[Chem. Pharm. Bull. 32(10)3880—3885(1984)]

# Studies on the Constituents of Cistanchis Herba. IV. Isolation and Structures of Two New Phenylpropanoid Glycosides, Cistanosides C and D

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(Received February 13, 1984)

Two new phenylpropanoid glycosides, named cistanosides C and D, were isolated from the whole plant of *Cistanche salsa* (C. A. MEY.) G. BECK (Orobanchaceae), together with 2′-acetyl acteoside and osmanthuside B. The structures of cistanosides C and D were established as 2-(4-hydroxy-3-methoxyphenyl)ethyl *O*-α-L-rhamnopyranosyl-(1→3)-*O*-(4-*O*-caffeoyl)-β-D-glucopyranoside (II) and 2-(4-hydroxy-3-methoxyphenyl)ethyl *O*-α-L-rhamnopyranosyl-(1→3)-*O*-(4-*O*-feruloyl)-β-D-glucopyranoside (III), respectively, on the basis of chemical and spectral data.

**Keywords**——*Cistanche salsa*; Cistanchis Herba; Orobanchaceae; phenylpropanoid glycoside; cistanoside C; cistanoside D; 2′-acetyl acteoside; osmanthuside B

In the preceding paper,[1] we reported the isolation and structure determination of two new phenylpropanoid glycosides, cistanosides A and B, from Cistanchis Herba, the whole plants of *Cistanche salsa* (C. A. MEY) G. BECK (Orobanchaceae).

As a continuation of our investigations on the constituents of this crude drug, this paper deals with the isolation and structure elucidation of two new phenylpropanoid glycosides, named cistanoside C (II) and cistanoside D (III), as well as the isolation of two known phenylpropanoid glycosides, 2′-acetyl acteoside (I)[2] and osmanthuside B (IV).[3] The *n*-butanol-soluble fraction of this crude drug exhibited eight spots detected by spraying ferric chloride on a thin-layer chromatography (TLC) plate, as shown in Fig. 1. The four compounds (I—IV) isolated in the present work are less polar than those (echinacoside, cistanoside A, acteoside and cistanoside B) reported in the previous paper.

The dried whole plants were extracted with hot methanol and the methanolic extract was suspended in water. This suspension was extracted with ethyl acetate and then *n*-butanol saturated with water, successively. The *n*-butanol-soluble fraction was chromatographed on polyamide, silica gel and Sephadex LH-20 columns successively to give four phenylpropanoid glycosides, 2′-acetyl acteoside (I), cistanoside C (II), cistanoside D (III) and osmanthuside B (IV). These compounds gave a positive coloration with ferric chloride and were assumed to be phenylpropanoid glycosides possessing phenolic hydroxyl groups from their ultraviolet (UV)

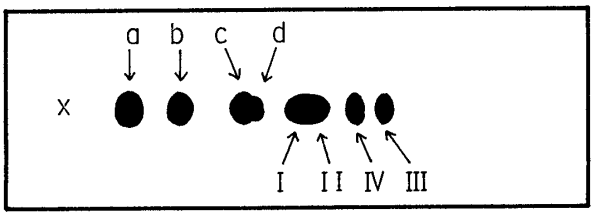


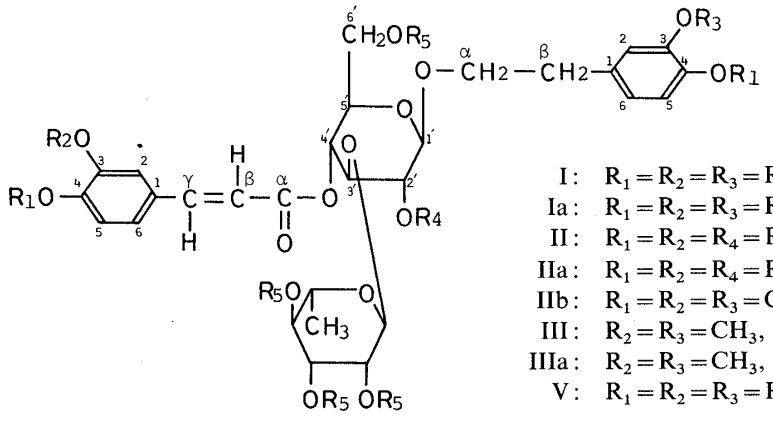

Fig. 1. Thin-Layer Chromatogram of Total Phenylpropanoid Glycosides

a, echinacoside; b, cistanoside A; c, acteoside; d, cistanoside B.
Plate, Silica gel 60 $F_{254}$; solvent, $CHCl_3$–MeOH–$H_2O$ (6:4:1).

