Reinvestigation of Absolute Stereostructure of (−)-Rosiridol: Structures of Monoterpene Glycosides, Rosiridin, Rosiridosides A, B, and C, from *Rhodiola sachalinensis*[1)]

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Three new (−)-rosiridol glycosides, rosiridosides A, B, and C, were isolated from the roots of *Rhodiola sachalinensis* together with rosiridin [(−)-rosiridol 1-*O*-*β*-D-glucopyranoside]. In the course of the structure elucidation of those new glycosides, the absolute configuration of the 4-position in (−)-rosiridol was reinvestigated. On the basis of the application of the modified Mosher's method for (−)- and (+)-rosiridol derivatives, the absolute configuration of the 4-position in (−)-rosiridol should be revised to be *S* orientation from the recently assigned *R* form, so that the absolute stereostructures of rosiridosides A, B, and C and rosiridin were determined.

Key words (−)-rosiridol; rosiridoside; rosiridin; (+)-rosiridol; *Rhodiola sachalinensis*; Rhodiolae Radix

In the course of our serial studies on bioactive constituents from Chinese natural medicines,[2—11)] we have characterized the structures of aliphatic alcohol and monoterpene oligoglycosides and cyanogenic glycosides from the roots of *Rhodiola* (*R*) *quadrifida*,[12,13)] *R. sacra*,[14)] and *R. crenulata*.[15)] Among the isolated compounds, several monoterpene oligoglycosides were found to show histamine release from rat exudate cells induced by an antigen–antibody reaction.[14)] Furthermore, from the extracts of *R. sachalinensis* with a protective effect an D-galactosamine-induced cytotoxicity in primary cultured mouse hepatocytes, we isolated monoterpene oligoglycosides, flavonol bisdesmosides, and cyanogenic glycoside named sachalosides I—VIII and reported their structures and hepatoprotective effects.[1,16)] As a continuing study on *R. sachalinensis*, we isolated three new (−)-rosiridol glycosides termed rosiridosides A (**3**), B (**4**), and C (**5**) together with rosiridin (**2**) [(−)-rosiridol 1-*O*-*β*-D-glucopyranoside].

The absolute configuration of the 4-position in (−)-rosiridol (**1**) was first reported to be *S* by the total enantioselective synthesis and chemical transformation to (−)-eldanolide.[17)] However, the absolute configuration of the 4-position in (−)-rosiridol and rosiridin was recently revised to be *R* (**1′**, **2′**) by the application of the modified Mosher's method.[18,19)] In order to determine the absolute stereostructures of rosiridosides (**3**—**5**), we began with a reinvestigation of the absolute configuration of (−)-rosiridol. This paper deals with the application of the modifiel Mosher's method for (−)- and (+)-rosiridol pivalate (**6**, **11**), which supported the previous synthetic evidence to be *S* configuration (**1**, **2**) rather than *R* form (**1′**, **2′**). Furthermore, we described the isolation and structure elucidation of three new (−)-rosiridol glycosides (**3**, **4**, **5**).

(–)-Rosiridol (**1**): R = H
Rosiridin (**2**): R = Glc

1′: R = H
2′: R = Glc

Rosiridoside A (**3**): R = Ara(p)
Rosiridoside B (**4**): R = Glc^6—Ara(f)
Rosiridoside C (**5**): R = Glc^6—Ac

Glc: *β*-D-glucopyranosyl
Ara(p): *α*-L-arabinopyranosyl
Ara(f): *α*-L-arabinofuranosyl

Chart 1

Isolation of Rosiridin and Rosiridosides A, B, and C The methanolic extract from the roots of *R. sachalinensis* was partitioned into an EtOAc–H_2O mixture to furnish an EtOAc-soluble phase (3.5% from the roots) and aqueous layer, which was further extracted with *n*-BuOH to give a *n*-BuOH-soluble phase. The EtOAc-soluble fraction was subjected to normal-phase and reverse-phase column chromatographies, and finally HPLC to give rosiridoside C (**5**, 0.0013%). The *n*-BuOH-soluble phase was subjected to Diaion HP-20 column chromatography to afford a methanol-eluted fraction (3.5%) as previously reported.[1,16)] The methanol-eluted fraction was separated by normal- and reversed-phase column chromatography, and finally HPLC to give rosiridosides A (**3**, 0.00019%) and B (**4**, 0.00032%) together with rosiridin (**2**, 0.49%).

