Monoterpene Constituents from *Cistanche tubulosa*—Chemical Structures of Kankanosides A—E and Kankanol—

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Four new iridoid glycosides, kankanosides A (1), B (2), C (3), and D (4), a chlorinated iridoid, kankanol (5), and an acyclic monoterpene glycoside, kankanoside E (6), were isolated from the methanolic extract of dried stems of *Cistanche tubulosa* (SCHRENK) R. WIGHT (Orobanchaceae) together with 16 known compounds. The structures of these new compounds (1—6) were determined on the basis of the chemical and physicochemical evidence.

Key words *Cistanche tubulosa*; kankanoside; kankanol; iridoid; monoterpene; Orobanchaceae

Cistanche tubulosa (SCHRENK) R. WIGHT (Orobanchaceae) is a perennial parasitic plant growing on the roots of *Salvadora* or *Calotropis* species, and distributed in North Africa, Arabia, and Asian countries.[1)] The stems of this plant (Kanka-nikujuyou in Japanese) have been used traditionally for treating impotence, sterility, lumbago, and body weakness.[2)] Previously, several iridoids, monoterpenoids, phenylethanoids, and lignans were isolated from Chinese and Pakistan *C. tubulosa*.[1,3–7)] In the course of our serial studies on bioactive constituents from Chinese natural medicines,[8–18)] four new iridoid glycosides, kankanosides A (**1**), B (**2**), C (**3**), and D (**4**), a chlorinated iridoid, kankanol (**5**), and an acyclic monoterpene glycoside, kankanoside E (**6**), were isolated from the methanolic extract of this herbal medicine together with 16 known compounds including 12 monoterpenes (**7**—**18**). This paper deals with the isolation and structure elucidation of new monoterpene constituents (**1**—**6**).

The methanolic extract from dried stems of *C. tubulosa* (26.8% from this herbal medicine) was subjected to normal-phase and reversed-phase silica gel column chromatography and repeated HPLC to give kankanosides A (**1**, 0.0054%), B (**2**, 0.030%), C (**3**, 0.0027%), and D (**4**, 0.0015%), kankanol (**5**, 0.0034%), and kankanoside E (**6**, 0.027%) together with mussaenosidic acid[19)] (**7**, 0.020%), geniposidic acid[19)] (**8**, 0.030%), 8-epiloganic acid[7)] (**9**, 0.033%), gluroside[19)] (**10**, 0.14%), antirrhide[20)] (**11**, 0.0079%), ajugol[19)] (**12**, 0.011%), bartsioside[19)] (**13**, 0.21%), 6-deoxycatalpol[19)] (**14**, 0.11%), argyol[21)] (**15**, 0.0030%), cistanin[22,23)] (**16**, 0.0040%), cistachlorin[22)] (**17**, 0.0035%), (2*E*,6*Z*)-8-*β*-D-glucopyranosyloxy-2,6-dimethyl-2,6-octadienoic acid[24)] (**18**, 0.0028%), D-mannitol[25)] (4.19%), uridine[25)] (0.0069%), (3*R*)-3-hydroxy-2-pyrrolidinone[26,27)] (0.0020%), and (3*R*)-3-hydroxy-1-methyl-2-pyrrolidinone[27)] (0.0059%).

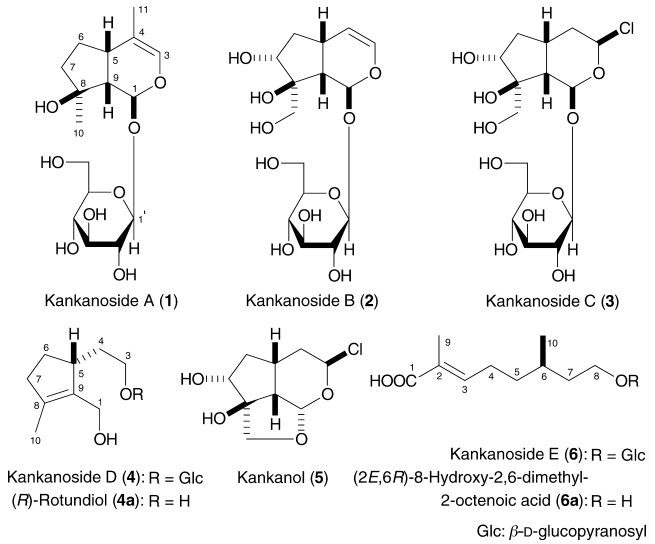

Chart 1

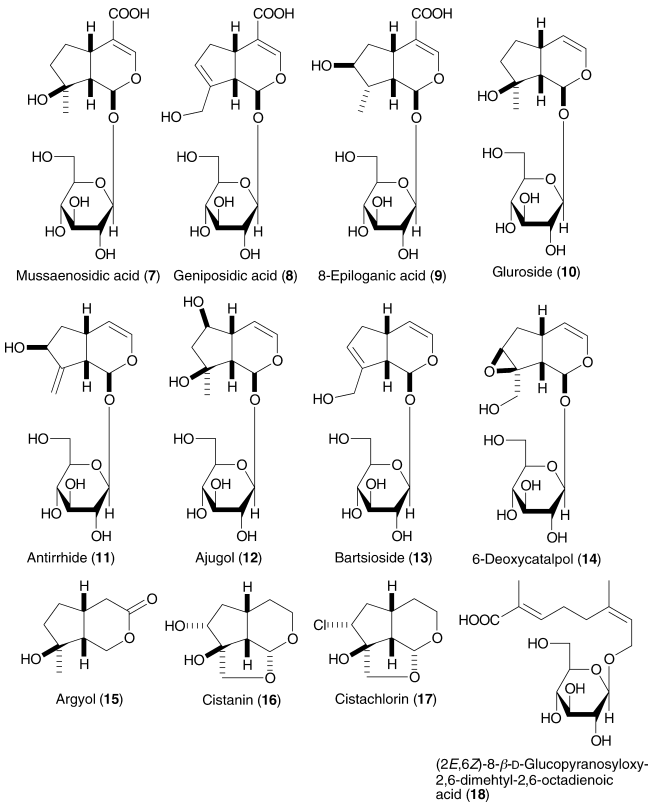

Chart 2

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Structure of Kankanoside A (1) Kankanoside A (**1**) was obtained as an amorphous powder and exhibited a negative optical rotation ($[\alpha]_D^{25}$ −107.4° in MeOH). The IR spectrum of **1** showed absorption band at 1647 cm^{-1} assignable to olefin function in addition to strong absorption bands at 3410 and 1076 cm^{-1} suggestive of a glycoside moiety. In the positive- and negative-ion fast atom bombardment (FAB)-MS of **1**, quasimolecular ion peaks were observed at *m/z* 369 $(M+Na)^+$ and 345 $(M-H)^-$, and high-resolution FAB-MS analysis revealed the molecular formula of **1** to be $C_{16}H_{26}O_8$. Acid hydrolysis of **1** with 1.0 M hydrochloric acid (HCl) liberated D-glucose, which was identified by HPLC analysis using an optical rotation detector.[8,10—12,15—18] The ^{1}H-(CD_3OD, Table 1) and ^{13}C-NMR (Table 2) spectra of **1**, which were assigned by various NMR experiments,[28] showed signals assignable to two methyls [δ 1.31 (s, 10-H_3), 1.51 (br s, 11-H_3)], two methylenes [δ 1.49, 2.02 (both m, 6α- and 6β-H), 1.64, 1.67 (both m, 7β- and 7α-H)], two methines [δ 2.21 (dd, J=2.7, 9.5 Hz, 9-H), 2.71 (m, 5-H)], and an α,β-unsaturated acetal group [δ 5.33 (d, J=2.7 Hz, 1-H), 5.95 (br s, 3-H)] together with a β-glucopyranosyl moiety [δ 4.62 (d, J=7.9 Hz, 1′-H)]. As shown in Fig. 1, the ^{1}H–^{1}H correlation spectroscopy (^{1}H–^{1}H COSY) experiment on **1** indicated the presence of partial structures written in bold lines. In the heteronuclear multiple-bond correlations (HMBC) experiment on **1**, long-range correlations were observed between the following protons and carbons (3-H, 1′-H and 1-C; 11-H_3 and 3-C; 3-H, 5-H, 6-H_2, 9-H, 11-H_3 and 4-C; 11-H_3 and 5-C; 10-H_3 and 7-C; 1-H, 7-H_2, 9-H, 10-H_3 and 8-C; 10-H_3 and 9-C; 7-H_2 and 10-C) as shown in Fig. 1. Enzymatic hydrolysis of **1** with β-glucosidase gave an aglycon, kankagenin a (**1a**) as shown in Fig. 3. Comparison of the ^{13}C-NMR spectrum for **1** with those for **1a** revealed the glycosylation shift around the 1-position in **1** [**1**: δ_C 94.1 (1-C), 134.6 (3-C), 53.3 (9-C); **1a**: δ_C 92.9 (1-C), 135.3 (3-C), 54.7 (9-C)]. Thus, the connectivity of the β-D-glucopyranosyl moiety in **1** was also clarified to be at the 1-position of **1a**. Next, the relative stereostructure of **1** was characterized by nuclear Overhauser enhancement spectroscopy (NOESY) experiment, which showed NOE correlations between the fol-

