Bioactive Constituents from Chinese Natural Medicines. XXXVI.1) Four New Acylated Phenylethanoid Oligoglycosides, Kankanosides J1, J2, K1, and K2, from Stems of Cistanche tubulosa

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During the course of our studies on bioactive constituents from Chinese natural medicines,1)–3) we found that methanolic extract of dried stems of Cistanche tubulosa (SCHRENK) R. WIGHT (Orobanchaceae) showed vasorelaxant4) and hepatoprotective activities.5) From the dried stems of C. tubulosa, five iridoids, kankanosides A—D and kankanol, a monoterpene glycoside, kankanoside E, two phenylethanoid oligoglycosides, kankanosides F and G, and an acylated oligosugar, kankanoside H, were isolated together with 30 known constituents.4,5) Recently, we additionally isolated four new acylated phenylethanoid oligoglycosides including kankanosides H1, H2, and J1,5) and two acylated oligosugars from fresh stems of C. tubulosa.5) Furthermore, principal phenylethanoid glycosides, echinacoside, acteoside, and isoacteoside, were found to inhibit α-galactosamine-induced cytotoxicity in primary cultured mouse hepatocytes.

Key words Cistanche tubulosa; kankanoside; phenylethanoid glycoside; Orobanchaceae; hepatoprotective activity

Four new acylated phenylethanoid oligoglycosides, kankanosides J1 (1), J2 (2), K1 (3), and K2 (4), were isolated from stems of Cistanche tubulosa (Orobanchaceae) together with isocampneoside I (5). Their structures were elucidated on the basis of chemical and physicochemical evidence. Among them, 3—5 were found to inhibit α-galactosamine-induced cytotoxicity in primary cultured mouse hepatocytes.

During the course of our studies on bioactive constituents from Chinese natural medicines,1)–3) we found that methanolic extract of dried stems of Cistanche tubulosa (SCHRENK) R. WIGHT (Orobanchaceae) showed vasorelaxant4) and hepatoprotective activities.5) From the dried stems of C. tubulosa, five iridoids, kankanosides A—D and kankanol, a monoterpene glycoside, kankanoside E, two phenylethanoid oligoglycosides, kankanosides F and G, and an acylated oligosugar, kankanoside H, were isolated together with 30 known constituents.4,5) Recently, we additionally isolated four new acylated phenylethanoid oligoglycosides including kankanosides H1, H2, and J1,5) and two acylated oligosugars from fresh stems of C. tubulosa.5) Furthermore, principal phenylethanoid glycosides, echinacoside, acteoside, and isoacteoside, were found to inhibit increase in serum aspartate aminotransferase (sAST) and alanine aminotransferase (sALT) levels in liver injured mice induced by α-galactosamine (α-GalN)/lipopolysaccharide at doses of 25—100 mg/kg per os (p.o.), and structural requirements of phenylethanoid glycosides for the hepatoprotective activity were elucidated.1) A continuing study on constituents from the fresh stems of C. tubulosa, we further isolated four new acylated phenylethanoid oligoglycosides, kankanosides J1 (1), J2 (2), K1 (3), and K2 (4). This paper deals with isolation and structure elucidation of 1—4.

Fresh stems of C. tubulosa (cultivated in Urumuqi, Xinjiang Province, China) were extracted with methanol under reflux to yield a methanolic extract (8.36% from the fresh stems). From the methanolic extract, H2O- and MeOH-eluted fractions (5.63% and 2.73%, respectively) were obtained by Diaion HP-20 column chromatography (H2O→MeOH) as was described previously.1) By the intensive chromatographies on the MeOH-eluted fraction, four new phenylethanoid oligoglycosides, kankanosides J1 (1, 0.0002%), J2 (2, 0.0002%), K1 (3, 0.0002%), and K2 (4, 0.0005%) together with isocampneoside I4) (5, 0.0006%) were isolated.

