Iridoid and Acyclic Monoterpenoid Glycosides, Kankanosides L, M, N, O, and P from *Cistanche tubulosa*  
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Three iridoid glycosides, kankanosides L, M, and N, and two acyclic monoterpenoid glycosides, kankanosides O and P, were isolated from fresh stems of *Cistanche tubulosa* (Orobanchaceae) together with eight iridoid glycosides, five acyclic monoterpenoid glycosides, three phenylpropanoid glycosides, and four lignan glycosides. Their structures were elucidated on the basis of chemical and physicochemical evidence.

**Key words** *Cistanche tubulosa*; kankanoside; iridoid; acyclic monoterpenoid; Orobanchaceae

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*Cistanche tubulosa* (SCHRENK) R. WIGHT (Orobanchaceae) is a perennial parasitic plant growing on roots of *Salvadora* or *Calotropis* species, distributed in North Africa, Arabia, and Asian countries.1 stems of this plant (Kanka-nikujyou in Japanese) have traditionally been used for treatment of impotence, sterility, lumbago, and body weakness as well as a promoting agent of blood circulation.1,2 During the course of our studies on bioactive constituents from stems of *C. tubulosa*,3–6 we previously reported twenty-four phenylethanoid oligoglycosides including kankanosides H1, H2, I, J1, J2, K1, K2, and K3, and two acylated oligosugars from fresh stems of *C. tubulosa*.5,6 Furthermore, principal phenylethanoid glycosides, echinacoside, acteoside, and isoacteoside, were found to inhibit increase in serum aspartate aminotransferase (sAST) and alanine aminotransferase (sALT) levels in the mice’s injured liver induced by d-galactosamine (d-GalN)/lipopolysaccharide at doses of 25—100 mg/kg per os (p.o.). Structural requirements of the phenylethanoid glycosides for the hepatoprotective activity were also elucidated.5,7 In this continuing study on constituents in the fresh stems of *C. tubulosa*, we further isolated eleven iridoid glycosides including kankanosides L (1), M (2), and N (3), seven acyclic monoterpenophyglycosides including kankanosides O (4) and P (5), three phenylpropanoids, and four lignans. This paper deals with isolation and structure elucidation of five new compounds (1—5).

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 described previously.5,8 The MeOH-eluted fraction was subjected to SiO2 and ODS column chromatographies and finally HPLC to furnish kankanosides L (1, 0.0026%), M (2, 0.0001%), N (3, 0.0007%), O (4, 0.0020%), and P (5, 0.0002%), 6-deoxycatalpol13,17 (6, 0.197%), bartsioside17 (7, 0.0583%), gluroside17 (8, 0.0443%), kankanoiside17 (9, 22.3 mg, 0.0010%), massaeasidic acid17 (10, 0.0056%), 8-epiloganic acid17 (11, 0.0023%), 8-epideoxyloganic acid17 (12, 0.0004%), geniposidic acid17 (13, 0.0040%), kankanoside17 (E3) (14, 0.0026%), (2E,6Z)-8-β-D-glucopyranosylxylo-2,6-dimethyl-2,6-octadienoic acid18 (15, 31.0 mg, 0.0014%), (2E,6E)-3,7-dimethyl-8-hydroxyoctadien-1-yl-O-β-D-glucopyranoside19 (16, 0.0082%), 8-hydroxygeraniol19 (8-β-D-glucopyranoside20 (17, 0.0044%), betulalbuside20 (A12, 0.0004%), coniferin13 (19, 0.0002%), syringin13 (20, 0.0015%), sinapic aldehyde14 (O-β-D-glucopyranoside21 (21, 0.0001%), (+)-pinoresinol14 (O-β-D-glucopyranoside22 (22, 0.010%), eucommia23 (23, 0.0002%), isoeucommia17 (24, 0.0010%), and (2E)-syringaresinol14 (O-β-D-glucopyranoside25 (25, 0.0044%).

