Bioactive Constituents from Chinese Natural Medicines. XXVIII.[1] Chemical Structures of Acyclic Alcohol Glycosides from the Roots of *Rhodiola crenulata*

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Five new glycosides, creosides I, II, III, IV, and V, were isolated from the methanolic extract of the roots of *Rhodiola crenulata*, together with 21 known compounds. The chemical structures of new constituents were elucidated on the basis of chemical and physicochemical evidence.

Key words Rhodiolae Radix; *Rhodiola crenulata*; creoside; glycoside; Chinese medicine; Crassulaceae

The roots of the alpine plant genus *Rhodiola* (Crassulaceae), Rhodiolae Radix, have been widely used as a hemostatic, antibechic, tonic, and endermic liniment for burns and contusions in traditional Chinese medicine. During the course of our serial studies on the bioactive constituents from Chinese natural medicines,[2—11] we have characterized the structures of several flavonol bisdesmosides, monoterpene glycosides, and cyanoglycosides from the roots of *Rhodiola quadrifida* (PALL.) FISCH. *et* MAY, *Rhodiola sacra* (PRAIN ex HAMET) S. H. FU, and *Rhodiola sachalinensis* A. BOR.[2,12,13] In particular, the flavonol bisdesmosides, sachalosides III and IV, from the roots of *R. sachalinensis* were found to show a protective effect against D-galactosamine-induced cytotoxicity in primary cultured mouse hepatocytes.[2] In addition, the monoterpene oligoglycosides, sacranosides A and B, from the roots of *R. sacra* were found to inhibit histamine release from rat exudate cells induced by an antigen–antibody reaction.[13] As part of our continuing study of the constituents of Rhodiolae Radix, five new glycosides, creosides I, II, III, IV, and V, were isolated from the methanolic extract of the roots of *Rhodiola crenulata* (HOOK. f. *et* THOMS.) H. OHBA, together with 21 known compounds. In this paper, we describe the isolation and structural elucidation of the new constituents (**1**—**5**) (Chart 1).

The dried roots of *R. crenulata* were extracted with MeOH under reflux. The methanolic extract (41.1% from the dried

