Bioactive Constituents from Chinese Natural Medicines. XXXVI.[1] Four New Acylated Phenylethanoid Oligoglycosides, Kankanosides J_1, J_2, K_1, and K_2, from Stems of *Cistanche tubulosa*

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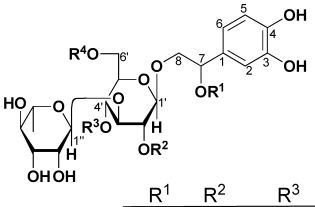
Four new acylated phenylethanoid oligoglycosides, kankanosides J_1 (1), J_2 (2), K_1 (3), and K_2 (4), were isolated from stems of *Cistanche tubulosa* (Orobanchaceae) together with isocampneoside I (5). Their structures were elucidated on the basis of chemical and physicochemical evidence. Among them, 3—5 were found to inhibit D-galactosamine-induced cytotoxicity in primary cultured mouse hepatocytes.

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

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 vasorelaxant[4] and hepatoprotective activities.[1] 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, kankanose, were isolated together with 30 known constituents.[4,5] Recently, we additionally isolated 19 phenylethanoid oligoglycosides including kankanosides H_1, H_2, and I[1] and two acylated oligosugars from fresh stems of *C. tubulosa*.[1] 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 D-galactosamine (D-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] As a continuing study on constituents from the fresh stems of *C. tubulosa*, we further isolated four new acylated phenylethanoid oligoglycosides, kankanosides J_1 (**1**), J_2 (**2**), K_1 (**3**), and K_2 (**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, H_2O- and MeOH-eluted fractions (5.63% and 2.73%, respectively) were obtained by Diaion HP-20 column chromatography ($H_2O \rightarrow$MeOH) as was described previously.[1] By the intensive chromatographies on the MeOH-eluted fraction, four new phenylethanoid oligoglycosides, kankanosides J_1 (**1**, 0.0002%), J_2 (**2**, 0.0002%), K_1 (**3**, 0.0002%), and K_2 (**4**, 0.0005%) together with isocampneoside I[6] (**5**, 0.0006%) were isolated.

Structures of Kankanosides J_1 (1) and J_2 (2) Kankanoside J_1 (**1**) was obtained as a white powder with negative optical rotation ($[\alpha]_D^{25}$ −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 carbonyls, 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 $C_{32}H_{40}O_{17}$ by high-resolution positive-ion FAB-MS measurement. The ^{1}H- and ^{13}C-NMR spectra of **1** (CD_3OD, Tables 1, 2), which were assigned by various NMR experiments,[7] showed signals assignable to a methoxy group [δ 3.21 (3H, s, 7-OCH_3)], a methylene and a methine bearing an oxygen function [δ 3.58, 4.00 (1H each, both m, 8-H_2), 4.18 (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-D-glucopyranosyl 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_3), 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, 8-, 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 ^{1}H- and ^{13}C-NMR spectra of **1** were superimposable on those of campneoside I[1,8—10] (**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-OCH_3 and 7-C (δ_C 83.3); Glc-1-H and 8-C (δ_C



	R^1	R^2	R^3	R^4
Kankanosides J_1 (**1**), J_2 (**2**):	CH_3	Ac	*trans*-Caf	H
Kankanosides K_1 (**3**), K_2 (**4**):	CH_3	H	*trans*-Caf	Glc
Isocampneoside I (**5**):	CH_3	H	H	*trans*-Caf
Campneoside I (**6**):	CH_3	H	*trans*-Caf	H
Campneoside II (**7**):	H	H	*trans*-Caf	H

trans-Caf: *trans*-caffeoyl

Chart 1

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Table 1. ^{1}H-NMR Data (600 MHz, CD_3OD) for Kankanosides J_1 (**1**), J_2 (**2**), K_1 (**3**), and K_2 (**4**)

