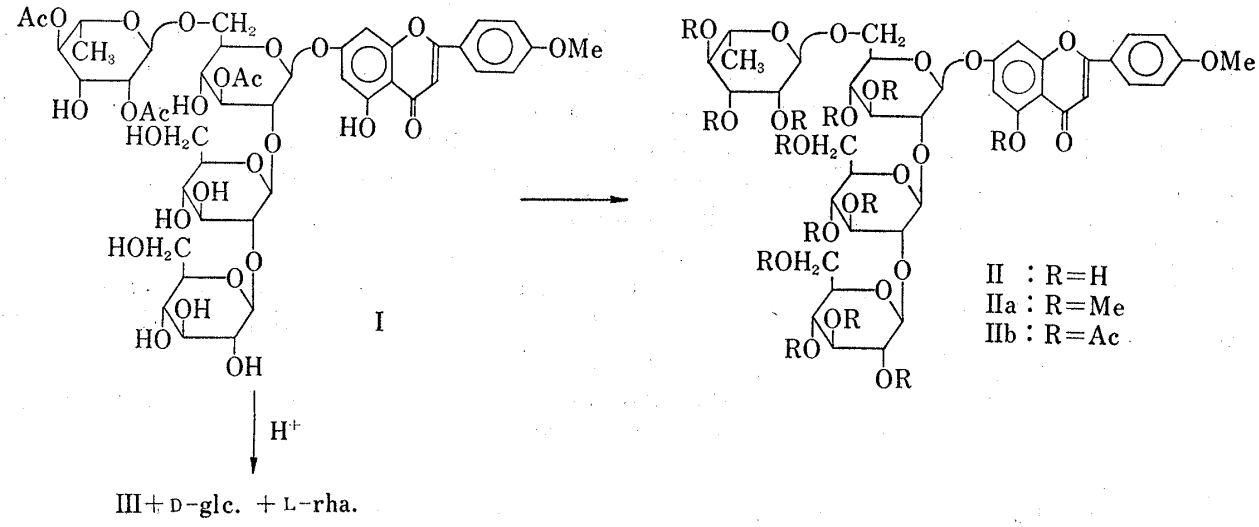


Chart 1

1) This work was presented at the Annual Meeting of Pharmacognosical Society of Japan, Osaka, October, 1974, and at the 95th Annual Meeting of the Pharmaceutical Society of Japan, Nishinomiya, April, 1975.
2) Location: *Maidashi, Higashi-ku, Fukuoka.*
3) N. Morita, "Wooren no kenkyu (黄連の研究)" ed. by M. Minabe, Kazama Shobô, Tokyo, 1970.

on the nuclear magnetic resonance (NMR) spectrum. These spectral data indicated that I was a new flavonoid glycoside having three acetyl groups.

When treated with 1N NaOH, I yielded a colorless amorphous hydrolysate (II), mp 290—291°, $C_{40}H_{52}O_{24}\cdot 2\frac{1}{2}H_2O$, and acetic acid which was directly identified with an authentic sample through *p*-bromophenacyl acetate. On complete acid hydrolysis I afforded an aglycone (III) (yellow needles), mp 260—262°, $C_{16}H_{12}O_5$ (M^+: *m/e* 284), which was identified to be acacetin by the direct comparison (UV and IR spectra).[4,5] D-Glucose and L-rhamnose were determined by paper chromatography.

The location of the sugar moiety in I was deduced to be at C_7 in acacetin from the UV spectra ($\lambda_{max}^{MeOH+AlCl_3}$: 279, 342 nm, $\lambda_{max}^{MeOH+NaOAc}$: 270, 326 nm).[6]

Methylation of II by Hakomori's method[7] and a subsequent purification by the preparative thin-layer chromatography (TLC) on silica gel provided a fully methylated compound (IIa) (colorless plates), mp 88—89°, which showed no hydroxyl absorption bands on the IR spectrum.