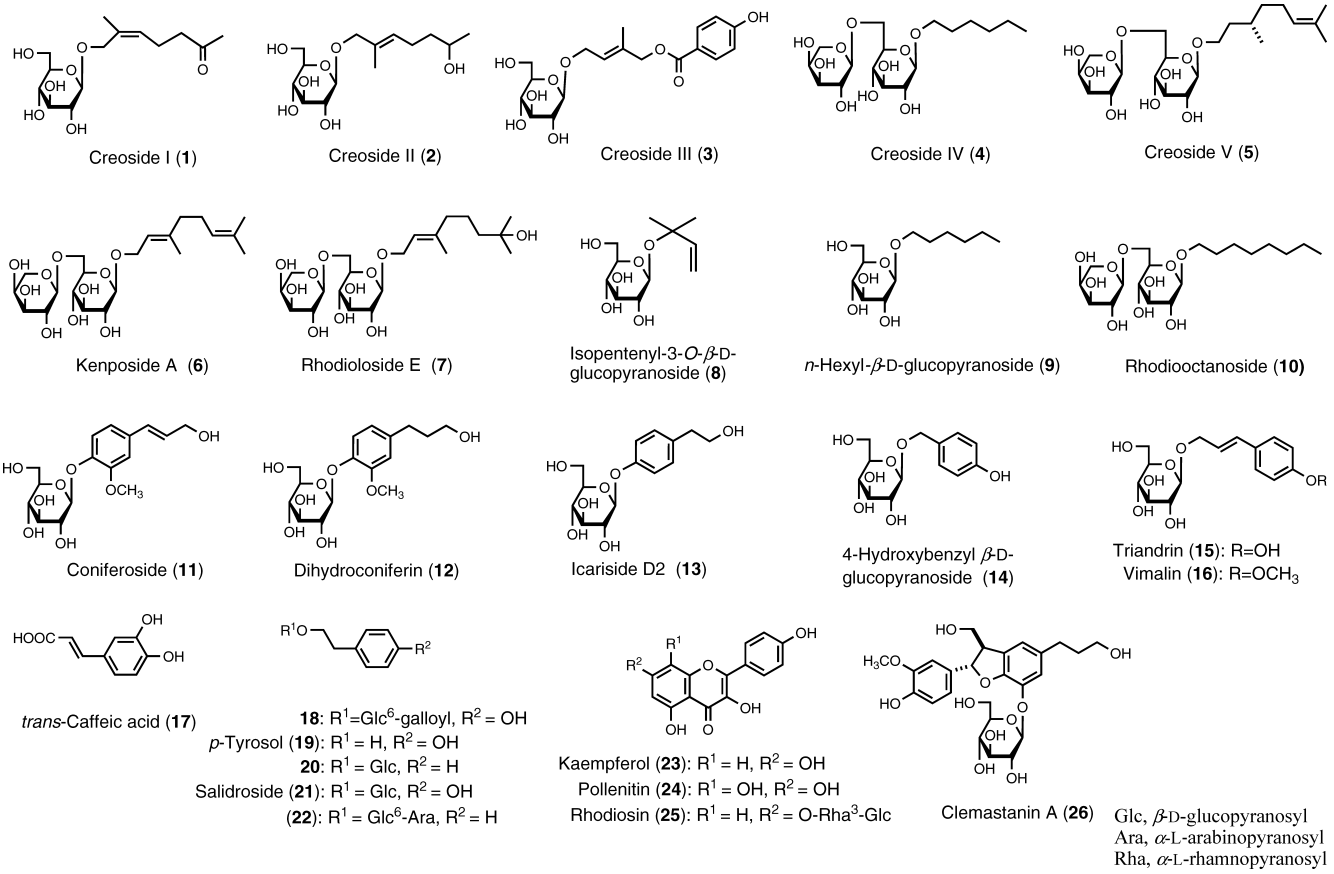

Chart 1. Structures of Constituents from the Roots of *R. crenulata*

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roots) was partitioned into an EtOAc–H_2O mixture to furnish an EtOAc-soluble fraction (11.6%) and aqueous layer. The aqueous layer was further extracted with *n*-BuOH to give *n*-BuOH- and H_2O-soluble fractions (12.4% and 15.9%, respectively). The EtOAc-soluble fraction was subjected to normal-phase and reversed-phase column chromatographies and finally to HPLC to give three known compounds, *trans*-caffeic acid (**17**, 0.036%),[14] *p*-tyrosol (**19**, 0.3183%),[15] and kaempferol (**23**, 0.0068%).[16] The *n*-BuOH-soluble fraction was also subjected to normal- and reversed-phase column chromatographies and HPLC to give creosides I (**1**, 0.0026%), II (**2**, 0.0011%), III (**3**, 0.0002%), IV (**4**, 0.0014%), and V (**5**, 0.0015%), together with 18 known compounds, kenposide A (**6**, 0.0195%),[17] rhodioloside E (**7**, 0.0058%),[18] isopentenyl-3-*O*-β-D-glucopyranoside (**8**, 1.8171%),[19] *n*-hexyl-β-D-glucopyranoside (**9**, 0.0005%),[20] rhodiooctanoside (**10**, 0.0017%),[12] coniferoside (**11**, 0.0027%),[21] dihydroconiferin (**12**, 0.0001%),[22] icariside D2 (**13**, 0.0026%),[23] 4-hydroxybenzyl β-D-glucopyranoside (**14**, 0.0004%),[24] triandrin (**15**, 0.0127%),[25] vimalin (**16**, 0.0017%),[25] 1-*O*-β-D-(6″-*O*-galloyl)glucopyranoside (**18**, 0.0020%),[26] 2-phenylethyl β-D-glucopyranoside (**20**, 0.0049%),[27] salidroside (**21**, 1.1063%),[28] 2-phenylethyl *O*-α-L-arabinopyranosyl(1→6)-β-D-glucopyranoside (**22**, 0.0027%),[13] pollenitin (**24**, 0.0010%),[29] rhodosin (**25**, 0.0295%),[30] and clemastanin A (**26**, 0.0018%).[31]

Creoside I (**1**) was isolated as a colorless amorphous powder with negative optical rotation ($[\alpha]_D^{27}$ −38.7° in MeOH). The IR spectrum of **1** showed strong, broad absorption bands at 3450 and 1043 cm^{-1}, suggestive of a glycoside structure, together with absorption bands at 1717 and 1653 cm^{-1} assignable to carbonyl and olefin functions, respectively. In the positive-ion fast atom bombardment (FAB) MS of **1**, a quasi-molecular ion peak was observed at *m/z* 327 $(M+Na)^+$. High-resolution (HR) FAB-MS analysis revealed the molecular formula of **1** to be $C_{14}H_{24}O_7$. Acid hydrolysis of **1** with aqueous HCl 1.0 M liberated D-glucose, which was identified in HPLC analysis using an optical rotation detector.[2] The ^{1}H-NMR (CD_3OD) and ^{13}C-NMR (Table 1) spectra of **1**, which were assigned based on the results of various NMR experiments,[32] showed signals assignable to an aglycon moiety [δ 1.77 (3H, s, H_3-8), 2.13 (3H, s, H_3-7), 2.32 (2H, m, H_2-4), 2.53 (2H, t, J=7.6 Hz, H_2-5), 4.21, 4.34 (1H each, both d, J=11.0 Hz, H_2-1), 5.37 (1H, t, J=6.9 Hz, H-3)] and a β-D-glucopyranosyl moiety [δ 4.23 (1H, d, J=7.6 Hz, H-1′)]. As shown in Fig. 1, the double-quantum filter correlation spectroscopy (DQF COSY) experiment on **1** indicated the presence of partial structures shown in boldface lines, and in the heteronuclear multiple-bond correlation (HMBC) experiment, long-range correlations were observed between the following protons and carbons: H-1 and C-8, 1′; H-3 and C-5, 8; H_2-4 and C-6; H_3-7 and C-5; H_3-8 and C-1, 3; and H-1′ and C-1. Finally, enzymatic hydrolysis of **1** with β-glucosidase afforded the known compound *cis*-2-methyl-6-oxo-2-hepten-1-ol.[33] On the basis of this evidence, the structure of creoside I (**1**) was determined as shown.

Creoside II (**2**), obtained as a colorless amorphous powder with a negative optical rotation ($[\alpha]_D^{26}$ −22.6° in MeOH), showed absorption bands at 3450, 1655, and 1041 cm^{-1} ascribable to hydroxyl, olefin, and ether functions, respectively, in the IR spectrum. The positive-ion FAB-MS of **2** exhibited a quasimolecular ion peak at *m/z* 329 $(M+Na)^+$. The molecular formula $C_{14}H_{26}O_7$ of **2** was determined from the quasi-molecular ion peak and by HR-FAB-MS measurement. The acid hydrolysis of **2** liberated D-glucose, which was identified using HPLC analysis.[2] The ^{1}H-NMR (CD_3OD) and ^{13}C-NMR (Table 1) spectra[32] of **2**, which were assigned based on the results of various NMR experiments, showed signals assignable to a aglycon moiety [δ 1.15 (3H, d, J=6.1 Hz, H_3-7), 1.46 (2H, m, H_2-4), 1.70 (3H, s, H_3-8), 2.21 (2H, m, H_2-5), 3.71 (1H, m, H-6), 4.04 (1H, d, J=11.6 Hz, H-1a), 4.20 (1H, d, J=11.6 Hz, H-1b), 5.39 (1H, t, J=7.3 Hz, H-3)] and a β-D-glucopyranosyl moiety [δ 4.25 (1H, d, J=8.0 Hz, H-1′)]. As shown in Fig. 1, long-range correlations in the HMBC experiment on **2** were observed between the following protons and carbons: H-1 and C-3, 8, 1′; H_3-7 and C-5; H_3-8 and C-

