New Phenylethanoid Glycosides from *Cistanche tubulosa* (SCHRENK) HOOK. f. I.

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Four new phenylethanoid glycosides, tubulosides A (II), B (VI), C (VII) and D (VIII), have been isolated from *Cistanche tubulosa* (SCHRENK) HOOK. f. (Orobanchaceae), together with four known phenylethanoid glycosides, echinacoside (I), acteoside (III), acteoside isomer (IV) and 2'-acetylacteoside (V). The structures of II, VI, VII and VIII were established on the basis of chemical evidence and spectral data. Compounds VII and VIII possess a triacetylrhamnosyl moiety as the terminal sugar.

Keywords—*Cistanche tubulosa*; Orobanchaceae; parasitic plant; phenylethanoid glycoside; tubuloside A; tubuloside B; tubuloside C; tubuloside D

In our series of investigations on the chemical constituents of *Cistanche* spp. (Orobanchaceae), the phenylethanoid glycosides1 -3) and iridoide4) from *Cistanche salsa* (C. A. MEY.) G. BECK have been reported. The present paper deals with the phenylethanoid glycosides from *Cistanche tubulosa* (SCHRENK) HOOK. f. collected in Pakistan. *Cistanche tubulosa* (SCHRENK) HOOK. f.5) is a parasitic plant growing on the roots of *Salvadora* and *Calotropis* spp., and occurs widely in North Africa, Arabia, West Asia to Pakistan and India. The whole plant is used medicinally in Pakistan as a remedy for diarrhea and sores.6)

We now wish to report the isolation of four new phenylethanoid glycosides, named tubulosides A (II), B (VI), C (VII) and D (VIII), as well as four known phenylethanoid glycosides, echinacoside (I), acteoside (III), acteoside isomer (IV) and 2'-acetylacteoside (V). The structures of these compounds were determined on the basis of chemical evidence and spectroscopic studies.

The ethanolic extract of the whole plants was suspended in water. This suspension was extracted with ethyl acetate and then with n-butanol saturated with water. The n-butanol-soluble fraction was chromatographed on polyamide and silica gel columns and subjected to high-performance liquid chromatography (HPLC) successively, to give eight phenylethanoid glycosides (I—VIII).

Compounds I, III, IV and V were isolated as amorphous powders, showing similar spectra to those of echinacoside,1) acteoside,1) acteoside isomer7) and 2'-acetylacteoside,2) respectively, and were identified by direct comparison with authentic samples [thin layer chromatography (TLC), infrared (IR), proton nuclear magnetic resonance (1H-NMR), and carbon-13 nuclear magnetic resonance (13C-NMR) spectra].

Tubuloside A (II) was isolated as an amorphous powder, [α]D -103.7° (MeOH), C37H48O21·3/2H2O. The IR spectrum suggested the presence of hydroxyl groups
(3440 cm\(^{-1}\)), a conjugated ester (1705 cm\(^{-1}\)), a double bond (1634 cm\(^{-1}\)) and aromatic rings (1608, 1522 cm\(^{-1}\)), and the ultraviolet (UV) spectrum showed absorption maxima at 220, 250 sh, 292 sh and 334 nm. The \(^1\)H-NMR spectrum of II showed signals of a methyl group of rhamnose \([\delta 1.07 (3H, d, J=6 Hz)]\), a methyl signal of an acetoxyl group \([\delta 1.98 (3H, s)]\), benzylic methylene protons \([\delta 2.70 (2H, t, J=7 Hz)]\), two glucose-anomeric protons \([\delta 4.32, 4.54 (1H each, d, J=8 Hz)]\), a rhamnose-anomeric proton \([\delta 5.11 (1H, br s)]\), two trans olefinic protons \([\delta 6.25, 7.64 (1H each, d, J=16 Hz)]\) and aromatic protons \([\delta 6.5–7.2 (6H)]\).