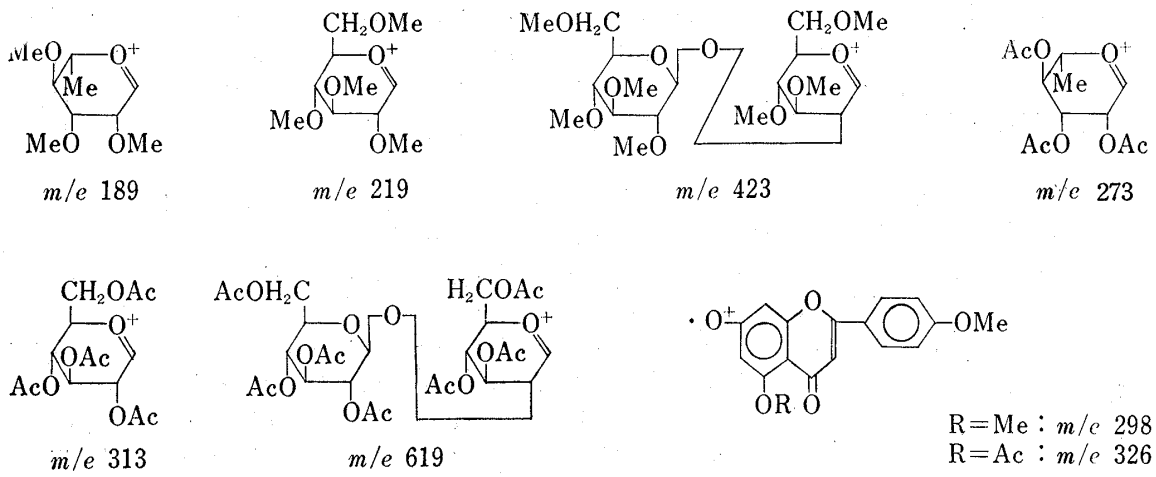


Fig. 1. Mass Fragment Ions of IIa and IIb

The high resolution mass spectrum of IIa exhibited the fragment ions at *m/e* 189 and 219 corresponding to $C_9H_{17}O_4$ and $C_{10}H_{19}O_5$, respectively,[8] besides the methylated aglycone (*m/e* 298). The formation of the fragment ions at *m/e* 189 and 219 can be explained by the sequence where the rhamnopyranose and glucopyranose units were located at the terminals in the oligosaccharide. These results were also supported by the mass spectral analyses on a fully acetylated compound (IIb), (colorless amorph.), mp 115—116°, presenting the fragment ions at *m/e* 273, 313 and 619, respectively.[9]

The NMR spectrum of IIa showed four signals attributable to anomeric protons at δ 4.68 (2H, d, J=6.0 Hz), 4.74 (1H, s) and 5.32 (1H, d, J=6.0 Hz) ppm, in addition to methyl protons due to L-rhamnopyranose at δ 1.16 (3H, d, J=6.0 Hz) ppm.

The above results led to the conclusion that II is a flavonoid glycoside consisting of acacetin and a tetrasaccharide composed of three moles of D-glucopyranose and one mole of L-rhamnose,

4) T.J. Marby, K.R. Markham, and M.B. Thomas, "The Systematic Identification of Flavonoids," Springer-Verlag, Berlin, Heiderberg, New York, 1970, p. 41.
5) C.J. Pouchert, "The Aldrich Library of Infrared Spectra," Aldrich Chemical Co., Inc., p. 669.
6) J.B. Harbone, "Comparative Biochemistry of the Flavonoids," Academic Press, London, 1967.
7) S. Hakomori, *J. Biochem.* (Tokyo), **55**, 255 (1964).
8) *a*) V. Kovácik, Š. Bauer, and J. Rosík, *Carbohyd. Res.*, **8**, 291 (1968); *b*) H. Okabe and T. Kawasaki, *Chem. Pharm. Bull.* (Tokyo), **20**, 514 (1970).
9) G.S. Johnson and W.S. RuLiffson, *Chem. Commn.*, **1970**, 587.

and that the tetrasaccharide is branched at a glucose unit having the rhamnose and the biose (D-glucose-D-glucose) residue at terminals.

Then, IIa was subjected to methanolysis and the products were examined by gas liquid chromatography (GLC). Methyl pyranosides of 2,3,4-tri-O-methyl-α-L-rhamnose; 2,3,4,6-tetra-O-methyl-β-D-glucose; 3,4,6-tri-O-methyl-β-(and-α-)-D-glucose; 3,4-di-O-methyl-β-(and-α-)-D-glucose were identified by co-chromatography (Fig. 2).

Consequently, the structure of II must be presented to be acacetin-7-O-β-D-glucopyranosyl-(1—2)-O-β-D-glucopyranosyl-(1—2)-O-[α-L-rhamnopyranosyl-(1—6)]-β-D-glucopyranoside or acacetin-7-O-β-D-glucopyranosyl-(1—2)-O-β-D-glucopyranosyl-(1—6)-O-[α-L-rhamnopyranosyl-(1—2)]-β-D-glucopyranoside.

In order to determine the sequence of each sugar in I, I was hydrolyzed with Takadiastase A[10] to give a main product (IV) together with acacetin. Since IV was unstable for the further purification, IV was treated with NH_4OH at room temperature for a few minutes to give a colorless product (IVa), mp 269—270°, $[\alpha]_D^{20}$ −88° (pyridine). On methylation by the Hakomori's method IVa provided a fully methylated compound (IVb) (colorless needles), mp 72—73°, $C_{35}H_{46}O_{14}$ (M^+: *m/e* 690). The NMR spectrum of IVb showed a singlet due to an anomeric proton of L-rhamnose residue at δ 4.78, and a doublet signal due to an anomeric proton of