Table 1. ^{13}C-NMR (150 MHz, CD_3OD) Data for **1**—**5** and **3a**

Position	1	2	3	3a	4	5
1	67.7	75.9	66.1	59.1	71.1	69.3
2	133.7	133.8	124.9	128.3	30.8	37.8
3	129.8	129.8	136.0	134.1	26.8	30.6
4	23.1	25.1	70.0	70.1	32.9	38.4
5	48.8	39.8	14.3	14.1	23.7	26.5
6	211.4	68.1			14.4	125.9
7	30.0	23.6				131.9
8	21.9	14.1				17.8
9						25.9
10						20.0
1′	102.4	102.6	122.2	122.3	104.4	104.1
2′	75.0	75.1	132.9	132.8	75.1	75.0
3′	78.1	78.2	116.3	116.2	77.9	77.9
4′	71.7	71.7	163.7	163.7	71.7	71.6
5′	77.9	77.9	116.3	116.2	76.8	76.8
6′	62.8	62.8	132.9	132.8	69.5	69.4
7′			167.8	167.8		
1″			103.3		105.2	105.1
2″			75.1		72.4	72.3
3″			78.1		74.2	74.1
4″			71.7		69.5	69.4
5″			78.1		66.7	66.7
6″			62.8			

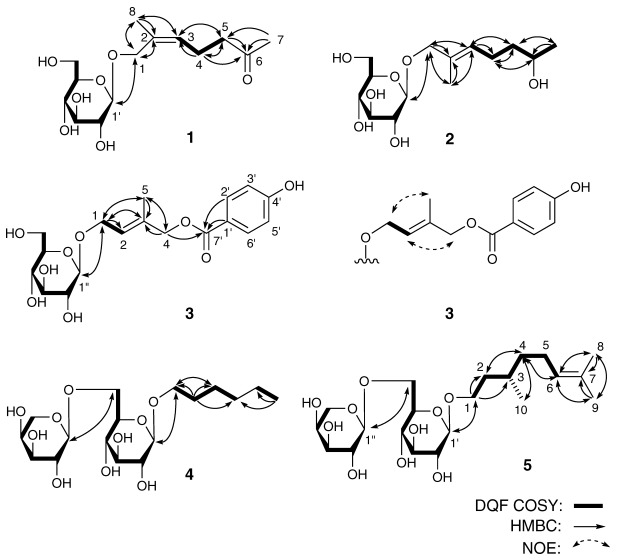

Fig. 1. Significant DQF COSY, HMBC, and NOE Correlations of New Compounds **1**—**5**

1, 3; H-3 and C-5, 8; and H-1′ and C-1. Finally, the enzymatic hydrolysis of **2** afforded an aglycon, (*E*)-2-methyl-2-heptene-1,6ξ-diol.[34,35] On the basis of this evidence, the structure of creoside II (**2**) was determined as shown.[36]

Creoside III (**3**), obtained as a colorless amorphous powder with a negative optical rotation ($[\alpha]_D^{24}$ −25.9° in MeOH), showed absorption bands at 3450, 1686, 1655, 1508, and 1040 cm^{-1} assignable to hydroxyl, ester, olefin, phenyl, and ether functions, respectively, in the IR spectrum. In the positive-ion FAB-MS of **3**, a quasimolecular ion peak was observed at *m/z* 407 $(M+Na)^+$. HR-FAB-MS analysis revealed the molecular formula of **3** to be $C_{18}H_{24}O_9$. The acid hydrolysis of **2** liberated D-glucose, which was identified using HPLC analysis.[2] The enzymatic hydrolysis of **3** afforded a new aglycon termed creol A (**3a**), of which the molecular formula $C_{12}H_{14}O_4$ was determined from a molecular ion peak [*m/z* 222 $(M)^+$] and by HR-EI-MS measurement. The ^{1}H-NMR (CD_3OD) and ^{13}C-NMR (Table 1) spectra[32] of **3a** showed the presence of a vinyl methyl [δ 1.76 (3H, s, H_3-5)], an allylic methylene bearing an oxygen function [δ 4.16 (2H, d, J=6.2 Hz, H_2-1)], an allylic methylene with an acyl group [δ 4.69 (2H, s, H_2-4)], a trisubstituted olefin [δ 5.71 (1H, t, J=6.2 Hz, H-2)], and a *p*-hydroxybenzoyl group [6.83 (2H, d, J=8.9 Hz, H-3′,5′), 7.89 (2H, d, J=8.9 Hz, H-2′,6′)]. The geometry of **3a** was characterized by the chemical shift value of the vinyl methyl carbon (5-C), *i.e.*, the ^{13}C-NMR signal of the vinyl methyl group (8-C) on a trisubstituted alkene with the *trans*-configuration like **2** (δ 14.1) was shifted upfield relative to that on a trisubstituted alkene with the *cis*-configuration like **1** (δ 21.9) as a result of the strong interaction between 4-C and 8-C of **2**. The vinyl methyl carbon (5-C) signal of **3a** was observed at δ 14.1, and thus the geometry of **3a** was determined to be *trans*. In addition, the geometry of **3a** was also confirmed based on the results of a nuclear Overhauser enhancement spectroscopy (NOESY) experiment, which showed NOE correlations between the following proton pairs: H_2-1 and H_3-5; and H-2 and H_2-4 (Fig. 1). This evidence led us to formulate the structure of a new aglycon, cresol A (**3a**), as shown. The ^{1}H-NMR (CD_3OD) and ^{13}C-NMR (Table 1) spectra[32] of **3** showed signals assignable to an aglycon moiety [δ 1.78 (3H, s, H_3-5), 4.30 (1H, dd, J=7.6, 11.7 Hz, H-1a), 4.46 (1H, dd, J=6.2, 11.7 Hz, H-1b), 4.70 (2H, s, H_2-4), 5.74 (1H, t-like, *ca.* J=6.9 Hz, H-2), 6.83 (2H, d, J=8.3 Hz, H_2-3′, 5′), 7.89 (2H, d, J=8.3 Hz, H_2-2′, 6′)] and a β-D-glucopyranosyl moiety [δ 4.30 (1H, d, J=8.2 Hz, H-1″)]. Comparison of the ^{13}C-NMR data of **3** with those of **3a** indicated the presence of a glycosidation shift around the 1-position of **3**. Furthermore, long-range correlations in the HMBC experiment on **3** were observed between the following protons and carbons: H-1 and C-3, 5, 1″; H_2-4 and C-5, 7′; H_3-5 and C-1, 3, 4; H-2′, 6′ and C-4′, 7′; and H-3′, 5′ and C-1′, 4′ (Fig. 1). On the basis of this evidence, the structure of creoside III (**3**) was determined as shown.