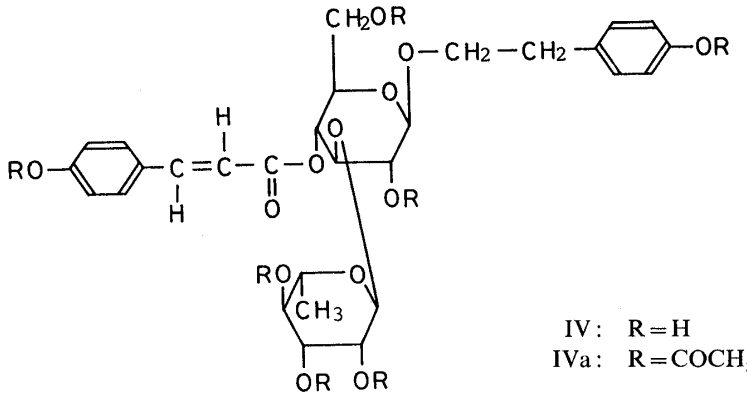
spectral data[4] as well as infrared (IR), proton nuclear magnetic resonance ($^1$H-NMR) and carbon-13 nuclear magnetic resonance ($^{13}$C-NMR) spectral analyses.

**2′-Acetyl Acteoside (I)**

Compound I was isolated as an amorphous powder, and the IR spectrum suggested the presence of hydroxyl groups (3450 cm$^{-1}$), a conjugated ester (1705 cm$^{-1}$), a double bond (1640 cm$^{-1}$) and aromatic rings (1610, 1535 cm$^{-1}$). The $^1$H-NMR spectrum of I showed signals of a methyl group of rhamnose [$\delta$ 1.01 (3H, d, $J$=6 Hz)], a methyl signal of an acetoxyl group [$\delta$ 1.99 (3H, s)], benzylic methylene protons [$\delta$ 2.69 (2H, t, $J$=7 Hz)], a glucose-anomeric proton [$\delta$ 4.50 (1H, d, $J$=8 Hz)], a rhamnose-anomeric proton [$\delta$ 5.16 (1H, br s)], two *trans* olefinic protons [$\delta$ 6.25, 7.59 (1H each, d, $J$=16 Hz)] and aromatic protons [$\delta$ 6.5—7.2 (6H)]. On acetylation, I afforded the octaacetate (Ia), $C_{47}H_{54}O_{24}$, amorphous powder, whose $^1$H-NMR spectrum showed five alcoholic [$\delta$ 1.87, 1.94, 2.02, 2.08 and 2.10 (3H each)] and four phenolic [$\delta$ 2.27, 2.28 (3H each) and 2.30 (6H)] methyl signals of acetoxyl groups. Compound Ia was found to be identical with the nonaacetate of acteoside[1,4-8] (=verbascoside) (V)[9,10] by direct comparison (TLC, IR and $^1$H-NMR) with an authentic sample. On the other hand, the $^{13}$C-NMR spectrum of I was almost identical with that of acteoside (V), except for the signals due to the glucose moiety and the acetoxyl group [$\delta$ 20.9 ($CH_3$), 171.5 (C=O)], suggesting that the acetoxyl group was attached to the glucose moiety. The location of the acetoxyl group in the glucose moiety of I was determined from its $^{13}$C-NMR spectrum by detailed comparison with that of V. The $^{13}$C-NMR spectrum of V showed signals at $\delta$ 104.1, 75.9 and 81.6 due to C-1′, C-2′ and C-3′ of glucose, respectively, whereas the spectrum of I showed signals of the corresponding carbons at $\delta$ 101.6, 75.1 and 80.3,



I: $R_1=R_2=R_3=R_5=H$, $R_4=COCH_3$
Ia: $R_1=R_2=R_3=R_4=R_5=COCH_3$
II: $R_1=R_2=R_4=R_5=H$, $R_3=CH_3$
IIa: $R_1=R_2=R_4=R_5=COCH_3$, $R_3=CH_3$
IIb: $R_1=R_2=R_3=CH_3$, $R_4=R_5=H$
III: $R_2=R_3=CH_3$, $R_1=R_4=R_5=H$
IIIa: $R_2=R_3=CH_3$, $R_1=R_4=R_5=COCH_3$
V: $R_1=R_2=R_3=R_4=R_5=H$