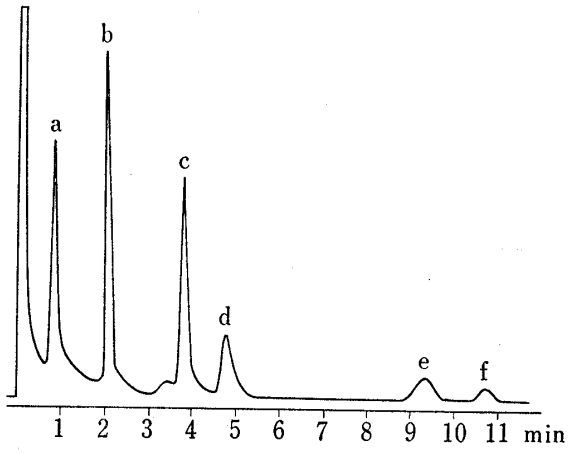


Fig. 2. Gas Chromatogram of Methanolysates of IIa

a) methyl 2,3,4-tri-O-methyl-α-L-rhamnopyranoside
b) methyl 2,3,4,6-tetra-O-methyl-β-D-glucopyranoside
c) methyl 3,4,6-tri-O-methyl-α-D-glucopyranoside
d) methyl 3,4,6-tri-O-methyl-β-D-glucopyranoside
e) methyl 3,4-di-O-methyl-α-D-glucopyranoside
f) methyl 3,4-di-O-methyl-β-D-glucopyranoside
condition: 5% 1,4-butanediol succinate on Shimalite (60—80 mesh), 1.5 m × 4 mmφ
temperature: 145°
carrier gas: N_2, 0.8 kg/cm²

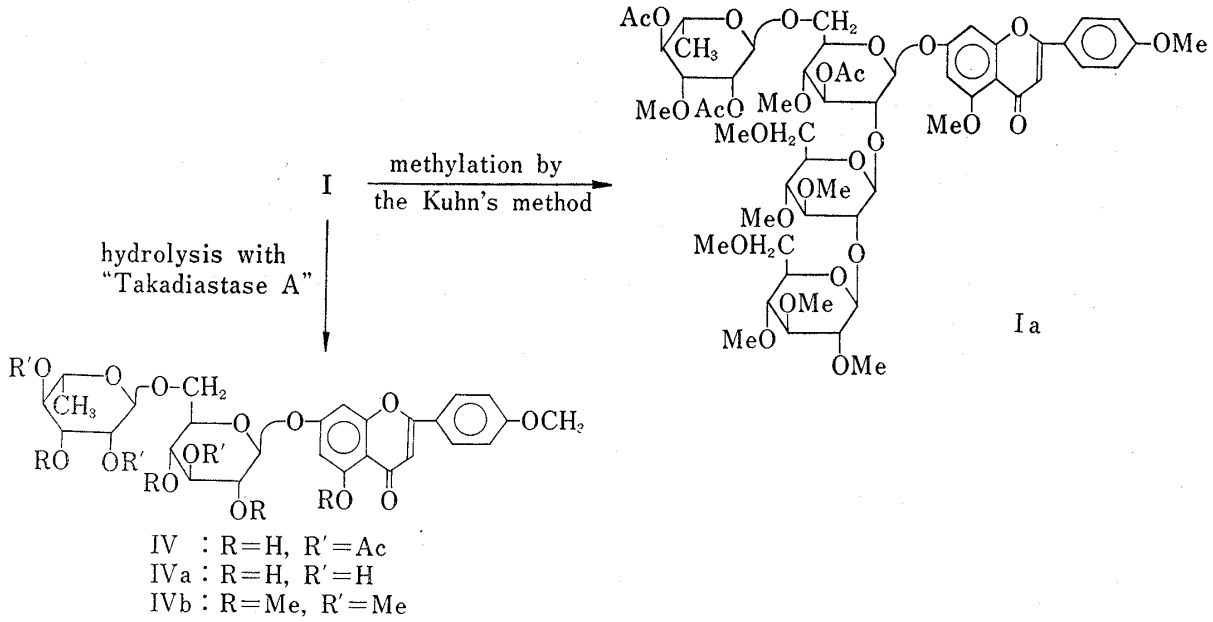


Chart 2

10) This enzyme was kindly supplied by Prof. O. Tanaka of Hiroshima University.

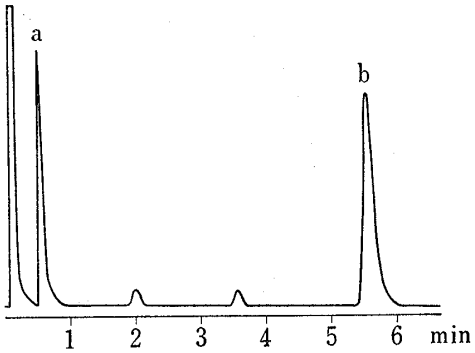


Fig. 3. Gas Chromatogram of Methanolysates of IVb

condition: 5% 1,4-butanediol succinate on Shimalite (60—80 mesh), 1.5 m×4 mmϕ
column temperature; 145°
carrier gas: N_2, 1.0 kg/cm²
a) methyl 2,3,4-tri-O-methyl-α-L-rhamnopyranoside
b) methyl 2,3,4-tri-O-methyl-β-D-glucopyranoside

