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# Studies on the Constituents of Cistanchis Herba. III. Isolation and Structures of New Phenylpropanoid Glycosides, Cistanosides A and B

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Two new phenylpropanoid glycosides, named cistanoside A (III) and cistanoside B (IV), were isolated from the whole plant of *Cistanche salsa* (C. A. MEY.) G. BECK (Orobanchaceae), together with acteoside (I) and echinacoside (II). The structures of III and IV were determined to be 2-(4-hydroxy-3-methoxyphenyl)ethyl *O*-α-L-rhamnopyranosyl-(1→3)-*O*-[β-D-glucopyranosyl-(1→6)]-(4-*O*-caffeoyl)-β-D-glucopyranoside and 2-(4-hydroxy-3-methoxyphenyl)ethyl *O*-α-L-rhamnopyranosyl-(1→3)-*O*-[β-D-glucopyranosyl-(1→6)]-(4-*O*-feruloyl)-β-D-glucopyranoside, respectively, on the basis of chemical and spectral data.

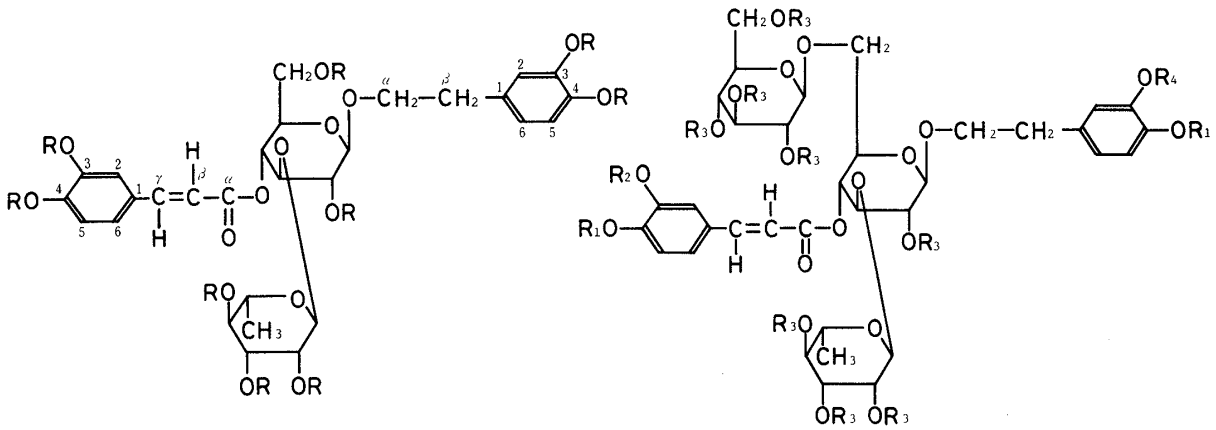
**Keywords**——*Cistanche salsa*; Cistanchis Herba; Orobanchaceae; phenylpropanoid glycoside; cistanoside A; cistanoside B; echinacoside; acteoside

We have already reported the isolation of a monoterpene glucoside, 8-hydroxygeraniol-1-β-D-glucoside,[1] and three iridoids, 8-epiloganic acid,[1] cistanin[2] and cistachlorin,[2] from Cistanchis Herba, dried whole plants of *Cistanche salsa* (C. A. MEY.) G. BECK (Orobanchaceae). As a continuation of our investigation on the constituents of this crude drug, we now wish to report the isolation and the structure elucidation of two new phenylpropanoid glycosides, named cistanosides A and B, as well as the isolation of two known phenylpropanoid glycosides, acteoside[3-7] (verbascoside)[8,9] and echinacoside.[10,11]

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. The *n*-butanol-soluble fraction was chromatographed on silica gel to give three fractions (fr. 1—3). After repeated chromatography (silica gel, Sephadex LH-20) of these fractions, four phenylpropanoid glycosides, acteoside (I), echinacoside (II), and cistanosides A (III) and B (IV), were isolated.

