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

Seikou NAKAMURA, Xuezheng LI, Hisashi MATSUDA, and Masayuki YOSHIKAWA*

Kyoto Pharmaceutical University; Misasagi, Yamashina-ku, Kyoto 607–8412, Japan.

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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 Rhodiola Radix; *Rhodiola crenulata*; creoside; glycoside; Chinese medicine; Crassulaceae

The roots of the alpine plant genus *Rhodiola* (Crassulaceae), *Rhodiola* Radix, have been widely used as a hemostatic, antiepileptic, 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 cytotoxic-

ity 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 *Rhodiola* 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*

* To whom correspondence should be addressed. e-mail: myoshika@mb.kyoto-phu.ac.jp

roots) was partitioned into an EtOAc–H₂O 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 H₂O-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⁾ rhodioidside E (**7**, 0.0058%),¹⁸⁾ isopentenyl-3-*O*- β -D-glucopyranoside (**8**, 1.8171%),¹⁹⁾ *n*-hexyl- β -D-glucopyranoside (**9**, 0.0005%),²⁰⁾ rhodioidoctanoside (**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⁾ salidoside (**21**, 1.1063%),²⁸⁾ 2-phenylethyl *O*- α -L-arabinopyranosyl(1 $\rightarrow$ 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 ($[\alpha]_D^{27} -38.7^\circ$ 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 C₁₄H₂₄O₇. 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 ¹H-NMR (CD₃OD) and ¹³C-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, H₃-8), 2.13 (3H, s, H₃-7), 2.32 (2H, m, H₂-4), 2.53 (2H, t, *J*=7.6 Hz, H₂-5), 4.21, 4.34 (1H each, both d, *J*=11.0 Hz, H₂-1), 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; H₂-4 and C-6; H₃-7 and C-5; H₃-8 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 ($[\alpha]_D^{26} -22.6^\circ$ 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

Table 1. ¹³C-NMR (150 MHz, CD₃OD) 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**

a quasimolecular ion peak at *m/z* 329 (M+Na)⁺. The molecular formula C₁₄H₂₆O₇ of **2** was determined from the quasimolecular ion peak and by HR-FAB-MS measurement. The acid hydrolysis of **2** liberated D-glucose, which was identified using HPLC analysis.²⁾ The ¹H-NMR (CD₃OD) and ¹³C-NMR (Table 1) spectra³²⁾ of **2**, which were assigned based on the results of various NMR experiments, showed signals assignable to an aglycon moiety [δ 1.15 (3H, d, *J*=6.1 Hz, H₃-7), 1.46 (2H, m, H₂-4), 1.70 (3H, s, H₃-8), 2.21 (2H, m, H₂-5), 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'; H₃-7 and C-5; H₃-8 and C-