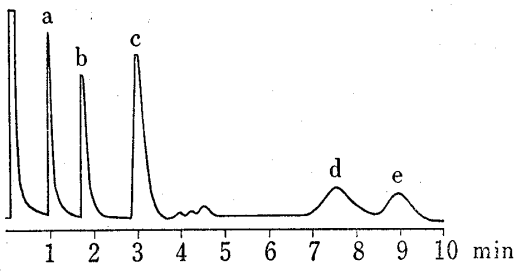


Fig. 4. Gas Chromatogram of Acetylated Methanolysates of Ia

condition: 3% OV-17 on Chromosorb W (AW) DMCS (60—80 mesh), 1.5 m×4 mmϕ
column temperature; 180°
carrier gas: N_2, 1.2 kg/cm²
a) methyl 2,4-di-O-acetyl-3-mono-methyl-α-L-rhamnopyranoside
b) methyl 2,3,4,6-tetra-O-methyl-β-D-glucopyranoside
c) methyl 2-mono-O-acetyl-3,4,6-tri-O-methyl-β-D-glucopyranoside
d) methyl 2,3,6-tri-O-acetyl-4-mono-O-methyl-D-β-glucopyranoside
e) methyl 2,3,6-tri-O-acetyl-4-mono-O-methyl-α-D-glucopyranoside

D-glucose residue combined with C_7 hydroxyl group in acacetin at δ 5.02 ppm.[11] Moreover, on methanolysis IVb afforded methyl pyranosides of 2,3,4-tri-O-methyl-α-L-rhamnose and 2,3,4-tri-O-methyl-β-D-glucose, which were identified with authentic samples by co-chromatography on GLC (Fig. 3).

From these results IVa was assumed to be linarin (acacetin-7-O-α-L-rhamno-(1—6)-β-D-glucoside). The identity of IVa and linarin was established by the direct comparison (mp, $[\alpha]_D$, UV and IR spectra).[12]

Therefore, the structure of II was determined to be acacetin-7-O-β-D-glucopyranosyl-(1—2)-O-β-D-glucopyranosyl-(1—2)-O-[α-L-rhamnopyranosyl-(1—6)]-β-D-glucopyranoside.

The locations of the acetyl groups in I were determined as follows. A colorless methylated compound (Ia), mp 102—103°, (M^+: *m/e* 1183), derived from I by the Kuhn's method[13] indicated ester absorption band at 1745 cm^{-1} on the IR spectrum, and the signals due to three acetyl methyl protons at δ 2.00, 2.08 and 2.15 ppm, besides two phenolic methoxyl groups (δ 3.92 and 3.96 ppm) and alcoholic methoxyl groups (δ 3.20—3.80 ppm) on the NMR spectrum. Therefore, three acetyl groups were combined with the hydroxyl groups in sugar moiety.[14]

The high resolution mass spectrum of Ia exhibited the fragment ions corresponding to $C_{10}H_{19}O_5$, $C_{11}H_{17}O_6$ and $C_{19}H_{35}O_{10}$ at *m/e* 219, 245 and 423, respectively, besides a fragment ion peak due to methyl acacetin at *m/e* 298 (Fig. 1). Thus, it was demonstrated that two acetyl groups were in the terminal L-rhamnose units, and that no acetyl group was in sophorose unit. Then the examination by GLC was carried out. The methanolysates of Ia were acetylated with Ac_2O and pyridine, and the products were subjected to GLC. Methyl pyranosides of 2,4-O-diacetyl-3-O-methyl-α-L-rhamnose; 2,3,4,6-tetra-O-methyl-β-D-glucose; 2-O-acetyl-3,4,6-tri-O-methyl-β-D-glucose; 2,3,6-O-triacetyl-4-O-methyl-β-D-glucose were identified by GLC (Fig. 4).

Accordingly, the structure of I was established to be acacetin-7-O-β-D-glucopyranosyl-(1—2)-O-β-D-glucopyranosyl-(1—2)-O-[2,4-O-diacetyl-α-L-rhamnopyranosyl-(1—6)]-3-O-acetyl-β-D-glucoside.

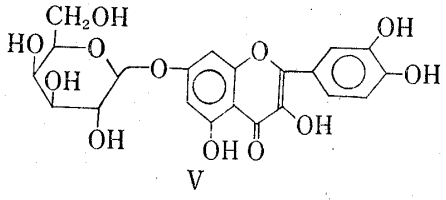
11) H. Rösler, T.J. Mabry, M.F. Cranmer, and J. Kagan, *J. Org. Chem.*, **30**, 4346 (1965).
12) *a*) G. Zemplén and R. Bognár, *Chem. Ber.*, **74**, 1818 (1941); *b*) H. Wagner, L. Hörhammer, and W. Kirchner, *Arch. Pharm.*, **293**, 1053 (1960).
13) R. Kuhn, H. Trischmann, and I. Löw, *Angew. Chem.*, **67**, 32 (1955).
14) S. Kawanishi, S. Sakuma, H. Okino, and J. Shoji, *Chem. Pharm. Bull.* (Tokyo), **20**, 93 (1972).