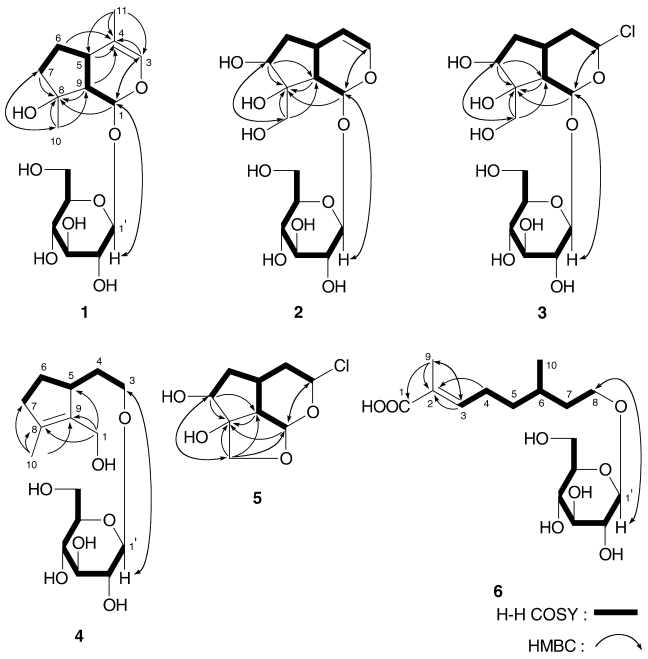

Fig. 1. H–H COSY and HMBC Correlations of **1**—**6**

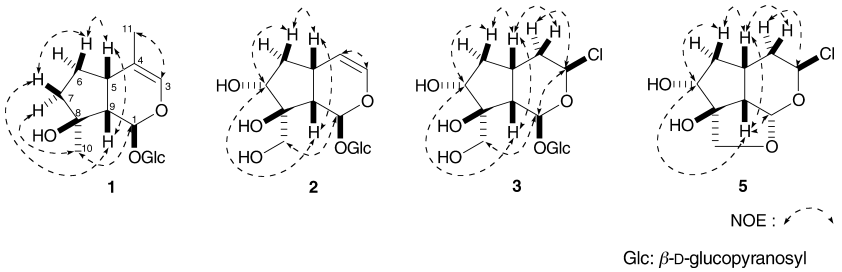

Fig. 2. NOE Correlations **1**—**3** and **5**

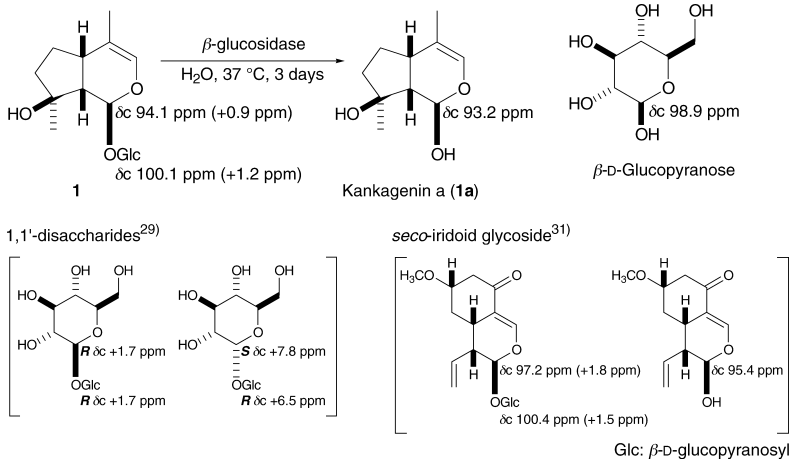

Fig. 3. Application of ^{13}C-NMR Glycosylation Shift (All in Pyridine-d_5)

Table 1. ^{1}H-NMR (500 MHz) Data of Kankanosides A—C (**1**—**3**), Kankanol (**5**), **19**, **1a**, **5a**, **14a**, and **14b**

H-	**1**[a] δ (*J* Hz)	**1**[b] δ (*J* Hz)	**2**[a] δ (*J* Hz)	**3**[a] δ (*J* Hz)	**19**[a] δ (*J* Hz)
1	5.33 (d, 2.7)	5.96 (d, 2.5)	5.49 (d, 6.4)	5.48 (d, 2.5)	5.27 (d, 5.2)
3	5.95 (br s)	6.22 (br s)	6.22 (dd, 1.8, 6.1)	5.10 (br d, *ca.* 3)	6.24 (dd, 2.2, 6.2)
4α			4.95 (dd, 4.0, 6.1)	1.70 (br dd, *ca.* 3, 13)	4.95 (dd, 3.4, 6.2)
4β				2.70 (br dd, *ca.* 6, 13)	
5	2.71 (m)	2.84 (m)	2.83 (m)	2.61 (m)	2.83 (m)
6α	1.49 (m)	1.49 (m)	1.40 (ddd, 5.2, 7.3, 13.5)	1.68 (br d, *ca.* 13)	1.86 (ddd, 4.3, 7.1, 13.1)
6β	2.02 (m)	2.09 (m)	2.52 (ddd, 7.0, 9.2, 13.5)	2.44 (m)	1.77 (ddd, 4.6, 8.5, 13.1)
7α	1.67 (m)	1.95 (m)			
7β	1.64 (m)	1.79 (m)	4.02 (dd, 5.2, 7.0)	3.94 (br s)	4.01 (dd, 4.3, 4.6)
8					
9	2.21 (dd, 2.7, 9.5)	2.84 (m)	2.21 (dd, 6.4, 8.6)	2.47 (dd, 2.5, 7.9)	2.29 (dd, 5.2, 9.5)
10	1.31 (3H, s)	1.42 (3H, s)	3.85 (d, 11.9)	4.01 (d, 11.3)	3.67 (d, 11.6)
			3.99 (d, 11.9)	4.04 (d, 11.3)	3.74 (d, 11.6)
11	1.51 (3H, br s)	1.66 (3H, br s)			
1′	4.62 (d, 7.9)	5.43 (d, 7.9)	4.72 (d, 7.9)	4.60 (d, 8.0)	4.68 (d, 8.0)
2′	3.18 (dd, 7.9, 8.5)	4.09 (dd. 7.9, 8.3)	3.20 (dd, 7.9, 9.2)	3.17 (dd, 8.0, 9.2)	3.22 (dd, 8.0, 9.2)
3′	3.36 (dd, 8.5, 8.9)	4.29 (dd, 8.3, 8.9)	3.38 (dd, 8.9, 9.2)	3.36 (dd, 8.9, 9.2)	3.38 (dd, 8.9, 9.2)
4′	3.26 (dd, 8.9, 9.5)	4.34 (dd, 8.9, 9.2)	3.27 (m)	3.27 (m)	3.29 (m)
5′	3.28 (m)	3.97 (m)	3.28 (m)	3.29 (m)	3.31 (m)
6′	3.65 (dd, 5.8, 11.9)	4.41 (dd, 4.9, 11.6)	3.65 (dd, 5.8, 11.9)	3.65 (dd, 5.8, 12.2)	3.67 (dd, 5.2, 12.2)
	3.89 (dd, 1.9, 11.9)	4.50 (dd, 2.2, 11.6)	3.88 (dd, 1.5,11.9)	3.88 (dd, 1.8, 12.2)	3.85 (dd, 1.9, 12.2)