On acetylation, II afforded the undecaacetate (IIa), which was identical with the dodecaacetate\(^1\) of echinacoside (I). The \(^13\)C-NMR spectrum of II was almost identical with that of I, except for the signals due to the glucose bonded directly to the aglycone and the acetoxyl group \([\delta 20.9 (CH\textsubscript{3}), 171.5 (C=O)]\), suggesting that the acetoxyl group is attached to the glucose moiety. In the \(^13\)C-NMR spectrum of II, the acylation shifts\(^8\) \([-2.3 (C-1), -0.9 (C-2)\) and \(-1.0 (C-3)\)] were observed at C-1, C-2 and C-3 of the inner glucose by detailed comparison with the spectrum of I, indicating that the acetoxyl group is linked to the C-2 hydroxyl group of the glucose moiety in II. On methanalysis of II with acetyl chloride in methanol, methyl caffeate and 3,4-dihydroxyphenethyl alcohol were detected by TLC and HPLC. Acid hydrolysis of II with 10% sulfuric acid afforded glucose and rhamnose in a ratio of 2 to 1.

On the basis of the above-mentioned evidence, the structure of tubuloside A was determined to be 2-(3,4-dihydroxyphenyl)ethyl O-\(\alpha\)-L-rhamnopyranosyl-(1\(\rightarrow\)3)-\(\beta\)-D-glucopyranosyl-(1\(\rightarrow\)6)-(4-O-caffeoyl)-2-O-acetyl-\(\beta\)-D-glucopyranoside (II).

Tubuloside B (VI) was isolated as an amorphous powder, \([\alpha]_D\) \(-39.0^\circ\) (MeOH), C\(_{31}\)H\(_{38}\)O\(_{16}\), whose \(^1\)H-NMR spectrum showed the presence of an aliphatic acetoxyl group \([\delta 1.98 (3H, s)]\). The \(^13\)C-NMR spectrum of VI was very similar to that of acteoside isomer (IV), but differed slightly in the signals due to the glucose moiety and the presence of the acetoxyl group \([\delta 20.9 (CH\textsubscript{3}), 171.6 (C=O)]\). The location of the acetoxyl group in the glucose moiety of VI was determined from its \(^13\)C-NMR spectrum by detailed comparison with that of IV.

\[\text{Chart 1}\]
The signals of C-1, C-2 and C-3 of the glucose moiety showed acylation shifts \([-2.5\text{ (C-1)}, -0.6\text{ (C-2) and } -1.4\text{ (C-3)}\], as in the case of II, indicating that the acetoxyl group is linked to the C-2 hydroxyl group of the glucose moiety in VI. On acetylation, VI afforded the octaacetate (VIa) which was identical with the nonaacetate of IV. On methanolysis of VI with acetyl chloride in methanol, methyl caffeate and 3,4-dihydroxyphenethyl alcohol were detected by TLC and HPLC. Acid hydrolysis of VI with 10\% sulfuric acid afforded glucose and rhamnose in a ratio of 1 to 1. These results led us to conclude that the structure of tubuloside B is 2-(3,4-dihydroxyphenyl)ethyl O-\(\alpha\)-L-rhamnopyranosyl-(1-3)-(6-\(\alpha\)-caffeoyl)-2-O-acetyl-\(\beta\)-D-glucopyranoside (VI).

Tubuloside C (VII) was isolated as an amorphous powder, \([\alpha]_D -104.8^\circ\text{ (MeOH)},\)
C₄₃H₅₄O₂₄·H₂O, whose ¹H-NMR spectrum showed the presence of four aliphatic acetoxy groups [δ 1.80, 1.92, 1.95 and 2.08 (3H each, s)]. The ¹³C-NMR spectrum of VII was almost identical with that of tubuloside A (II), which possesses an aliphatic acetoxy group in the inner glucose, except for the signals due to the rhamnose moiety. Furthermore, in the ¹³C-NMR spectrum of VII, acylation shifts were observed in the signals due to C-2, C-3 and C-4 of the rhamnose moiety by detailed comparison with the data for II. Consequently, the locations of the four acetoxy groups were determined to be C-2 of the inner glucose and C-2, C-3, C-4 of the rhamnose moiety in VII. On acetylation, VII afforded the octaacetate which was identical with tubuloside A undecaacetate (IIa). On methanolysis of VII with acetyl chloride in methanol, methyl caffeate and 3,4-dihydroxyphenethyl alcohol were detected by TLC and HPLC. Acid hydrolysis of VII with 10% sulfuric acid afforded glucose and rhamnose in a ratio of 2 to 1.