IV: R=H
IVa: R=$COCH_3$

Chart 1

Table I. $^{13}$C-NMR Chemical Shifts of I, II, III, IV and V (in Methanol-$d_4$)

| | | I | II | III | IV | V |
|---|---|---|---|---|---|---|
| Aglycone | 1 | 131.9 | 131.6 | 131.6 | 130.7 | 131.6 |
| | 2 | 116.6 | 113.9 | 114.0 | 116.1 | 116.6 |
| | 3 | 144.4 | 148.8 | 148.8 | 131.2 | 144.4 |
| | 4 | 145.9 | 145.9 | 145.9 | 156.6 | 146.0 |
| | 5 | 117.2 | 116.6 | 116.6 | 131.2 | 117.2 |
| | 6 | 121.4 | 122.4 | 122.4 | 116.1 | 121.4 |
| | α | 72.5 | 72.3 | 72.3 | 72.1 | 72.3 |
| | β | 36.2 | 36.6 | 36.7 | 36.2 | 36.5 |
| Caffeic acid | 1 | 127.7 | 127.7 | 127.2 | 127.1 | 127.7 |
| (ferulic) | 2 | 114.7 | 114.8 | 112.1 | 116.8 | 114.8 |
| (*p*-coumaric) | 3 | 149.6 | 149.6 | 150.7 | 130.8 | 149.5 |
| | 4 | 146.6 | 146.7 | 149.3 | 161.1 | 146.6 |
| | 5 | 116.4 | 116.2 | 116.2 | 130.8 | 116.4 |
| | 6 | 123.2 | 123.2 | 124.2 | 116.8 | 123.2 |
| | α | 168.1 | 168.3 | 168.2 | 168.2 | 168.3 |
| | β | 115.5 | 115.5 | 115.2 | 114.8 | 115.5 |
| | γ | 148.1 | 147.9 | 147.8 | 147.5 | 148.0 |
| Glucose | 1 | 101.6 | 104.1 | 104.2 | 104.1 | 104.1 |
| | 2 | 75.1 | 76.0 | 76.0 | 76.0 | 75.9 |
| | 3 | 80.3 | 81.6 | 81.4 | 81.5 | 81.6 |
| | 4 | 70.7 | 70.3[b] | 70.3[d] | 70.2[f] | 70.3[h] |
| | 5 | 76.0 | 76.1 | 76.0 | 75.9 | 76.1 |
| | 6 | 62.2 | 62.4 | 62.5 | 62.3 | 62.3 |
| Rhamnose | 1 | 103.1 | 102.8 | 102.8 | 102.7 | 102.8 |
| | 2 | 72.0[a] | 72.1[c] | 72.2[e] | 72.2[g] | 72.1 |
| | 3 | 71.7[a] | 72.0[c] | 72.1[e] | 72.1[g] | 72.1 |
| | 4 | 73.6 | 73.8 | 73.8 | 73.7 | 73.8 |
| | 5 | 70.7 | 70.7[b] | 70.8[d] | 70.7[f] | 70.7[h] |
| | 6 | 18.4 | 18.4 | 18.4 | 18.4 | 18.4 |
| Others | $OCH_3$ | | 56.6 | 56.6 (×2) | | |
| | $CH_3$ | 20.9 | | | | |
| | CO | 171.5 | | | | |

*a*—*h*) Assignments may be interchanged in each column.

respectively. The differences in the chemical shifts of the corresponding carbon atoms of V and I, [−2.5 (C-1′), −0.8 (C-2′) and −1.3 (C-3′)], indicated that the acylation shifts[11] were caused by the linkage of an acetoxyl group to the C-2′ hydroxyl group of the glucose moiety in V. On methanolysis of I with acetyl chloride in methanol, methyl caffeate and 3,4-dihydroxyphenethyl alcohol were detected by TLC and high-performance liquid chromatography (HPLC). From the above results, the structure of I was established as 2′-acetyl acteoside, which has previously been isolated from *Aeginetia indica* L. (Orobanchaceae).[2]