H-	**5**[a] δ (*J* Hz)	**1a**[a] δ (*J* Hz)	**1a**[b] δ (*J* Hz)	**5a**[a] δ (*J* Hz)	**14a**[a] δ (*J* Hz)	**14b**[a] δ (*J* Hz)
1	5.26 (d, 4.3)	4.93 (d, 3.4)	5.54 (d, 5.5)	5.27 (d, 4.6)	5.57 (d, 5.5)	5.59 (d, 5.2)
3	5.17 (br d, *ca.* 3)	5.97 (br s)	6.34 (br s)	5.20 (m)	5.25 (dd, 4.0, 8.8)	6.26 (dd, 6.1, 8.3)
4α	1.60 (ddd, 2.5, 5.5, 13.1)			1.64 (ddd, 2.2, 5.5, 12.5)	1.57 (ddd, 6.1, 8.8, 14.4)	1.71 (ddd, 5.2, 8.3, 13.8)
4β	1.80 (br dd, *ca.* 3, 13)			1.82 (br dd, *ca.* 3, 13)	1.82 (ddd, 4.0, 6.5, 14.4)	2.04 (ddd, 2.8, 6.1, 13.8)
5	2.50 (m)	2.71 (m)	3.09 (m)	2.55 (dd, 3.2, 13.4)	2.53 (m)	2.75 (m)
6α	1.83 (br d, *ca.* 12)	1.58 (m)	1.55 (m)	1.85 (br d, *ca.* 13)	1.89 (m)	1.95 (m)
6β	2.57 (m)	2.06 (m)	2.04 (m)	2.32 (m)	2.16 (ddd, 0.9, 5.5, 12.5)	2.30 (br dd, *ca.* 7, 13)
7α		1.68 (m)	2.25 (m)			
7β	3.80 (br s)	1.64 (m)	1.90 (m)	4.33 (br s)	4.24 (ddd, 1.2, 6.4, 12.5)	4.87 (dd, 7.0, 13.4)
8						
9	2.76 (dd, 4.3, 8.0)	2.04 (m)	2.77 (dd, 5.5, 8.9)	3.08 (dd, 4.6, 6.7)	2.33 (dd, 5.5, 9.2)	3.08 (dd, 5.2, 9.5)
10	3.75 (d, 9.2)	1.33 (3H, s)	1.57 (3H, s)	3.98 (d, 11.0)	3.58 (dd, 1.2, 10.4)	3.99 (d, 10.8)
	3.88 (d, 9.2)			4.30 (d, 11.0)	4.31 (d, 10.4)	4.31 (d, 10.8)
11		1.54 (3H, br s)	1.84 (3H, br s)			
Ac				2.04 (3H, s)		2.05 (3H, s)
						2.07 (3H, s)

Measured in *a*) CD_3OD and *b*) pyridine-d_5.

lowing proton pairs (1-H and 10-H_3; 3-H and 11-H_3; 5-H and 6β-H, 9-H; 6β-H and 7β-H; 7α-H and 10-H_3; 7β-H and 9-H) as shown in Fig. 2. Finally, the absolute configuration of the 1-position in **1** was determined by application of the ^{13}C-NMR glycosylation shift rule of 1,1′-disaccharide,[29] which was found to be applicable to the dihemiacetal compounds.[30,31] The stereostructure of the 1-position in **1** was confirmed to be maintained in **1a** by comparison of the ^{1}H-NMR analysis including the NOESY experiment. The glycosylation shift values [$\Delta\delta$+1.2 ppm (1′-C) and +0.9 ppm (1-C), in pyridine-d_5] were found to be characteristic of the *R*,*R*-dihemiacetal combination, which corresponded to the absolute stereostructure of **1** as shown in Fig. 3. Consequently, the absolute configuration at the 1-position of **1** was determined to be *S* configuration and the absolute stereostructure of **1** was elucidated as shown.

Structures of Kankanosides B (2) and C (3) Kankanoside B (**2**) was also isolated as an amorphous powder with negative optical rotation ($[\alpha]_D^{26}$ −118.7° in MeOH). The IR spectrum of **2** showed absorption bands at 3410, 1647, and 1085 cm^{-1} ascribable to hydroxyl, olefin, and ether functions. The molecular formula $C_{15}H_{24}O_{10}$ of **2** was determined by the quasimolecular ion peaks in positive-ion FAB-MS and by high-resolution FAB-MS. Acid hydrolysis of **2** with 1.0 M HCl liberated D-glucose, which was identified by HPLC analysis using an optical rotation detector.[8,10—12,15—18] The ^{1}H- (CD_3OD, Table 1) and ^{13}C-NMR (Table 2) spectra[28] of **2** showed signals assignable to two methylenes [δ 1.40 (ddd, *J*=5.2, 7.3, 13.5 Hz, 6α-H), 2.52 (ddd, *J*=7.0, 9.2, 13.5 Hz, 6β-H), 3.85, 3.99 (both d, *J*=11.9 Hz, 10-H_2)], four methines [δ 2.21 (dd, *J*=6.4, 8.6 Hz, 9-H), 2.83 (m, 5-H), 4.02 (dd, *J*=5.2, 7.0 Hz, 7-H), 5.49 (d, *J*=6.4 Hz, 1-H)], and a *cis*-olefin pair [δ 4.95 (dd, *J*=4.0, 6.1 Hz, 4-H), 6.22 (dd, *J*=1.8, 6.1 Hz, 3-H)], together with a β-glucopyranosyl moiety [δ 4.72 (d, *J*=7.9 Hz, 1′-H)]. The proton and carbon signals in the ^{1}H- and ^{13}C-NMR data of **2** were similar to those of 6-deoxycatalpol (**14**), except for the signals due to the 7- and 8-positions. As shown in Fig. 1, the ^{1}H–^{1}H COSY exper-

Table 2. ^{13}C-NMR (125 MHz) Data of Kankanosides A—C (**1**—**3**), Kankanol (**5**), **19**, **1a**, **5a**, **14a**, and **14b**

C-	**1**[a] δ_C (mult.)	**1**[b] δ_C (mult.)	**2**[a] δ_C (mult.)	**3**[a] δ_C (mult.)	**19**[a] δ_C (mult.)
1	94.1 (d)	94.1 (d)	94.1 (d)	93.3 (d)	95.8 (d)
3	134.6 (d)	134.5 (d)	139.9 (d)	96.1 (d)	140.7 (d)
4	115.0 (s)	113.7 (s)	108.9 (d)	34.7 (t)	109.0 (d)
5	36.2 (d)	35.7 (d)	30.8 (d)	28.5 (d)	32.0 (d)
6	28.2 (t)	27.8 (t)	40.6 (t)	38.2 (t)	40.3 (t)
7	41.4 (t)	41.4 (t)	80.0 (d)	82.0 (d)	79.5 (d)
8	80.2 (s)	78.8 (s)	84.1 (s)	83.0 (s)	84.3 (s)
9	53.3 (d)	53.2 (d)	50.5 (d)	50.4 (d)	46.1 (d)
10	24.6 (q)	25.3 (q)	64.6 (t)	65.1 (t)	67.0 (t)
11	16.2 (q)	16.2 (q)			
1′	99.4 (d)	100.1 (d)	99.8 (d)	99.0 (d)	100.6 (d)
2′	74.9 (d)	75.0 (d)	74.9 (d)	74.8 (d)	74.8 (d)
3′	78.1 (d)	78.6 (d)	78.0 (d)	78.2 (d)	77.9 (d)
4′	71.8 (d)	71.5 (d)	71.8 (d)	71.8 (d)	71.4 (d)
5′	78.2 (d)	78.7 (d)	78.3 (d)	78.3 (d)	78.3 (d)
6′	62.9 (t)	62.6 (t)	62.9 (t)	62.9 (t)	62.5 (t)