Compounds, I, II, III and IV gave a brown coloration with ferric chloride, indicating the presence of phenolic hydroxyl groups, and were assumed to be phenylpropanoid glycosides from their ultraviolet (UV) spectral data[7] as well as infrared (IR), proton nuclear magnetic resonance ($^1$H-NMR) and $^{13}$C-nuclear magnetic resonance ($^{13}$C-NMR) spectral analyses.

Acteoside (I) was isolated as an amorphous powder and gave the amorphous non-aacetate (Ia), $C_{47}H_{54}O_{24}$, upon acetylation with acetic anhydride and pyridine. Compound I was suggested to possess five alcoholic and four phenolic hydroxyl groups [in acetate, $\delta$ 1.87, 1.94, 2.02, 2.08, 2.10, 2.27, 2.28 (3H, each) and 2.30 (6H)], a conjugated ester group (1696 cm$^{-1}$), a double bond (1634 cm$^{-1}$) and aromatic rings (1606, 1520 cm$^{-1}$) from the IR, UV and $^1$H-NMR spectral data for I and Ia. On methanolysis of I with acetyl chloride in methanol,[7] methyl caffeate and 3,4-dihydroxyphenethyl alcohol were detected. Acid hydrolysis of I with 10% sulfuric acid afforded glucose and rhamnose in a ratio of 1 to 1. Based



I: R=H
Ia: R=COCH$_3$

II: $R_1=R_2=R_3=R_4=H$
IIa: $R_1=R_2=R_3=R_4=COCH_3$
III: $R_1=R_2=R_3=H$, $R_4=CH_3$
IIIa: $R_1=R_2=R_3=COCH_3$, $R_4=CH_3$
IIIb: $R_1=R_2=R_4=CH_3$, $R_3=H$
IV: $R_1=R_3=H$, $R_2=R_4=CH_3$
IVa: $R_1=R_3=COCH_3$, $R_2=R_4=CH_3$
IVb: $R_1=R_2=R_4=CH_3$, $R_3=H$

Chart 1

on the foregoing results, I was assumed to be acteoside and this was confirmed by direct comparison [thin layer chromatography (TLC), IR and $^1$H-NMR] of the nonaacetate (Ia) with an authentic sample.

Echinacoside (II) was isolated as an amorphous powder and gave the dodecaacetate (IIa), $C_{59}H_{70}O_{32}$, mp 130—131 °C, as colorless needles upon acetylation with acetic anhydride and pyridine. Acid hydrolysis of II with 10% sulfuric acid afforded glucose and rhamnose in a ratio of 2 to 1. Furthermore, on methanolysis of II with acetyl chloride in methanol, methyl caffeate and 3,4-dihydroxyphenethyl alcohol were detected. The $^{13}$C-NMR spectrum of II was very similar to that of I, except that six more carbon signals due to a glucose moiety were found in the sugar carbon region. The signal at $\delta$ 69.2 due to the C-6 of glucose bonded directly to the aglycone showed a downfield shift by 6.9 ppm, while the chemical shifts of other carbons were almost identical with those of I, indicating that the glucose moiety was attached to the C-6 of glucose bonded directly to the aglycone. From the above results, II was assumed to be echinacoside and this was confirmed by direct comparison (TLC, IR and $^1$H-NMR) with an authentic sample.

Cistanoside A (III) was isolated as an amorphous powder and gave an undecaacetate (IIIa), $C_{58}H_{70}O_{31}$, upon acetylation with acetic anhydride and pyridine. The IR spectrum suggested the presence of hydroxyl groups (3420 cm$^{-1}$), a conjugated ester (1696 cm$^{-1}$), a double bond (1632 cm$^{-1}$) and aromatic rings (1606, 1518 cm$^{-1}$), and the UV spectrum showed absorption maxima at 223, 246 sh, 290 and 333 nm. The molecular weight was confirmed by the observation of the peak at $m/z$ 823 ($M^+ + ^{23}Na$) in field desorption mass spectrometry (FD-MS). The $^1$H-NMR spectrum of III showed signals due to a methyl group of rhamnose [$\delta$ 1.08 (3H, d, $J$=6 Hz)], benzylic methylene protons [$\delta$ 2.84 (2H, t, $J$=7 Hz)], a methoxyl group [$\delta$ 3.84 (3H, s)], two glucose-anomeric protons [$\delta$ 4.28, 4.38 each (1H, d, $J$=8 Hz)], a rhamnose-anomeric proton [$\delta$ 5.16 (1H, s)], two *trans*-olefinic protons [$\delta$ 6.26, 7.60 each (1H, d, $J$=16 Hz)] and aromatic protons [$\delta$ 6.4—7.2 (6H)]. The IR and UV spectra of III are very similar to those of II, whereas in the $^1$H-NMR spectrum III exhibited the signal assignable to an aromatic methoxyl group at $\delta$ 3.84. Furthermore, the $^1$H-NMR spectrum of