Coptiside II (V), a yellow amorphous powder, mp 243—244°, $[\alpha]_D^{25}$ −96° (pyridine), $C_{21}H_{20}O_{11}\cdot 2\frac{1}{2}H_2O$, indicated positive colorlations for $FeCl_3$ and H_2SO_4 reagents. On the UV (λ_{max}^{MeOH}: 258, 364 nm; $\lambda_{max}^{MeOH+AlCl_3}$: 270, 405 nm; $\lambda_{max}^{MeOH+NaOAc}$: 264, 366 nm) and IR ν_{max}^{KBr}: 3460, 3300 (broad), 1655, 1610 cm^{-1} spectra, V was assumed to be a flavonoid glycoside having a hydroxyl group at C_5 and no free hydroxyl group at C_7 in aglycone. Since V revealed a signal due to an anomeric proton at δ 5.39 ppm (1H, d, J=8.0 Hz), besides the signals due to protons of C_6, C_8, $C_{5'}$, $C_{2'}$ and $C_{6'}$ at δ 6.22 (1H, d, J=2.0 Hz), 6.40 (1H, d, J=2.0 Hz), 6.84 (1H, d, J=8.0 Hz), 7.56 (1H, d, J=2.0 Hz) and 7.70 (1H, d.d, J=2.0, 8.0 Hz), respectively, V was suggested to be quercetin-7-O-monoglycoside.

On acid hydrolysis V afforded an aglycone (VI) (yellow needles), mp >300°, and as a sugar portion D-galactose was determined on PPC. The UV (λ_{max}^{MeOH}: 256, 375 nm) and IR (ν_{max}^{KBr}: 1655, 1600 cm^{-1}) spectra of VI were superimposed with those of an authentic sample of quercetin.

Therefore, the structure of V was determined to be quercetin-7-O-β-D-galactopyranoside.

Recently, linariin (pectolinarigenin-7-O-(4‴-monoacetyl-rhamno-(1—6)-glucoside), boehmerin (quercetin-3-O-(3″-O-acetyl)-α-L-arabinofuranoside) and a mixture of mono - and di-acetate of 7-O-β-D-glucosyl-8-O-β-D-glucosyl-4′-O-methylapigenin were isolated from the leaves of *Linaria* spp.,[15] *Boehmeria tricuspis*[16] and *Trema aspera*,[17] respectively. Coptiside I is the first tetraglycoside having three acetyl groups in the sugar moiety.



V

Fig. 5

Experimental

Melting points were determined by a Yanagimoto micro melting point apparatus and were uncorrected. Optical rotations were taken with a JASCO DIP-SL automatic polarimeter. IR and UV spectra were recorded on a Nihon Bunko Model DS-301 and Shimadzu SV-50A spectrometers, respectively, and NMR spectra were measured at 100 MHz with JNR-4H-100 and chemical shifts are given on δ (ppm) scale with tetramethylsilane as the internal standard (s, singlet; d, doublet; t, triplet; q, quartet). Mass spectra were taken on a JMS-OlSG mass spectrometer with a direct inlet system. Gas–liquid chromatography (GLC) was run on a Shimadzu GC-5A with flame ionization detector. Paper partition chromatography (PPC) for sugar was carried out on Tôyô Roshi No. 50 using upper layer of *n*–BuOH–AcOH–H_2O (4: 1: 5) (solvent 1) and *n*–BuOH–pyridine–H_2O (6: 2: 3)+pyridine (1) (solvent 2) as solvents and aniline hydrogen phthalate for staining. Thin–layer chromatography (TLC) was conducted on Kieselgel G nach Stahl (Merck) using $FeCl_3$ reagent and 10% H_2SO_4 as the detector. Column chromatography was carried out with Kieselgel (70—200 mesh) (Merck) and polyamide C-200 (Wakô).

Isolation——Powdered leaves (3.95 kg) collected in Tottori prefecture were percolated with MeOH. The MeOH extract (120 g) was separated with H_2O into soluble and insoluble portions. The H_2O soluble portion was successively extracted with ether (1 liter, 3 times), AcOEt (2 liters, 3 times) and *n*–BuOH saturated with H_2O (2 liters, 3 times). The BuOH extract (80 g) was chromatographed over polyamide (120 g) using H_2O and then MeOH. The H_2O eluate (40 g) was repeatedly chromatographed over silica gel with $CHCl_3$–MeOH–H_2O (7: 3: 0.6) to give berberine and coptiside I (I, 900 mg). The MeOH eluate was rechromatographed over silica gel using $CHCl_3$–MeOH–H_2O (7: 3: 0.6) to give coptiside II (V, 200 mg).

Berberine was identified as its hydrochloride, yellow needles, mp 213° (decomp.) by the comparison of IR (KBr) spectrum with an authentic sample.