Reinvestigation of the Absolute Configuration of (−)-Rosiridol Rosiridin (**2**) was obtained as a colorless viscous oil with a negative optical rotation {$[\alpha]_D^{25}$ −32.1° (*c*=2.06 in MeOH), $[\alpha]_D^{22}$ −31.3° (*c*=4.65 in acetone)} and the IR spectrum of **2** showed absorption bands at 3450, 1638, and 1045 cm^{-1} assignable to hydroxyl, olefin, and ether functions. The positive-ion first atom bombardment (FAB)-MS of **2** exhibited a quasimolecular ion peak at *m*/*z* 355 $(M+Na)^+$. The high resolution (HR) MS analysis revealed the molecular formula of **2** to be $C_{16}H_{28}O_7$. Acid hydrolysis of **2** with HCl 1 M liberated D-glucose, which was identified by HPLC analysis using an optical rotation detector.[3,7,16)] On the basis of this evidence and by comparison of the physical data with reported values {$[\alpha]_D^{25}$ −16.9° (*c*=0.087 in MeOH),[18)] $[\alpha]_D^{20}$ −32.7° (*c*=1.1 in acetone),[20)] ^{1}H- and ^{13}C-NMR,[18)] MS[18)]}, **2** was identified to be rosiridin, which was isolated from *R. rosea*[20)] and *R. sachalinensis*, in which the absolute stereostructure of rosiridin was reported to be **2′**.[18)] Enzymatic hydrolysis of **2** with *β*-glucosidase furnished (−)-rosiridol, {**1**, $[\alpha]_D^{25}$ −7.7° (*c*=1.98 in acetone), $[\alpha]_D^{22}$ −14.2° (*c*=0.42 in $CHCl_3$)}, whose molecular formula $C_{10}H_{18}O_2$ was determined from a molecular ion peak at *m*/*z* 170 $(M)^+$ and by HR-MS measurement. The ^{1}H-NMR (CD_3OD) and ^{13}C-NMR (Table 1) spectra[21)] of **1** showed signals assignable to three methyls [*δ* 1.65, 1.69, 1.74, (3H each, both s, H_3-8, 10,

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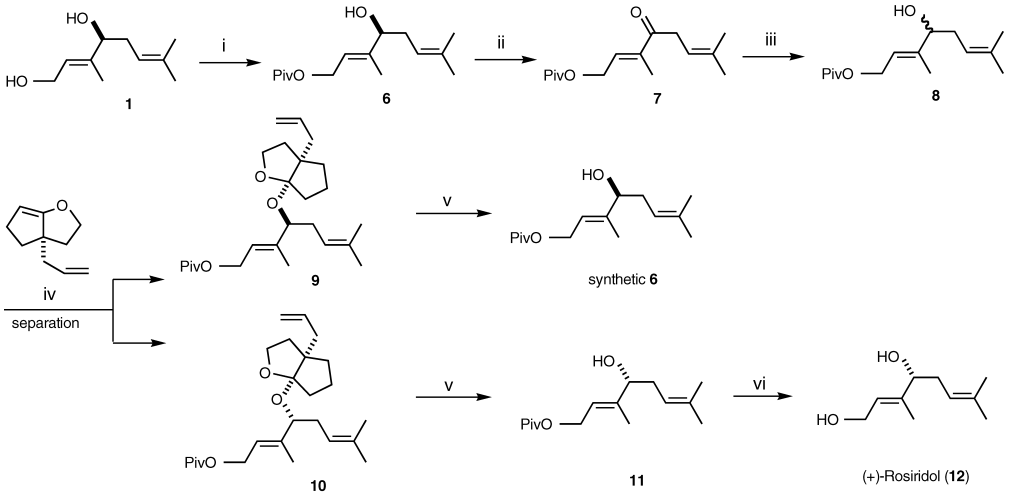
Table 1. ^{13}C-NMR (150 MHz) Data for **1** ($CDCl_3$), **2**—**5** (CD_3OD)

Position	1	2	3	4	5
1	59.2	66.0	65.9	66.1	65.8
2	124.5	122.8	122.9	122.7	122.6
3	140.4	142.9	142.9	143.3	143.5
4	76.3	78.0	78.1	78.0	78.0
5	34.2	34.8	34.8	34.8	34.8
6	119.7	121.6	121.6	121.6	121.6
7	135.4	134.0	134.1	134.1	134.2
8	18.0	18.1	18.0	18.1	18.1
9	25.9	26.0	26.0	26.1	26.1
10	12.2	12.1	12.0	12.2	12.0
1′		102.8	103.5	102.8	102.5
2′		75.0	72.5	75.0	75.0
3′		77.9	74.4	78.1	78.0
4′		71.6	69.6	72.0	71.7
5′		78.1	66.9	76.8	75.3
6′		62.7		68.1	64.9
1″				110.2	
2″				83.3	
3″				79.0	
4″				85.9	
5″				63.1	
6′-*O*-Ac					172.8
					20.8