**Structures of Kankanosides**  
1 (L, M, and N)  
Kankanoside L (1) was obtained as a white powder with negative optical rotation ([α]D25 −45.7 in MeOH). Its IR spectrum showed strong absorption band at 3433 and 1080 cm−1 suggestive of a glycoside moiety. The fast atom bombardment (FAB)-MS of 1 run in the positive- and negative-ion modes showed quasimolecular ion peaks at [M −H]+ 371 and 347 [M −H]−, respectively, and the molecular formula was determined as C16H20O8 by high-resolution FAB-MS measurement. Acid hydrolysis of 1 with 1.0 M hydrochloric acid (HCl) liberated β-glucose, which was identified by 1H-NMR and 13C-NMR spectra. The 1H- and 13C-NMR spectra of 1 (CD3OD, Tables 1, 2) were assigned by various NMR experiments,10,19 showed signals assignable to four methylenes (δ 1.43 (1H, br dd, J= ca. 5, 13 Hz, 4α-H), 1.73 (1H, br dd, J= ca. 12, 14 Hz, 6α-H), 1.85 (1H, m, 4β-H), 1.93 (1H, br dd, J= ca. 8, 14 Hz, 6β-H), 3.50 (1H, dd, J= 2.4, 12.5, 13.0 Hz, 3α-H), 3.83 (1H, br dd, J=...
correlation spectroscopy (1H–1H COSY) experiment on 4.70 (d, 5, 13 Hz, 3 ca. 1404 V ol. 58, No. 10 10-H2), two methines [J (HMBC) experiment on 6.16 (1H, m, 5-H)], and an acetal group [d (ca. 12, 14)] together with a 3-glucopyranosyl moiety [d (both d, 13.1)] as shown in Fig. 1. Next, the relative stereostructure of 1 was characterized by phase-sensitive nuclear Overhauser enhancement spectroscopy (phase-sensitive NOESY) experiment, which showed NOE correlations between the following proton pairs (1-H and 3-C, 8-C; 1-H and 4-C; 3-H and 1-C; 7-H and 8-C, 10-C; 9-H and 8-C; as shown in Fig. 1. The 1H- and 13C-NMR spectra of 1 were superimposable on those of principal iridoid constituent 6-deoxy-catalpol (6), except for the signals due to the saturated —3 dihydro-6-deoxycatalpol (1). Kankanoside M (2) was obtained as a white powder with negative optical rotation ([\(\alpha\)]D 26° 18.7 in MeOH). The IR spectrum of 2 showed absorption bands at 3433, 1736, 1655, 1176 cm\(^{-1}\) ascribable to hydroxyl, \(\delta\)-lactone, olefin, and ether moieties. The positive-ion FAB-MS spectrum of 2 gave [M + Na\(^+\)] as shown in Table 2. The 13C-NMR spectra of 2 (CD\(_3\)OD, Tables 1, 2) showed signals assignable to four methylenes [\(\delta\) 1.66, 2.11 (1H each, both 3 ca. 120)] and 1076 cm\(^{-1}\) ascribable to hydroxyl, \(\delta\)-lactone, olefin, and ether moieties. The positive-ion FAB-MS spectrum of 2 gave [M + Na\(^+\)] as shown in Table 2. The 13C-NMR spectra of 2 (CD\(_3\)OD, Tables 1, 2) showed signals assignable to four methylenes [\(\delta\) 1.66, 2.11 (1H each, both
correlation spectroscopy (1H–1H COSY) experiment on 4.70 (d, 5, 13 Hz, 3 ca. 1404 V ol. 58, No. 10 10-H2), two methines [J (HMBC) experiment on 6.16 (1H, m, 5-H)], and an acetal group [d (ca. 12, 14)] together with a 3-glucopyranosyl moiety [d (both d, 13.1)] as shown in Fig. 1. Next, the relative stereostructure of 1 was characterized by phase-sensitive nuclear Overhauser enhancement spectroscopy (phase-sensitive NOESY) experiment, which showed NOE correlations between the following proton pairs (1-H and 3-C, 8-C; 1-H and 4-C; 3-H and 1-C; 7-H and 8-C, 10-C; 9-H and 8-C; as shown in Fig. 1. The 1H- and 13C-NMR spectra of 1 were superimposable on those of principal iridoid constituent 6-deoxycatalpol (6), except for the signals due to the saturated —3 dihydro-6-deoxycatalpol (1). Kankanoside M (2) was obtained as a white powder with negative optical rotation ([\(\alpha\)]D 26° 18.7 in MeOH). The IR spectrum of 2 showed absorption bands at 3433, 1736, 1655, 1176 cm\(^{-1}\) ascribable to hydroxyl, \(\delta\)-lactone, olefin, and ether moieties. The positive-ion FAB-MS spectrum of 2 gave [M + Na\(^+\)] as shown in Table 2. The 13C-NMR spectra of 2 (CD\(_3\)OD, Tables 1, 2) showed signals assignable to four methylenes [\(\delta\) 1.66, 2.11 (1H each, both
correlation spectroscopy (1H–1H COSY) experiment on 4.70 (d, 5, 13 Hz, 3 ca. 1404 V ol. 58, No. 10 10-H2), two methines [J (HMBC) experiment on 6.16 (1H, m, 5-H)], and an acetal group [d (ca. 12, 14)] together with a 3-glucopyranosyl moiety [d (both d, 13.1)] as shown in Fig. 1. Next, the relative stereostructure of 1 was characterized by phase-sensitive nuclear Overhauser enhancement spectroscopy (phase-sensitive NOESY) experiment, which showed NOE correlations between the following proton pairs (1-H and 3-C, 8-C; 1-H and 4-C; 3-H and 1-C; 7-H and 8-C, 10-C; 9-H and 8-C; as shown in Fig. 1. The 1H- and 13C-NMR spectra of 1 were superimposable on those of principal iridoid constituent 6-deoxycatalpol (6), except for the signals due to the saturated —3 dihydro-6-deoxycatalpol (1). Kankanoside M (2) was obtained as a white powder with negative optical rotation ([\(\alpha\)]D 26° 18.7 in MeOH). The IR spectrum of 2 showed absorption bands at 3433, 1736, 1655, 1176 cm\(^{-1}\) ascribable to hydroxyl, \(\delta\)-lactone, olefin, and ether moieties. The positive-ion FAB-MS spectrum of 2 gave [M + Na\(^+\)] as shown in Table 2. The 13C-NMR spectra of 2 (CD\(_3\)OD, Tables 1, 2) showed signals assignable to four methylenes [\(\delta\) 1.66, 2.11 (1H each, both
m, 4-H2), 2.15, 2.75 (1H each, both m, 6-H2), 4.29 (1H, ddd, J=2.8, 8.4, 14.3 Hz, 3J-H), 4.32, 4.51 (1H each, both d, J=13.1 Hz, 10-H2), 4.35 (1H, ddd, J=3.1, 6.7, 14.3 Hz, 3a-H), two methines [δ 2.97 (1H, m, 5-H)], 3.82 (1H, brs, 9-H)], an olefin [δ 3.94 (1H, m, 7-H)], and a saturated lactone group (δc 174.9) together with a β-D-glucopyranosyl moiety [δ 4.32 (1H, d, J=7.9 Hz, 1a-H)].  As shown in Fig. 1, the 1H-1H COSY experiment 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 and 1-C; 7-H and 9-C; 10-H and 1-C; 8-C and 9-C; 1-C and 8-C; 2-C and 3-C; 1-C and 7-C), the relative stereostructure of 2 was characterized by phase-sensitive NOESY experiment, which showed NOE correlations between the following proton pairs (3a-H and 4a-H; 3b-H and 4b-H; 4b-H and 5-H; 5-H and 6b-H; 9-H) as shown in Fig. 1. Thus, the stereostructure of 2 was elucidated as shown.