**Cistanoside C (II)**

Compound II was isolated as an amorphous powder and gave the octaacetate (IIa), $C_{46}H_{54}O_{23}$, amorphous powder, on acetylation. The $^1$H-NMR spectrum of II was very similar to that of V except for the appearance of the signal assignable to an aromatic methoxyl group at $\delta$ 3.86. Furthermore, the $^1$H-NMR spectrum of IIa revealed the presence of eight methyl signals of acetoxyl groups, including five alcoholic [$\delta$ 1.88, 1.95, 1.98, 2.09 and 2.10 (3H each)] and three phenolic [$\delta$ 2.30 (3H), 2.31 (6H)] acetoxyl groups. On the other hand, the $^{13}$C-NMR spectrum of II showed almost the same chemical shifts as those of V, except for signals due to

the aglycone moiety attached to the C-1′ hydroxyl group of the glucose, indicating that rhamnose and caffeic acid are linked to the C-3′ and C-4′ hydroxyl groups, respectively, of the glucose bonded directly to the aglycone. The field-desorption mass spectrum (FD-MS) of II exhibited an ion peak at *m/z* 661 $(M+Na)^+$. On methanolysis of II with acetyl chloride in methanol, methyl caffeate and 4-hydroxy-3-methoxyphenethyl alcohol were detected by TLC and HPLC. Acid hydrolysis of II with 10% sulfuric acid afforded glucose and rhamnose in a ratio of 1 to 1. Partial methylation of II with dimethyl sulfate and potassium carbonate in acetone afforded the methyl ether (IIb), and its $^1$H-NMR spectrum showed the presence of four aromatic methoxyl signals [$\delta$ 3.77, 3.79, 3.86 and 3.88 (3H each)]. Compound IIb was identical with the tetramethyl ether of acteoside (V). These results led us to conclude that the structure of cistanoside C is 2-(4-hydroxy-3-methoxyphenyl)ethyl *O*-α-L-rhamnopyranosyl-(1→3)-*O*-(4-*O*-caffeoyl)-β-D-glucopyranoside (II).

**Cistanoside D (III)**

Compound III was isolated as an amorphous powder and gave the heptaacetate (IIIa), $C_{45}H_{54}O_{22}$, amorphous powder, on acetylation. The IR, UV and $^1$H-NMR spectra of III were similar to those of II, indicating a close structural relationship of the two glycosides. The $^{13}$C-NMR spectrum of III also showed almost the same chemical shifts as those of II, except for signals due to the caffeic acid moiety. The signal at $\delta$ 3.84 (6H, s) in the $^1$H-NMR spectrum, and that at $\delta$ 56.6 in the $^{13}$C-NMR spectrum showed the presence of two aromatic methoxyl groups. Five alcoholic [$\delta$ 1.88, 1.96, 1.98 (3H each) and 2.10 (6H)] and two phenolic [$\delta$ 2.31, 2.33 (3H each)] methyl signals of acetoxyl groups were apparent in the $^1$H-NMR spectrum of IIIa. Acid hydrolysis of III with 10% sulfuric acid afforded glucose and rhamnose in a ratio of 1 to 1. Furthermore, on methanolysis of III with acetyl chloride in methanol, methyl ferulate and 4-hydroxy-3-methoxyphenethyl alcohol were detected by TLC and HPLC. The FD-MS of III exhibited an ion peak at *m/z* 675 $(M+Na)^+$. On the basis of the above-mentioned observation and the fact that partial methylation of III in the same way as for II afforded the methyl ether, which was identical with IIb, the structure of cistanoside D was determined to be 2-(4-hydroxy-3-methoxyphenyl)ethyl *O*-α-L-rhamnopyranosyl-(1→3)-*O*-(4-*O*-feruloyl)-β-D-glucopyranoside (III).

**Osmanthuside B (IV)**

Compound IV showed absorptions due to hydroxyl groups (3450 $cm^{-1}$), a conjugated ester (1705 $cm^{-1}$), a double bond (1640 $cm^{-1}$) and aromatic rings (1612, 1522 $cm^{-1}$) in the IR spectrum. On acetylation, IV afforded the heptaacetate (IVa), $C_{43}H_{50}O_{20}$, colorless needles, mp 135—136 °C, whose $^1$H-NMR spectrum showed five alcoholic [$\delta$1.87, 1.94, 2.00 (3H each) and 2.09 (6H)] and two phenolic [$\delta$ 2.28, 2.31 (3H each) methyl signals of acetoxyl groups. On methanolysis of IV with acetyl chloride in methanol, methyl *p*-coumarate and *p*-hydroxyphenethyl alcohol were detected by TLC and HPLC. Acid hydrolysis of IV with 10% sulfuric acid afforded glucose and rhamnose in a ratio of 1 to 1. The molecular weight was confirmed by the observation of an ion peak at *m/z* 615 $(M+Na)^+$ on FD-MS. Based on the foregoing results, IV was assumed to be osmanthuside B, which has been isolated from leaves of *Osmanthus fragrans* LOUR. var. *aurantiacus* MAKINO (Oleaceae),[3] and this was confirmed by direct comparison (TLC, IR and $^1$H-NMR) of the heptaacetate (IVa) with the heptaacetate of an authentic sample.