TABLE I. $^{13}C$ Chemical Shifts of I, II, III and IV in Methanol-$d_4$

| Carbon No. | | I | II | III | IV |
|---|---|---|---|---|---|
| Aglycone | 1 | 131.6 | 131.4 | 131.5 | 131.6 |
| | 2 | 116.6 | 116.4 | 113.9 | 113.9 |
| | 3 | 144.4 | 144.3 | 148.7 | 148.7 |
| | 4 | 146.0 | 145.7 | 145.7 | 145.9 |
| | 5 | 117.2 | 117.1 | 116.1 | 116.2 |
| | 6 | 121.4 | 121.3 | 122.4 | 122.4 |
| | α | 72.3 | 72.1 | 72.1 | 72.2 |
| | β | 36.5 | 36.4 | 36.6 | 36.6 |
| Caffeic acid (ferulic) | 1 | 127.7 | 127.5 | 127.6 | 127.6 |
| | 2 | 114.8 | 114.7 | 114.7 | 112.1 |
| | 3 | 149.5 | 149.4 | 149.5 | 150.7 |
| | 4 | 146.6 | 146.5 | 146.6 | 149.3 |
| | 5 | 116.4 | 116.4 | 116.5 | 116.5 |
| | 6 | 123.2 | 123.1 | 123.1 | 124.2 |
| | α | 168.3 | 168.3 | 168.3 | 168.3 |
| | β | 115.5 | 115.3 | 115.4 | 115.1 |
| | γ | 148.0 | 148.1 | 148.1 | 148.0 |
| Glucose | 1 | 104.1 | 103.9 | 104.1 | 104.1 |
| | 2 | 75.9 | 75.9 | 75.9 | 76.1 |
| | 3 | 81.6 | 81.5 | 81.5 | 81.4 |
| | 4 | 70.3[a)] | 70.2[b)] | 70.3[c)] | 70.3[d)] |
| | 5 | 76.1 | 74.5 | 74.6 | 74.7 |
| | 6 | 62.3 | 69.2 | 69.3 | 69.4 |
| Rhamnose | 1 | 102.8 | 102.7 | 102.8 | 102.8 |
| | 2 | 72.1 | 72.1 | 72.1 | 72.2 |
| | 3 | 72.1 | 72.1 | 72.1 | 72.2 |
| | 4 | 73.8 | 73.7 | 73.7 | 73.7 |
| | 5 | 70.7[a)] | 70.5[b)] | 70.6[c)] | 70.7[d)] |
| | 6 | 18.4 | 18.3 | 18.4 | 18.4 |
| Glucose | 1 | | 104.4 | 104.5 | 104.6 |
| | 2 | | 74.9 | 74.9 | 75.0 |
| | 3 | | 77.6 | 77.6 | 77.7 |
| | 4 | | 71.3 | 71.4 | 71.5 |
| | 5 | | 77.6 | 77.6 | 77.7 |
| | 6 | | 62.5 | 62.6 | 62.6 |
| | $OCH_3$ | | | 56.6 | 56.6 |
| | | | | | 56.6 |

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

IIIa revealed the presence of eleven acetoxyl signals belonging to eight alcoholic [$\delta$ 1.87, 1.94, 2.00, 2.10 (3H, each), 1.98 and 2.03 (6H, each)] and three phenolic [2.29 (3H) and 2.31 (6H)] acetoxyl groups. On the other hand, the $^{13}C$-NMR spectrum of III showed almost the same chemical shifts as those of II, except for signals due to the aglycone moiety, indicating that rhamnose, caffeic acid and glucose were linked to the C-3, C-4 and C-6 hydroxyl groups, respectively, of the glucose bonded directly to the aglycone.