Creoside IV (**4**), obtained as a colorless amorphous powder with a negative optical rotation ($[\alpha]_D^{24}$ −32.2° in MeOH), showed absorption bands at 3450 and 1040 cm^{-1} assignable to hydroxyl and ether functions, respectively, in the IR spectrum. The molecular formula $C_{17}H_{32}O_{10}$ was determined based on positive-ion FAB-MS [*m/z* 419 $(M+Na)^+$] and HR-FAB-MS measurement. The acid hydrolysis of **4** liberated L-arabinose and D-glucose.[2] The ^{1}H- and ^{13}C-NMR (Table 1) spectra[32] of **4** indicated the presence of a methyl [δ 0.97 (3H, t, J=6.8 Hz, H_3-6)], a methylene bearing an oxygen function [δ 4.20 (1H, dd, J=7.6, 12.4 Hz, H-1a), 4.35 (1H, dd, J=6.9, 12.4 Hz, H-1b)], four methylenes [*d* 1.32 (4H, m, H_2-4, 5), 1.38 (2H, m, H_2-3), 1.60 (2H, m, H_2-2)], a β-D-glucopyranosyl moiety [δ 4.24 (1H, d, J=8.2 Hz, H-1′)], and an α-L-arabinopyranosyl moiety [δ 4.30 (1H, d, J=6.9 Hz, H-1″)]. The proton and carbon signals due to the 1-*O*-glycoside moiety in the ^{1}H- and ^{13}C-NMR spectra of **4** were superimposable on those of rhodiooctanoside (**10**).[12] Finally, the enzymatic hydrolysis of **4** afforded *n*-hexanol. On the basis of this evidence, the structure of creoside IV (**4**) was determined as shown.

Creoside V (**5**) was isolated as a colorless amorphous powder with a negative optical rotation ($[\alpha]_D^{27}$ −24.3° in MeOH). The molecular formula $C_{21}H_{38}O_{10}$ of **5** was determined based on positive- and negative-ion FAB-MS at *m/z* 473 $(M+Na)^+$, *m/z* 449 $(M-H)^-$, and *m/z* 317 $(M-C_5H_8O_4)^-$ and HR-FAB-MS measurement. The acid hydrolysis of **5** liberated L-arabinose and D-glucose.[2] The ^{1}H-NMR (CD_3OD) and ^{13}C-NMR (Table 1) spectra[32] of **5** showed signals assignable to a monoterpene moiety [δ 0.91 (3H, J=6.5 Hz, H_3-10), 1.17, 1.35 (1H each, both m, H_2-4), 1.41, 1.69 (1H each , both m, H_2-2), 1.59 (1H, m, H-3), 1.60 (3H, s, H_3-8), 1.67 (3H, s, H_3-9), 2.00 (1H, m, H-5a), 2.53 (1H, t like, J=7.6 Hz, H-5b), 3.54, 3.93 (1H each, both m, H_2-1), 5.10 (1H, t-like, *ca.* J=7.6 Hz, H-6)], a β-D-glucopyranosyl moiety [δ 4.25 (1H, d, J=8.0 Hz, H-1′)], and an α-L-arabinopyranosyl moiety [δ 4.31 (1H, d, J=7.0 Hz, H-1″)]. The proton and carbon signals due to the 1-*O*-glycoside moiety in the ^{1}H- and ^{13}C-NMR spectra of **5** were superimposable on those of rhodiooctanoside (**10**)[12] and **4**, whereas the proton and carbon signals due to the monoterpene part were similar to those of (−)-citronellol.[37] In addition, the detailed ^{1}H- and ^{13}C-NMR data (Table 1) analysis including DQF COSY and HMBC experiments (Fig. 1) led us to confirm the planar structure of **5**. Finally, the enzymatic hydrolysis of **5** afforded the monoterpene (−)-citronellol.[37] On the basis of this evidence, the structure of creoside V (**5**) was determined as shown.

Experimental

General Experimental Procedures The following instruments were used to obtain physical data: specific rotations, Horiba SEPA-300 digital polarimeter (l=5 cm); IR spectra, Shimadzu FTIR-8100 spectrometer; EI-MS and HR-EI-MS, JEOL JMS-GCMATE mass spectrometer; FAB-MS and HR-FAB-MS, JEOL JMS-SX 102A mass spectrometer; ^{1}H-NMR spectra, JEOL EX-270 (270 MHz), JNM-LA500 (500 MHz), and JEOL ECA-600K (600 MHz) spectrometers; ^{13}C-NMR spectra, JEOL EX-270 (68 MHz) JNM-LA500 (125 MHz), and JEOL ECA-600K (150 MHz) spectrometers with tetramethylsilane as an internal standard; HPLC detector, Shimadzu RID-6A refractive index detector; and HPLC column, YMC-Pack ODS-A (YMC, 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 materials were used for chromatography: normal-phase silica gel column chromatography, Silica gel BW-200 (Fuji Silysia Chemical, Ltd., 150—350 mesh); reversed-phase silica gel column chromatography, Chromatorex ODS DM1020T (Fuji Silysia Chemical, Ltd., 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) (reversed phase); reversed-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 The dried roots of *R. crenulata* were collected at Tibet

of China in 2005 and identified by one of authors (M.Y.). A voucher of the plant is on file in our laboratory (2007. China-07-RC).