## Experimental

Melting points were determined on a Mitamura micro-melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-140 digital polarimeter. IR spectra were recorded with a Hitachi 270-30 infrared spectrophotometer and UV spectra with a Hitachi 200-20 spectrometer. $^1$H-NMR and $^{13}$C-NMR spectra

were recorded with a JEOL FX-90Q machine (89.55 and 22.5 MHz, respectively). Chemical shifts are given on the $\delta$ (ppm) scale with tetramethylsilane as an internal standard (s, singlet; d, doublet; t, triplet; br, broad). FD-MS were measured with a JEOL JMS-01-SG2 mass spectrometer. Gas chromatography (GC) was run on a Shimadzu GC-4CM apparatus with a flame ionization detector. HPLC was performed on a Kyowa Seimitsu KHP-010 machine equipped with a UV detector operated at 250 nm. Polyamide (Polyamide C-200, Wako Pure Chemical) and silica gel (Wako gel C-300, Wako Pure Chemical) were used for column chromatography. Kieselgel 60 $F_{254}$ (Merck) precoated plates were used for TLC and detection was carried out by spraying ethanolic $FeCl_3$ solution or 10% $H_2SO_4$ followed by heating.

**Extraction and Isolation**——The dried whole plants of *Cistanche salsa* (C. A. MEY.) G. BECK (10 kg, commercial crude drug produced in China) were chopped and extracted with MeOH (36 l × 2) under reflux. The extract was concentrated under reduced pressure and the residue was suspended in water. This suspension was extracted with EtOAc and then *n*-BuOH saturated with water, successively. The *n*-BuOH-soluble fraction was concentrated *in vacuo* to afford a residue (292 g). This residue was chromatographed on a polyamide column using $H_2O$ and then MeOH. The fraction eluted with MeOH was concentrated to give the phenolic crude glucosides, which were chromatographed on a silica gel column using $CHCl_3$–MeOH–$H_2O$ (70 : 20 : 1) to give a mixture of I, II, III and IV. The mixture of glucosides was rechromatographed on a Sephadex LH-20 column using $H_2O$–MeOH (1 : 1) to give cistanoside D (III) (250 mg), cistanoside C (II) (3.5 g), osmanthuside B (IV) (300 mg) and 2′-acetyl acteoside (I) (3.5 g).

**2′-Acetyl Acteoside (I)**——Amorphous powder, $[\alpha]_D^{19}$ −117.6° ($c$=1.25, MeOH). IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3450, 1735, 1705, 1640, 1610, 1535. UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 222 (4.05), 245 sh (3.89), 295 sh (3.99), 334 (4.05). $^1$H-NMR (methanol-$d_4$) $\delta$: 1.07 (3H, d, $J$=6 Hz, $CH_3$ of rhamnose), 1.99 (3H, s, $COCH_3$), 2.69 (2H, t, $J$=7 Hz, Ar–$CH_2$–), 4.50 (1H, d, $J$=8 Hz, H-1 of glucose), 5.16 (1H, br s, H-1 of rhamnose), 6.25 (1H, d, $J$=16 Hz, Ar–CH=C<u>H</u>–), 6.5—7.2 (6H, aromatic H), 7.59 (1H, d, $J$=16 Hz, Ar–C<u>H</u>=CH–). $^{13}$C-NMR: Table I.

**Cistanoside C (II)**——Amorphous powder, $[\alpha]_D^{19}$ −88.4° ($c$=0.86, MeOH). IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3430, 1705, 1638, 1610, 1525. UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 222 (4.18), 245 sh (3.90), 290 sh (4.00), 332 (4.15). FD-MS $m/z$: 661 $(M+Na)^+$. $^1$H-NMR (methanol-$d_4$) $\delta$: 1.11 (3H, d, $J$=6 Hz, $CH_3$ of rhamnose), 2.88 (2H, t, $J$=7 Hz, Ar–$CH_2$–), 3.86 (3H, s, $OCH_3$), 4.40 (1H, d, $J$=8 Hz, H-1 of glucose), 5.20 (1H, br s, H-1 of rhamnose), 6.27 (1H, d, $J$=16 Hz, Ar–CH=C<u>H</u>–), 6.6—7.2 (6H, aromatic H), 7.61 (1H, d, $J$=16 Hz, Ar–C<u>H</u>=CH–). $^{13}$C-NMR: Table I.