On methanolysis of III with acetyl chloride in methanol, methyl caffeate and 3-methoxy-4-hydroxyphenethyl alcohol were detected. Acid hydrolysis of III with 10% sulfuric acid afforded glucose and rhamnose in a ratio of 2 to 1. Partial methylation of III with dimethyl sulfate and potassium carbonate in acetone afforded the methyl ether (IIIb), and its $^1H$-NMR spectrum showed the presence of four aromatic methoxyl signals [$\delta$ 3.69, 3.73 (3H, each) and 3.77 (6H)]. Compound IIIb was identical with the tetramethyl ether of echinacoside (II).

These results led us to conclude that the structure of cistanoside A is 2-(4-hydroxy-3-methoxyphenyl)ethyl *O*-α-L-rhamnopyranosyl-(1→3)-*O*-[β-D-glucopyranosyl-(1→6)]-(4-*O*-caffeoyl)-β-D-glucopyranoside (III).

Cistanoside B (IV) was isolated as an amorphous powder and gave a decaacetate (IVa), $C_{57}H_{70}O_{30}$, upon acetylation with acetic anhydride and pyridine. The IR, UV and $^1$H-NMR spectra of IV were similar to those of III, indicating a close structural relationship of the two glycosides. The $^{13}$C-NMR spectrum of IV showed almost the same chemical shifts as those of III, except for signals due to the caffeic acid moiety. The signals at $\delta$ 3.75 and 3.78 (each 3H, 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. Eight alcoholic [$\delta$ 1.86, 1.93, 1.98, 2.08 (3H, each), 1.96 and 2.00 (6H, each)] and two phenolic [$\delta$ 2.28 and 2.31 (3H, each)] acetoxyl groups were showed in the $^1$H-NMR spectrum of IVa.

Acid hydrolysis of IV with 10% sulfuric acid afforded glucose and rhamnose in a ratio of 2 to 1. Furthermore, on methanolysis of IV with acetyl chloride in methanol, methyl ferulate and 3-methoxy-4-hydroxyphenethyl alcohol were detected. The molecular weight was confirmed by the observation of the peak at *m/z* 836 [$(M^+ - 1) + {}^{23}Na$] on FD-MS.

On the basis of the above-mentioned observations and the fact that partial methylation of IV in the same way as described for III afforded the methyl ether (IVb), which was identical with IIIb, the structure of cistanoside B was determined to be 2-(4-hydroxy-3-methoxyphenyl)ethyl *O*-α-L-rhamnopyranosyl-(1→3)-*O*-[β-D-glucopyranosyl-(1→6)]-(4-*O*-feruloyl)-β-D-glucopyranoside (IV).

## 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 on 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). MS and FD-MS were measured with a Hitachi RMU-7L and a JEOL JMS-01-SG2 mass spectrometer, respectively. Gas chromatography (GC) was run on a Shimadzu GC-4CM instrument with a flame ionization detector. High performance liquid chromatography (HPLC) was performed on a Kyowa Seimitsu KHP-010 machine equipped with a UV detector operated at 250 nm. Silica gel (Wako gel C-300, Wako Pure Chemical) was used for column chromatography. Kieselgel 60 $F_{254}$ (Merck) precoated plates were used for TLC and detection was achieved 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 with *n*-BuOH saturated with water. The *n*-BuOH-soluble fraction was concentrated *in vacuo* to afford the residue (292 g). This residue was chromatographed on a silica gel column using $CHCl_3$–MeOH–$H_2O$ (6 : 4 : 1) and the eluate was separated into three fractions (fr. 1—3). Fraction 1 was rechromatographed on a silica gel column using $CHCl_3$–MeOH–$H_2O$ (7 : 3 : 0.5), and on a Sephadex LH-20 column [$H_2O$–MeOH (1 : 1)] repeatedly to give cistanoside B (200 mg) and acteoside (7.2 g). After repeated chromatography (silica gel and Sephadex LH-20) of fr. 2 and fr. 3, cistanoside A (8.5 g) and echinacoside (10.6 g) were isolated, respectively.