Extraction and Isolation The dried roots of *R. crenulata* (5.0 kg) were powdered and extracted 3 times with MeOH under reflux for 3 h. Evaporation of the solvent under reduced pressure provided a methanolic extract (2054 g, 41.1% from the dried roots), and the extract was partitioned into an EtOAc–H_2O (1 : 1, v/v) mixture to furnish an EtOAc-soluble fraction (580 g, 11.6%) and an aqueous phase. The aqueous phase was further extracted with *n*-BuOH to give an *n*-BuOH-soluble fraction (618 g, 12.4%) and an H_2O-soluble fraction (796 g, 15.9%). The EtOAc fraction (110 g) was subjected to ordinary-phase silica gel column chromatography [3.0 kg, $CHCl_3$→$CHCl_3$–MeOH (20 : 1)→$CHCl_3$–MeOH–H_2O (20 : 3 : 1, lower layer→15 : 3 : 1, lower layer→10 : 3 : 1, lower layer→7 : 3 : 1, lower layer)→MeOH] to give nine fractions [Fr. 1 (16.7 g), Fr. 2 (7.5 g), Fr. 3 (11.6 g), Fr. 4 (3.3 g), Fr. 5 (2.6 g), Fr. 6 (1.2 g) , Fr. 7 (2.2 g) , Fr. 8 (12.2 g), Fr. 9 (40.4 g)]. Fraction 4 (3.3 g) was subjected to reversed-phase silica gel column chromatography [100 g, MeOH–H_2O (20 : 80→30 : 70→40 : 60→50 : 50→60 : 40→70 : 30, v/v)→MeOH] to give seven fractions [Fr. 4-1 (=*p*-tyrosol, 2.1 g, 0.32%), Fr. 4-2 (205 mg), Fr. 4-3 (71 mg), Fr. 4-4 (73 mg), Fr. 4-5 (=kaempferol, 99 mg, 0.0068%), Fr. 4-6 (177 mg), Fr. 4-7 (179 mg)]. Fraction 6 (1.2 g) was subjected to reversed-phase silica gel column chromatography [50 g, MeOH–H_2O (30 : 70→40 : 60→50 : 50→60 : 40→70 : 30→90 : 10, v/v)→MeOH] to give seven fractions [Fr. 6-1 (115 mg), Fr. 6-2 (=*trans*-caffeic acid, 342 mg, 0.036%), Fr. 6-3 (182 mg), Fr. 6-4 (72 mg), Fr. 6-5 (64 mg), Fr. 6-6 (131 mg), Fr. 6-7 (117 mg). The *n*-BuOH-soluble fraction (180.0 g) was subjected to ordinary-phase silica gel column chromatography [3.0 kg, $CHCl_3$–MeOH–H_2O (20 : 3 : 1, lower layer→15 : 3 : 1, lower layer→10 : 3 : 1, lower layer→7 : 3 : 1, lower layer→6 : 4 : 1, v/v/v)→MeOH] to give seven fractions [Fr. 1 (1.9 g), Fr. 2 (4.1 g), Fr. 3 (1.6 g), Fr. 4 (34.7 g), Fr. 5 (31.1 g), Fr. 6 (6.1 g), and Fr. 7 (80.2 g)]. Fraction 4 (34.7 g) was subjected to reversed-phase silica gel column chromatography [1000 g, MeOH–H_2O (10 : 90→20 : 80→30 : 70→40 : 60→50 : 50→60 : 40, v/v)→MeOH] to afford six fractions [Fr. 4-1 (506 mg), Fr. 4-2 (=isopentenyl-3-*O*-*β*-D-glucopyranoside, 2.7 g, 1.82%), Fr. 4-3 (6.1 g), Fr. 4-4 (321 mg), Fr. 4-5 (412 mg), Fr. 4-6 (238 mg)]. Fraction 4-3 (754 mg) was separated by HPLC [MeOH–H_2O (20 : 80, v/v)] to give creoside I (**1**, 35.8 mg, 0.0026%). Fraction 4-4 (321 mg) was separated by HPLC [MeOH–H_2O (35 : 65, v/v)] to give 2-phenylethyl *β*-D-glucopyranoside (71.2 mg, 0.0049%). Fraction 4-5 (412 mg) was separated by HPLC [MeCN–H_2O (20 : 80, v/v)] to give creoside III (**3**, 21.4 mg, 0.0002%), *n*-hexyl-*β*-D-glucopyranoside (7.6 mg, 0.0005%) and vimalin (24.0 mg, 0.0017%). Fraction 5 (31.1 g) was subjected to reversed-phase silica gel column chromatography [900 g, MeOH–H_2O (20 : 80→30 : 70→50 : 50→70 : 30, v/v)→MeOH] to afford ten fractions [Fr. 5-1 (456 mg), Fr. 5-2 (=rhodiocyanoside, 1.61 g, 1.11%), Fr. 5-3 (168 mg), Fr. 5-4 (995 mg), Fr. 5-5 (217 mg), Fr. 5-6 (1.1 g), Fr. 5-7 (121 mg), Fr. 5-8 (674 mg) , Fr. 5-9 (10.0 g) , Fr. 5-10 (197 mg)]. Fraction 5-3 (168 mg) was separated by HPLC [MeOH–H_2O (20 : 80, v/v)] to give coniferoside (39.1 mg, 0.0027%) and dihydroconiferin (14.4 mg, 0.0001%). Fraction 5-4 (995 mg) was separated by HPLC [MeOH–H_2O (25 : 75, v/v)] to give creoside II (**2**, 15.5 mg, 0.0011%), triandrin (184.2 mg, 0.0127%) and 2-phenylethyl *O*-*α*-L-arabinopyranosyl(1→6)-*β*-D-glucopyranoside (39.2 mg, 0.0027%). Fraction 5-6 (533 mg) was separated by HPLC [MeOH–H_2O (35 : 65, v/v)] to give creoside IV (**4**, 20.1 mg, 0.0014%). Fraction 5-9 (361 mg) was separated by HPLC [MeOH–H_2O (55 : 45, v/v)] to give kenposide A (235.1 mg, 0.0162%) and rhodiooctanoside (24.0 mg, 0.0017%). Fraction 5-10 (197 mg) was separated by HPLC [MeOH–H_2O (60 : 40, v/v)] to give creoside V (**5**, 22.3 mg, 0.0015%). Fraction 6 (6.1 g) was subjected to reversed-phase silica gel column chromatography [200 g, MeOH–H_2O (20 : 80→30 : 70→50 : 50→70 : 30, v/v)→MeOH] to afford ten fractions [Fr. 6-1 (1.1 g), Fr. 6-2 (312 mg), Fr. 6-3 (1.1 g), Fr. 6-4 (792 mg), Fr. 6-5 (636 mg), Fr. 6-6 (199 mg), Fr. 6-7 (167 mg), Fr. 6-8 (rhodosin, 429 mg, 0.0295%), Fr. 6-9 (426 mg) , Fr. 6-10 (369 mg)]. Fraction 6-2 (312 mg) was separated by HPLC [MeOH–H_2O (15 : 85, v/v)] to give icariside D2 (38.4 mg, 0.0026%) and 4-hydroxybenzyl *β*-D-glucopyranoside (5.4 mg, 0.0004%). Fraction 6-5 (636 mg) was separated by HPLC [MeOH–H_2O (35 : 65, v/v)] to give rhodioloside E (83.8 mg, 0.00576%), clemastanin A (16.8 mg, 0.0018%) and 1-*O*-*β*-D-(6″-*O*-galloyl)glucopyranoside (18.8 mg, 0.0020%). Fraction 6-9 (426 mg) was separated by HPLC [MeOH–H_2O (50 : 50, v/v)] to give pollenitin (15.3 mg, 0.0010%) and kenposide A (48.5 mg, 0.0033%).