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-1,6 ξ -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 ($[\alpha]_D^{24} -25.9^\circ$ 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 ¹H-NMR (CD_3OD) and ¹³C-NMR (Table 1) spectra³²⁾ of **3a** showed the presence of a vinyl methyl [δ 1.76 (3H, s, H₃-5)], an allylic methylene bearing an oxygen function [δ 4.16 (2H, d, $J=6.2$ Hz, H₂-1)], an allylic methylene with an acyl group [δ 4.69 (2H, s, H₂-4)], 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 ¹³C-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: H₂-1 and H₃-5; and H-2 and H₂-4 (Fig. 1). This evidence led us to formulate the structure of a new aglycon, cresol A (**3a**), as shown. The ¹H-NMR (CD_3OD) and ¹³C-NMR (Table 1) spectra³²⁾ of **3** showed signals assignable to an aglycon moiety [δ 1.78 (3H, s, H₃-5), 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, H₂-4), 5.74 (1H, t-like, *ca.* $J=6.9$ Hz, H-2), 6.83 (2H, d, $J=8.3$ Hz, H₂-3', 5'), 7.89 (2H, d, $J=8.3$ Hz, H₂-2', 6')] and a β -D-glucopyranosyl moiety [δ 4.30 (1H, d, $J=8.2$ Hz, H-1'')]. Comparison of the ¹³C-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''; H₂-4 and C-5, 7'; H₃-5 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 ($[\alpha]_D^{24} -32.2^\circ$ 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 ¹H- and ¹³C-NMR (Table 1) spectra³²⁾ of **4** indicated the presence of a methyl [δ 0.97 (3H, t, $J=6.8$ Hz, H₃-6)], 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 [δ 1.32 (4H, m, H₂-4, 5), 1.38 (2H, m, H₂-3), 1.60 (2H, m, H₂-2)], 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 ¹H- and ¹³C-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 ($[\alpha]_D^{27} -24.3^\circ$ 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 ¹H-NMR (CD_3OD) and ¹³C-NMR (Table 1) spectra³²⁾ of **5** showed signals assignable to a monoterpene moiety [δ 0.91 (3H, $J=6.5$ Hz, H₃-10), 1.17, 1.35 (1H each, both m, H₂-4), 1.41, 1.69 (1H each, both m, H₂-2), 1.59 (1H, m, H-3), 1.60 (3H, s, H₃-8), 1.67 (3H, s, H₃-9), 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, H₂-1), 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 ¹H- and ¹³C-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 ¹H- and ¹³C-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; ¹H-NMR spectra, JEOL EX-270 (270 MHz), JNM-LA500 (500 MHz), and JEOL ECA-600K (600 MHz) spectrometers; ¹³C-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 $\times$ 4.6 mm i.d.) and (250 $\times$ 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 60F₂₅₄ (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(SO₄)₂–10% aqueous H₂SO₄ 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-H₂O (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 H₂O-soluble fraction (796 g, 15.9%). The EtOAc fraction (110 g) was subjected to ordinary-phase silica gel column chromatography [3.0 kg, CHCl₃→CHCl₃-MeOH (20:1)→CHCl₃-MeOH-H₂O (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-H₂O (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-H₂O (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, CHCl₃-MeOH-H₂O (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-H₂O (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-H₂O (20:80, v/v)] to give creoside I (1, 35.8 mg, 0.0026%). Fraction 4-4 (321 mg) was separated by HPLC [MeOH-H₂O (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-H₂O (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-H₂O (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-H₂O (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-H₂O (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 $\rightarrow$ 6)- β -D-glucopyranoside (39.2 mg, 0.0027%). Fraction 5-6 (533 mg) was separated by HPLC [MeOH-H₂O (35:65, v/v)] to give creoside IV (4, 20.1 mg, 0.0014%). Fraction 5-9 (361 mg) was separated by HPLC [MeOH-H₂O (55:45, v/v)] to give kenposide A (235.1 mg, 0.0162%) and rhodiocyanoside (24.0 mg, 0.0017%). Fraction 5-10 (197 mg) was separated by HPLC [MeOH-H₂O (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-H₂O (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 (rhodoin, 429 mg, 0.0295%), Fr. 6-9 (426 mg), Fr. 6-10 (369 mg)]. Fraction 6-2 (312 mg) was separated by HPLC [MeOH-H₂O (15:85, v/v)] to give icaraside 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-H₂O (35:65, v/v)] to give rhodiolide 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-H₂O (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 ($[\alpha]_D^{25}$, ¹H-NMR, ¹³C-NMR, MS) with those of reported values.

Creoside I (1): A colorless amorphous powder; $[\alpha]_D^{25}$ -38.7° (*c*=2.58, MeOH); IR (film) $\nu_{\max}$ 3450, 1717, 1653, 1043 cm⁻¹; ¹H-NMR (CD₃OD,

600 MHz) δ : 1.77 (3H, s, H₃-8), 2.13 (3H, s, H₃-7), 2.32 (2H, m, H₂-4), 2.53 (2H, t, *J*=7.6 Hz, H₂-5), 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, H₂-6'), 3.86 (1H, dd, *J*=2.0, 12.4 Hz, H₂-6'), 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); ¹³C-NMR data see Table 1; positive-ion FAB-MS *m/z* 327 [M+Na]⁺; HR-FAB-MS: *m/z* 327.1413 (Calcd for C₁₄H₂₄O₇Na [M+Na]⁺, 327.1420).

Creoside II (2): A colorless amorphous powder; $[\alpha]_D^{25}$ -22.6° (*c*=0.57, MeOH); IR (KBr) $\nu_{\max}$ 3450, 1655, 1041 cm⁻¹; ¹H-NMR (CD₃OD, 600 MHz) δ : 1.15 (1H, d, *J*=6.1 Hz, H-7), 1.46 (2H, m, H₂-4), 1.70 (3H, s, H₃-8), 2.21 (2H, m, H₂-5), 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, H₂-6'), 3.71 (1H, m, H-6), 3.86 (1H, dd, *J*=2.1, 12.4 Hz, H₂-6'), 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); ¹³C-NMR data see Table 1; positive-ion FAB-MS *m/z* 329 [M+Na]⁺; HR-FAB-MS: *m/z* 329.1581 (Calcd for C₁₄H₂₆O₇Na [M+Na]⁺, 329.1576).