**Acteoside (I)**——Amorphous powder, $[\alpha]_D^{24}$ −80.9° (*c* = 1.23, MeOH). IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3420, 1696, 1634, 1606, 1520. UV $\lambda_{max}^{MeOH}$ nm (log ε): 218 sh (4.27), 247 sh (4.03), 292 (4.13), 334 (4.25). $^1$H-NMR (methanol-$d_4$) $\delta$: 1.10 (3H, d, *J* = 6 Hz, $CH_3$ of rhamnose), 2.78 (2H, t, *J* = 7 Hz, Ar–C$\underline{H}_2$–$CH_2$–), 4.36 (1H, d, *J* = 8 Hz, H-1 of glucose), 5.17 (1H, d, *J* = 1 Hz, H-1 of rhamnose), 6.25 (1H, d, *J* = 16 Hz, Ar–CH=C$\underline{H}$–), 6.4—7.1 (6H, aromatic H), 7.58 (1H, d, *J* = 16 Hz, Ar–C$\underline{H}$=CH–). $^{13}$C-NMR: Table I.

**Echinacoside (II)**——Amorphous powder, $[\alpha]_D^{27}$ −69.9° (*c* = 1.42, MeOH). IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3400, 1690, 1625, 1600, 1518. UV $\lambda_{max}^{MeOH}$ nm (log ε): 220 (4.23), 244 sh (3.98), 292 (4.07), 334 (4.21). $^1$H-NMR (methanol-$d_4$) $\delta$: 1.09 (3H, d, *J* = 6 Hz, $CH_3$ of rhamnose), 2.79 (2H, t, *J* = 7 Hz, Ar–C$\underline{H}_2$–$CH_2$–), 4.29, 4.37 (each 1H, d, *J* = 8 Hz, H-1 of glucose), 5.16 (1H, d, *J* = 1 Hz, H-1 of rhamnose), 6.26 (1H, d, *J* = 16 Hz, Ar–CH=C$\underline{H}$–), 6.4—7.1 (6H, aromatic H), 7.59 (1H, d, *J* = 16 Hz, Ar–C$\underline{H}$=CH–). $^{13}$C-NMR: Table I.

**Cistanoside A (III)**——Amorphous powder, $[\alpha]_D^{27}$ −65.5° (*c* = 1.34, MeOH). IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3420, 1696, 1632,

1606, 1518. UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 223 (4.11), 246 sh (3.93), 290 (4.00), 333 (4.11). $^1$H-NMR (methanol-$d_4$) $\delta$: 1.08 (3H, d, $J$=6 Hz, $CH_3$ of rhamnose), 2.84 (2H, t, $J$=7 Hz, Ar–C$\underline{H}_2$–$CH_2$–), 3.84 (3H, s, $OCH_3$), 4.28, 4.38 (each 1H, d, $J$=8 Hz, H-1 of glucose), 5.16 (1H, s, H-1 of rhamnose), 6.26 (1H, d, $J$=16 Hz, Ar–CH=C$\underline{H}$–), 6.4—7.2 (6H, aromatic H), 7.60 (1H, d, $J$=16 Hz, Ar–C$\underline{H}$=CH–). $^{13}$C-NMR: Table I. FD-MS $m/z$: 823 ($M^+ + {}^{23}$Na).