Position	**1** δ_H (J Hz)	**2** δ_H (J Hz)	**3** δ_H (J Hz)	**4** δ_H (J Hz)
2	6.74 (d, 1.8)	6.73 (br s)	6.80 (d, 2.0)	6.78 (d, 1.9)
5	6.74 (d, 8.2)	6.73 (d, 8.2)	6.76 (d, 8.1)	6.76 (d, 8.2)
6	6.63 (dd, 1.8, 8.2)	6.62 (dd, 1.8, 8.2)	6.68 (dd, 2.0, 8.1)	6.67 (dd, 1.9, 8.2)
7	4.18 (dd-like, *ca.* 4, 8)	4.22 (dd, 3.2, 8.2)	4.34 (dd, 3.4, 8.1)	4.37 (dd, 3.1, 9.1)
8	3.58 (m)	3.63 (m)	3.62 (dd, 3.4, 11.0)	3.72 (dd, 9.1, 11.0)
	4.00 (m)	3.83 (dd, 3.2, 11.0)	4.02 (dd, 8.1, 11.0)	3.84 (dd, 3.1, 11.0)
7-OC$\underline{H}_3$	3.21 (3H, s)	3.24 (3H, s)	3.23 (3H, s)	3.25 (3H, s)
8-*O*-Glc				
1′	4.54 (d, 7.8)	4.64 (d, 8.2)	4.40 (d, 7.9)	4.44 (d, 7.9)
2′	4.91 (dd, 7.8, 9.2)	4.91 (dd, 8.2, 9.2)	3.43 (dd, 7.9, 9.2)	3.43 (dd, 7.9, 9.1)
3′	4.01 (dd, 9.2, 9.6)	4.04 (dd, 9.2, 9.6)	3.81 (dd, 9.2, 9.7)	3.82 (dd, 9.1, 9.7)
4′	4.99 (dd, 9.2, 9.6)	5.00 (dd, 9.6, 9.6)	5.00 (dd, 9.7, 9.7)	5.02 (dd, 9.7, 9.8)
5′	3.56 (m)	3.61 (m)	3.77 (m)	3.77 (ddd, 2.2, 5.5, 9.8)
6′	3.51 (m)	3.52 (m)	3.64 (dd, 5.5, 11.6)	3.62 (dd, 5.5, 11.9)
	3.61 (m)	3.61 (m)	3.92 (dd, 2.0, 11.6)	3.94 (dd, 2.2, 11.9)
3′-*O*-Rha				
1″	4.80 (br s)	4.82 (br s)	5.19 (d, 1.7)	5.20 (d, 1.6)
2″	3.25 (m)	3.65 (m)	3.91 (dd, 1.7, 3.3)	3.92 (dd, 1.6, 3.2)
3″	3.61 (m)	3.52 (m)	3.57 (dd, 3.3, 9.5)	3.57 (dd, 3.2, 9.4)
4″	3.25 (m)	3.26 (m)	3.28 (m)	3.28 (m)
5″	3.55 (m)	3.52 (m)	3.55 (dq, 9.5, 6.4)	3.55 (dq, 9.1, 6.2)
6″	1.07 (3H, d, 6.4)	1.07 (3H, d, 6.4)	1.08 (3H, d, 6.4)	1.08 (3H, d, 6.2)
6′-*O*-Glc				
1‴			4.31 (d, 7.9)	4.26 (d, 7.7)
2‴			3.19 (dd, 7.9, 9.1)	3.19 (dd, 7.7, 9.3)
3‴			3.34 (dd, 8.8, 9.1)	3.32 (m)
4‴			3.26 (dd, 8.8, 9.6)	3.25 (m)
5‴			3.22 (m)	3.22 (m)
6‴			3.63 (dd, 5.7, 12.1)	3.62 (dd, 5.5, 12.0)
			3.83 (dd, 2.2, 12.1)	3.82 (dd, 2.1, 12.0)
2′-*O*-Ac	2.00 (3H, s)	2.11 (3H, s)		
4′-*O*-*trans*-Caf				
2	7.04 (d, 1.8)	7.04 (d, 1.8)	7.05 (d, 1.9)	7.05 (d, 1.9)
5	6.77 (d, 8.2)	6.77 (d, 8.2)	6.78 (d, 8.3)	6.78 (d, 8.4)
6	6.95 (dd, 1.8, 8.2)	6.95 (dd, 1.8, 8.2)	6.96 (dd, 1.9, 8.3)	6.96 (dd, 1.9, 8.4)
7	7.59 (d, 16.0)	7.54 (d, 16.0)	7.60 (d, 15.8)	7.60 (d, 15.8)
8	6.26 (d, 16.0)	6.27 (d, 16.0)	6.27 (d, 15.8)	6.28 (d, 15.8)