From the above results, the structure of tubuloside C was determined to be 2-(3,4-dihydroxyphenyl)ethyl 2,3,4-tri-O-acetyl-a-L-rhamnopyranosyl-(1→3)-β-D-glucopyranosyl-(1→6) [(4-O-caffeoyl)-2-O-acetyl-β-D-glucopyranoside (VII)].

Tubuloside D (VIII) was isolated as an amorphous powder, [α]D −91.4° (MeOH), C₄₃H₅₄O₂₃·H₂O, whose ¹H-NMR spectrum showed the presence of four aliphatic acetoxy groups [δ 1.81, 1.93, 1.96 and 2.09 (3H each, s)]. On acetylation, VIII afforded the heptaacetate (VIIIa), whose ¹H-NMR spectrum showed eight aliphatic [δ 1.87, 1.94, 1.96, 1.99, 2.10 (3H each, s) and 2.02 (9H, s)] and three aromatic [δ 2.27, 2.30 and 2.32 (3H each, s)] acetoxy methyl signals. The ¹³C-NMR spectrum of VIII was almost identical with that of tubuloside C (VII), except for the signals due to the p-coumaric acid moiety. On methanolysis of VIII with acetyl chloride in methanol, methyl p-coumarate and 3,4-dihydroxyphenethyl alcohol were detected by TLC and HPLC. Acid hydrolysis of VIII with 10% sulfuric acid afforded glucose and rhamnose in a ratio of 2 to 1.

On the basis of these results, the structure of tubuloside D was determined to be 2-(3,4-dihydroxyphenyl)ethyl 2,3,4-tri-O-acetyl-a-L-rhamnopyranosyl-(1→3)-β-D-glucopyranosyl-(1→6) [(4-O-p-coumaryl)-2-O-acetyl-β-D-glucopyranoside (VIII)].

Many phenylethanoid glycosides such as forsythoside A, leucosceptoside A, isomarintynoside and so on, having a rhamnose moiety as the terminal sugar, have been reported. In these cases, the rhamnose moiety is not acetylated. Tubulosides C (VII) and D (VIII) contain an acetylated rhamnose moiety and are the first naturally occurring compounds having a triacetylrhamnose moiety to be reported.

**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 Hitachi 270-30 infrared spectrophotometer and UV spectra with a Hitachi 200-20 spectrometer. ¹H-NMR and ¹³C-NMR spectra were recorded with a JEOL FX-90Q machine (89.55 and 22.5 MHz, respectively). Chemical shifts are given on the δ (ppm) scale with tetramethylsilane (TMS) as an internal standard (s, singlet; d, doublet; t, triplet; br, broad). Gas-liquid chromatography (GC) was run on a Shimadzu GC-4CM apparatus with a flame ionization detector. HPLC was performed on a Hitachi 655A-11 machine. Silica gel (Wako gel C-300, Wako Pure Chemical) was used for column chromatography. Kieselgel 60 F₂₅₄ (Merck) precoated plates were used for TLC and detection was carried out by spraying 10% H₂SO₄ followed by heating.

**Isolation**—Fresh whole plants of *C. tubulosa* (22 kg), collected in December 1984, in Karachi, Pakistan, were extracted with EtOH. The ethanolic extract was suspended in H₂O, and extracted with EtOAc and then with n-BuOH saturated with H₂O. The n-BuOH extract (99.1 g) was absorbed on a Diaion HP-20 (Nippon Rensui Co.) column and the resin was eluted with MeOH after being washed with H₂O. The MeOH eluate (15.4 g) was chromatographed on a polyamide C-200 (Wako Pure Chemical) column using H₂O and then MeOH. The fraction eluted with MeOH was concentrated to give a residue (phenolic crude glycosides) (8.0 g). After repeated chromatography of the residue on silica gel with CHCl₃-MeOH-H₂O (70:30:5) and HPLC with a H₂O-CH₂CN or H₂O-MeOH solvent system, eight glycosides (I—VIII) were isolated. I, 230 mg; II, 200 mg; III, 240 mg; IV, 60 mg; V, 210 mg; VI, 100 mg; VII, 60 mg;
VIII, 65 mg. Conditions for HPLC: column, Develosil ODS-10 (20 × 250 mm); solvent, I, II (17% CH₂CN), III, IV (20%, CH₂CN), V (22% CH₂CN), VI (25% CH₂CN), VII (53% MeOH), VIII (55% MeOH); detector (UV), 220 nm; flow rate, 6.9 ml/min.