Kankanoside N (3) was isolated as a white powder with negative optical rotation ([α]D25 = -24.6 in MeOH). In the positive-ion FAB-MS of 3, a quasimolecular ion peak was observed at m/z 371 [M+Na]+. The molecular formula C16H28O8 was determined by high-resolution FAB-MS measurement. Acid hydrolysis of 3 with 1.0 M HCl liberated α-glucose. The 1H- and 13C-NMR data (CD3OD, Tables 1, 2) showed signals assignable to a methyl [δ 1.07 (3H, d, J=7.2 Hz, 10-H3)], four methylenes [δ 1.37, 1.87 (1H each, both m, 7-H2), 1.65, 1.81 (1H each, both m, 6-H2)], [3.65 (1H, dd, J=9.1, 9.8 Hz), 3.90 (1H, dd, J=5.9, 9.8 Hz), 11-H2], and [3.70 (1H, dd, J=3.3, 12.0 Hz), 3.88 (1H, m, 3-H)], four methylenes [δ 1.69 (1H, m, 4-H), 1.75 (1H, m, 9-H)], 2.06 (1H, m, 8-H), 2.16 (1H, m, 5-H)], and a hemiacetal group [δ 4.67 (1H, d, J=7.4 Hz, 1H)], together with a β-D-glucopyranosyl moiety [δ 4.26 (1H, d, J=7.9 Hz, 1a-H)]. The iridoid structure of 3 was clarified by 1H-1H COSY and HMBC experiments and the relative stereostructure was characterized by phase-sensitive NOESY experiment as shown in Fig. 1. Consequently, the stereostructure of 3 was elucidated as shown.