Coptiside I (I)——A pale yellow powder (MeOH), mp 190—191°, $[\alpha]_D^{25}$ −93° (c=1.0, pyridine). *Anal.* Calcd. for $C_{46}H_{58}O_{27}\cdot 4H_2O$: C, 49.46; H, 6.14. Found: C, 49.13; H, 5.78. UV λ_{max}^{MeOH} nm (log ε): 270 (4.15), 326 (4.16). UV $\lambda_{max}^{MeOH+AlCl_3}$ nm: 279, 342. UV $\lambda_{max}^{MeOH+AcONa}$ nm: 270, 326. IR ν_{max}^{KBr} cm^{-1}: 3440, 1745, 1655, 1610. NMR (DMSO–d_6) δ: 1.96, 1.98, 2.00 (each 3H, s, $-COCH_3$), 3.86 (3H, s, $-OCH_3$), 6.54 (1H, d, J=2.0 Hz, C_6–H),

15) *a*) N. Morita, M. Shimizu, M. Arisawa, and K. Kobayashi, *Yakugaku Zasshi*, **94**, 913 (1974); *b*) L.P. Kuptsova and Bankovskii, *Khim. Prir. Soedin*, **6**, 128 (1970) [*C. A.*, **73**, 106300 (1970)].
16) T. Takemoto and T. Miyase, *Yakugaku Zasshi*, **94**, 1597 (1974).
17) P. Oelrichs, J.T.B. Marshall, and D.H. Williams, *J. Chem. Soc.*, (*C*), **1968**, 941.

6.84 (1H, d, J=2.0 Hz, C_8–H), 6.93 (1H, s, C_3–H), 7.12 (2H, d, J=8.0 Hz, $C_{3'}$–H, $C_{5'}$–H), 8.04 (2H, d, J=8.0 Hz, $C_{2'}$–H, $C_{6'}$–H).

Alkaline Hydrolysis of I——A solution of I (150 mg) in 1N NaOH–MeOH (10 ml) was kept at room temperature overnight. The geratinous compound precipitated was filtered and crystallized from MeOH–dioxane to give a colorless powder (II, 106 mg); mp 290—291°. *Anal.* Calcd. for $C_{40}H_{52}O_{24}\cdot 2\frac{1}{2}H_2O$: C, 49.95 H, 5.97. Found: C, 50.00; H, 5.91. UV λ_{max}^{MeOH} nm: 270, 324. IR ν_{max}^{KBr} cm^{-1}: 1655, 1610. UV $\lambda_{max}^{MeOH+AlCl_3}$ nm: 279, 342. UV $\lambda_{max}^{MeOH+AcONa}$ nm: 270, 324.

The filtrate was adjusted with 1N HCl–MeOH to pH 6.0—6.5. To this solution was added *p*-bromophenacyl bromide (70 mg) in EtOH and refluxed for 2 hr. The solvent was evaporated *in vacuo* and the residue was extracted with benzene. The benzene soluble portion was subjected to preparative TLC over silica gel using benzene to give colorless needles (5 mg); mp 82—83°. Mass Spectrum: Calcd. for $[M]^+$, $C_{10}H_9O_3Br$: 255.973. Found: C, 255.974. IR ν_{max}^{KBr} cm^{-1}: 1750, 1705. This compound was identified with *p*-bromophenacyl acetate by the IR spectrum and mixed melting point determination.

Acid Hydrolysis of Coptiside I——I (100 mg) was refluxed with 1N H_2SO_4 (H_2O: EtOH=7: 3) for 4 hr, and the solution was condensed *in vacuo*. The mixture was diluted with H_2O, and extracted with AcOEt. The AcOEt layer was evaporated to give yellow needles (III, 13 mg); mp 260—262°. Mass Spectrum: Calcd. for $[M]^+$, $C_{16}H_{12}O_5$: 284.068. Found: 284.064. UV λ_{max}^{MeOH} nm: 270, 330. IR ν_{max}^{KBr} cm^{-1}: 1650, 1610. NMR (DMSO-d_6) δ: 3.86 (3H, s, $-OCH_3$), 6.20 (1H, d, J=2.0 Hz, C_6–H), 6.51 (1H, d, J=2.0 Hz, C_8–H), 6.87 (1H, s, C_3–H), 7.12 (2H, d, J=8.0 Hz, $C_{3'}$–H, $C_{5'}$–H), 8.04 (2H, d, J=8.0 Hz, $C_{2'}$–H, $C_{6'}$–H), 12.90 (1H, s, –OH). III was identified with acacetin by the UV and IR spectra.

The aqueous layer was neutralized with $BaCO_3$ and condensed *in vacuo*. The precipitates were filtered off, and the filtrate was condensed and examined by PPC. D-Glucose (0.10; solvent 1. 0.45; solvent 2) and L-rhamnose (0.33; solvent 1. 0.70; solvent 2) were identified by PPC.