Structures of Kankanosides J1 (1) and J2 (2) Kankanoside J1 (1) was obtained as a white powder with negative optical rotation ([α]D−0.35 to −6.5° in MeOH). The IR spectrum of 1 showed absorption bands at 3414, 1734, 1719, 1701, 1638, 1508, 1159, 1067, and 1046 cm−1 ascribable to hydroxyls, ester carboxyls, ether functions, and aromatic rings. The positive- and negative-ion FAB-MS spectra of 1 showed quasimolecular ion peaks at m/z 719 (M+Na)+ and m/z 695 (M−H)−, and the molecular formula was determined as C32H40O17 by high-resolution positive-ion FAB-MS measurement. The 1H- and 13C-NMR spectra of 1 (CD3OD, Tables 1, 2), which were assigned by various NMR experiments,7) showed signals assignable to a methoxy group [δ 3.21 (3H, s, 7-OCH3)] and a methylene and a methine bearing an oxygen function [δ 3.58, 4.00 (1H each, both m, H8, H9)]. 18 (1H, dd-like, J = ca. 4, 8 Hz, 7-H]), ortho- and meta-coupled ABC-type aromatic protons [δ 6.63 (1H, dd, J = 1.8, 8.2 Hz, 6-H), 6.74 (1H, d, J = 1.8 Hz, 2-H), 6.74 (1H, d, J = 8.2 Hz, 5-H)], a β,γ-diglucopyranosyl moiety [δ 4.54 (1H, d, J = 7.8 Hz, Glc-1-H)] and an α-L-rhamnopyranosyl moiety [δ 1.07 (3H, d, J = 6.4 Hz, Rha-6-H)], 4.80 (1H, br s, Rha-1-H)] together with an acetyl group [δ 2.00 (3H, s)] and a trans-caffeoyl group [an trans-olefin (δ 6.26, 7.59 (1H each, both d, J = 16.0 Hz, 8s, 7-H)] and ortho- and meta-coupled ABC-type aromatic protons [δ 6.77 (1H, d, J = 8.2 Hz, 5-H), 6.95 (1H, dd, J = 1.8, 8.2 Hz, 6-H), 7.04 (1H, d, J = 1.8 Hz, 2-H)]. The 1H- and 13C-NMR spectra of 1 were superimposable on those of campneoside I3)–6) (6), except for the signals due to the acetyl group. Connectivities of the oligoglycoside and acyl moieties in 1 were confirmed by the heteronuclear multiple bond correlation (HMBC) experiment, which showed long-range correlations between the following proton and carbon pairs: 7-OCH3 and 7-C (δ C 83.3); Glc-1-H and 8-C (δ C
\[ \text{Glc-2-H} \; \delta \; 4.91 \; (1H, \; dd, \; J = 7.8, \; 9.2 \; Hz) \]
\[ \text{and} \; \text{the} \; \text{acetyl} \; \text{carbonyl} \; \text{carbon} \; (\delta \_C \; 171.4) \; \text{Glc-4-H} \; \delta \; 4.99 \; (1H, \; dd, \; J = 9.2, \; 9.6 \; Hz) \; \text{and} \; \text{the} \; \text{trans-caffeoyl} \; \text{carbonyl} \; \text{carbon} \; (\delta \_C \; 168.1); \; \text{and} \; \text{Rha-1-H} \; \text{and} \; \text{Glc-3-C} \; (\delta \_C \; 80.5) \; (\text{Fig. 1}). \]

Finally, alkaline hydrolysis of \( \text{I} \) with 5\% potassium hydroxide (KOH) liberated trans-caffeic acid, which was identified by HPLC analysis, together with a deacylated product. The deacylated product was successively treated with 1.0M hydrochloric acid (HCl) to liberate L-rhamnose and D-glucose, which were identified by HPLC analysis using an optical rotation detector.\(^1\)–\(^5\) Thus, the structure of kankanoside J\(_1\) was elucidated to be 2-methoxy-2-(3,4-dihydroxyphenyl)ethyl \( O-\alpha-L-\) rhamnopyranosyl-(1\( \rightarrow \)3)-2-O-acetyl-4-\( O\)-trans-caffeoyl-\( \beta\)-D-glucopyranoside \((\text{I}). \)

Kankanoside J\(_2\) \((\text{2})\) was isolated as a white powder with negative optical rotation \((\lbrack \alpha \rbrack \text{D}_{25} = 18.1° \; \text{in MeOH})\). By high-resolution positive-ion FAB-MS measurement, the molecular formula of \( \text{2} \) was found to be the same as that of \( \text{I} \). The \( ^1\)H- and \( ^13\)C-NMR data of \( \text{2} \) \((\text{CD}_{3}\text{OD}, \text{Tables 1, 2})\) were very similar to those of \( \text{I} \), except for the signals due to the ethyl bridge of the aglycone moiety {a methoxy group \( \delta \; 3.24 \; (3H, \; s, \; 7-O\text{-CH}_3) \)}; a methylene \( \delta \; 3.63 \; (1H, \; m, \; 3.83 \; (1H, \; dd, \)