9)], a methylene bearing an oxygen function [δ 4.23 (2H, dd, J=3.5, 6.9 Hz H_2-1)], a methine bearing an oxygen function [δ 4.03 (1H, dd, J=5.5, 7.6 Hz, H-4)], two olefinic protons [δ 5.12 (1H, t-like, J=*ca.* 7.0 Hz, H-6), 5.67 (1H, t-like, J=*ca.* 7 Hz, H-2)], and a methylene [δ 2.27 (2H, m, H_2-5)]. These results supported that the planner structure of (−)-rosiridol (**1**) was the same as the reported structure.[17,19] On the other hand, although all the optical rotations of reported (−)-rosiridol showed negative values {$[\alpha]_D^{20}$ −7.7° (c=1.3 in acetone),[20] $[\alpha]_D^{25}$ −7.1° (c=0.4 in acetone),[17] $[\alpha]_D^{24}$ −7.1° (c=0.1 in acetone),[19] $[\alpha]_D^{24}$ −21.1° (c=0.1 in $CHCl_3$)[19]}, the absolute configuration at the 4-position was first reported to be S[17] and then changed to R form.[18,19] In order to confirm the absolute configuration of (−)-rosiridol, the application of the modified Mosher's method for (−)- and (+)-rosiridol was carried out. (−)- And (+)-rosiridol 1-pivalates (**6**, **11**) having the different configulation at the 4-position were prepared from (−)-rosiridol (**1**) in 5-steps and applied the modified Mosher's method. Namely, selective acylation of **1** with pivaloyl chloride yielded the 1-pivalate (**6**), which was treated with CrO_3 in pyridine to afford the 4-ketone (**7**). Reduction of **7** with $NaBH_4$ in the presence of $CeCl_3$ furnished an enantiomeric mixture (**8**), which was treated with (5*S*)-allyl-2-oxabicyclo[3,3,0]oct-8-ene (ALBO)[22] followed by separation to give **9** (21%) and **10** (39%). Acid treatment of **9** and **10** with *p*-TsOH furnished **6** and **11**, respectively. Synthetic **6** was identified by comparison with authentic (−)-rosiridol 1-pivalate (**6**) {$[\alpha]_D^{25}$ −5.9° (c=0.45 in acetone), IR, ^{1}H- and ^{13}C-NMR, MS}. Deacylation of **11** with 1% NaOMe yielded (+)-rosiridol (**12**). The physical data (IR, ^{1}H- and ^{13}C-NMR, positive FAB-MS, HR-MS) of **11** and **12** were in accord with those of **6** and **1**, respectively, except for the optical rotation {**11**: $[\alpha]_D^{25}$ +4.4° (c=0.45 in acetone); **12**: $[\alpha]_D^{25}$ +6.1° (c=0.32 in acetone), $[\alpha]_D^{22}$ +14.1° (c=0.32 in $CHCl_3$)}. Treatment of **6** and **11** with (−)-MTPA-Cl in pyridine yielded the (*S*)-MTPA ester (**6a**, **11a**), respectively. Whereas, the (*R*)-MTPA ester (**6b**, **11b**) were derived from **6** and **11** by treatment with (+)-MTPA-Cl in pyridine. In the case of MTPA esterification using *S*-(−)- or *R*-(+)-MTPA in the presence of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and 4-dimethylaminopyridine (4-DMAP), **6a** and **6b** were also obtained from **6**. As shown in Fig. 2, the signals due to the protons attached to the 5, 6, 8 and 9-positions in the (*S*)-MTPA ester (**6a**) were observed at higher fields compared with those of the (*R*)-MTPA ester (**6b**) [$\Delta\delta$: negative], while the signals due to the protons on the 1, 2, and 10-positions in **6a** were observed at lower fields compared with those of **6b** [$\Delta\delta$: positive]. The same result was obtained in the application case of the modified Mosher's method using synthetic **6**. On the other hand, the signals due to the protons attached to the 1, 2, and 10-positions in the (*S*)-MTPA ester (**11a**) were observed at higher fields compared with those of the (*R*)-MTPA ester (**11b**) [$\Delta\delta$: negative], while the signals due to the protons on the 5, 6, 8, and 9-positions in **11a** were observed at lower fields compared with those of **11b** [$\Delta\delta$: positive]. On the basis of those findings, the absolute configuration of the 4-position in (−)-rosiridol (**1**) was clarified to be *S* and also the absolute stereostructure of (−)-rosiridol (**1**) and rosiridin (**2**) was determined as shown.

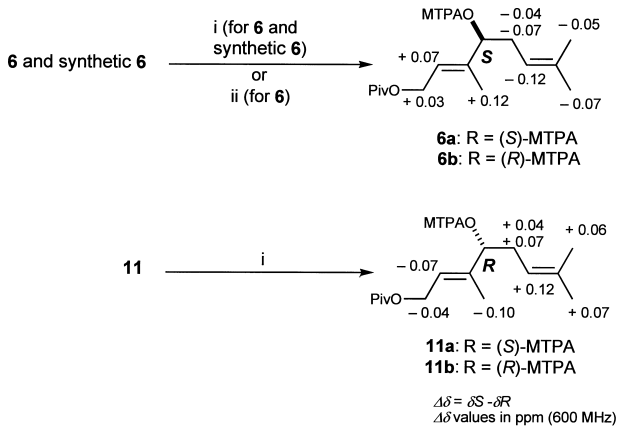
Absolute Stereostructures of Rosiridosides A, B, and C Rosiridoside A (**3**) was obtained as a colorless viscous oil with negative optical rotation ($[\alpha]_D^{27}$ −10.4° in MeOH). The IR spectrum of **3** showed absorption bands at 3545, 1653, and 1040 cm^{-1} assignable to hydroxyl, olefin, and ether functions. The positive-ion FAB-MS of **3** showed a pseudomolecular ion peak at m/z 325 $(M+Na)^+$ and the HR-MS analysis revealed the molecular formula of **3** to be $C_{15}H_{26}O_6$. Acid hydrolysis of **3** with HCl 1 M liberated L-arabinose, which was identified by HPLC analysis using an optical rotation detector.[3,7,16] Enzymatic hydrolysis of **3** with naringinase yielded (−)-rosiridol (**1**). The ^{1}H- (CD_3OD) and ^{13}C-NMR (Table 1) spectra[21] of **3** exhibited the presence of a (−)-rosiridol moiety [δ 1.62, 1.65, 1.68 (3H each, both s, H_3-8, 10, 9), 2.24 (2H, t like, J=*ca.* 7 Hz, H_2-5), 4.23, 4.30 (1H each, both dd, J=6.8, 12.4 Hz, H_2-1), 3.96 (1H, t, J=6.9 Hz, H-4), 5.10 (1H, t, J=6.8 Hz, H-6), 5.56 (1H, t, J=6.8 Hz, H-2)] and an α-L-arabinopyranosyl part [4.23 (1H, d, J=6.8 Hz, H-1′)]. As shown in Fig. 1, the double quantum filter correlation spectroscopy (DQF COSY) experiment on **3** indicated the presence of partial structures written in bold lines, and in the heteronuclear multiple-bond correlations (HMBC) experiment, long-range correlation was observed between the 1′-proton and 1-carbon. Furthermore, comparison of the ^{13}C-NMR data of **3** with those of **1** indicated the presence of a glycosylation shift around the 1-position. Consequently, the absolute stereostructure of rosiridoside A (**3**) was determined as shown.