The known compounds were identified by comparison of their physical data ($[\alpha]_D$, ^{1}H-NMR, ^{13}C-NMR, MS) with those of reported values.

Creoside I (**1**): A colorless amorphous powder; $[\alpha]_D^{27}$ −38.7° (*c*=2.58, MeOH); IR (film) ν_{max} 3450, 1717, 1653, 1043 cm^{-1}; ^{1}H-NMR (CD_3OD, 600 MHz) *δ*: 1.77 (3H, s, H_3-8), 2.13 (3H, s, H_3-7), 2.32 (2H, m, H_2-4), 2.53 (2H, t, *J*=7.6 Hz, H_2-5), 3.18 (1H, dd, *J*=7.6, 8.9 Hz, H-2′), 3.25 (1H, m, H-4′), 3.30 (1H, m, H-3′), 3.35 (1H, m, H-5′), 3.67 (1H, dd, *J*=5.5, 12.4 Hz, H_2-6′), 3.86 (1H, dd, *J*=2.0, 12.4 Hz, H_2-6′), 4.21, 4.34 (1H each, both d, *J*=11.0 Hz, H-1), 4.23 (1H, d, *J*=7.6 Hz, H-1′), 5.37 (1H, t, *J*=6.9 Hz, H-3); ^{13}C-NMR data see Table 1; positive-ion FAB-MS *m/z* 327 $[M+Na]^+$; HR-FAB-MS: *m/z* 327.1413 (Calcd for $C_{14}H_{24}O_7Na$ $[M+Na]^+$, 327.1420).

Creoside II (**2**): A colorless amorphous powder; $[\alpha]_D^{26}$ −22.6° (*c*=0.57, MeOH); IR (KBr) ν_{max} 3450, 1655, 1041 cm^{-1}; ^{1}H-NMR (CD_3OD, 600 MHz) *δ*: 1.15 (1H, d, *J*=6.1 Hz, H-7), 1.46 (2H, m, H_2-4), 1.70 (3H, s, H_3-8), 2.21 (2H, m, H_2-5), 3.19 (1H, dd, *J*=8.0, 8.9 Hz, H-2′), 3.30 (2H, m, H-3′, 4′), 3.35 (1H, m, H-5′), 3.67 (1H, dd, *J*=5.5, 12.4 Hz, H_2-6′), 3.71 (1H, m, H-6), 3.86 (1H, dd, *J*=2.1, 12.4 Hz, H_2-6′), 4.04 (1H, d, *J*=11.6 Hz, H-1a), 4.20 (1H, d, *J*=11.6 Hz, H-1b), 4.25 (1H, d, *J*=8.0 Hz, H-1′), 5.39 (1H, t, *J*=7.3 Hz, H-3); ^{13}C-NMR data see Table 1; positive-ion FAB-MS *m/z* 329 $[M+Na]^+$; HR-FAB-MS: *m/z* 329.1581 (Calcd for $C_{14}H_{26}O_7Na$ $[M+Na]^+$, 329.1576).

Creoside III (**3**): A colorless amorphous powder; $[\alpha]_D^{24}$ −25.9° (*c*=1.31, MeOH); IR (film) ν_{max} 3450, 1686, 1655, 1508, 1040 cm^{-1}; ^{1}H-NMR (CD_3OD, 600 MHz) *δ*: 1.78 (3H, s, H_3-5), 3.18 (1H, dd, *J*=8.2, 8.9 Hz, H-2″), 3.27 (1H, m, H-4″), 3.30 (1H, m, H-3″), 3.35 (1H, m, H-5″), 3.67 (1H, dd, *J*=5.5, 11.7 Hz, H_2-6″), 3.86 (1H, dd, *J*=2.1, 11.7 Hz, H_2-6″), 4.30 (1H, dd, *J*=7.6, 11.7 Hz, H-1a), 4.30 (1H, d, *J*=8.2 Hz, H-1″), 4.46 (1H, dd, *J*=6.2, 11.7 Hz, H-1b), 4.70 (2H, s, H_2-4), 5.74 (1H, t-like, *ca.* *J*=6.9 Hz, H-2), 6.83 (2H, d, *J*=8.9 Hz, H-3′, 5′), 7.89 (2H, d, *J*=8.3 Hz, H-2′, 6′); ^{13}C-NMR data see Table 1; positive-ion FAB-MS *m/z* 407 $[M+Na]^+$; HR-FAB-MS: *m/z* 407.1326 (Calcd for $C_{18}H_{24}O_9Na$ $[M+Na]^+$, 407.1318).

Creoside IV (**4**): A colorless amorphous powder; $[\alpha]_D^{24}$ −32.2° (*c*=1.45, MeOH); IR (KBr) ν_{max} 3450, 1040 cm^{-1}; ^{1}H-NMR (CD_3OD, 600 MHz) *δ*: 0.97 (3H, t, *J*=6.8 Hz, H_3-6), 1.32 (4H, m, H_2-4, 5), 1.38 (2H, m, H_2-3), 1.60 (2H, m, H_2-2), 3.18 (1H, dd, *J*=8.2, 8.9 Hz, H-2′), 3.34 (2H, m, H-3′, 4′), 3.42 (1H, m, H-5′), 3.52 (1H, m, H_2-5″), 3.53 (1H, m, H-3″), 3.59 (1H, m, H-2″), 3.73 (1H, dd, *J*=5.5, 11.7 Hz, H_2-6′), 3.80 (1H, m, H-4″), 3.85 (1H, m, H_2-5″), 4.08 (1H, dd, *J*=2.0, 11.7 Hz, H_2-6′), 4.20 (1H, dd, *J*=12.4, 7.6 Hz, H-1a), 4.24 (1H, d, *J*=8.2 Hz, H-1′), 4.30 (1H, d, *J*=6.9 Hz, H-1″), 4.35 (1H, dd, *J*=12.4, 6.9 Hz, H-1b); ^{13}C-NMR data see Table 1; positive-ion FAB-MS *m/z* 419 $[M+Na]^+$; negative-ion FAB-MS *m/z* 395 $[M-H]^-$, HR-FAB-MS: *m/z* 419.1898 (Calcd for $C_{17}H_{32}O_{10}Na$ $[M+Na]^+$, 419.1893).