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### Table 1. \(^1\)H-NMR Data (600 MHz, CD\(_3\)OD) for Kankanosides J\(_1\) \((1)\), J\(_2\) \((2)\), K\(_1\) \((3)\), and K\(_2\) \((4)\)

<table>
<thead>
<tr>
<th>Position</th>
<th>( \delta _H (\text{Hz}) )</th>
<th>( \delta _H (\text{Hz}) )</th>
<th>( \delta _H (\text{Hz}) )</th>
<th>( \delta _H (\text{Hz}) )</th>
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<td>6.76 (d, 8.1)</td>
<td>6.76 (d, 8.2)</td>
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<td>( 6 )</td>
<td>6.63 (dd, 1.8, 8.2)</td>
<td>6.62 (dd, 1.8, 8.2)</td>
<td>6.68 (dd, 2.0, 8.1)</td>
<td>6.67 (dd, 1.9, 8.2)</td>
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<td>( 7 )</td>
<td>4.18 (dd-like, ca. 4, 8)</td>
<td>4.22 (dd, 3.2, 8.2)</td>
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<td>( 8 )</td>
<td>3.58 (m)</td>
<td>3.63 (m)</td>
<td>3.62 (dd, 3.4, 11.0)</td>
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<td>( 7\text{-OCH}_3 )</td>
<td>4.00 (m)</td>
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<td>3.84 (dd, 3.1, 11.0)</td>
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<td>( 8\text{-O-Glc} )</td>
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<td>3.24 (3H, s)</td>
<td>3.23 (3H, s)</td>
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<td>( 2' )</td>
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<td>4.91 (dd, 8.2, 9.2)</td>
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<td>( 3' )</td>
<td>4.01 (dd, 9.2, 9.6)</td>
<td>4.04 (dd, 9.2, 9.6)</td>
<td>3.81 (dd, 9.2, 9.7)</td>
<td>3.82 (dd, 9.1, 9.7)</td>
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<td>( 4' )</td>
<td>4.99 (dd, 9.2, 9.6)</td>
<td>5.00 (dd, 9.6, 9.6)</td>
<td>5.00 (dd, 9.7, 9.7)</td>
<td>5.02 (dd, 9.7, 9.8)</td>
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<td>( 5' )</td>
<td>3.56 (m)</td>
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<td>3.77 (m)</td>
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<td>3.52 (m)</td>
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<td>( 3'\text{-O-Rha} )</td>
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<td>3.61 (m)</td>
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<td>3.91 (dd, 1.7, 3.3)</td>
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<td>( 3'' )</td>
<td>3.61 (m)</td>
<td>3.52 (m)</td>
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<td>3.26 (m)</td>
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<td>1.07 (3H, d, 6.4)</td>
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<td>3.32 (m)</td>
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<td>3.25 (m)</td>
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<td>3.22 (m)</td>
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<tr>
<td>( 6'\text{-O-trans-Caf} )</td>
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<td>( 3'\text{-O-trans-Caf} )</td>
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<td>3.82 (dd, 2.1, 12.0)</td>
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Fig. 1. \(^1\)H–\(^1\)H COSY and HMBC Correlations for \( 1-4 \)
Table 2. $^{13}$C-NMR Data (150 MHz, CD$_3$OD) for Kankanosides J$_1$ (1), J$_2$ (2), K$_1$ (3), and K$_2$ (4).