Structures of Kankanosides O (4) and P (5) Kankanosides O (4) and P (5), C16H28O8, were also obtained as white powders with negative optical rotations (4: [α]D25 = -26.1; 5: [α]D25 = -32.7 both in MeOH). The IR spectra of 4 and 5 showed absorption bands at 3433, 1696, 1647, and 1076 cm⁻¹ for 4, and at 3434, 1701, 1647, and 1076 cm⁻¹ for 5, ascribable to glycosidic, carbonyl, and olefin functions. Their UV spectra showed common absorption maximum at 217 nm indicating the presence of an α,β-unsaturated carboxylic acid moiety in both of them. Acid hydrolysis of 4 and 5 liberated α-glucose, whereas by the enzymatic hydrolysis with β-glucosidase, 4 and 5 gave (2E,6E)-8-hydroxy-2,6-dimethyl-2,6-octadienoic acid (4a) and (2E,6E)-8-hydroxy-3,7-dimethyl-2,6-octadienoic acid (5a), respectively. 1H- and 13C-NMR data of 4 (CD3OD, Tables 2, 3) showed signals assignable to two methyls [δ 1.71 (3H, brs, 10-H3)], 1.70 (3H, brs) with those of 4a, a glycosylation shift was observed at the 8-position (δc 4: 65.5; 4a: 59.4). The position of the glucoside linkage was also confirmed by HMBC experiments as shown in Fig. 2. Consequently, the stereostructure of 4 was clarified to be (2E,6E)-8-β-D-glucopyranosyloxy-2,6-dimethyl-2,6-octadienoic acid. On the other hand, the 1H- and 13C-NMR data of 5 (CD3OD, Tables 2, 3) indicated the presence of a (2E,6E)-8-hydroxy-3,7-dimethyl-2,6-octadienoic acid moiety (δ 1.70 (3H, brs, 10-H3), 2.14 (3H, brs, 9-H2), 2.24 (2H, m, 4-H2), 2.27 (2H, m, 5-H2), 4.05, 4.20 (1H each, both br d, J=ca. 12 Hz, 8-H2), 5.47 (1H, t, J=7.1, 0.9 Hz, 6-H2), 5.67 (1H, brs, 1-H)] together with a β-D-glucopyranosyl part [δ 4.23 (d, J=7.7 Hz, 1a-H)]. The connectivity of the β-D-glucopyranosyl moiety in 5 was elucidated on the basis of HMBC experiments as shown in Fig. 2. Furthermore, a typical glycosylation shift was observed for the signals at 8-position (δc 5: 75.6; 5a: 68.5). On the basis of the above-mentioned evidence, the stereostructure of 5 was determined to be (2E,6E)-8-β-D-glucopyranosyloxy-3,7-dimethyl-2,6-octadienoic acid.

Effects of the Constituents on Tumor Necrosis Factor-α (TNF-α)-induced Cytotoxicity in L929 Cells TNF-α is known to mediate a variety of organ injury through its induction of cellular apoptosis. In the case of liver, the biological effects of TNF-α have been implicated in hepatic injury induced by hepatic toxins, ischemia/reperfusion, viral hepatitis, and alcohol. Therefore, TNF-α is considered to be an important target to discover anti-inflammatory and hepatoprotective agents. On the basis of above-mentioned concept, we investigated protective constituents from naturally occurring products on TNF-α-induced cell death in L929 cells, a TNF-α-sensitive cell line. Previously, we have reported