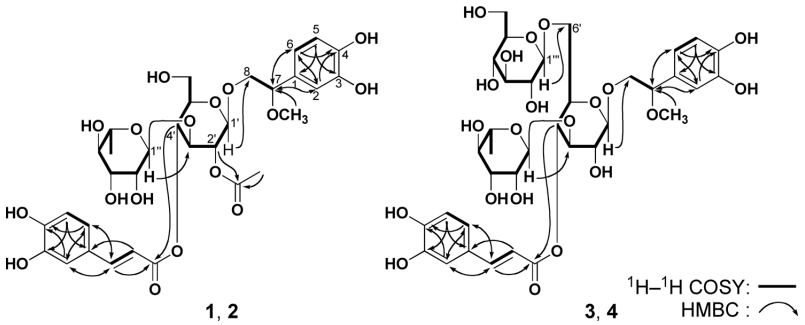

Fig. 1. ^{1}H–^{1}H COSY and HMBC Correlations for **1**—**4**

74.1); Glc-2-H [δ 4.91 (1H, dd, J=7.8, 9.2 Hz)] and the acetyl carbonyl carbon (δ_C 171.4); Glc-4-H [δ 4.99 (1H, dd, J=9.2, 9.6 Hz)] and the *trans*-caffeoyl carbonyl carbon (δ_C 168.1); and Rha-1-H and Glc-3-C (δ_C 80.5) (Fig. 1). Finally, alkaline hydrolysis of **1** 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 1.0 M 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*-α-L-rhamnopyranosyl-(1→3)-2-*O*-acetyl-4-*O*-*trans*-caffeoyl-β-D-glucopyranoside (**1**).

Kankanoside J_2 (**2**) was isolated as a white powder with negative optical rotation ($[\alpha]_D^{25}$ −18.1° in MeOH). By high-resolution positive-ion FAB-MS measurement, the molecular formula of **2** was found to be the same as that of **1**. The ^{1}H- and ^{13}C-NMR data of **2** (CD_3OD, Tables 1, 2) were very similar to those of **1**, except for the signals due to the ethyl bridge of the aglycone moiety {a methoxy group [δ 3.24 (3H, s, 7-OCH_3)], a methylene [δ 3.63 (1H, m), 3.83 (1H, dd,

Table 2. ^{13}C-NMR Data (150 MHz, CD_3OD) for Kankanosides J_1 (**1**), J_2 (**2**), K_1 (**3**), and K_2 (**4**)

Position	**1** δ_C	**2** δ_C	**3** δ_C	**4** δ_C
1	131.7	131.2	131.2	130.7
2	115.3	115.0	115.2	115.1
3	146.5	146.5	146.5	146.5
4	146.4	146.3	146.4	146.5
5	116.3	116.2	116.3	116.4
6	120.2	119.7	120.2	119.9
7	83.3	84.3	83.5	84.4
8	74.1	74.9	74.7	75.2
7-O$\underline{C}$H$_3$	56.8	57.1	56.7	56.7
8-*O*-Glc				
1′	101.7	102.2	104.1	104.5
2′	75.1	75.3	76.0	76.3
3′	80.5	80.5	81.5	81.4
4′	70.7	70.7	70.6	70.5
5′	76.3	76.2	74.9	74.7
6′	62.3	62.2	69.4	69.5
3′-*O*-Rha				
1″	103.4	103.3	103.0	103.0
2″	72.7	72.6	72.4	72.4
3″	71.9	71.9	72.1	72.0
4″	73.7	73.6	73.8	73.8
5″	70.8	70.8	70.4	70.4
6″	18.5	18.5	18.4	18.4
6′-*O*-Glc				
1‴			104.6	104.7
2‴			75.1	75.1
3‴			77.8	77.8
4‴			71.5	71.5
5‴			77.9	77.9
6‴			62.6	62.6
2′-*O*-Ac				
1	20.9	21.0		
2	171.4	171.5		
4′-*O*-Caf				
1	127.7	127.6	127.6	127.6
2	115.3	115.2	115.3	115.3
3	146.9	146.5	146.9	146.8
4	149.9	149.9	149.9	149.9
5	116.6	116.5	116.5	116.5
6	123.3	123.2	123.3	123.3
7	148.2	148.2	148.2	148.3
8	114.6	114.6	114.7	114.7
9	168.1	168.1	168.5	168.5