**Cistanoside D (III)**——Amorphous powder, $[\alpha]_D^{20}$ −71.0° ($c$=1.00, MeOH). IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3450, 1710, 1638, 1605, 1520. UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 237 (4.03), 285 (4.02), 328 (4.02). FD-MS $m/z$: 675 $(M+Na)^+$. $^1$H-NMR (methanol-$d_4$) $\delta$: 1.12 (3H, d, $J$=6 Hz, $CH_3$ of rhamnose), 2.85 (2H, t, $J$=7 Hz, Ar–$CH_2$–), 3.84 (6H, s, $OCH_3$ × 2), 4.38 (1H, d, $J$=8 Hz, H-1 of glucose), 5.22 (1H, br s, H-1 of rhamnose), 6.37 (1H, d, $J$=16 Hz, Ar–CH=C<u>H</u>–), 6.6—7.2 (6H, aromatic H), 7.67 (1H, d, $J$=16 Hz, Ar–C<u>H</u>=CH–). $^{13}$C-NMR: Table I.

**Osmanthuside B (IV)**——Amorphous powder, $[\alpha]_D^{19}$ −82.5° ($c$=1.34, MeOH). IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3450, 1705, 1640, 1612, 1522. UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 225 (4.28), 313 (4.29). FD-MS $m/z$: 615 $(M+Na)^+$. $^1$H-NMR (methanol-$d_4$) $\delta$: 1.10 (3H, d, $J$=6 Hz, $CH_3$ of rhamnose), 2.84 (2H, t, $J$=7 Hz, Ar–$CH_2$–), 4.38 (1H, d, $J$=8 Hz, H-1 of glucose), 5.20 (1H, br s, H-1 of rhamnose), 6.33 (1H, d, $J$=16 Hz, Ar–CH=C<u>H</u>–), 6.70 (2H, d, $J$=9 Hz, H-3, H-5 of aglycone), 6.80 (2H, d, $J$=9 Hz, H-3, H-5 of *p*-coumaric acid), 7.07 (2H, d, $J$=9 Hz, H-2, H-6 of aglycone), 7.45 (2H, d, $J$=9 Hz, H-2, H-6 of *p*-coumaric acid), 7.67 (1H, d, $J$=16 Hz, Ar–C<u>H</u>=CH–). $^{13}$C-NMR: Table I.