C-	**5**[a] δ_C (mult.)	**1a**[a] δ_C (mult.)	**1a**[b] δ_C (mult.)	**5a**[a] δ_C (mult.)	**14a**[a] δ_C (mult.)	**14b**[a] δ_C (mult.)
1	98.1 (d)	92.9 (d)	93.2 (d)	96.5 (d)	103.0 (d)	101.9 (d)
3	95.7 (d)	135.3 (d)	135.1 (d)	95.7 (d)	93.7 (d)	91.0 (d)
4	34.3 (t)	114.3 (s)	112.7 (s)	34.0 (t)	31.9 (t)	28.3 (t)
5	26.1 (d)	37.9 (d)	37.8 (d)	25.9 (d)	31.2 (d)	31.1 (d)
6	42.3 (t)	28.9 (t)	28.8 (t)	41.7 (t)	40.6 (t)	40.4 (t)
7	79.6 (d)	40.6 (t)	40.6 (t)	76.5 (d)	66.9 (d)	61.8 (d)
8	92.7 (s)	81.0 (s)	79.7 (s)	98.7 (s)	89.4 (s)	97.6 (s)
9	54.9 (d)	54.7 (d)	55.0 (d)	53.5 (d)	50.6 (d)	51.0 (d)
10	71.5 (t)	25.1 (q)	26.1 (q)	69.0 (t)	74.6 (t)	74.4 (t)
11		16.4 (q)	16.6 (q)			
Ac				21.1 (q)		21.0 (q)
				172.1 (s)		172.4 (s)
						21.7 (q)
						171.5 (s)

Measured in *a*) CD_3OD and *b*) pyridine-d_5.

iment on **2** indicated the presence of partial structures written in bold lines and, in the HMBC experiment, long-range correlations were observed between the following proton and carbon pairs (3-H, 1′-H and 1-C; 1-H and 3-C; 10-H_2 and 7-C; 1-H, 7-H, 9-H, 10-H_2 and 8-C; 7-H, 10-H_2 and 9-C; 7-H and 10-C). The relative stereostructure of **2** was characterized by NOESY experiment, which showed NOE correlations between the following proton pairs (1-H and 10-H_2; 3-H and 4-H; 5-H and 6β-H, 9-H; 6β-H and 7-H; 7-H and 9-H) as shown in Fig. 2. Finally, alkaline treatment of **14** with 5% aqueous potassium hydroxide (KOH) yielded **2** and **19**,[32] so that the stereostructure of **2** was clarified.

Kankanoside C (**3**) was isolated as an amorphous powder with negative optical rotation ($[\alpha]_D^{26}$ −34.0° in MeOH). In the negative-ion FAB-MS of **3**, a pair of isotope quasimolecular ion peaks were observed at *m/z* 399 and 401 $(M-H)^-$. The molecular formula of **3** was determined to be $C_{15}H_{25}ClO_{10}$ by high resolution FAB-MS measurement. Acid hydrolysis of **3** with 1.0 M HCl liberated D-glucose, which was identified by HPLC analysis using an optical rotation detector.[8,10—12,15—18] The ^{1}H- (CD_3OD, Table 1) and ^{13}C-NMR (Table 2) spectra[28] of **3** showed signals assignable to three methylenes [δ 1.70 (br dd, *J*=*ca.* 3, 13 Hz, 4α-H), 2.70 (br dd, *J*=*ca.* 6, 13 Hz, 4β-H), 1.68 (br d, *J*=*ca.* 13 Hz, 6α-H), 2.44 (m, 6β-H), 4.01, 4.04 (both d, *J*=11.3 Hz, 10-H_2)], five methines [δ 2.47 (dd, *J*=2.5, 7.9 Hz, 9-H), 2.61 (m, 5-H), 3.94 (br s, 7-H), 5.10 (br d, *J*=*ca.* 3 Hz, 3-H), 5.48 (d, *J*=2.5 Hz, 1-H)], and a β-glucopyranosyl moiety [δ 4.60 (d, *J*=8.0 Hz, 1′-H)]. The proton and carbon signals in the ^{1}H- and ^{13}C-NMR spectra of **3** were superimposable on those of **2**, except for the signals due to the 3- and 4-positions. The positions of β-D-glucopyranosyl and chlorine function in **3** was elucidated from the H–H COSY and HMBC experiments as shown in Fig. 1. Consequently, the planar structure of **3** was constructed to be as shown. The relative stereostructure of **3** was determined by a NOESY experiment, in which NOE correlations were observed between the following proton pairs (1-H and 3-H, 10-H_2; 3-H and 4α-H; 4β-H and 5-H; 5-H and 6β-H, 9-H; 6β-H and 7-H; 7-H and 9-H) as shown in Fig. 2.

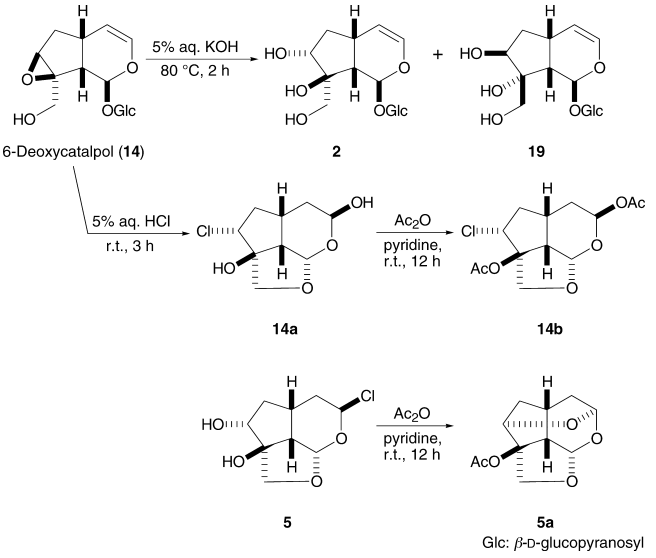

Chart 3

Structures of Kankanoside D (4) and Kankanol (5) Kankanoside D (**4**) was isolated as an amorphous powder with negative optical rotation ($[\alpha]_D^{25}$ −30.6° in MeOH). The IR spectrum of **4** showed an absorption band at 1655 cm^{-1} ascribable to olefin function and strong absorption bands at 3410 and 1078 cm^{-1} suggestive of its glycosidic structure. In the positive-ion FAB-MS of **4**, a quasimolecular ion peak was observed at *m/z* 341 $(M+Na)^+$. The molecular formula $C_{15}H_{26}O_7$ of **4** was determined by high-resolution FAB-MS measurement. Acid hydrolysis of **4** with 1.0 M HCl liberated D-glucose,[8,10—12,15—18] whereas (*R*)-rotundiol (**4a**)[33,34] was obtained by enzymatic hydrolysis of **4** with β-glucosidase. The ^{1}H-NMR (Table 3, CD_3OD) and ^{13}C-NMR (Table 4) spectra[28] of **4** showed signals assignable to a methyl [δ 1.69 (s, 10-H_3)], three methylenes [δ 1.41, 2.06 (both m, 4-H_2), 1.51, 2.01 (both m, 6α- and 6β-H), 2.23 (br dd, *J*=*ca.* 8, 15 Hz, 7α-H), 2.37 (br dd, *J*=*ca.* 8, 15 Hz, 7β-H)], a methine [δ 2.90 (m, 5-H)], and two methylenes bearing an oxygen function {δ [3.56 (ddd, *J*=2.8, 7.4, 13.2 Hz), 3.97 (ddd, *J*=4.9, 8.0, 13.2 Hz), 3-H_2], 4.04, 4.18 (both d, *J*=12.2 Hz, 1-H_2)} together with a β-glucopyranosyl part [δ 4.25 (d, *J*=7.7 Hz, 1′-H)]. The position of the β-D-glucopyranosyl part in **4** was clarified by HMBC experiment to be 3-position (Fig. 1). On the basis of this evidence, the absolute stere-