J=3.2, 11.0 Hz), 8-H_2] and a methine bearing an oxygen function [δ 4.22 (1H, dd, J=3.2, 8.2 Hz, 7-H)]}. Alkaline hydrolysis of **2** with 5% KOH liberated *trans*-caffeic 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 same long-range correlations as in the case of **1** were observed in the HMBC experiment. Consequently, the planar structure of kankanoside J_2 (**2**) was revealed to be the same as that of **1**, and was elucidated to be 7-isomer of **1**.[11]

Structures of Kankanosides K_1 (3) and K_2 (4) Kankanosides K_1 (**3**) and K_2 (**4**), $C_{36}H_{48}O_{21}$, were also obtained as white powders with negative optical rotations (**3**: $[\alpha]_D^{25}$ −75.3°; **4**: $[\alpha]_D^{25}$ −7.4° both in MeOH). The ^{1}H- and ^{13}C-NMR spectra of **3** and **4** (CD_3OD, Tables 1, 2) showed signals assignable to a methoxy group [**3**: δ 3.23 (3H, s, 7-OCH_3); **4**: δ 3.25 (3H, s, 7-OCH_3)], a methylene and a methine bearing an oxygen function {**3**: δ [3.62 (1H, dd, J=3.4, 11.0 Hz), 4.02 (1H, dd, J=8.1, 11.0 Hz), 8-H_2], 4.34 (1H, dd, J=3.4, 8.1 Hz, 7-H); **4**: δ [3.72 (1H, dd, J=9.1, 11.0 Hz), 3.84 (1H, dd, J=3.1, 11.0 Hz), 8-H_2], 4.37 (1H, dd, J=3.1, 9.1 Hz, 7-H)}, *ortho*- and *meta*-coupled ABC-type aromatic protons [**3**: δ 6.68 (1H, dd, J=2.0, 8.1 Hz, 6-H), 6.76 (1H, d, J=8.1 Hz, 5-H), 6.80 (1H, d, J=2.0 Hz, 2-H); **4**: δ 6.67 (1H, dd, J=1.9, 8.2 Hz, 6-H), 6.76 (1H, d, J=8.2 Hz, 5-H), 6.78 (1H, d, J=1.9 Hz, 2-H)], two β-D-glucopyranosyl moieties [**3**: δ 4.31 (1H, d, J=7.9 Hz, *terminal*-Glc-1-H), 4.40 (1H, d, J=7.9 Hz, *inner*-Glc-1-H); **4**: δ 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 α-L-rhamnopyranosyl moiety [**3**: δ 1.08 (3H, d, J=6.4 Hz, Rha-6-H_3), 5.19 (1H, d, J=1.7 Hz, Rha-1-H); **4**: δ 1.08 (3H, d, J=6.2 Hz, Rha-6-H_3), 5.20 (1H, d, J=1.6 Hz, Rha-1-H)] together with a *trans*-caffeoyl group {an *trans*-olefin [**3**: δ 6.27, 7.60 (1H each, both d, J=15.8 Hz, 8-, 7-H); **4**: δ 6.28, 7.60 (1H each, both d, J=15.8 Hz, 8-, 7-H)] and *ortho*- and *meta*-coupled ABC-type aromatic protons [**3**: δ 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**: δ 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 ^{1}H- and ^{13}C-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*-caffeoyl 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*-caffeic 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 *O*-α-L-rhamnopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→6)]-4-*O*-*trans*-caffeoyl-β-D-glucopyranoside (**3** and **4**).[11,13]