**Acetylation of I, II, III and IV**——Compound I (100 mg), II (100 mg), III (50 mg) or IV (50 mg) was dissolved in pyridine (1 ml) and acetic anhydride (1 ml) and the solution was left at room temperature overnight. The reaction mixture was poured into ice-water, and then extracted with EtOAc. Each EtOAc extract was concentrated *in vacuo* and the residue was chromatographed on a silica gel column using benzene–acetone (10 : 1) to give the octaacetate (Ia) (75 mg), octaacetate (IIa) (82 mg), heptaacetate (IIIa) (40 mg) or heptaacetate (IVa) (45 mg). 2′-Acetyl acteoside octaacetate (Ia): amorphous powder, *Anal.* Calcd for $C_{47}H_{54}O_{24}$: C, 56.29; H, 5.43. Found: C, 56.25; H, 5.38. IR $\nu_{max}^{KBr}$ cm$^{-1}$: 1750, 1644, 1506, 1430. UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 281 (4.51). $^1$H-NMR ($CDCl_3$) $\delta$: 1.04 (3H, d, $J$=6 Hz, $CH_3$ of rhamnose), 1.87, 1.94, 2.02, 2.08, 2.10 (3H each, s, OAc), 2.27, 2.28 (3H each, s, Ar–OAc), 2.30 (6H, s, Ar–OAc × 2), 2.88 (2H, t, $J$=7 Hz, Ar–$CH_2$–), 6.35 (1H, d, $J$=16 Hz, Ar–CH=C<u>H</u>–), 7.0—7.5 (6H, aromatic H), 7.66 (1H, d, $J$=16 Hz, Ar–C<u>H</u>=CH–). Cistanoside C octaacetate (IIa): amorphous powder, *Anal.* Calcd for $C_{46}H_{54}O_{23}$: C, 56.67; H, 5.58. Found: C, 56.75; H, 5.54. IR $\nu_{max}^{KBr}$ cm$^{-1}$: 1760, 1650, 1617, 1518, 1435. UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 225 (4.17), 272 (4.18). $^1$H-NMR ($CDCl_3$) $\delta$: 1.04 (3H, d, $J$=6 Hz, $CH_3$ of rhamnose), 1.88, 1.95, 1.98, 2.09, 2.10 (3H each, s, OAc), 2.30 (3H, s, Ar–OAc), 2.31 (6H, s, Ar–OAc × 2), 2.87 (2H, t, $J$=7 Hz, Ar–$CH_2$–), 6.33 (1H, d, $J$=16 Hz, Ar–CH=C<u>H</u>–), 6.7—7.4 (6H, aromatic H), 7.65 (1H, d, $J$=16 Hz, Ar–C<u>H</u>=CH–). Cistanoside D heptaacetate (IIIa): amorphous powder, *Anal.* Calcd for $C_{45}H_{54}O_{22}$: C, 57.08; H, 5.75. Found: C, 57.12; H, 5.76. IR $\nu_{max}^{KBr}$ cm$^{-1}$: 1760, 1645, 1613, 1522, 1435. UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 228 (3.88), 270 (3.88). $^1$H-NMR ($CDCl_3$) $\delta$: 1.04 (3H, d, $J$=6 Hz, $CH_3$ of rhamnose), 1.88, 1.96, 1.98 (3H each, s, OAc), 2.10 (6H, s, OAc × 2), 2.31, 2.33 (3H each, s, Ar–OAc), 2.87 (2H, t, $J$=7 Hz, Ar–$CH_2$–), 3.86 (6H, s, $OCH_3$ × 2), 6.34 (1H, d, $J$=16 Hz, Ar–CH=C<u>H</u>–), 6.7—7.4 (6H, aromatic H), 7.68 (1H, d, $J$=16 Hz, Ar–C<u>H</u>=CH–). Osmanthuside B heptaacetate (IVa): colorless needles from MeOH, mp 135—136 °C, *Anal.* Calcd for $C_{43}H_{50}O_{20}$: C, 58.24; H, 5.68. Found: C, 58.34; H, 5.76. IR $\nu_{max}^{KBr}$ cm$^{-1}$: 1765, 1640, 1608, 1515, 1440. UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 218 (4.39), 282 (4.43). $^1$H-NMR ($CDCl_3$) $\delta$: 1.03 (3H, d, $J$=6 Hz, $CH_3$ of rhamnose), 1.87, 1.94, 2.00 (3H each, s, OAc), 2.09 (6H, s, OAc × 2), 2.28, 2.31 (3H each, s, Ar–OAc), 2.87 (2H, t,

$J=7$ Hz, Ar–$CH_2$–), 6.34 (1H, d, $J=16$ Hz, Ar–CH=C<u>H</u>–), 6.97 (2H, d, $J=9$ Hz, H-3, H-5 of aglycone), 7.12 (2H, d, $J=9$ Hz, H-3, H-5 of *p*-coumaric acid), 7.21 (2H, d, $J=9$ Hz, H-2, H-6 of aglycone), 7.53 (2H, d, $J=9$ Hz, H-2, H-6 of *p*-coumaric acid), 7.70 (1H, d, $J=16$ Hz, Ar–C<u>H</u>=CH–).

**Acid Hydrolysis of II, III and IV**——A solution of a glycoside (*ca.* 2 mg) in 10% $H_2SO_4$ (1 ml) was heated in a boiling water bath for 30 min. The solution was passed through an Amberlite IR-45 column and concentrated to give a residue, which was reduced with sodium borohydride (*ca.* 3 mg) for 1 h. The reaction mixture was passed through an Amberlite IR-120 column and concentrated to dryness. Boric acid was removed by distillation with MeOH and the residue was acetylated with acetic anhydride (1 drop) and pyridine (1 drop) at 100 °C for 1 h. The reagents were evaporated off *in vacuo*. Glucitol acetate and rhamnitol acetate were detected in a ratio of 1 to 1 from each glycoside by GC. Conditions: column, 1.5% OV-17, 3 mm i.d. × 1.5 m; column temp., 180 °C; carrier gas, $N_2$ (30 ml/min). $t_R$ (min) 2.0 (rhamnitol acetate), 5.5 (glucitol acetate).