Methylation of II by the Hakomori's Method——A solution of II (50 mg) and sodium hydride (100 mg) in dimethyl sulfoxide (DMSO) (4 ml) was stirred for 1 hr under cooling with H_2O. To the mixture was added CH_3I (1 ml). After stirring for 1 hr under cooling with H_2O, the mixture was diluted with H_2O and extracted with $CHCl_3$. The $CHCl_3$ layer was washed with H_2O, dried and evaporated. The colorless oily residue was purified by preparative TLC over silica gel using AcOEt–EtOH (24: 1) to give colorless plates (IIa, 25 mg), mp 88—89°. Mass Spectrum *m/e*: 423.2187 (Calcd. for $C_{19}H_{39}O_{10}$: 423.2330), 298.0880 ($C_{17}H_{14}O_5$: 298.0841), 219.1181 ($C_{10}H_{19}O_5$: 219.1233), 189.1137 ($C_9H_{17}O_4$: 189.1127). NMR ($CDCl_3$) δ: 1.16 (3H, d, J=6.0 Hz, rhamnose C_6–CH_3), 3.90 (3H, s, $-OCH_3$), 3.96 (3H, s, $-OCH_3$), 4.68 (2H, d, J=6.0 Hz, anomeric proton), 4.74 (1H, s, anomeric proton), 5.32 (1H, d, J=6.0 Hz, anomeric proton), 6.48 (1H, d, J=2.0 Hz, C_6–H), 6.60 (1H, s, C_3–H), 6.73 (1H, d, J=2.0 Hz, C_8–H), 7.02 (2H, d.d, J=2.0, 8.0 Hz, $C_{3'}$–H, $C_{5'}$–H), 7.82 (2H, d.d, J=2.0, 8.0 Hz, $C_{2'}$–H, $C_{6'}$–H).

Acetylation of II——II was acetylated with Ac_2O and pyridine, and the usual working up gave a colorless amorphous powder (IIb), mp 115—116°, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1750, 1655, 1610. Mass Spectrum *m/e*: 619, 313, 273. NMR ($CDCl_3$) δ: 1.15 (3H, d, J=6.0 Hz, rhamnose C_6–CH_3), 3.92 (3H, s, $-OCH_3$), 6.52 (1H, d, J=2.0 Hz, C_6–H), 6.60 (1H, d, J=2.0 Hz, C_8–H), 6.61 (1H, s, C_3–H), 7.04 (2H, d, J=8.0 Hz, $C_{3'}$–H, $C_{5'}$–H), 7.88 (2H, d, J=8.0 Hz, $C_{2'}$–H, $C_{6'}$–H).

Methanolysis of IIa——A mixture of IIa (6 mg) and 0.5 N NaOH–MeOH (5 ml) was kept at room temperature overnight. The mixture was acidified with 1.5N HCl–MeOH (5 ml) and refluxed on a water bath for 1.5 hr. The reaction mixture was neutralized with Ag_2O and the precipitates were filtered off. The filtrate was treated with H_2S gas, filtered and the solvent was evaporated *in vacuo*. The residue was treated with Ac_2O (1 ml) and pyridine (1 ml) at room temperature overnight, and the solvent was evaporated *in vacuo*. The residue was extracted with $CHCl_3$. The $CHCl_3$ solution was washed with H_2O, dried and evaporated to give a colorless oil, which was examined by GLC (Fig. 2).

Partial Hydrolysis of I with Takadiastase A——To a solution of I (140 mg) in McIlvaine buffer (pH 4.0) (30 ml) was added Takadiastase A (140 mg), and the mixture was incubated at 37° for 96 hr. The solution was evaporated *in vacuo*, and the residue was extracted with MeOH. The MeOH extract was purified by the preparative TLC over silica gel using AcOEt–MeOH–H_2O (20: 3: 2). The band at *Rf* value: 0.80 was extracted with MeOH and the residue (IV) was treated with 28% NH_4OH (1 ml) at room temperature for 10 min. The solvent was evaporated *in vacuo* to give colorless needles (IVa, 18 mg), mp 269—270°, $[\alpha]_D^{20}$ −88° (c=0.28, pyridine). IR ν_{max}^{KBr} cm^{-1}: 1660, 1610. This compound was identified with linarin by the comparisons of mp, $[\alpha]_D$ and IR spectrum.

Methylation of IVa by the Hakomori's Method——IVa (10 mg) was methylated by the similar procedure as II to afford colorless needles (IVb, 7 mg), mp 72—73°. Mass Spectrum *m/e*: 690.2916 (Calcd. for $C_{35}H_{46}O_{14}$: 690.2892), 392.1998 ($C_{18}H_{32}O_9$: 392.2046), 298.0868 ($C_{17}H_{14}O_5$: 298.0841), 189.1145 ($C_9H_{17}O_4$: 189.1127). NMR ($CDCl_3$) δ: 1.16 (3H, d, rhamnose C_6–CH_3), 3.88 (3H, s, $-OCH_3$), 3.96 (3H, s, $-OCH_3$), 4.78 (1H, s, anomeric proton), 5.02 (1H, d, J=6.0 Hz, anomeric proton), 6.47 (1H, d, J=2.0 Hz, C_6–H), 6.60 (1H, s, C_3–H), 6.67 (1H, d, J=2.0 Hz, C_8–H), 7.00 (2H, d.d, J=2.0, 8.0 Hz, $C_{3'}$–H, $C_{5'}$–H), 7.80 (2H, d.d, J=2.0, 8.0 Hz, $C_{2'}$–H, $C_{6'}$–H).