Previously, methanolic extract from stems of *C. tubulosa* and several phenylethanoid constituents such as echinacoside, acteoside, and isoacteoside 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 isocampneoside I (**5**) on D-GalN-induced cytotoxicity in primary cultured hepatocytes. Although their activities were weaker than those of echinacoside (IC_{50}=10.2 μM), acteoside (4.6 μM), and isoacteoside (5.3 μM), the principle phenylethanoid constituents from stems of *C. tubulosa*,[1] **3**—**5** showed moderate activity.[14]

Experimental

The following instruments were used to obtain spectral and physical data: specific rotations, Horiba SEPA-300 digital polarimeter (l=5 cm); UV spectra, Shimadzu UV-1600 spectrometer; IR spectra, Shimadzu FTIR-8100 spectrometer; ^{1}H- and ^{13}C-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 5C_{18}-MS-II and πNAP (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 Chromatorex ODS DM1020T (Fuji Silysia Chemical, Ltd., 100—200 mesh); normal-phase TLC, pre-coated TLC plates with silica gel $60F_{254}$ (Merck, 0.25 mm); reversed-phase TLC, pre-coated TLC plates with silica gel RP-18 F_{254S} (Merck, 0.25 mm); reversed-phase HPTLC, pre-coated TLC plates with silica gel RP-18 WF_{254S} (Merck, 0.25 mm), detection was achieved by spraying with 1% $Ce(SO_4)_2$–10% aqueous H_2SO_4, followed by heating.

Plant Material This item was described in a previous report.[1)]

Extraction and Isolation Fresh stems 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, H_2O→MeOH) to give H_2O- 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, $CHCl_3$–MeOH–H_2O (15 : 3 : 0.4→10 : 3 : 0.5→6 : 4 : 1, v/v/v)→MeOH] to give seven fractions [Fr. 1 (1.12 g), 2 (9.56 g), 3 (0.89 g), 4 (10.69 g), 5 (8.84 g), 6 (12.52 g), and 7 (4.60 g)], as was described previously.[1)] The fraction 4 (10.69 g) was separated by reversed-phase silica gel CC [500 g, MeOH–H_2O (30 : 70, v/v)→MeOH→acetone] to give four fractions [Fr. 4-1 (878.2 mg), 4-2 (7.06 g), 4-3 (1.57 g), and 4-4 (792.8 mg)]. The fraction 4-3 (1.57 g) was purified by HPLC [Cosmosil $5C_{18}$-MS-II, CH_3CN–1% aqueous AcOH (20 : 80, v/v)] to give 11 fractions {Fr. 4-3-1 (30.4 mg), 4-3-2 (55.2 mg), 4-3-3 [=campneoside I (**6**, 22.1 mg, 0.0010%)], 4-3-4 [=acteoside (224.6 mg, 0.010%)], 4-3-5 (27.4 mg), 4-3-6 (43.6 mg), 4-3-7 [=isoacteoside (825.0 mg, 0.037%)], 4-3-8 [=syringalide A 3′-*O*-α-L-rhamnopyranoside (37.6 mg, 0.0017%)], 4-3-9 (39.8 mg), 4-3-10 [=2′-acetylacteoside (85.4 mg, 0.0038%)], and 4-3-11 (64.6 mg)}, as was described previously.[1)] The fraction 4-3-5 (27.4 mg) was further purified by HPLC [Cosmosil πNAP, CH_3CN–1% aqueous AcOH (18 : 82, v/v)] to give isocampneoside I (**5**, 8.5 mg, 0.0004%). The fraction 4-3-6 (43.6 mg) was further purified by HPLC [Cosmosil πNAP, CH_3CN–1% aqueous AcOH (18 : 82, v/v)] to give **5** (3.7 mg, 0.0002%) together with kankanosides H_1[1)] (17.0 mg, 0.0008%) and H_2[1)] (3.3 mg, 0.0001%). The fraction 4-3-9 (39.8 mg) was further purified by HPLC [Cosmosil πNAP, CH_3CN–1% aqueous AcOH (18 : 82, v/v)] to give kankanosides J_1 (**1**, 3.7 mg, 0.0002%) and J_2 (**2**, 3.5 mg, 0.0002%) together with kankanoside I[1)] (15.4 mg, 0.0007%) and isoacteoside[1)] (3.1 mg, 0.0001%). The fraction 5 (8.84 g) was separated by reversed-phase silica gel CC [400 g, MeOH–H_2O (20 : 80→30 : 70, v/v)→MeOH→acetone] to give seven fractions [Fr. 5-1 (870.2 mg), 5-2 (478.9 mg), 5-3 (3.72 g), 5-4 (979.9 mg), 5-5 (1.19 g), 5-6 (1.27 g), and 5-7 (130.1 mg)]. The fraction 5-3-4 (72.3 mg) was further purified by HPLC [Cosmosil πNAP, CH_3CN–1% aqueous AcOH (10 : 90, v/v)] to give kankanosides K_1 (**3**, 5.1 mg, 0.0002%) and K_2 (**4**, 10.6 mg, 0.0005%) together with campneoside II[1)] (**7**, 10.6 mg, 0.0005%).