Creoside V (**5**): A colorless amorphous powder; $[\alpha]_D^{27}$ −24.3° (*c*=2.79, MeOH); IR (KBr) ν_{max} 3450, 1649, 1084 cm^{-1}; ^{1}H-NMR (CD_3OD, 600 MHz) *δ*: 0.91 (3H, *J*=6.5 Hz, H_3-10), 1.17, 1.35 (1H each, both m, H_2-4), 1.41, 1.69 (1H each, both m, H_2-2), 1.60 (3H, s, H_3-8), 1.59 (1H, m, H-3), 1.67 (3H, s, H_3-9), 2.00 (1H, m, H-5b), 2.53 (1H, t like, *J*=7.6 Hz, H-5b), 3.18 (1H, dd, *J*=8.0, 8.9 Hz, H-2′), 3.35 (1H, m, H-3′), 3.42 (1H, m, H-5′), 3.43 (1H, m, H-4′), 3.53 (1H, m, H_2-5″), 3.54 (1H, m, H-3″), 3.54, 3.93 (1H each, both m, H_2-1), 3.59 (1H, m, H-2″), 3.74 (1H, dd, *J*=5.2, 11.6 Hz, H_2-6′), 3.81 (1H, m, H-4″), 3.87 (1H, dd, *J*=3.3, 12.4 Hz, H_2-5″), 4.09 (1H, dd, *J*=2.2, 11.6 Hz, H_2-6′), 4.25 (1H, d, *J*=8.0 Hz, H-1′), 4.31 (1H, d, *J*=7.0 Hz, H-1″), 5.10 (1H, t-like, *ca.* *J*=7.6 Hz, H-6); ^{13}C-NMR data see Table 1; positive-ion FAB-MS *m/z* 473 $[M+Na]^+$; negative-ion FAB-MS *m/z* 449 $[M-H]^-$, 317 $[M-C_5H_8O_4]^-$, HR-FAB-MS: *m/z* 473.2368 (Calcd for $C_{21}H_{38}O_{10}Na$ $[M+Na]^+$, 473.2363).

Acid Hydrolysis of 1—5 Solution of **1**—**5** (each 2.0 mg) in 1 M HCl (1.0 ml) were each heated under reflux for 3 h. After cooling, each reaction mixture was neutralized with Amberlite IRA-400 (OH^- form) and filtrated, and the solution was partitioned with EtOAc to give two layers. The aqueous layer was evaporated and then subjected to HPLC analysis using Kaseisorb LC NH_2-60-5 column (4.6 mm×250 mm i.d., Tokyo Kasei Co., Ltd., Tokyo, Japan) and an optical rotation detector (Shodex OR-2, Showa Denko Co., Ltd., Tokyo, Japan). D-Glucose and L-arabinose were confirmed by comparison of the retention times with the authentic samples (Wako Pure Chemicals Ltd., Osaka, Japan) [D-glucose and L-arabinose, mobile phase: CH_3CN–H_2O (75 : 25, v/v), flow rate: 0.5 ml/min, t_R: 13.8 min (D-glucose, positive optical rotation); t_R: 12.3 min (L-arabinose, positive optical rotation)].

Enzymatic Hydrolysis of 1, 2 and 3 with β-Glucosidase Solution of **1** (6.7 mg) in H_2O (1.0 ml) was treated with *β*-glucosidase (from Almond, Oriental yeast Co., Ltd., Japan, 1 unit) and the solution was stirred at 37 °C for 24 h. 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 (50 : 50, v/v)] to furnish *cis*-2-methyl-6-oxo-2-hepten-1-ol (2.3 mg, 74.2%). Through a similar procedure, enzymatic hydrolysis of **2** (8.6 mg) and **3** (9.8 mg) were carried out to afford (*E*)-2-methyl-2-heptene-1,6*ξ*-diol (2.7 mg, 66.7%) and **3a** (5.0 mg, 88.3%), respectively. The obtained compounds, *cis*-2-methyl-6-oxo-2-hepten-1-ol and (*E*)-2-methyl-2-

heptene-1,6ξ-diol were identified by comparison of their physical data (^{1}H-NMR, ^{13}C-NMR, MS) with reported values.

Compound 3a A colorless amorphous powder; IR (film) ν_{max} 3450, 1686, 1608, 1514, 1040 cm^{-1}; ^{1}H-NMR (CD_3OD, 600 MHz) δ: 1.76 (3H, s, H_3-5), 4.16 (2H, d, J=6.2 Hz, H_2-1), 4.69 (2H, s, H_2-4), 5.71 (1H, t, J=6.2 Hz, H-2), 6.83 (2H, d, J=8.9 Hz, H-3′, 5′) , 7.89 (2H, d, J=8.9 Hz, H-2′, 6′); ^{13}C-NMR data see Table 1; positive-ion EI-MS m/z 222 $[M]^+$; HR-EI-MS: m/z 222.0899 (Calcd for $C_{12}H_{14}O_4$ $[M]^+$, 222.0892).

Enzymatic Hydrolysis of 4 and 5 with Naringinase A solution of **4** (8.8 mg) in 0.1 M acetate buffer (pH 3.8, 1.0 ml) was treated with narigninase (Sigma Chemical Co., 2 units), and the solution was stirred at 40 °C for 24 h. 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 (50 : 50, v/v)] to furnish *n*-hexanol (1.5 mg, 66.2%), which was identified by comparison of the ^{1}H- and ^{13}C-NMR data with authentic sample values. By a similar procedure, enzymatic hydrolysis of **5** (12.5 mg) was carried out to afford (−)-citronellol (1.8 mg, 41.9%). The obtained compound, (−)-citronellol, was identified by comparison of the physical data ($[\alpha]_D$, ^{1}H-NMR, ^{13}C-NMR, MS) with reported values.