**Cistanoside B (IV)**——Amorphous powder, $[\alpha]_D^{24}$ −66.7° ($c$=0.90, MeOH). IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3435, 1714, 1634, 1602, 1516. UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 220 sh (4.02), 232 sh (3.95), 289 (3.78), 330 (3.93). $^1$H-NMR (methanol-$d_4$) $\delta$: 1.00 (3H, d, $J$=6 Hz, $CH_3$ of rhamnose), 2.76 (2H, t, $J$=7 Hz, Ar–C$\underline{H}_2$–$CH_2$–), 3.75, 3.78 (each 3H, s, $OCH_3$), 4.18, 4.30 (each 1H, d, $J$=8 Hz, H-1 of glucose), 5.10 (1H, s, H-1 of rhamnose), 6.27 (1H, d, $J$=16 Hz, Ar–CH=C$\underline{H}$–), 6.4—7.2 (6H, aromatic H), 7.56 (1H, d, $J$=16 Hz, Ar–C$\underline{H}$=CH–). $^{13}$C-NMR: Table I. FD-MS $m/z$: 836 [$(M^+ - 1) + {}^{23}$Na].

**Acetylation of I, II, III and IV**——Compound I (100 mg), II (100 mg), III (100 mg) or IV (50 mg) was dissolved in pyridine (1 ml) and acetic anhydride (1 ml) and left at room temperature overnight. The reaction mixture was poured into ice–water, and then extracted with EtOAc. The EtOAc extract was concentrated *in vacuo* and the residue was chromatographed on a silica gel column using benzene–acetone (5 : 1) to give the nonaacetate (Ia) (85 mg), dedecaacetate (IIa) (80 mg), undecaacetate (IIIa) (87 mg) or decaacetate (IVa) (35 mg), respectively. Acteoside nonaacetate (Ia): amorphous powder, *Anal.* Calcd for $C_{47}H_{54}O_{24}$: C, 56.29; H, 5.43. Found: C, 56.41; H, 5.41. 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 (each 3H, s, OAc), 2.27, 2.28 (each 3H, s, Ar–OAc), 2.30 (6H, s, Ar–OAc×2), 2.88 (2H, t, $J$=7 Hz, Ar–C$\underline{H}_2$–$CH_2$–), 6.35 (1H, d, $J$=16 Hz, Ar–CH=C$\underline{H}$–), 7.0—7.5 (6H, aromatic H), 7.66 (1H, d, $J$=16 Hz, Ar–C$\underline{H}$=CH–). Echinacoside dodecaacetate (IIa): colorless needles from MeOH, mp 130—131 °C, *Anal.* Calcd for $C_{59}H_{70}O_{32}$: C, 54.88; H, 5.46. Found: C, 54.77; H, 5.38. IR $\nu_{max}^{KBr}$ cm$^{-1}$: 1775, 1660, 1523, 1450. UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 283 (4.25). $^1$H-NMR ($CDCl_3$) $\delta$: 1.05 (3H, d, $J$=6 Hz, $CH_3$ of rhamnose), 1.89, 1.96, 1.97, 2.01, 2.11 (each 3H, s, OAc), 2.03 (9H, s, OAc×3), 2.29 (3H, s, Ar–OAc), 2.31 (9H, s, Ar–OAc×3), 2.88 (2H, t, $J$=7 Hz, Ar–C$\underline{H}_2$–$CH_2$–), 6.35 (1H, d, $J$=16 Hz, Ar–CH=C$\underline{H}$–), 7.0—7.4 (6H, aromatic H), 7.66 (1H, d, $J$=16 Hz, Ar–C$\underline{H}$=CH–). Cistanoside A undecaacetate (IIIa): colorless needles from MeOH, mp 121—122 °C. *Anal.* Calcd for $C_{58}H_{70}O_{31}$: C, 55.15; H, 5.59. Found: C, 54.90; H, 5.50. IR $\nu_{max}^{KBr}$ cm$^{-1}$: 1758, 1642, 1512, 1432. UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 280 (4.31). $^1$H-NMR ($CDCl_3$) $\delta$: 1.04 (3H, d, $J$=6 Hz, $CH_3$ of rhamnose), 1.87, 1.94, 2.00, 2.10 (each 3H, s, OAc), 1.98, 2.03 (each 6H, s, OAc×2), 2.29 (3H, s, Ar–OAc), 2.31 (6H, s, Ar–OAc×2), 2.87 (2H, t, $J$=7 Hz, Ar–C$\underline{H}_2$–$CH_2$–), 6.35 (1H, d, $J$=16 Hz, Ar–CH=C$\underline{H}$–), 6.8—7.4 (6H, aromatic H), 7.65 (1H, d, $J$=16 Hz, Ar–C$\underline{H}$=CH–). Cistanoside B decaacetate (IVa): colorless needles from MeOH, mp 177—178 °C, *Anal.* Calcd for $C_{57}H_{70}O_{30}$: C, 55.41; H, 5.72. Found: C, 55.29; H, 5.56. IR $\nu_{max}^{KBr}$ cm$^{-1}$: 1756, 1636, 1604, 1514, 1420. UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 281 (4.01). $^1$H-NMR ($CDCl_3$) $\delta$: 1.04 (3H, d, $J$=6 Hz, $CH_3$ of rhamnose), 1.86, 1.93, 1.98, 2.08 (each 3H, s, OAc), 1.96, 2.00 (each 6H, s, OAc×2), 2.28, 2.31 (each 3H, s, Ar–OAc), 2.86 (2H, t, $J$=7 Hz, Ar–C$\underline{H}_2$–$CH_2$–), 6.34 (1H, d, $J$=16 Hz, Ar–CH=C$\underline{H}$–), 6.8—7.3 (6H, aromatic H), 7.66 (1H, d, $J$=16 Hz, Ar–C$\underline{H}$=CH–).