Rosiridoside B (**4**) was also isolated as a colorless viscous oil with negative optical rotation ($[\alpha]_D^{27}$ −73.7° in MeOH) and its IR spectrum showed absorption bands due to hydroxyl, olefin, and ether functions. In the positive and negative-ion FAB-MS of **4**, quasimolecular ion peaks were observed at m/z 487 $(M+Na)^+$ and m/z 463 $(M-H)^-$ and the molecular formula $C_{21}H_{36}O_{11}$ was determined based on the results of HR-FAB-MS measurement. The acid hydrolysis of **4** liberated D-glucose and L-arabinose, whereas the enzymatic hydrolysis of **4** afforded (−)-rosiridol (**1**). The ^{1}H- (CD_3OD)



Reagents: i) pivaloyl chloride, pyridine, **6** (78%); ii) CrO_3, pyridine, **7** (30%); iii) $NaBH_4$, $CeCl_3 \cdot 7H_2O$, **8** (96%); iv) *p*-TsOH, **9** (21%), **10** (39%); v) *p*-TsOH, synthetic **6** (97% from **9**), **11** (87% from **10**); vi) 1% NaOMe, (+)-rosiridol (**12**, 32%)

Fig. 1. Synthesis of (−)- and (+)-Rosiridol



Reagents: i) (–)- or (+)-MTPACl, pyridine, **6a** (79% from **6**; 65% from synthetic **6**), **6b** (69% from **6**; 73% from synthetic **6**), **11a** (54% from **11**), **11b** (72% from **11**); ii) *S*-(–)- or *R*-(+)-MTPA, EDC, 4-DMAP, CH_2Cl_2, **6a** (16% from **6**), **6b** (18% from **6**).

Fig. 2

and ^{13}C-NMR (Table 1) spectra[21] of **4** showed signals due to a (−)-rosiridol moiety together with β-D-glucopyranosyl [δ 4.29 (1H, d, J=7.6 Hz, H-1′)] and α-L-arabinofuranosyl [δ 4.96 (1H, br s, H-1″)] parts. The proton and carbon signals in the ^{1}H- and ^{13}C-NMR data of **4** were superimposable on those of **3**, except for the signals due to the α-L-arabinopyranosyl part of **3**. The HMBC experiment on **4** showed long-range correlations between the 1′-proton and 1-carbon and between the 1″-proton and 6-carbon. On the basis of this evidence and detail comparison of ^{13}C-NMR data of **4** with those of **1** and **2**, the absolute stereostructure of rosiridoside B (**4**) was characterized as shown.

Rosiridoside C (**5**), a colorless viscous oil, $[\alpha]_D^{25}$ −16.7° in MeOH, showed absorption bands at 3566, 1734, 1647 and 1037 cm^{-1} assignable to hydroxyl, carbonyl, olefin, and ether functions in the IR spectrum. The molecular formula $C_{18}H_{30}O_8$ was determined based on the results of the positive-ion FAB-MS at m/z 397 $(M+Na)^+$ and HR-FAB-MS measurement. The acid hydrolysis of **5** liberated D-glucose,[3,7,16] while alkaline hydrolysis of **5** furnished rosiridin (**2**) and acetic acid, which was identified by HPLC analysis of its *p*-nitrobenzyl derivative.[23,24] The proton and carbon signals in the ^{1}H- and ^{13}C-NMR spectra[21] of **5** was very similar to those of **2**, except for the signal due to the acetyl group. In the HMBC experiment of **5**, a long-range correlation was observed between the 6′-proton and acetyl carbonyl carbon. Furthermore, comparison of the ^{13}C-NMR data of **5** with those of **2** indicated an acetylation shift around the 6′-position of **5**. Those findings led us to formulate the stereostructure of rosiridoside C (**5**) 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); Diaion HP-20 column chromatography (Nippon Rensui); 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. sachalinensis* were collected at Jilin and Heilongjiang provinces of China in 2005 and identified by one of authors (X. Li). A voucher of the plant is on file in our laboratory (2006. China-06).

Extraction and Isolation The methanolic extract (1457 g, 14.6%) was obtained from dried roots of *R. sachalinensis* (10.0 kg) as reported previously.[16] The aliquot (1395 g) from the extract was partitioned into an EtOAc–H_2O (1 : 1, v/v) mixture to furnish an EtOAc-soluble fraction (336 g, 3.5%) and an aqueous phase. The aqueous phase was further extracted with *n*-BuOH to give an *n*-BuOH-soluble fraction (418 g, 4.4%) and an H_2O-sol-