Table 3. ^{1}H-NMR (500 MHz, CD_3OD) Data of Kankanosides D (**4**) and E (**6**)

H-	**4** δ (J Hz)	H-	**6** δ (J Hz)
1	4.04 (d, 12.2)	3	6.78 (dd, 1.2, 7.3)
	4.18 (d, 12.2)	4	2.22 (2H, m)
3	3.56 (ddd, 2.8, 7.4, 13.2)	5	1.30 (m)
	3.97 (ddd, 4.9, 8.0, 13.2)		1.48 (m)
4	1.41 (m)	6	1.65 (m)
	2.06 (m)	7	1.45 (m)
5	2.90 (m)		1.70 (m)
6α	1.51 (m)	8	3.61 (m)
6β	2.01 (m)		3.94 (m)
7α	2.23 (br dd, *ca.* 8, 15)	9	1.81 (3H, s)
7β	2.37 (br dd, *ca.* 8, 15)	10	0.95 (3H, d, 6.4)
10	1.69 (3H, s)		
1′	4.25 (d, 7.7)	1′	4.26 (d, 7.6)
2′	3.16 (dd, 7.7, 9.2)	2′	3.18 (dd, 7.6, 9.2)
3′	3.34 (dd, 8.9, 9.2)	3′	3.37 (dd, 8.6, 9.2)
4′	3.26 (m)	4′	3.27 (m)
5′	3.27 (m)	5′	3.28 (m)
6′	3.66 (dd, 5.2, 12.2)	6′	3.68 (dd, 5.2, 11.9)
	3.86 (dd, 1.8, 12.2)		3.87 (dd, 2.1, 11.9)

Table 4. ^{13}C-NMR (125 MHz, CD_3OD) Data of Kankanosides D (**4**) and E (**6**)

C-	**4** δ_C (mult.)	**6** δ_C (mult.)
1	57.1 (t)	171.7 (s)
2		128.7 (s)
3	70.0 (t)	144.1 (d)
4	34.9 (t)	27.1 (t)
5	44.2 (d)	36.8 (t)
6	29.6 (t)	30.6 (d)
7	38.1 (t)	37.5 (t)
8	137.4 (s)	68.9 (t)
9	138.4 (s)	12.4 (q)
10	14.0 (q)	19.8 (q)
1′	104.7 (d)	104.2 (d)
2′	75.2 (d)	75.0 (d)
3′	78.0 (d)	77.8 (d)
4′	71.7 (d)	71.5 (d)
5′	78.1 (d)	78.0 (d)
6′	62.8 (t)	62.7 (t)

ostructure of **4** was determined to be as shown.

Kankanol (**5**) was obtained as an amorphous powder with positive optical rotation ($[\alpha]_D^{25}$ +11.1°). The chemical ionization (CI)-MS of **5** showed a pair of isotope ion peaks at *m/z* 221 and 223 due to a quasimolecular ion $(M+H)^+$. The high-resolution CI-MS measurement of **5** revealed the molecular formula to be $C_9H_{13}ClO_4$. The ^{1}H-NMR (Table 1, CD_3OD) and ^{13}C-NMR (Table 2) spectra[28] of **5** showed the presence of the following functions: three methylenes [δ 1.60 (ddd, J=2.5, 5.5, 13.1 Hz, 4α-H), 1.80 (br dd, J=*ca.* 3, 13 Hz, 4β-H), 1.83 (br d, J=*ca.* 12 Hz, 6α-H), 2.57 (m, 6β-H), 3.75, 3.88 (both d, J=9.2 Hz, 10-H_2)], five methines [δ 2.76 (dd, J=4.3, 8.0 Hz, 9-H), 2.50 (m, 5-H), 3.80 (br s, 7-H), 5.17 (br d, J=*ca.* 3 Hz, 3-H), 5.26 (d, J=4.3 Hz, 1-H)]. The planar structure of **5** was confirmed by ^{1}H–^{1}H COSY and HMBC experiments. That is, the ^{1}H–^{1}H COSY experiment on **5** indicated the presence of the partial structures written in bold lines, and in the HMBC experiment, long-range correlations were observed as shown in Fig. 1. The relative stereostructure of **5** was determined by the NOESY experiment, in which NOE correlations were observed between the following proton pairs (1-H and 9-H; 3-H and 4α-H; 4β-H and 5-H; 5-H and 6β-H, 9-H; 6β-H and 7-H; 7-H and 9-H) as shown in Fig. 2. By comparison of the ^{1}H- and ^{13}C-NMR data for **5** with those for **14a**, which was obtained by the treatment of **14** with 5% aqueous HCl as shown in Chart 1,[35] the position of the chlorine group in **5** was supported to be the 3-position. Furthermore, acetylation of **5** with acetic anhydride (Ac_2O) and pyridine yielded the 3,7-oxide (**5a**), while **14a** gave the diacetate (**14b**) under the same acetylation condition. This evidence also led us to confirm the position of chlorine function to be the 3β-position (Chart 3). Consequently, the stereostructure of **5** was determined to be as shown.

Structure of Kankanoside E (6) Kankanoside E (**6**) was isolated as an amorphous powder with negative optical rotation ($[\alpha]_D^{25}$ −20.0° in MeOH). The IR spectrum of **6** showed absorption bands at 3410, 1647, 1085 cm^{-1} ascribable to glycosidic and carbonyl functions, while its UV spectrum showed absorption maximum at 211 nm (log ε 4.63), indicating the presence of an α,β-unsaturated carboxylic acid. The molecular formula $C_{16}H_{28}O_8$ of **6** was characterized by the positive- and negative-ion FAB-MS and by high-resolution MS measurement. Acid hydrolysis of **6** liberated D-glucose,[8,10—12,15—18] whereas (2*E*,6*R*)-8-hydroxy-2,6-dimethyl-2-octenoic acid (**6a**)[36] was obtained by enzymatic hydrolysis of **6** with β-glucosidase. The ^{1}H-NMR (Table 3, CD_3OD) and ^{13}C-NMR (Table 4) spectra[28] of **6** indicated the presence of a (2*E*,6*R*)-8-hydroxy-2,6-dimethyl-2-octenoic acid moiety [δ 0.95 (d, J=6.4 Hz, 10-H_3), 1.30, 1.48 (both m, 5-H_2), 1.45, 1.70 (both m, 7-H_2), 1.65 (m, 6-H), 1.81 (s, 9-H_3), 2.22 (2H, m, 4-H_2), 3.61, 3.94 (both m, 8-H_2), 6.78 (dd, J=1.2, 7.3 Hz, 3-H)] together with a β-D-glucopyranosyl part [δ 4.26 (d, J=7.6 Hz, 1′-H)]. By comparison of the carbon signals in the ^{13}C-NMR spectrum of **6** with those of **6a**, the glycosylation shift was observed around the 8-position of **6**. The position of the glucoside linkage was also confirmed by HMBC experiments as shown in Fig. 1. Consequently, the absolute stereostructure of **6** was clarified to be (2*E*,6*R*)-8-β-D-glucopyranosyloxy-2,6-dimethyl-2-octenoic acid.