**Acid Hydrolysis of I, 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 ratios of 1 to 1 from I, and 2 to 1 from II, III and IV by GC. Conditions: column, 1.5% OV-17, 3 mm×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**——Compounds I, II, III and IV (*ca.* 1 mg) were each 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 residues of I and II, methyl caffeate and 3,4-dihydroxyphenethyl alcohol in the residues of I and II, methyl caffeate and 3-methoxy-4-hydroxyphenethyl alcohol in that of III, and methyl ferulate and 3-methoxy-4-hydroxyphenethyl alcohol in that of 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, 1.5 ml/min]. 3,4-Dihydroxyphenethyl alcohol: *Rf* 0.06, $t_R$ (min) 2.8. Methyl caffeate: *Rf* 0.20, $t_R$ (min) 10.8. 3-Methoxy-4-hydroxyphenethyl alcohol: *Rf* 0.31, $t_R$ (min) 3.8. Methyl ferulate: *Rf* 0.58, $t_R$ (min) 22.4.

**Partial Methylation of III and IV**——$(CH_3)_2SO_4$ (3 drops) was added to a solution of III or IV (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 (5 : 1) to give the methyl ether (IIIb) (23 mg) from III or (IVb) (20 mg) from IV, as an amorphous powder. $[\alpha]_D^{25}$ −46.2° ($c$=0.92, MeOH). IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3450, 1708, 1634, 1600, 1516, 1456, 1424, 1264, 1140, 1096, 1024. UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 220 (4.04), 232 (4.03), 288 (3.88), 327 (4.02). MS $m/z$: 650 [$M^+ - (\cdot\underset{\underset{O}{\|}}{CH}$–CH=CH–$\phi$–$(OMe)_2$], 577, 503, 430, 369, 365, 341, 296, 281, 212, 207, 147. $^1$H-NMR (methanol-$d_4$) $\delta$: 0.98 (3H, d, $J$=6 Hz, $CH_3$ of rhamnose), 2.80 (2H, t, $J$=7 Hz, Ar–C$\underline{H}_2$–$CH_2$–), 3.69, 3.73 (each 3H, s, $OCH_3$), 3.77 (6H, s,

$OCH_3 \times 2$), 4.18, 4.30 (each 1H, d, $J=8$ Hz, H-1 of glucose), 5.08 (1H, s, H-1 of rhamnose), 6.31 (1H, d, $J=16$ Hz, Ar–CH=CH–), 6.7—7.2 (6H, aromatic H), 7.59 (1H, d, $J=16$ Hz, Ar–CH=CH–). These products were found to be identical with the tetramethyl ether of echinacoside (II) by direct comparison (TLC, IR and $^1$H-NMR).

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