Echinacoside (I) — Amorphous powder. IR ν max cm⁻¹: 3450, 1690, 1625, 1600, 1518. ¹H-NMR (methanol-d₄) δ: 1.09 (3H, d, J = 6 Hz, CH₃ of rhamnose), 2.79 (2H, t, J = 7 Hz, Ar-CH₂-), 4.29, 4.37 (1H each, 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=CH-), 6.4—7.1 (6H, aromatic H), 7.59 (1H, d, J = 16 Hz, Ar-CH=CH-). ¹³C-NMR: Table I.

Tubuloside A (II) — Amorphous powder, [α]D₂⁰ = −103.7° (c = 1.08, MeOH). Anal. Calc'd for C₃₇H₄₁O₁₆·C₅H₅O₄: C, 58.65; H, 5.75. Found: C, 58.91; H, 5.82. ¹H-NMR (methanol-d₄) δ: 1.07 (3H, d, J = 6 Hz, CH₃ of rhamnose), 2.77 (2H, t, J = 7 Hz, Ar-CH₂-), 4.36 (1H, d, J = 8 Hz, H-1 of glucose), 5.18 (1H, d, J = 1 Hz, H-1 of rhamnose), 6.25 (1H, d, J = 16 Hz, Ar-CH=CH-), 6.4—7.1 (6H, aromatic H), 7.58 (1H, d, J = 16 Hz, Ar-CH=CH-). ¹³C-NMR: Table I.

Acteoside (III) — Amorphous powder. IR ν max cm⁻¹: 3240, 1696, 1634, 1606, 1520. ¹H-NMR (methanol-d₄) δ: 1.10 (3H, d, J = 6 Hz, CH₃ of rhamnose), 2.78 (2H, t, J = 7 Hz, Ar-CH₂-), 3.03, 3.10, 3.18, 3.22 (3H each, s, OAc), 2.29 (3H, s, Ar-OAc), 2.31 (9H, s, Ar-OAc x 3), 2.87 (2H, t, J = 7 Hz, Ar-CH₂-), 6.35 (1H, d, J = 16 Hz, Ar-CH=CH-), 6.4—7.1 (6H, aromatic H), 7.59 (1H, d, J = 16 Hz, Ar-CH=CH-). ¹³C-NMR: Table I.

Acetylation of II and VII Compound VIII (35 mg) was acetylated in the same manner as described for II to give the undecaacetate (VIIIa) (30 mg) as an amorphous powder. IR ν max cm⁻¹: 1760, 1638, 1604, 1510. ¹H-NMR (CDCl₃) δ: 1.03 (3H, d, J = 6 Hz, CH₃ of rhamnose), 1.87, 1.94, 1.96, 1.99, 2.10 (3H each, s, OAc), 2.02 (9H, s, OAc x 3), 2.27, 2.30, 2.32 (3H each, s, Ar-OAc), 2.88 (2H, t, J = 7 Hz, Ar-CH₂-), 6.36 (1H, d, J = 16 Hz, Ar-CH=CH-), 7.0—7.2 (6H, aromatic H). ¹³C-NMR: Table I.

Acetylation of VI and VIII — Treatment of VII (30 mg) with Ac₂O (1 ml) and pyridine (1 ml) at room temperature overnight followed by the usual work-up afforded a crude acetate, which was purified by chromatography on silica gel with benzene-acetone (5:1) to give the dodecaacetate (VIIa) (22 mg) from VII, as colorless needles from MeOH, mp 130—131°C. IR ν max cm⁻¹: 1775, 1660, 1523, 1450. ¹H-NMR (CDCl₃) δ: 1.05 (3H, d, J = 6 Hz, CH₃ of rhamnose), 1.89, 1.96, 1.97, 2.01, 2.11 (3H each, s, OAc), 2.03 (9H, s, OAc x 3), 2.29 (3H, s, Ar-OAc), 2.31 (9H, s, Ar-OAc x 3), 2.88 (2H, t, J = 7 Hz, Ar-CH₂-), 6.35 (1H, d, J = 16 Hz, Ar-CH=CH-), 7.0—7.4 (6H, aromatic H), 7.66 (1H, d, J = 16 Hz, Ar-CH=CH-). ¹³C-NMR: Table I.