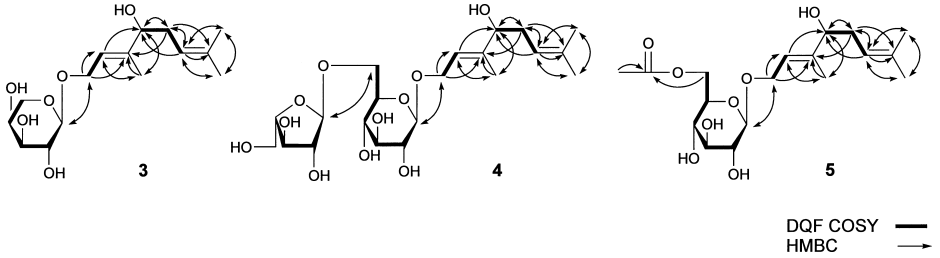


Fig. 3. Significant DQF COSY and HMBC Correlations of New Constituents **3**—**5**

uble fraction (620 g, 6.5%). The *n*-BuOH-soluble fraction (366 g) was subjected to Diaion HP-20 column chromatography (4.0 kg, H_2O→MeOH) to give H_2O- and MeOH-eluted fractions 65.9 g, 0.8% and 290.7 g, 3.5%), respectively. The EtOAc fraction (139 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 (15 : 3 : 1, lower layer→10 : 3 : 1, lower layer→7 : 3 : 1, lower layer)→MeOH] to give six fractions [Fr. 1 (18.0 g), Fr. 2 (38.7 g), Fr. 3 (19.8 g), Fr. 4 (13.1 g), Fr. 5 (15.1 g), Fr. 6 (17.4 g)]. Fraction 4 (13.0 g) was subjected to reversed-phase silica gel column chromatography [450 g, MeOH–H_2O (30 : 70→40 : 60→50 : 50→60 : 40→70 : 30→90 : 10, v/v)→ MeOH] to give twelve fractions [Fr. 4-1 (1157 mg), Fr. 4-2 (241 mg), Fr. 4-3 (689 mg), Fr. 4-4 (538 mg), Fr. 4-5 (752 mg), Fr. 4-6 (2058 mg), Fr. 4-7 (540 mg), Fr. 4-8 (479 mg), Fr. 4-9 (1701 mg), Fr. 4-10 (976 mg), Fr. 4-11 (248 mg), Fr. 4-12 (2391 mg)]. Fr. 4-9 (1771 mg) was further purified by HPLC [MeOH–H_2O (55 : 45, v/v)] to give rosiridoside C (**5**, 38.5 mg). The MeOH-eluted fraction (188.8 g) was subjected to ordinary-phase silica gel column chromatography [3.0 kg, $CHCl_3$–MeOH–H_2O (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 (428 mg), Fr. 2 (1.8 g), Fr. 3 (60.5 g), Fr. 4 (13.4 g), Fr. 5 (16.6 g), Fr. 6 (12.6 g), Fr. 7 (64.1 g)]. Fraction 3 (60.5 g) was subjected to reversed-phase silica gel column chromatography [1.5 kg, MeOH–H_2O (10 : 90→20 : 80→30 : 70→40 : 60→50 : 50→60 : 40→70 : 30, v/v)→MeOH] to give 11 fractions [Fr. 3-1 (12.1 g), Fr. 3-2 (3.4 g), Fr. 3-3 (2.3 g), Fr. 3-4 (822 mg), Fr. 3-5 (572 mg), Fr. 3-6 (26.5 g), Fr. 3-7 (956 mg), Fr. 3-8 (221 mg), Fr. 3-9 (347 mg), Fr. 3-10 (626 mg), Fr. 3-11 (356 mg). Fr. 3-6 (26.5044 g) was identified as rosiridin (**2**, 26.5044 g). Fr. 3-7 (956.3 mg) was separated by HPLC [MeOH–H_2O (45 : 55, v/v)] to give rosiridoside A (**3**, 10.5 mg). Fraction 5 (15.1 g) was subjected to reversed-phase silica gel column chromatography [450 g, MeOH–H_2O (20 : 80→30 : 70→50 : 50→ 70 : 30→90 : 10, v/v)→MeOH] to afford nine fractions [Fr. 5-1 (142 mg), Fr. 5-2 (5021 mg), Fr. 5-3 (860 mg), Fr. 5-4 (761 mg), Fr. 5-5 (6560 mg), Fr. 5-6 (212 mg), Fr. 5-7 (381 mg), Fr. 5-8 (386 mg), Fr. 5-9 (143 mg)]. Fraction 5-5 (446 mg) was separated by HPLC [MeOH–H_2O (35 : 65, v/v)] to give rosiridoside B (**4**, 17.6 mg).

Rosiridin (**2**): Obtained as colorless viscous oil; $[\alpha]_D^{25}$ −32.1° (*c*=2.06, MeOH); $[\alpha]_D^{22}$ −31.3° (*c*=4.65, acetone); IR (Film) ν_{max} 3450, 1638, 1045 cm^{-1}; ^{1}H-NMR (CD_3OD, 600 MHz) δ: 1.62, 1.66, 1.68, (3H each, both s, H_3-8, 9, 10), 2.23 (2H, t, *J*=6.8 Hz, H_2-5), 3.97 (1H, t, *J*=6.8 Hz, H-4), 4.28 (1H, dd, *J*=6.3, 11.7 Hz, H-1a), 4.32 (1H, m, H-1b), 4.30 (1H, d, *J*=7.7 Hz, H-1′), 5.12 (1H, t, *J*=6.8 Hz, H-6), 5.56 (1H, t like, *J*=*ca*. 7 Hz, H-2); ^{13}C-NMR data see Table 1; positive-ion FAB-MS *m*/*z*: 355 $[M+Na]^+$; HR-FAB-MS *m*/*z*: 355.1742 (Calcd for $C_{16}H_{28}O_7Na$ $[M+Na]^+$, 355.1733).