Experimental

The following instruments were used to obtain physical data: specific rotations, Horiba SEPA-300 digital polarimeter (l=5 cm); UV spectra, Shimadzu UV-1600 spectrometer; IR spectra, Shimadzu FTIR-8100 spectrometer; EI-MS, CI-MS and high-resolution CI-MS, JEOL JMS-GCMATE mass spectrometer; FAB-MS and high-resolution MS, JEOL JMS-SX 102A mass spectrometer; ^{1}H-NMR spectra, JEOL EX-270 (270 MHz) and JNM-LA500 (500 MHz) spectrometers; ^{13}C-NMR spectra, JEOL EX-270 (68 MHz) and JNM-LA500 (125 MHz) spectrometers with tetramethylsilane as an internal standard; and HPLC detector, Shimadzu RID-6A refractive index and SPD-10A*vp* UV-VIS detectors. HPLC column, YMC-Pack ODS-A (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: ordinary-phase silica gel column chromatography, Silica gel BW-200 (Fuji Silysia Chemical, Ltd., Aichi, Japan, 150—350 mesh); reverse-phase silica gel column chromatography, Chromatorex ODS DM1020T (Fuji Silysia Chemical, Ltd., Aichi, Japan, 100—200 mesh); TLC, precoated TLC plates with Silica gel 60F_{254} (Merck, 0.25 mm) (ordinary phase) and Silica gel RP-18 F_{254S} (Merck, 0.25 mm) (reverse phase); reverse-phase HPTLC, precoated TLC plates with Silica gel RP-18 WF_{254S} (Merck, 0.25 mm); and detection was achieved by spraying with 1% $Ce(SO_4)_2$–10% aqueous H_2SO_4 followed

by heating.

Plant Material Dried stems of *Cistanche tubulosa* (SCHRENK) R. WIGHT were purchased at Urumuqi, Xinjiang Province, China in January 2005 *via* Eishin Trading Co., Ltd. Osaka, Japan, and botanical identification was undertaken by professor Jia Xiaoguang in Xinjiang Institute of Traditional Chinese and Ethnologic Medicines. A voucher specimen (2005.01. Xinjiang-01) of this plant is on file in our laboratory.

Extraction and Isolation Dried stems of *C. tubulosa* (5.0 kg) was powdered and extracted three times with methanol under reflux for 3 h. Evaporation of the solvent under reduced pressure provided the methanolic extract (1340 g, 26.8% from this herbal medicine). The methanolic extract (160 g) was subjected to normal-phase silica gel column chromatography [3.2 kg, $CHCl_3$–MeOH–H_2O (10 : 3 : 1→7 : 3 : 1, lower layer→6 : 4 : 1)MeOH] to give six fractions [Fr. 1 (5.04 g), Fr. 2 (9.84 g), Fr. 3 (7.80 g), Fr. 4 (13.28 g), Fr. 5 (113.60 g), and Fr. 6 (7.61 g)]. Fraction 1 (5.00 g) was separated by reversed-phase silica gel column chromatography [150 g, MeOH–H_2O (40 6: 60→50 : 50→60 : 40, v/v)→MeOH] to afford five fractions [Fr. 1-1 (830 mg), Fr. 1-2 (590 mg), Fr. 1-3 (180 mg), Fr. 1-4 (124 mg), and Fr. 1-5 (3200 mg)]. Fr. 1-1 (830 mg) was further separated by HPLC [MeOH–H_2O (10 : 90, v/v)] to give kankanol (**5**, 20 mg, 0.0034%), argyol (**15**, 18 mg, 0.0030%), cistanin (**16**, 24 mg, 0.0040%), and Fr. 1-1-2 (62 mg), which was further separated by HPLC [MeOH–H_2O (2 : 98, v/v)] to give (3*R*)-3-hydroxy-2-pyrrolidinone (0.0020%) and (3*R*)-3-hydroxy-1-methyl-2-pyrrolidinone (0.0059%). Fr. 1-2 (590 mg) was purified by HPLC [MeOH–H_2O (35 : 65, v/v) and CH_3CN–H_2O (20 : 80, v/v)] to give cistanchlorin (**17**, 21 mg, 0.0035%). Fraction 2 (9.72 g) was subjected to reversed-phase silica gel column chromatography [290 g, MeOH–H_2O (20 : 80→30 : 70→40 : 60→60 : 40, v/v)→MeOH] to afford seven fractions [Fr. 2-1 (1986 mg), Fr. 2-2 (1563 mg), Fr. 2-3 (3931 mg), Fr. 2-4 (375 mg), Fr. 2-5 (486 mg), Fr. 2-6 (460 mg), and Fr. 2-7 (336 mg)]. Fr. 2-1 (466 mg) was separated by HPLC [MeOH–H_2O (5 : 95, v/v)] to give uridine (0.0069%). Fr. 2-2 (535 mg) was separated by HPLC [MeOH–H_2O (10 : 90, v/v)] to give antirrhide (**11**, 15 mg, 0.0079%) and 6-deoxycatalpol (**14**, 214 mg, 0.11%). Fr. 2-3 (535 mg) was separated by HPLC [MeOH–H_2O (20 : 80, v/v)] to furnish gluroside (**10**, 110 mg, 0.14%) and bartsioside (**13**, 164 mg, 0.21%). Fr. 2-4 (375 mg) was separated by HPLC [MeOH–H_2O (30 : 70, v/v)] to give kankanosides A (**1**, 32 mg, 0.0054%) and D (**4**, 9 mg, 0.0015%). Fr. 2-6 (460 mg) was further separated by HPLC [MeOH–H_2O (45 : 55, v/v)] to provide kankanoside E (**6**, 161 mg, 0.027%) and (2*E*,6*Z*)-8-*β*-D-glucopyranosyloxy-2,6-dimethyl-2,6-octadienoic acid (**18**, 17 mg, 0.0028%). Fraction 3 (7.60 g) was subjected to reversed-phase silica gel column chromatography [230 g, MeOH–H_2O (20 : 80→40 : 60→50 : 50→60 : 40, v/v)→MeOH] to give five fractions [Fr. 3-1 (2652 mg), Fr. 3-2 (593 mg), Fr. 3-3 (3610 mg), Fr. 3-4 (190 mg), and Fr. 3-5 (336 mg)]. Fr. 3-1 (480 mg) was purified by HPLC [MeOH–H_2O (10 : 90, v/v)] to give kankanoside B (**2**, 19 mg, 0.018%), geniposidic acid (**8**, 32 mg, 0.030%), and ajugol (**12**, 12 mg, 0.011%). Fraction 4 (13.10 g) was subjected to reversed-phase silica gel column chromatography [390 g, MeOH–H_2O (10 : 90→20 : 80→30 : 70→40 : 60→50 : 50, v/v)→MeOH] to give seven fractions [Fr. 4-1 (6114 mg), Fr. 4-2 (430 mg), Fr. 4-3 (1058 mg), Fr. 4-4 (170 mg), Fr. 4-5 (2595 mg), Fr. 4-6 (1635 mg), and Fr. 4-7 (1064 mg)]. Fr. 4-2 (430 mg) was further separated by HPLC [MeOH–H_2O (5 : 95, v/v)] to afford **2** (70 mg, 0.012%) and kankanoside C (**3**, 16 mg, 0.0027%). Fr. 4-6 (1058 mg) was also separated by HPLC [MeOH–H_2O (15 : 85, v/v)] to furnish mussaenosidic acid (**7**, 116 mg, 0.020%) and 8-epiloganic acid (**9**, 193 mg, 0.033%). Fraction 5 (15.15 g) was subjected to reversed-phase silica gel column chromatography [455 g, MeOH–H_2O (0 : 100→10 : 90→20 : 80→40 : 60→50 : 50, v/v)→MeOH] to give seven fractions [Fr. 5-1 (9311 mg), Fr. 5-2 (1114 mg), Fr. 5-3 (306 mg), Fr. 5-4 (347 mg), Fr. 5-5 (1620 mg), Fr. 5-6 (1453 mg), and Fr. 5-7 (1106 mg)]. Fr. 5-1 (9311 mg) was crystallized from MeOH to give D-mannitol (3337 mg, 4.19%).

The known compounds (**7**—**18**) were identified by comparison of their physical data ($[\alpha]_D$, IR, ^{1}H-NMR, ^{13}C-NMR, MS) with reported values[1,7,19—24,26,27] or those of commercial samples.[25]

Kankanoside A (**1**): An amorphous powder, $[\alpha]_D^{25}$ −107.4° (*c*=1.50, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{16}H_{26}O_8Na$ $(M+Na)^+$ 369.1525; Found 369.1522. IR (KBr): 3410, 2940, 1647, 1076 cm^{-1}. ^{1}H-NMR (500 MHz, CD_3OD and pyridine-d_5) *δ*: given in Table 1. ^{13}C-NMR (125 MHz, CD_3OD and pyridine-d_5) δ_C : given in Table 2. Positive-ion FAB-MS: *m/z* 369 $(M+Na)^+$. Negative-ion FAB-MS: *m/z* 345 $(M-H)^-$.