<table>
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<td>133.0</td>
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<td>130.9</td>
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J=3.2, 11.0 Hz), 8-H$_3$, and a methine bearing an oxygen function [J 4.22 (1H, dd, J=3.2, 8.2 Hz, 7-H)]. Alkaline hydrolysis of 2 with 5% KOH liberated transcaffeic acid together with a deacylated product, and the deacylated product was successively treated with 1.0 M HCl to liberate L-rhamnose and D-glucose. As shown in Fig. 1, the long-range correlations as in the case of 1 were observed in the HMBC experiment. Consequently, the planar structure of kankanoside J$_1$ (2) was revealed to be the same as that of 1, and was elucidated to be 7-isomer of K$_1$.11,12)

Structures of Kankanosides K$_1$ (3) and K$_2$ (4)
Kankanosides K$_1$ (3) and K$_2$ (4), C$_{13}$H$_{20}$O$_{13}$, were also obtained as white powders with negative optical rotations (3: [a]$_D^{25}$= -75.3$^\circ$, 4: [a]$_D^{25}$ = -7.4$^\circ$ both in MeOH). The 1H- and $^{13}$C-NMR spectra of 3 and 4 (CD$_3$OD, Tables 1, 2) showed signals assignable to a methoxy group [3: $\delta$ 3.23 (3H, s, 7-OCH$_3$); 4: $\delta$ 3.25 (3H, s, 7-OCH$_3$)], a methine and a methine bearing an oxygen function [3: $\delta$ 3.62 (1H, dd, J=3.4, 11.0 Hz), 4.02 (1H, dd, J=8.1, 11.0 Hz), 8-H$_3$], 4.34 (1H, dd, J=3.4, 8.1 Hz, 7-H); 4: [3.72 (1H, dd, J=9.1, 11.0 Hz), 3.84 (1H, dd, J=3.1, 11.0 Hz), 8-H$_3$], 4.37 (1H, dd, J=3.1, 9.1 Hz, 7-H)], ortho- and meta-coupled ABC-type aromatic protons [3: $\delta$ 6.68 (1H, dd, J=2.0, 8.1 Hz, 6-H), 6.76 (1H, d, J=8.1 Hz, 5-H), 6.60 (1H, d, J=2.0 Hz, 2-H); 4: $\delta$ 6.73 (1H, dd, J=1.9, 8.2 Hz, 6-H), 6.76 (1H, d, J=8.2 Hz, 5-H), 6.58 (1H, d, J=1.9 Hz, 2-H)], two $\beta$-D-glucopyranosyl moieties [3: $\delta$ 4.31 (1H, d, J=7.9 Hz, terminal-Glc-1-H), 4.41 (1H, d, J=7.9 Hz, inner-Glc-1-H); 4: $\delta$ 4.26 (1H, d, J=7.7 Hz, terminal-Glc-1-H), 4.44 (1H, d, J=7.9 Hz, inner-Glc-1-H), and an $\alpha$-L-rhamnopyranosyl moiety [3: $\delta$ 4.08 (3H, d, J=6.4 Hz, Rha-6-H), 5.19 (1H, d, J=1.7 Hz, Rha-1-H); 4: $\delta$ 1.08 (3H, d, J=6.2 Hz, Rha-6-H), 5.20 (1H, d, J=1.6 Hz, Rha-1-H)] together with a transcaffeoyl group [an trans-olefin [3: $\delta$ 6.27, 7.60 (1H each, both J=15.8 Hz, 8-, 7-H); 4: $\delta$ 6.28, 7.60 (1H each, both J=15.8 Hz, 8-, 7-H)] and ortho- and meta-coupled ABC-type aromatic protons [3: $\delta$ 6.78 (1H, d, J=8.3 Hz, 5-H), 6.96 (1H, dd, J=1.9, 8.3 Hz, 6-H), 7.05 (1H, d, J=1.9 Hz, 2-H)]; 4: $\delta$ 6.78 (1H, d, J=8.4 Hz, 5-H), 6.96 (1H, dd, J=1.9, 8.4 Hz, 6-H), 7.05 (1H, d, J=1.9 Hz, 2-H)].

The proton and carbon signals in the 1H- and 13C-NMR spectra of 3 and 4 were superimposable on those of echinacoside,1,4,12) except for the signals due to the 7-methoxy group. The connectivities of the trans-ceaffeoyl group and the glycosyl moieties in 3 and 4 were elucidated on the basis of HMBC experiments as shown in Fig. 1. Finally, alkaline hydrolysis of 3 and 4 with 5% KOH gave the deacylated products together with trans-ceaffeic acid. Those deacylated products were successively treated with 1.0 M HCl to liberate L-rhamnose and D-glucose, respectively. Consequently, the structure of kankanosides K$_1$ and K$_2$ were determined to be 2-methoxy-2(3,4-dihydroxyphenyl)ethyl $\alpha$-L-rhamnopyranosyl-(1→3)-[\beta$\beta$-D-glucopyranosyl-(1→6)]-4-O-trans-caffeoyl-$\beta$-D-glucopyranoside (3 and 4).11,13)