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Table 3. 1H-NMR Data (600 MHz, CD3OD) for Kankanosides O (4) and P (5)
that several constituents from *Piper chaba*, Boesenbergia rotundata, *Panica granatium*, Helichrysum arenarium, and Sapindus rarak were found to show inhibitory effects of TNF-α-induced cytotoxicity in L929 cells. Since the phenylethanoid constituents of *C. tubulosa* (e.g. echinacoside, acetoxyis, and isoacetoxyis, etc.) were also inhibited this cytotoxicity, we further examined iridoid, phenylpropanoid, and lignin constituents as shown in Table 4. As the result, kankanoside A (9), inhibition: 16.3 ± 2.0% at (μm), masuenaesic acid (10, 44.7 ± 8.7%), 8-epilagionic acid (11, 10.7 ± 0.4%), 8-hydroxygeraniol 8-β-O-glucopyranoside (17, 21.3 ± 2.4%), and (+)-pinoresinol O-β-glucopyranoside (22, 22.3 ± 1.6%), were found to show significant activity. Although their activities were weaker than those of echinacoside (IC50 = 31.1 μm), acetoxyis (17.8 μm), and isoacetoxyis (22.7 μm), the principle phenylethanoid constituents.

**Experimental**

The following instruments were used to obtain spectral and physical data: specific rotations, Horiba SEPA-300 digital polarimeter (l=5 cm); UV spectr., 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 5C18-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 Chromatograph ODS DM1020T (Fujii Sylux 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–NAP, CH3CN–1% aqueous AcOH (10:90, v/v) 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.

**Plant Material** This item was described in a previous report.

**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, H2O→MeOH) to give H2O- and MeOH-eluted fractions (167.84 g, 6.53% 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 (15:3.0:4.1:0:5→6:4:1, v/v/v)) 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. The fraction 1 (1.12 g) was separated by reversed-phase silica gel CC [55 g, MeOH–H2O (40:60, v/v→MeOH)] to give five fractions [Fr. 1–1 (13.28 g), 1–2 (267.0 mg), 1–3 (182.8 mg), 1–4 (310.9 mg), and 1–5 (91.4 mg)]. The fraction 1–1 (13.28 mg) was further purified by HPLC [Cosmosil 5C18-MS-II, CH3CN–1% aqueous AcOH (10:90, v/v)] to give kankanoside M (2, 2.8 mg, 0.0001%), bistetoside (7, 5.2 mg, 0.0002%), sinapiolic acid 4-O-β-D-glucopyranoside (21, 1.4 mg, 0.0001%). The fraction 1–2 (267.0 mg) was further purified by HPLC [Cosmosil 5C18-MS-II, CH3CN–1% aqueous AcOH (7:93, v/v) to give (2E,6E)-3,7-dimethyl-8-hydroxyoctadec-1-yl-O-β-D-glucopyranoside (16, 15.8 mg, 0.0003%), 8-hydroxygeraniol 8-β-D-glucopyranoside (17, 5.2 mg, 0.0002%), (+)-pinoresinol O-β-D-glucopyranoside (25, 98.0 mg, 0.0044%). The fraction 2 (9.56 g) was separated by reversed-phase silica gel CC [400 g, MeOH–H2O (10:90, v/v→MeOH→acetone)] to give five fractions [Fr. 2–1 (55.9 mg), 2–2 (4.48 mg), 2–3 (3.42 mg), 2–4 (1.16 g), and 2–5 (31.9 mg)], as was described previously. The fraction 2–2 (500.0 mg) was subjected to HPLC [Cosmosil 5C18-MS-II, CH3CN–1% aqueous AcOH (7:93, v/v)] to give seven fractions [Fr. 2–3-1 (66.5 mg), 2–3-2 (20.4 mg), 2–3-3 (26.0 mg), 2–3-4 (136.6 mg), 2–3-5 (785.6 mg), 2–3-6 (825.1 mg, 0.045%), and 2–3-7 (849.3 mg)]. The fraction 2–3-3 (136.6 mg) was further purified by HPLC [Cosmosil πNAP, CH3CN–1% aqueous AcOH (7:93, v/v→MeOH→acetone)] to give six fractions [Fr. 2–3-3-1 (64.8 mg, 0.0130%) by hydrolysis with salicidioside (13.7 mg, 0.0027%). The fraction 2–4 (1.16 g) was subjected to HPLC [Cosmosil 5C18-MS-II, CH3CN–1% aqueous AcOH (15:85, v/v→MeOH→acetone) and CH3CN πNAP, CH3CN–1% aqueous AcOH (10:90 or 15:85, v/v→MeOH→acetone)] to give kankanoside N (3, 15.6 mg, 0.0007%), O (4, 44.1 mg, 0.0020%), and P (5, 4.2 mg, 0.0002%), 6 (24.0 mg, 0.0011%), 8 (17.7 mg, 0.0008%), kankanoside A (9, 22.3 mg, 0.0001%), 8-epi-octoylacetic acid (12, 8.1 mg, 0.0004%), kankanoside E (14, 58.1 mg, 0.0026%), (2E,6Z)-8-β-D-glucopyranosylgeraniol (15, 310.9 mg, 0.0001%), (16, 168.6 mg, 0.0075%), 17 (94.3 mg, 0.0002%), betulatin A (18, 8.1 mg, 0.0004%), coniferin (19, 3.8 mg, 0.0002%), and syringin (20, 33.9 mg, 0.0015%). The fraction 4 (10.69 g) was separated by reversed-phase silica gel CC [500 g, MeOH–H2O (30:70, v/v→MeOH→acetone)] to give four fractions [Fr. 4–1 (878.2 mg), 4–2 (706.7 mg), 4–3 (1.57 g), and 4–4 (792.8 mg)], as was described previously. The fraction 4–1 (500.0 mg) was purified by HPLC [Cosmosil 5C18-MS-II, CH3CN–1% aqueous AcOH (100:90, v/v→MeOH→acetone)] to give masuenaesic acid (10, 39.1 mg, 0.0031%) and geniposide acid (13, 51.2 mg, 0.0040%). The fraction 5 (8.84 g) was separated by reversed-phase silica gel CC [400 g, MeOH–H2O (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 (7.32 g), 5–4
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Japan. High-resolution positive-ion FAB-MS: C_{4}H_{8}O_{3}Na [M+Na]^{+} 371.1318; Found 371.1309. 1H-NMR (600 MHz, CD_{3}OD) δc: given in Table 1. Positive-ion FAB-MS m/z: 371 [M+Na]^{+}. Negative-ion FAB-MS m/z 347 [M–H].