Kankanoside B (**2**): An amorphous powder, $[\alpha]_D^{26}$ −118.7° (*c*=0.10, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{15}H_{24}O_{10}Na$ $(M+Na)^+$ 387.1267; Found 387.1261. IR (KBr): 3410, 2940, 1647, 1085 cm^{+1}. ^{1}H-NMR (500 MHz, CD_3OD) *δ*: given in Table 1. ^{13}C-NMR (125 MHz, CD_3OD) δ_C: given in Table 2. Positive-ion FAB-MS: *m/z* 387 $(M+Na)^+$. Negative-ion FAB-MS: *m/z* 363 $(M-H)^-$.

Kankanoside C (**3**): An amorphous powder, $[\alpha]_D^{26}$ −34.0° (*c*=1.00, MeOH). High-resolution negative-ion FAB-MS: Calcd for $C_{15}H_{24}ClO_{10}$ $(M-H)^-$ 399.1058; Found 399.1077. IR (KBr): 3410, 2964, 1159, 1078, 1048, 949 cm^{-1}. ^{1}H-NMR (500 MHz, CD_3OD) *δ*: given in Table 1. ^{13}C-NMR (125 MHz, CD_3OD) δ_C: given in Table 2. Negative-ion FAB-MS: *m/z* 399, 401 $(M-H)^-$.

Kankanoside D (**4**): An amorphous powder, $[\alpha]_D^{25}$ −30.6° (*c*=0.50, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{15}H_{26}O_7Na$ $(M+Na)^+$ 341.1204; Found 341.1210. IR (KBr): 3410, 2940, 1655, 1078, 1040 cm^{-1}. ^{1}H-NMR (500 MHz, CD_3OD) *δ*: given in Table 3. ^{13}C-NMR (125 MHz, CD_3OD) δ_C: given in Table 4. Positive-ion FAB-MS: *m/z* 341 $(M+Na)^+$. Negative-ion FAB-MS: *m/z* 317 $(M-H)^-$.

Kankanol (**5**): An amorphous powder, $[\alpha]_D^{25}$ +11.1° (*c*=1.40, MeOH). High-resolution CI-MS: Calcd for $C_9H_{14}ClO_4$ $(M+H)^+$ 221.0580; Found 221.0582. IR (KBr): 3399, 3004, 1165, 1096, 1059, 1048, 955 cm^{-1}. ^{1}H-NMR (500 MHz, CD_3OD) *δ*: given in Table 1. ^{13}C-NMR (125 MHz, CD_3OD) δ_C: given in Table 2. CI-MS *m/z* (%): 221 $(M+H)^+$ (5), 223 $(M+H)^+$ (2), 185 (88), 167 (100), 149 (71), and 57 (49).

Kankanoside E (**6**): An amorphous powder, $[\alpha]_D^{25}$ −20.0° (*c*=2.00, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{16}H_{28}O_8Na$ $(M+Na)^+$ 371.1682; Found 371.1690. UV [MeOH, nm (log *ε*)]: 215 (4.16). IR (KBr): 3410, 2940, 1647, 1085, 1043 cm^{-1}. ^{1}H-NMR (500 MHz, CD_3OD) *δ*: given in Table 3. ^{13}C-NMR (125 MHz, CD_3OD) δ_C: given in Table 4. Positive-ion FAB-MS: *m/z* 371 $(M+Na)^+$. Negative-ion FAB-MS: *m/z* 347 $(M-H)^-$.

Acid Hydrolysis of 1—4 and 6 with 1 M HCl A solution of **1**—**4** or **6** (each 1.5 mg) in 1 M HCl (0.5 ml) was heated under reflux for 3 h. After cooling, the reaction mixture was poured into ice-water and neutralized with Amberlite IRA-400 (OH^- form), and the resin was removed by filtration. Then, the filtrate was extracted with EtOAc. The aqueous layer was subjected to HPLC analysis under the 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 (75 : 25, v/v); flow rate 0.8 ml/min; column temperature, room temperature. Identification of D-glucose present in the aqueous layer was carried out by comparison of its retention time and optical rotation with those of an authentic sample: t_R=12.3 min (positive optical rotation).

Enzymatic Hydrolysis of 1, 4, and 6 with *β*-Glucosidase A solution of **1** (7.7 mg) in H_2O (1.5 ml) was treated with *β*-glucosidase (5.0 mg, from almond, Oriental Yeast Co., Tokyo, Japan) and the solution was stirred at 37 °C for 3 d. After EtOH was added to the reaction mixture, the solvent was removed under reduced pressure and the residue was purified by HPLC [MeOH–H_2O (55 : 45, v/v)] to furnish kankagenin a (**1a**, 2.3 mg, 56%). Through a similar procedure, (*R*)-rotundiol[33,34] (**4a**, 1.2 mg, 69%) and (2*E*,6*R*)-8-hydroxy-2,6-dimethyl-2-octenoic acid[36] (**6a**, 6.8 mg, 62%) were obtained from **4** (3.5 mg) and **6** (20.4 mg), respectively.

Kankagenin a (**1a**): A white powder, $[\alpha]_D^{25}$ −18.4° (*c*=0.20, MeOH). High-resolution EI-MS: Calcd for $C_{10}H_{16}O_3$ (M^+) 184.1099; Found 184.1106. IR (KBr): 3410, 2940, 1684 cm^{-1}. ^{1}H-NMR (500 MHz, CD_3OD) *δ*: given in Table 1. ^{13}C-NMR (125 MHz, CD_3OD) δ_C: given in Table 2. EI-MS: *m/z* (%): 184 $(M^+, 37)$, 95 (100).

Alkaline Treatment of 14 with 5% aqueous KOH A solution of **14** (23.0 mg) in 5% aqueous KOH (1.0 ml) was stirred at 80 °C for 2 h. The reaction mixture was neutralized with Amberlite HCR-W2 (H^+ form). Removal of the solvent from the filtrate under reduced pressure furnished a residue, which was purified by HPLC [MeOH–H_2O (5 : 95, v/v)] to give **2** (4.0 mg, 16%) and **19**[32] (10.5 mg, 43%).

Acid Treatment of 14 with 5% aqueous HCl A solution of **14** (25.0 mg) in 5% aqueous HCl (2.0 ml) was stirred at room temperature for 3 h. The reaction mixture was poured into ice-water and the whole reaction mixture was extracted with EtOAc. The EtOAc extract was successively washed with saturated aqueous $NaHCO_3$ and brine, then dried over anhydrous $MgSO_4$ powder and filtered. Removal of the solvent from the filtrate under reduced pressure furnished a residue, which was separated by HPLC [MeOH–H_2O (20 : 80, v/v)] to give **14a** (4.0 mg, 25%).

14a: A white powder, $[\alpha]_D^{20}$ +21.5° (*c*=0.30, MeOH). High-resolution CI-MS: Calcd for $C_9H_{14}ClO_4$ $(M+H)^+$ 221.0580; Found 221.0587. IR (KBr): 3410, 2962, 1365, 1152, 1055, 945 cm^{-1}. ^{1}H-NMR (500 MHz, CD_3OD) *δ*: given in Table 1. ^{13}C-NMR (125 MHz, CD_3OD) δ_C: given in Table 2. CI-MS: *m/z* (%): 221 $(M+H)^+$ (7), 223 $(M+H)^+$ (3), 203 $(M-H_2O)^+$ (97), 205 $(M-H_2O)^+$ (33), 185 (7), 167 (12), 159 (32), 121

(27), 110 (48), 95 (64), 85 (100), 67 (56), and 57 (65).