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References and Notes

1) Part XXVII: Morikawa T., Xie H., Wang T., Matsuda H., Yoshikawa M., *Helv. Chim. Acta*, in press.
2) Nakamura S., Li X., Matsuda H., Ninomiya K., Morikawa T., Yamaguti K., Yoshikawa M., *Chem. Pharm. Bull.*, **55**, 1505—1511 (2007).
3) Zhang Y., Morikawa T., Nakamura S., Ninomiya K., Matsuda H., Muraoka O., Yoshikawa M., *Heterocycles*, **71**, 1565—1576 (2007).
4) Morikawa T., Zhang Y., Nakamura S., Matsuda H., Muraoka O., Yoshikawa M., *Chem. Pharm. Bull.*, **55**, 435—441 (2007).
5) Ninomiya K., Morikawa T., Zhang Y., Nakamura S., Matsuda H., Muraoka O., Yoshikawa M., *Chem. Pharm. Bull.*, **55**, 1185—1191 (2007).
6) Yoshikawa M., Wang T., Morikawa T., Xie H., Matsuda H., *Chem. Pharm. Bull.*, **55**, 1308—1315 (2007).
7) Yoshikawa M., Morikawa T., Zhang Y., Nakamura S., Muraoka O., Matsuda H., *J. Nat. Prod.*, **70**, 575—583 (2007).
8) Yoshikawa M., Matsuda H., Morikawa T., Xie H., Nakamura S., Muraoka O., *Bioorg. Med. Chem.*, **14**, 7468—7475 (2006).
9) Xie H., Morikawa T., Matsuda H., Nakamura S., Muraoka O., Yoshikawa M., *Chem. Pharm. Bull.*, **54**, 669—675 (2006).
10) Morikawa T., Xie H., Matsuda H., Yoshikawa M., *J. Nat. Prod.*, **69**, 881—886 (2006).
11) Morikawa T., Xie H., Matsuda H., Wang T., Yoshikawa M., *Chem. Pharm. Bull.*, **54**, 506—513 (2006).
12) Yoshikawa M., Shimada H., Shimoda H., Murakami N., Yamahara J., Matsuda H., *Chem. Pharm. Bull.*, **44**, 2086—2091 (1996).
13) Yoshikawa M., Shimada H., Horikawa S., Murakami T., Shimoda H., Yamahara J., Matsuda H., *Chem. Pharm. Bull.*, **45**, 1498—1503 (1997).
14) Kimura Y., Okuda H., Okuda T., Hatano T., Agata I., Arichi S., *Chem. Pharm. Bull.*, **33**, 2028—2034 (1985).
15) Nakata H., Sashida Y., Shimomura H., *Chem. Pharm. Bull.*, **30**, 4554—4556 (1982).
16) Okuyama T., Hosoyama K., Hiraga Y., Kurono G., Takemoto T., *Chem. Pharm. Bull.*, **26**, 3071—3074 (1978).
17) Yoshikawa K., Nagai M., Wakabayashi M., Arihara S., *Phytochemistry*, **34**, 1431—1433 (1993).
18) Ma G., Li W., Dou D., Chang X., Bai H., Satou T., Li J., Sun D., Kang T., Nikaido T., Koike K., *Chem. Pharm. Bull.*, **54**, 1229—1233 (2006).
19) Wang S., Wang F. P., *Yaoxue Xuebao*, **27**, 117—120 (1992).
20) Matsubara Y., Mizuno T., Sawabe A., Iizuka Y., Okamoto K., *Nippon Nogei Kagaku Kaishi*, **63**, 1373—1377 (1989).
21) Sano K., Sanada S., Ida Y., Shoji J., *Chem. Pharm. Bull.*, **39**, 865—870 (1991).
22) Pan H. F., Lundgren L. N., *Phytochemistry*, **42**, 1185—1189 (1996).
23) Miyase T., Ueno A., Takizawa N., Kobayashi H., Oguchi H., *Phytochemistry*, **28**, 3483—3485 (1989).
24) Ishikawa T., Kudo M., Kitajima J., *Chem. Pharm. Bull.*, **50**, 501—507 (2002).
25) Julkunen-Tiitto R., *Phytochemistry*, **28**, 2115—2125 (1989).
26) Nonaka G., Nishimura H., Nishioka I., *Chem. Pharm. Bull.*, **30**, 2061—2067 (1982).
27) Schwab W., Schreier P., *Phytochemistry*, **27**, 1813—1816 (1988).
28) Nishimura H., Nonaka G., Nishioka I., *Chem. Pharm. Bull.*, **32**, 1735—1740 (1984).
29) Hussein S. A. M., Barakat H. H., Nawar M. A. M. Willuhn G., *Phytochemistry*, **45**, 1529—1532 (1997).
30) Peng J. N., Ma C. Y., Ge Y. C., *Zhongcaoyao*, **26**, 177—179 (1995).
31) Kizu H., Shimana H., Tomimori T., *Chem. Pharm. Bull.*, **43**, 2187—2194 (1995).
32) The ^{1}H- and ^{13}C-NMR spectra of **1**—**5** were assigned with the aid of distortionless enhancement by polarization transfer (DEPT), DQF COSY, heteronuclear multiple quantum coherence (HMQC), and HMBC experiments.
33) Taylor W. G., Coutts R. T., *Can. J. Chem.*, **57**, 2103—2107 (1979).
34) Singh J., Kaur I., Kaur J., Bhalla A., Kad G. L., *Synth. Commun.*, **33**, 191—197 (2003).
35) The stereostructures of the 6-position in (*E*)-2-methyl-2-heptene-1,6-diol, which was synthesized as a racemic form by Singh *et al.*[34]
36) The stereostructure of the 6-position in **2** have not been characterized yet.
37) Uesato S., Kobayashi K., Inouye H., *Chem. Pharm. Bull.*, **30**, 927—940 (1982).