Rosiridoside A (**3**): Obtained as colorless viscous oil; $[\alpha]_D^{27}$ −10.4° (*c*=0.52, MeOH); IR (Film) ν_{max} 3545, 1653, 1040 cm^{-1}; ^{1}H-NMR (CD_3OD, 600 MHz) δ: 1.62, 1.65, 1.68 (3H each, both s, H_3-8, 10, 9), 2.24 (2H, t like, *J*=*ca.* 7 Hz, H_2-5), 4.23, 4.30 (1H each, both dd, *J*=6.8, 12.4 Hz, H_2-1), 3.96 (1H, t, *J*=6.9 Hz, H-4), 4.23 (1H, d, *J*=6.8 Hz, H-1′), 5.10 (1H, t, *J*=6.8 Hz, H-6), 5.56 (1H, t, *J*=6.8 Hz, H-2); ^{13}C-NMR data see Table 1; positive-ion FAB-MS *m*/*z*: 325 $[M+Na]^+$; HR-FAB-MS *m*/*z*: 325.1633 (Calcd for $C_{15}H_{26}O_6Na$ $[M+Na]^+$, 325.1627).

Rosiridoside B (**4**): Obtained as colorless viscous oil; $[\alpha]_D^{27}$ −73.7° (*c*=0.57, MeOH); IR (Film) ν_{max} 3565, 1634, 1041 cm^{-1}; ^{1}H-NMR (CD_3OD, 600 MHz) δ: 1.62, 1.67, 1.69 (3H each, both s, H_3-8, 10, 9), 2.24 (2H, t like, *J*=*ca.* 7 Hz, H_2-5), 4.30, 4.32 (1H each, both m, H_2-1), 3.96 (1H, m, H-4), 4.29 (1H, d, *J*=7.6 Hz, H-1′), 4.96 (1H, br s, H-1″), 5.10 (1H, t, *J*=7.0 Hz,, H-6), 5.56 (1H, t like, *J*=*ca*. 7 Hz, H-2); ^{13}C-NMR data see Table 1; positive-ion FAB-MS *m*/*z*: 487 $[M+Na]^+$; negative-ion FAB-MS *m*/*z*: 463 $[M-H]^-$; HR-FAB-MS *m*/*z*: 487.2162 (Calcd for $C_{21}H_{36}O_{11}Na$ $[M+Na]^+$, 487.2155).

Rosiridoside C (**5**): Obtained as colorless viscous oil; $[\alpha]_D^{25}$ −16.7° (*c*=0.94, MeOH); IR (Film) ν_{max} 3566, 1734, 1647, 1037 cm^{-1}; ^{1}H-NMR (CD_3OD, 600 MHz) δ: 1.62, 1.67, 1.69, 2.06 (3H each, both s, H_3-8, 10, 9, Ac), 2.24 (2H, t like, *J*=*ca.* 7 Hz, H_2-5), 3.97 (1H, t, *J*=6.9 Hz, H-4), 4.30, 4.32 (1H each, both m, H_2-1), 4.30 (1H, d, *J*=7.6 Hz, H-1′), 5.10 (1H, t, *J*=6.9 Hz, H-6), 5.55 (1H, t like, *J*=*ca*. 7 Hz, H-2); ^{13}C-NMR data see Table 1; positive-ion FAB-MS *m*/*z*: 397 $[M+Na]^+$; HR-FAB-MS *m*/*z*: 397.1841 (Calcd for $C_{18}H_{30}O_8Na$ $[M+Na]^+$, 397.1838).

Acid Hydrolysis of 2—5 A solution of **2**—**5** (each 1.0 mg) in HCl 1 M (2.0 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) with CH_3CN–H_2O [(75 : 25, v/v), 0.5 ml/min]. D-glucose and L-arabinose were confirmed by comparison of the retention times with the authentic samples (Wako Pure Chemicals Ltd., Osaka, Japan); t_R: 13.8 min (D-(+)-glucose, positive optical rotation); t_R: 12.3 min (L-(+)-arabinose, positive optical rotation).

Alkaline Hydrolysis of 5 A solution of **5** (7.0 mg) in 5% aqueous KOH (1.0 ml) was stirred at 37 °C for 5 h. The reaction mixture was neutralized with Amberlite HCR-W2 (H^+ form) and filtrated. The evaporated reaction mixture was subjected to SiO_2 column chromatography [$CHCl_3$–MeOH–H_2O (20 : 3 : 1, lower layer→15 : 3 : 1, lower layer)] to give **2** (4.6 mg, 74.2%) and the organic acid fraction. The obtained compound **2** was identified by comparison of their physical data ($[\alpha]_D$, ^{1}H-NMR, ^{13}C-NMR, MS) with isolated compound **2**. The organic acid fraction was dissolved in $(CH_2)_2Cl_2$ (2.0 ml) and the solution was treated with *p*-nitrobenzyl-*N*,*N*′-diisopyopylisourea (10 mg), then stirred at 80 °C for 1 h. The reaction solution was subjected to HPLC analysis [column: YMC-pack ODS-A, 250×4.6 mm i.d.; mobile phase: CH_3CN–H_2O (45 : 55, v/v); detection: UV (254 nm); flow rate: 1.0 ml/min] to identify the *p*-nitrobenzyl ester of acetic acid (t_R 28.5 min).