Kankanoside J_1 (**1**): A white powder, $[\alpha]_D^{25}$ −6.5° (c=0.25, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{32}H_{40}O_{17}Na$ $(M+Na)^+$ 719.2163; Found 719.2170. UV [λ_{max} (log ε), MeOH]: 291 (sh, 3.68), 333 (3.83) nm. IR (KBr, cm^{-1}): 3413, 1734, 1719, 1701, 1638, 1508, 1159, 1067, 1046. ^{1}H-NMR (600 MHz, CD_3OD) δ: given in Table 1. ^{13}C-NMR (150 MHz, CD_3OD) δ_C: given in Table 2. Positive-ion FAB-MS *m/z*: 719 $(M+Na)^+$. Negative-ion FAB-MS *m/z*: 695 $(M-H)^-$.

Kankanoside J_2 (**2**): A white powder, $[\alpha]_D^{25}$ −18.1° (c=0.23, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{32}H_{40}O_{17}Na$ $(M+Na)^+$ 719.2163; Found 719.2167. UV [λ_{max} (log ε), MeOH]: 291 (sh, 3.99), 333 (4.15) nm. IR (KBr, cm^{-1}): 3418, 1734, 1717, 1638, 1509, 1159, 1070, 1046. ^{1}H-NMR (600 MHz, CD_3OD) δ: given in Table 1. ^{13}C-NMR (150 MHz, CD_3OD) δ_C: given in Table 2. Positive-ion FAB-MS *m/z*: 719 $(M+Na)^+$. Negative-ion FAB-MS *m/z*: 695 $(M-H)^-$.

Kankanoside K_1 (**3**): A white powder, $[\alpha]_D^{25}$ −75.3° (c=0.16, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{36}H_{48}O_{21}Na$ $(M+Na)^+$ 839.2586; Found 839.2589. UV [λ_{max} (log ε), MeOH]: 289 (sh, 4.05), 335 (4.24) nm. IR (KBr, cm^{-1}): 3415, 1734, 1717, 1686, 1636, 1614, 1509, 1159, 1074. ^{1}H-NMR (600 MHz, CD_3OD) δ: given in Table 1. ^{13}C-NMR (150 MHz, CD_3OD) δ_C: given in Table 2. Positive-ion FAB-MS *m/z*: 839 $(M+Na)^+$.