Acetylation of 5 and 14a A solution of **5** (2.5 mg) in pyridine (0.5 ml) was treated with acetic anhydride (Ac_2O, 0.4 ml) and the mixture was stirred at room temperature for 12 h. The reaction mixture was poured into ice-water and the whole reaction mixture was extracted with EtOAc. The EtOAc extract was successively washed with 5% aqueous HCl, saturated aqueous $NaHCO_3$ and brine, then dried over anhydrous $MgSO_4$ powder and filtered. Removal of the solvent from the filtrate under reduced pressure furnished a residue, which was purified by HPLC [MeOH–H_2O (35 : 65, v/v)] to give **5a** (2.3 mg, 77%). Through the similar procedure, **14b** (2.7 mg, 87%) was also prepared and purified by HPLC [MeOH–H_2O (55 : 45, v/v)] from **14a** (2.1 mg).

5a: A white powder, $[\alpha]_D^{20}$ +1.8° (c=0.18, MeOH). High-resolution CI-MS: Calcd for $C_{11}H_{15}O_5$ $(M+H)^+$ 227.0919; Found 227.0925. IR (KBr): 2962, 1734, 1374, 1258, 1237, 1169, 1103, 1053, 947 cm^{-1}. ^{1}H-NMR (500 MHz, CD_3OD) δ: given in Table 1. ^{13}C-NMR (125 MHz, CD_3OD) δ_C: given in Table 2. CI-MS m/z (%): 227 $(M+H)^+$ (28), 209 $(M-H_2O)^+$ (4), 184 (3), 166 (45), 149 (22), 138 (38), 122 (44), 94 (31), 85 (100), and 57 (34).

14b: A white powder, $[\alpha]_D^{20}$ +23.7° (c=0.06, MeOH). High-resolution CI-MS: Calcd for $C_{13}H_{18}ClO_6$ $(M+H)^+$ 305.0792; Found 305.0790. IR (KBr): 1744, 1376, 1243, 1231, 1001, 941 cm^{-1}. ^{1}H-NMR (500 MHz, CD_3OD) δ: given in Table 1. ^{13}C-NMR (125 MHz, CD_3OD) δ_C: given in Table 2. CI-MS: m/z (%): 305 $(M+H)^+$ (2), 307 $(M+H)^+$ (1), 263 $(M+H-C_2H_3O)^+$ (6), 265 $(M+H-C_2H_3O)^+$ (3), 245 (30), 203 (8), 185 (100), 167 (7), 149 (33), 121 (26), 95 (15), 85 (37), and 57 (93).

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References and Notes

1) Kobayashi H., Oguchi H., Takizawa N., Miyase T., Ueno A., Usmanghani K., Ahmad M., *Chem. Pharm. Bull.*, **35**, 3309—3314 (1987).
2) Xinjiang Science and Technology Press, "Culture Techniques of Xinjiang Staple Medicinal Plants," Xinjiang Institute of Traditional Chinese and Ethnologic Medicines Ed., 2004, pp. 84—88.
3) Du N., Zhou P., Wang J., Liu C., Li W., *Zhongguo Yaoke Daxue Xuebao*, **24**, 46—48 (1993).
4) Xue D., *Zhongguo Zhongyao Zazhi*, **22**, 170—171 (1997).
5) Song Z., Mo S., Chen Y., Tu P., Li W., Zhao Y., Zheng J., *Zhongguo Zhongyao Zazhi*, **25**, 728—730 (2000).
6) Song Z., Tu P., Zhao Y., Zheng J., *Zhongcaoyao*, **31**, 808—810 (2000).
7) Yoshizawa F., Deyama T., Takizawa N., Usmanghani K., Ahmad M., *Chem. Pharm. Bull.*, **38**, 1927—1930 (1990).
8) Matsuda H., Morikawa T., Tao J., Ueda K., Yoshikawa M., *Chem. Pharm. Bull.*, **50**, 208—215 (2002).
9) Morikawa T., Matsuda H., Toguchida I., Ueda K., Yoshikawa M., *J. Nat. Prod.*, **65**, 1468—1474 (2002).
10) Tao J., Morikawa T., Toguchida I., Ando S., Matsuda H., Yoshikawa M., *Bioorg. Med. Chem.*, **10**, 4005—4012 (2002).
11) Morikawa T., Tao J., Ando S., Matsuda H., Yoshikawa M., *J. Nat. Prod.*, **66**, 638—645 (2003).
12) Tao J., Morikawa T., Ando S., Matsuda H., Yoshikawa M., *Chem. Pharm. Bull.*, **51**, 654—662 (2003).
13) Matsuda H., Morikawa T., Xie H., Yoshikawa M., *Planta Med.*, **70**, 847—855 (2004).
14) Sun B., Morikawa T., Matsuda H., Tewtrakul S., Harima S., Yoshikawa M., *J. Nat. Prod.*, **67**, 1464—1469 (2004).
15) Morikawa T., Sun B., Matsuda H., Wu L. J., Harima S., Yoshikawa M., *Chem. Pharm. Bull.*, **52**, 1194—1199 (2004).
16) Xie H., Wang T., Matsuda H., Morikawa T., Yoshikawa M., Tani T., *Chem. Pharm. Bull.*, **53**, 1416—1422 (2005).
17) Morikawa T., Xie H., Matsuda H., Yoshikawa M., *J. Nat. Prod.*, **69** (2006) in press.
18) Morikawa T., Xie H., Matsuda H., Wang T., Yoshikawa M., *Chem. Pharm. Bull.*, **54**, 506—513 (2006).
19) Kobayashi H., Karasawa H., Miyase T., Fukushima S., *Chem. Pharm. Bull.*, **33**, 3645—3650 (1985).
20) Otsuka H., *Phytochemistry*, **33**, 617—622 (1993).
21) Zhao W., Yang G., Xu R., Qin G., *Phytochemistry*, **41**, 1553—1555 (1996).
22) Kobayashi H., Karasawa H., Miyase T., Fukushima S., *Chem. Pharm. Bull.*, **32**, 1729—1734 (1984).
23) Xu Z., Yang S., Yang J., Lu R., *Zhongcaoyao*, **30**, 244—246 (1999).
24) Wang S. J., Pei Y. H., Hua H. M., *Chin. Chem. Lett.*, **12**, 343—344 (2001).
25) Those known compounds were identified by comparison of their physical data with commercially obtained samples.
26) Pires R., Burger K., *Tetrahedron*, **53**, 9213—9218 (1997).
27) Kamal A., Ramana K. V., Ramana A. V., Babu A. H., *Tetrahedron: Asymmetry*, **14**, 2587—2594 (2003).
28) The ^{1}H- and ^{13}C-NMR spectra of **1**—**6** and related compounds (**19**, **1a**, **5a**, **14a**, **14b**) were assigned with the aid of distortionless enhancement by polarization transfer (DEPT), double quantum filter correlation spectroscopy (DQF COSY), heteronuclear multiple quantum coherence (HMQC), and heteronuclear multiple bond connectivity (HMBC) experiments.
29) Nishizawa M., Kodama S., Yamase Y., Kayano K., Hatakeyama S., Yamada H., *Chem. Pharm. Bull.*, **42**, 982—984 (1994).
30) Yoshikawa M., Ueda T., Matsuda H., Yamahara J., Murakami N., *Chem. Pharm. Bull.*, **42**, 1691—1693 (1994).
31) Matsuda H., Shimoda H., Uemura T., Ueda T., Yamahara J., Yoshikawa M., *Chem. Pharm. Bull.*, **47**, 1753—1758 (1999).
32) Damtoft S., Jensen S. R., Nielsen B. J., *Phytochemistry*, **24**, 2281—2283 (1985).
33) Watanabe K., Takada Y., Matsuo N., Nishimura H., *Biosci. Biotech. Biochem.*, **59**, 1979—1980 (1995).
34) Takikawa H., Yamazaki Y., Mori K., *Eur. J. Org. Chem.*, 229—232 (1998).
35) Kitagawa I., Fukuda Y., Taniyama T., Yoshikawa M., *Chem. Pharm. Bull.*, **39**, 1171—1176 (1991).
36) Yamaguchi K., Shinohara C., Kojima S., Sodeoka M., Tsuji T., *Biosci. Biotech. Biochem.*, **63**, 731—735 (1999).