Kankanoside M (2): A white powder, [α]_{D}^{23} = –18.7 (c = 0.11, MeOH). High-resolution positive-ion FAB-MS: C_{4}H_{8}O_{3}Na [M+Na]^{+} 369.1525; Found 369.1528. UV [λ_{max} (log ε), MeOH, nm]: 279 (4.04). IR (KBr, cm$^{-1}$): 3433, 3292, 2928, 1720, 1076, 1028, 977, 696, 658. 1C-NMR (150 MHz, CD_{3}OD) δc: given in Table 3. Positive-ion FAB-MS m/z: 369 [M+Na]^{+}. A fraction 5-1 (870.2 mg) was further purified by HPLC (Shodex OR-2, 10% palladium carbon (5.0 mg) in MeOH (2.0 ml) was stirred at room temperature for 2.5 h, and the filtrate was condensed under reduced pressure to give 1 (12.0 mg, quant.).

**Enzymatic Hydrolysis of Kankanosides O and P with β-Glucosidase** To a solution of 4 (8.0 mg) in H_{2}O (1.5 ml) was added β-glucosidase (4.7 mg, from almond, Orient Yeast Co., Tokyo, Japan), and the solution was stirred at 37°C for 24 h. The reaction was quenched by the addition of EtOH (5.0 ml), the mixture was condensed under reduced pressure. The residue was extracted with EtOAc and evaporation of the solvent gave (2E,6E)-8-hydroxy-2,6-dimethyl-2,6-octadienocic acid(30) (4a, 3.9 mg, 92%). Through a similar procedure, (2E,6S)-8-hydroxy-3,7-dimethyl-2,6-octadienocic acid(31) (5a, 0.9 mg, 94%) was obtained from kankanoside P (5, 1.8 mg).

**Bioassay Method** Inhibitory effect on TNF-α-induced cytotoxicity in L929 cells was assayed by the method described in a previous paper. The fraction 1–5 was assigned with the aid of distortionless enhancement by polarization transfer (DEPT), hydrogen multiple quantum coherence (HMQC), and heteronuclear multiple bond connectivity (HMBC) experiments.

**References and Notes**


19) The 1H- and 13C-NMR spectra of Kankanoside L—P (1—5) were previously. The 1H- and 13C-NMR spectra of Kankanoside L—P (1—5) were previously. The 1H- and 13C-NMR spectra of Kankanoside L—P (1—5) were previously. The 1H- and 13C-NMR spectra of Kankanoside L—P (1—5) were previously.