Enzymatic Hydrolysis of 2 with β-Glucosidase A solution of **2** (35.2 mg) in H_2O (2 ml) was treated with β-glucosidase (from Almond, Oriental Yeast Co., Ltd., Japan, 25 mg) and the solution was stirred at 37 °C for 12 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 (45 : 55, v/v)] to furnish (−)-rosiridol (**1**, 14.7 mg, 82%).

(−)-Rosiridol (**1**): Obtained as colorless viscous oil; $[\alpha]_D^{25}$ −7.7° (*c*=1.98, acetone); $[\alpha]_D^{22}$ −14.2° (*c*=0.42, $CHCl_3$); IR (Film) ν_{max} 3500, 2971, 1655 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 1.65, 1.69, 1.74, (3H each, both s, H_3-8, 10, 9), 2.27 (2H, m, H_2-5), 4.03 (1H, dd, *J*=5.5, 7.6 Hz, H-4), 4.23 (2H, dd, *J*=3.5, 6.9 Hz H_2-1), 5.12 (1H, t-like, *J*=*ca*. 7 Hz, H-6), 5.67 (1H, t-like, *J*=*ca*. 7 Hz, H-2); ^{13}C-NMR data see Table 1; EI-MS *m*/*z*: 170 $[M]^+$, HR-EI-MS *m*/*z*: 170.1300 (Calcd for $C_{10}H_{18}O_2$ $[M]^+$, 170.1307).

Enzymatic Hydrolysis of 3 and 4 with Naringinase A solution of **3** (4.9 mg) in 0.1 M acetate buffer (pH 3.8, 2.0 ml) was treated with naringinase (Sigma Chemical Co., 10 mg), 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 (45 : 55, v/v)] to furnish (−)-rosiridol (**1**, 1.5 mg, 54%). Through a similar procedure, enzymatic hydrolysis of **4** (7.8 mg) was carried out to afford (−)-rosiridol (**1**, 1.8 mg, 63%).

Pivaloylation of (−)-Rosiridol (1) A Solution of **1** (337 mg, 1.98 mmol) in pyridine (2.0 ml) was treated with pivaloyl chloride (0.23 ml, 1.98 mmol) and the mixture was stirred at 0 °C for 2 h. The reaction mixture was poured into water (2.0 ml) and the whole was extracted with EtOAc (10 ml). The EtOAc extract was successively washed with H_2O and brine, then dried over Na_2SO_4 powder and filtered. Removal of the solvent from the

filtrate under reduced pressure furnished a residue, which was purified by normal-phase silica gel column chromatography [*n*-hexane–EtOAc (2 : 1, v/v)] to give **6** (395 mg, 78%).

The 1-Pivalate (**6**): Obtained as colorless viscous oil; $[\alpha]_D^{25}$ −7.1° (*c*=0.24, acetone); IR (Film) ν_{max} 3500, 2972, 1732, 1655, 1049 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 1.20 (9H, s, ($C\underline{H}_3$)$_3$CCOO), 1.64, 1.71, 1.72, (3H each, both s, H_3-8, 10, 9), 2.27 (2H, m, H_2-5), 4.04 (1H, dd-like, H-4), 4.62 (2H, d, *J*=6.1 Hz, H_2-1), 5.09 (1H, t, *J*=7.3 Hz, H-6), 5.58 (1H, t, *J*=6.1 Hz, H-2); ^{13}C-NMR ($CDCl_3$, 150 MHz) δ: 12.4 (C-10), 18.0 (C-8), 25.9 (C-9), 27.2 [($\underline{C}H_3$)$_3$CCOO], 34.1 (C-5), 38.7 [(CH_3)$_3\underline{C}$COO], 61.0 (C-1), 76.1 (C-4), 119.5 (C-2), 119.8 (C-6), 135.4 (C-7), 142.3 (C-3), 178.6 [(CH_3)$_3\underline{C}\underline{C}$OO]; positive-ion FAB-MS *m/z*: 277 $[M+Na]^+$; HR-FAB-MS *m/z*: 277.1786 (Calcd for $C_{15}H_{26}O_3Na$ $[M+Na]^+$, 277.1780).

Oxidation of 6 A solution of **6** (395 mg, 1.56 mmol) in pyridine (3 ml) was treated with CrO_3 (315 mg, 3.15 mmol) and the mixture was stirred at rt for 2 h. The reaction mixture was poured into water (10 ml) and the whole was extracted with EtOAc (10 ml). The EtOAc extract was washed with H_2O and brine, then dried over Na_2SO_4 powder and filtered. Removal of the solvent from the filtrate under reduced pressure furnished a residue, which was purified by normal-phase silica gel column chromatography [*n*-hexane–EtOAc (50 : 1→30 : 1, v/v)] to give **7** (117 mg, 30%).

The 4-Ketone (**7**): Obtained as colorless viscous oil; IR (Film) ν_{max} 1734, 1684, 1653, 1076 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 1.24 (9H, s, ($C\underline{H}_3$)$_3$CCOO), 1.59, 1.75, 1.83 (3H each, both s, H_3-8, 10, 9), 3.40 (2H, d, *J*=7.4 Hz, H_2-5), 4.79 (2H, d, *J*=6.1 Hz, H_2-1), 5.29 (1H, t, *J*=7.4 Hz, H-6), 6.57 (1H, t, *J*=6.1 Hz, H-2); EI-MS *m/z*: 252 $[M]^+$; HR-EI-MS *m/z*: 252.1731 (Calcd for $C_{15}H_{24}O_3$ $[M]^+$, 252.1725).