Creoside III (3): A colorless amorphous powder; $[\alpha]_D^{24}$ -25.9° (*c*=1.31, MeOH); IR (film) $\nu_{\max}$ 3450, 1686, 1655, 1508, 1040 cm⁻¹; ¹H-NMR (CD₃OD, 600 MHz) δ : 1.78 (3H, s, H₃-5), 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, H₂-6''), 3.86 (1H, dd, *J*=2.1, 11.7 Hz, H₂-6''), 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, H₂-4), 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'); ¹³C-NMR data see Table 1; positive-ion FAB-MS *m/z* 407 [M+Na]⁺; HR-FAB-MS: *m/z* 407.1326 (Calcd for C₁₈H₂₄O₉Na [M+Na]⁺, 407.1318).

Creoside IV (4): A colorless amorphous powder; $[\alpha]_D^{24}$ -32.2° (*c*=1.45, MeOH); IR (KBr) $\nu_{\max}$ 3450, 1040 cm⁻¹; ¹H-NMR (CD₃OD, 600 MHz) δ : 0.97 (3H, t, *J*=6.8 Hz, H₃-6), 1.32 (4H, m, H₂-4, 5), 1.38 (2H, m, H₂-3), 1.60 (2H, m, H₂-2), 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, H₂-5''), 3.53 (1H, m, H-3''), 3.59 (1H, m, H-2''), 3.73 (1H, dd, *J*=5.5, 11.7 Hz, H₂-6'), 3.80 (1H, m, H-4''), 3.85 (1H, m, H₂-5''), 4.08 (1H, dd, *J*=2.0, 11.7 Hz, H₂-6'), 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); ¹³C-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 C₁₇H₃₂O₁₀Na [M+Na]⁺, 419.1893).

Creoside V (5): A colorless amorphous powder; $[\alpha]_D^{27}$ -24.3° (*c*=2.79, MeOH); IR (KBr) $\nu_{\max}$ 3450, 1649, 1084 cm⁻¹; ¹H-NMR (CD₃OD, 600 MHz) δ : 0.91 (3H, *J*=6.5 Hz, H₃-10), 1.17, 1.35 (1H each, both m, H₂-4), 1.41, 1.69 (1H each, both m, H₂-2), 1.60 (3H, s, H₃-8), 1.59 (1H, m, H₂-4), 1.67 (3H, s, H₃-9), 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, H₂-5''), 3.54 (1H, m, H-3''), 3.54, 3.93 (1H each, both m, H₂-1), 3.59 (1H, m, H-2''), 3.74 (1H, dd, *J*=5.2, 11.6 Hz, H₂-6'), 3.81 (1H, m, H-4''), 3.87 (1H, dd, *J*=3.3, 12.4 Hz, H₂-5''), 4.09 (1H, dd, *J*=2.2, 11.6 Hz, H₂-6'), 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); ¹³C-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-C₂H₅O₄]⁻; HR-FAB-MS: *m/z* 473.2368 (Calcd for C₂₁H₃₈O₁₀Na [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 NH₂-60-5 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: CH₃CN-H₂O (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 H₂O (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-H₂O (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-1,6- ξ -diol were identified by comparison of their physical data (^1H -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} ; ^1H -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 -3', 5'), 7.89 (2H, d, $J=8.9$ Hz, H_2 -2', 6'); ^{13}C -NMR data see Table 1; positive-ion EI-MS m/z 222 $[\text{M}]^+$; HR-EI-MS: m/z 222.0899 (Calcd for $\text{C}_{12}\text{H}_{14}\text{O}_4$ $[\text{M}]^+$, 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 naringinase (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 [$\text{MeOH}:\text{H}_2\text{O}$ (50:50, v/v)] to furnish *n*-hexanol (1.5 mg, 66.2%), which was identified by comparison of the ^1H - 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 ($[\alpha]_D$, ^1H -NMR, ^{13}C -NMR, MS) with reported values.

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