Methanolysis of IVb——IVb (2 mg) was refluxed with 1N HCl–MeOH (3 ml) for 1.5 hr. The reaction mixture was neutralized with Ag_2O and the precipitates were filtered off. The filtrate was treated with H_2S

gas, filtered and the solvent was evaporated *in vacuo*. The residue was subjected to the GLC analysis (Fig. 3).

Methylation of I by the Kuhn's Method——To a solution of I (50 mg) in N,N-dimethylformamide (1 ml) were added freshly prepared Ag_2O (100 mg) and methyl iodide (1 ml). The mixture was stirred under cooling with H_2O for 24 hr. The precipitates were filtered off, and the filtrate was evaporated *in vacuo*. A pale yellow powder was purified by preparative TLC over silica gel using AcOEt–EtOH (24: 1) to give colorless plates (Ia, 11.8 mg); mp 102—103°, IR ν_{max}^{KBr} cm^{-1}: 3340 (nil.), 1745, 1640, 1610. Mass Spectrum *m/e*: 423.2258 (Calcd. for $C_{19}H_{35}O_{10}$: 423.223), 298.0841 ($C_{17}H_{14}O_5$: 298.0841), 245.1072 ($C_{11}H_{17}O_6$: 245.103), 219.126 ($C_{10}H_{19}O_5$: 219.123). NMR ($CDCl_3$) δ: 2.00 (3H, s, CH_3CO), 2.08 (3H, s, CH_3CO), 2.15 (3H, s, CH_3CO), 3.92 (3H, s, $-OCH_3$), 3.96 (3H, s, $-OCH_3$), 6.50 (1H, broad s, C_6–H), 6.61 (1H, s, C_3–H), 6.72 (1H, broad s, C_8–H), 7.02 (2H, d, J=8.0 Hz, $C_{3'}$–H, $C_{5'}$–H), 7.92 (2H, d, J=8.0 Hz, $C_{2'}$–H, $C_{6'}$–H).

Methanolysis of Ia——A mixture of Ia (6 mg) and 0.5N NaOH–MeOH (5 ml) was kept at room temperature overnight. After acidification with 1.5N HCl–MeOH (5 ml), the precipitates were removed by filtration, and H_2S gas was passed into the filtrate. After filtration of black precipitates, the solvent was evaporated *in vacuo*. The residue was acetylated with Ac_2O (1 ml) and pyridine (1 ml) overnight. The solvent was evaporated *in vacuo*, and the residue was extracted with $CHCl_3$. The $CHCl_3$ layer was washed, dried and evaporated. The residue was subjected to GLC analysis (Fig. 4).

Coptiside II (V)——A yellow amorphous powder (MeOH), mp 243—244°, $[\alpha]_D^{25}$ −96.0° (c=1.0, pyridine). *Anal.* Calcd. for $C_{21}H_{20}O_{11}\cdot 2\frac{1}{2}H_2O$: C, 51.12; H, 5.14. Found: C, 51.18; H, 4.66. UV λ_{max}^{MeOH} nm (log ε): 258 (4.18), 364 (4.16). UV $\lambda_{max}^{MeOH+AlCl_3}$ nm: 270, 405. UV $\lambda_{max}^{MeOH+NaOAc}$ nm: 264, 366. IR ν_{max}^{KBr} cm^{-1}: 3460, 3300, 1655, 1610. NMR (DMSO-d_6) δ: 5.39 (1H, d, J=8.0 Hz, anomeric proton), 6.22 (1H, d, J=2.0 Hz, C_6–H), 6.40 (1H, d, J=2.0 Hz, C_8–H), 6.84 (1H, d, J=8.0 Hz, $C_{5'}$–H), 7.56 (1H, d, J=2.0 Hz, $C_{2'}$–H), 7.70 (1H, d.d, J=2.0, 8.0 Hz, $C_{6'}$–H), 11.18 (1H, s, –OH).

Acid Hydrolysis of Coptiside II (V)——V (30 mg) was heated with 1N H_2SO_4 for 1 hr on a water bath. After cooling, the precipitates were filtered and crystallized from MeOH to give yellow needles (VI); mp >300°. UV λ_{max}^{MeOH} nm (log ε): 256 (4.25), 375 (4.25). IR ν_{max}^{KBr} cm^{-1}: 3350, 3450, 1655, 1610. NMR (DMSO-d_6) δ: 6.20 (1H, d, J=2.0 Hz, C_6–H), 6.42 (1H, d, J=2.0 Hz, C_8–H), 6.90 (1H, d, J=8.0 Hz, $C_{5'}$–H), 7.56 (1H, d.d, J=2.0, 8.0 Hz, $C_{6'}$–H), 7.70 (1H, d, J=2.0 Hz, $C_{2'}$–H), 9.34 (2H, s, –OH), 12.42 (1H, s, –OH). VI was identified with quercetin by IR and NMR spectra.

The filtrate was treated similarly as in coptiside I, and D-galactose (0.28; solvent 1. 0.19; solvent 2) was identified.

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