**Methanolysis of I, II, III and IV with Methanolic Acetyl Chloride**——Compound I, II, III or IV (*ca.* 1 mg) was refluxed with methanolic 5% $CH_3COCl$ (2 ml) for 30 min, and then the reagents were evaporated off *in vacuo*. The presence of methyl caffeate and 3,4-dihydroxyphenethyl alcohol in the residue of I, methyl caffeate and 4-hydroxy-3-methoxyphenethyl alcohol in II, methyl ferulate and 4-hydroxy-3-methoxyphenethyl alcohol in III, and methyl *p*-coumarate and *p*-hydroxyphenethyl alcohol in IV was demonstrated by TLC [$CHCl_3$–MeOH (20 : 1)] and HPLC [column, TSK GEL LS-410AK (4 mm i.d. × 300 mm); solvent, $H_2O$–MeOH (4 : 6); flow rate, 1.0 ml/min]. 3,4-Dihydroxyphenethyl alcohol: *Rf* 0.06, $t_R$ (min) 3.6. *p*-Hydroxyphenethyl alcohol: *Rf* 0.18, $t_R$ (min) 4.0. Methyl caffeate: *Rf* 0.20, $t_R$ (min) 6.1. 4-Hydroxy-3-methoxyphenethyl alcohol: *Rf* 0.31, $t_R$ (min) 4.1. Methyl *p*-coumarate: *Rf* 0.40, $t_R$ (min) 8.75. Methyl ferulate: *Rf* 0.58, $t_R$ (min) 8.7.

**Partial Methylation of II and III**——$(CH_3)_2SO_4$ (3 drops) was added to a solution of II or III (100 mg) in dry acetone (3 ml) containing anhydrous potassium carbonate (200 mg). The reaction mixture was stirred at room temperature for 20 h, then filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography using $CHCl_3$–MeOH (20 : 1) to give the methyl ether (IIb), 25 mg from II, 20 mg from III, as an amorphous powder. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3450, 1720, 1635, 1605, 1520, 1472, 1430, 1270, 1165, 1150, 1030. $^1$H-NMR (acetone-$d_6$): 1.11 (3H, d, $J=6$ Hz, $CH_3$), 2.85 (2H, t, $J=7$ Hz, Ar–$CH_2$–), 3.77, 3.79, 3.86, 3.88 (3H each, s, $OCH_3$), 4.45 (1H, d, $J=8$ Hz, H-1 of glucose), 5.28 (1H, br s, H-1 of rhamnose), 6.42 (1H, d, $J=16$ Hz, Ar–CH=C<u>H</u>–), 6.7—7.4 (6H, aromatic H), 7.65 (1H, d, $J=16$ Hz, Ar–C<u>H</u>=CH–). These products were found to be identical with the tetramethyl ether of acteoside (V) by direct comparison (TLC, IR and $^1$H-NMR).

**Acknowledgement** We are grateful to Dr. M. Kikuchi, Tohoku College of Pharmacy, for providing an authentic sample.

## References and Notes

1) Part III: H. Kobayashi, H. Karasawa, T. Miyase, and S. Fukushima, *Chem. Pharm. Bull.*, **32**, 3009 (1984).
2) T. Endo, H. Taguchi, and I. Yoshioka, Abstracts of Papers, the 98th Annual Meeting, Pharmaceutical Society of Japan, Okayama, April 1978, p. 312.
3) M. Kikuchi and Y. Yamauchi, Abstracts of Papers, the 22nd Meeting of the Tohoku Branch, Pharmaceutical Society of Japan, Morioka, October 1983, p. 38.
4) T. Miyase, A. Koizumi, A. Ueno, T. Noro, M. Kuroyanagi, S. Fukushima, Y. Akiyama, and T. Takemoto, *Chem. Pharm. Bull.*, **30**, 2732 (1982).
5) L. Birkofer, C. Kaiser, and U. Thomas, *Z. Naturforsch.*, **1968**, 1051.
6) H. Sasaki, H. Taguchi, T. Endo, I. Yoshioka, K. Higashiyama, and H. Otomasu, *Chem. Pharm. Bull.*, **26**, 2111 (1978).
7) G. Nonaka and I. Nishioka, *Phytochemistry*, **16**, 1265 (1977).
8) R. Cooper, P. H. Solomon, I. Kubo, K. Nakanishi, J. N. Shoolery, and J. L. Occolowitz, *J. Am. Chem. Soc.*, **102**, 7953 (1980).
9) C. Andary, R. Wylde, C. Laffite, G. Privat, and F. Winternitz, *Phytochemistry*, **21**, 1123 (1982).
10) G. Schilling, M. Hügel, and W. Mayer, *Z. Naturforsch.*, **1982**, 1633.
11) K. Yoshimoto, Y. Itatani, and Y. Tsuda, *Chem. Pharm. Bull.*, **28**, 2065 (1980).