Previously, methanolic extract from stems of C. tubulosa and several phenylethanoid constituents such as echinacoside, acetyl-, and isoacetoside were found to show hepatoprotective effects on D-galactosamine (D-GalN)/lipopolysaccharide-induced liver injury in mice and inhibitory effect on D-GalN-induced cytotoxicity in primary cultured mouse hepatocytes.1) We further examined inhibitory effects of kankanosides K$_1$ (3) and K$_2$ (4), and isocampeoside I (5) on D-GalN-induced cytotoxicity in primary cultured hepatocytes. Although their activities were weaker than those of echinacoside (IC$_{50}$=10.2 $\mu$m), acetoside (4.6 $\mu$m), and isoacetoside (5.3 $\mu$m), the principle phenylethanoid constituents from stems of C. tubulosa,13—5 showed moderate activity.14)

Experimental
The following instruments were used to obtain spectral and physical data: specific rotations, Horiba SEPA-300 digital polarimeter (1=5 cm); UV spectra, Shimadzu UV-1600 spectrometer; IR spectra, Shimadzu FTIR-8100 spectrometer; 1H- and 13C-NMR spectra, JEOL JNM-ECA600 (600, 150 MHz) and JEOL JNM-ECS400 (400, 100 MHz) spectrometers with tetramethylsilane as an internal standard; FAB-MS and high-resolution FAB-MS, JEOL JMS-SX 102A mass spectrometer; HPLC detector, Shimadzu RID-10A refractive index, Shimadzu SPD-10A UV–VIS, and Shodex OR-2 optical rotation detectors. HPLC column, Cosmosil SC$_{18}$-MS-II and $\mu$PAP (Nacalai Tesque Inc., 250×4.6 mm i.d.) and (250×20 mm i.d.) columns were used for analytical and preparative purposes, respectively.

The following experimental conditions were used for chromatography: normal-phase silica gel column chromatography (CC), silica gel 60N (Kanto
Chemical Co., Ltd., 63—210 mesh, spherical, neutral); reversed-phase silica gel CC, Diaion HP-20 (Nippon Rensui) and Chromatored ODS DM1020T (Fuji Silysia Chemical, Ltd., 100—200 mesh); normal-phase TLC, pre-coated TLC plates with silica gel 60F254 (Merck, 0.25 mm); reversed-phase TLC, pre-coated TLC plates with silica gel RP-18 F254s (Merck, 0.25 mm); reversed-phase HPTLC, pre-coated TLC plates with silica gel RP-18 WF254s (Merck, 0.25 mm), detection was achieved by spraying with 1% Ce(SO4)2–10% aqueous H2SO4, followed by heating.

**Plant Material**

This item was described in a previous report. 

**Extraction and Isolation**

Isolation of C. tubulosa (2.98 kg) were finely cut and extracted three times with methanol under reflux for 3 h. Evaporation of the solvent under reduced pressure provided a methanolic extract (249.1 g, 8.36%). The methanolic extract was subjected to Diaion HP-20 CC (5.0 kg, H2O–MeOH) to give H2O- and MeOH-eluted fractions (167.84 g, 5.63% and 81.21 g, 2.73%, respectively). The MeOH-eluted fraction (61.00 g) was subjected to normal-phase silica gel CC [1.8 kg, CHCl3–MeOH–H2O (15: 3: 0.4: 10), 0.0001%], 4-3-5 (27.4 mg), 4-3-6 (43.6 mg), 4-3-7 (12.0 mg) to give kankanosides J1 (1): 33415, 1734, 1717, 1686, 1636, 1614, 1509, 1159, 1074. 1H-NMR (600 MHz, CD3OD) δ: given in Table 1. 13C-NMR (150 MHz, CD3OD) δ: given in Table 1. Positive-ion FAB-MS: Calcd for C32H40O17Na (M+Na) 719.2163; Found 719.2167. UV λmax (log ε): 695 (M+), 531 (M+Na), 440 (M+Na2).

**Alkaline and Acid Hydrolysis of Kankanosides J 1 (1), J 2 (2), K 1 (3), and K 2 (4)**

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**References and Notes**