Kankanoside K_2 (**4**): A white powder, $[\alpha]_D^{25}$ −7.4° (c=0.35, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{36}H_{48}O_{21}Na$ $(M+Na)^+$ 839.2586; Found 8392594. UV [λ_{max} (log ε), MeOH]: 291 (sh 4.06), 334 (4.23) nm. IR (KBr, cm^{-1}): 3415, 1734, 1717, 1686, 1636, 1614, 1509, 1159, 1079. ^{1}H-NMR (600 MHz, CD_3OD) δ: given in Table 1. ^{13}C-NMR (150 MHz, CD_3OD) δ_C: given in Table 2. Positive-ion FAB-MS *m/z*: 839 $(M+Na)^+$.

Alkaline and Acid Hydrolysis of Kankanosides J_1 (1), J_2 (2), K_1 (3), and K_2 (4) Solutions of **1**—**4** (each 1.0 mg) in 5% aqueous potassium hydroxide (KOH, 0.5 ml) were stirred at 40 °C for 1 h. Each solution was neutralized with Dowex HCR W2 (H^+ form), and the resin was removed by filtration. Evaporation of the solvent from the filtrates under reduced pressure yielded the corresponding deacylated products, which were subjected to HPLC analysis [column: Cosmosil πNAP, 250×4.6 mm i.d.; mobile phase: CH_3CN–1% aqueous AcOH (15 : 85, v/v); detection: UV (254 nm); flow rate: 1.0 ml/min] to give *trans*-caffeic acid (t_R 9.9 min from **1**—**4**). Then each was dissolved in 1.0 M HCl (1.0 ml) and heated at 80 °C for 3 h. After being cooled, the reaction mixture was neutralized with Amberlite IRA-400 (OH^- form), and the resins were removed by filtration. After removal of the solvent under reduced pressure, the residue was separated by Sep-Pak C18 cartridge column (H_2O→MeOH). The H_2O-eluted fraction was subjected to HPLC analysis under following conditions: HPLC column, Kaseisorb LC NH_2-60-5, 4.6 mm i.d.×250 mm (Tokyo Kasei Co., Ltd., Tokyo, Japan); detection, optical rotation [Shodex OR-2 (Showa Denko Co., Ltd., Tokyo, Japan); mobile phase, CH_3CN–H_2O (85 : 15, v/v); flow rate 0.8 ml/min]. Identification of L-rhamnose (**i**) and D-glucose (**ii**) from **1**—**4** present in the H_2O-eluted fractions were carried out by comparison of their retention times and optical rotation with those of authentic samples [**i**, t_R 9.9 min (negative)] and [**ii**, t_R 17.9 min (positive)].

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References and Notes

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13) Campneoside I (**6**), a methylated version of campneoside II (**7**), was reported to be isolated from water extracts of leaves of *Campsis chinensis*[8)] and fruit of *Forsythia viridissima*.[10)] In the present study, **6** could not be produced by treatment of campneoside II (**7**) with methanol under reflux for more than 24 h. These findings suggest that **1**—**4** are not artificially produced during the extraction procedure.
14) Inhibitory effects of presently isolated kankanosides K_1 (**3**) and K_2 (**4**), and isocampneoside I (**5**, IC_{50}=81.6 μM) on D-GalN-induced cytotoxicity in primary cultured mouse hepatocytes were examined [inhibition (%) **3**: 0.0±1.1, 5.0±1.2, 10.2±2.3*, 16.8±2.9**, and 31.0±3.7**; **4**: 0.0±0.6, 5.2±0.1, 10.3±0.7**, 18.0±1.1**, and 24.9±3.4**; **5**: 0.0±1.0, 6.0±0.4*, 16.6±0.4**, 31.7±0.9**, and 53.7±2.5** at 0, 3, 10, 30, and 100 μM, respectively]. Values are expressed as means±S.E.M. (n=4). For statistical analysis, one-way analysis of variance (ANOVA) followed by Dunnett's test was used. Probability (p) values less than 0.05 were considered significant (* $p<0.05$, ** $p<0.01$). The bioassay method was described previously.[1)]