Acetylation of VII — Compound VIII (35 mg) was acetylated in the same manner as described for II to give the heptaacetate (VIIa) (30 mg) as an amorphous powder. IR ν max cm⁻¹: 1760, 1638, 1604, 1510. ¹H-NMR (CDCl₃) δ: 1.03 (3H, d, J = 6 Hz, CH₃ of rhamnose), 1.87, 1.94, 1.96, 1.99, 2.10 (3H each, s, OAc), 2.02 (9H, s, OAc x 3), 2.27, 2.30, 2.32 (3H each, s, Ar-OAc), 2.88 (2H, t, J = 7 Hz, Ar-CH₂-), 6.36 (1H, d, J = 16 Hz, Ar-CH=CH-), 7.0—7.2 (6H, aromatic H). ¹³C-NMR: Table I.

Acetylation of VIII — Compound VIII (35 mg) was acetylated in the same manner as described for II to give the octaacetate (VIIIa) (30 mg) as an amorphous powder. IR ν max cm⁻¹: 1760, 1638, 1604, 1510. ¹H-NMR (CDCl₃) δ: 1.03 (3H, d, J = 6 Hz, CH₃ of rhamnose), 1.87, 1.94, 1.96, 1.99, 2.10 (3H each, s, OAc), 2.02 (9H, s, OAc x 3), 2.27, 2.30, 2.32 (3H each, s, Ar-OAc), 2.88 (2H, t, J = 7 Hz, Ar-CH₂-), 6.36 (1H, d, J = 16 Hz, Ar-CH=CH-), 7.0—7.2 (6H, aromatic H). ¹³C-NMR: Table I.
(3H, aromatic H), 7.25 (2H, d, J = 9 Hz, H-3, H-5 of p-coumaric acid), 7.57 (2H, d, J = 9 Hz, H-2, H-6 of p-coumaric acid), 7.72 (1H, d, J = 16 Hz, Ar-CH = CH-).

**Methanolsysis of II, VI, VII and VIII**—Compound II, VI, VII or VIII (ca. 1 mg) was refluxed with methanolic 5% CH₃COCl (2 ml) for 30 min, and then the reagents were evaporated off. The presence of methyl caffeate and 3,4-dihydroxyphenethyl alcohol in the residue of II, VI and VII, and methyl p-coumarate and 3,4-dihydroxyphenethyl alcohol in that of VIII, was demonstrated by TLC [CHCl₃—MeOH (20 : 1)] and HPLC [column, TSK GEL LS-410AK (4 × 300 mm); solvent, H₂O—MeOH (4 : 6); detector (UV), 250 nm; flow rate, 1.0 ml/min]. Methyl caffeate [Rᵣ 0.20, tᵣ (min) 10.8], methyl p-coumarate [Rᵣ 0.40, tᵣ (min) 15.6], 3,4-dihydroxyphenethyl alcohol [Rᵣ 0.06, tᵣ (min) 2.8].

**Acid Hydrolysis of II, VI, VII and VIII**—A solution of a glycoside (ca. 2 mg) in 10% H₂SO₄ (1 ml) was heated in a boiling water bath for 30 min. The solution was passed through an Amberlite IR-45 column and the eluate was 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 Ac₂O (1 drop) and pyridine (1 drop) at 100°C for 1 h. The reagents were evaporated off. Glucitol acetate and rhamnitol acetate were detected in a ratio of 2 to 1 from II, VII and VIII, and 1 to 1 from VI by GC. tᵣ (min): 2.0 (rhamnitol acetate), 5.5 (glucitol acetate). Conditions for GC: column, 1.5% OV-17 (3 mm × 1.5 m); column temp., 180°C; carrier gas, N₂ (30 ml/min).

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**References and Notes**