Reduction of 7 A solution of **7** (117 mg, 0.46 mmol) in methanol (2 ml) was treated with $NaBH_4$ (17.4 mg, 0.46 mmol) and $CeCl_3$ $7H_2O$ (17.3 mg, 0.05 mmol), and the mixture was stirred at rt for 1 h. The reaction mixture was quenched in acetone, and then removal of the solvent under reduced pressure gave a residue, which was purified by normal-phase silica gel column chromatography [*n*-hexane–EtOAc (30 : 1, v/v)] to give **8** (112 mg, 96%).

The 4-Enantiomeric mixture (**8**). Obtained as colorless viscous oil; $[\alpha]_D^{24}$ +0.14° (*c*=1.47, acetone); IR (Film) ν_{max} 3500, 2971, 1655, 1048 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 1.20 (9H, s, ($C\underline{H}_3$)$_3$CCOO), 1.64, 1.71, 1.72 (3H each, both s, H_3-8, 10, 9), 2.27 (2H, dd like, H_2-5), 4.04 (1H, t, *J*=6.4 Hz, H-4), 4.62 (2H, d, *J*=6.6 Hz, H_2-1), 5.09 (1H, t-like, *J*=*ca*. 7 Hz, H-6), 5.58 (1H, t, *J*=6.6 Hz, H-2); positive-ion FAB-MS *m/z*: 277 $[M+Na]^+$; HR-FAB-MS *m/z*: 277.1776 (Calcd for $C_{15}H_{26}O_3Na$ $[M+Na]^+$, 277.1780).

Isolations of 9 and 10 by Chiral Resolution A solution of **8** (69.0 mg, 0.27 mmol) in CH_2Cl_2 (2.0 ml) was treated with (5*S*)-allyl-2-oxabicyclo[3.3.0]oct-8-ene (ALBO, 0.04 ml, 0.326 mmol) and *p*-TsOH (1 mg), and the mixture was stirred slowly from 0 °C to rt for 1 h. The reaction mixture was poured into saturated aqueous solution of sodium hydrogen carbonate and extracted with EtOAc (10 ml×3). The EtOAc extract was successively washed with H_2O and brine, then dried over Na_2SO_4 powder and filtered. Removal of the solvent under reduced pressure gave a residue, which was purified by normal-phase silica gel column chromatography [*n*-hexane–EtOAc (100 : 1→90 : 1→70 : 1, v/v)] to give **9** (42.8 mg, 21%) and **10** (42.6 mg, 39%).

Compound **9**: Obtained as colorless viscous oil; $[\alpha]_D^{24}$ −25.9° (*c*=1.28, acetone); IR (Film) ν_{max} 1732, 1653, 1047 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 1.18 (9H, s, ($C\underline{H}_3$)$_3$CCOO), 1.43—1.56 (6H, m, $CH_2CH_2CH_2$), 1.61, 1.68, 1.68, (3H each, both s, H_3-8, 10, 9), 1.68 (1H, m, $OCH_2CH_a\underline{H}_b$), 1.93 (1H, m, $OCH_2C\underline{H}_aH_b$), 2.07 (1H, dd, *J*=7.6, 13.7 Hz, CH_2=CH–$CH_a\underline{H}_b$), 2.09 (2H, m, H-5a), 2.24 (1H, dd, *J*=7.6, 14.5 Hz, H-5b), 2.30 (1H, dd, *J*=6.8, 13.7 Hz, CH_2=CH–$C\underline{H}_aH_b$), 3.76 (1H, dd like, *J*=8.2, 15.8 Hz, $OCH_a\underline{H}_bCH_2$), 3.81 (1H, ddd, *J*=4.1, 7.6, 15.8 Hz, $OC\underline{H}_aH_bCH_2$), 4.14 (1H, dd, *J*=6.2, 7.7 Hz, H-4), 4.55 (1H, dd, *J*=6.8, 12.4 Hz, H-1a), 4.61 (1H, dd, *J*=7.6, 12.4 Hz, H-1b), 5.03 (2H, m, $C\underline{H}_2$=$CHCH_2$), 5.08 (1H, dd-like, H-6), 5.49 (1H, dd, *J* =6.8, 7.6 Hz, H-2), 5.85 (1H, m, CH_2=$C\underline{H}CH_2$); ^{13}C-NMR ($CDCl_3$, 150 MHz) δ: 12.0 (C-10), 17.9 (C-8), 21.6, 34.8, 36.4 ($C\underline{C}H_2\underline{C}H_2\underline{C}H_2$), 25.8 (C-9), 27.2 [($\underline{C}H_3$)$_3$CCOO], 34.1 (C-5), 38.2 ($OCH_2\underline{C}H_2$), 38.7 [(CH_3)$_3\underline{C}$COO], 40.4 (CH_2=$CH\underline{C}H_2$), 54.5 ($OCH_2CH_2\underline{C}$), 60.8 (C-1), 65.8 ($O\underline{C}H_2CH_2$), 77.9 (C-4), 116.7 ($\underline{C}H_2$=CH), 117.6 [$O\underline{C}O$(C)(CH_2)], 120.0 (C-2), 121.1 (C-6), 132.6 (C-7), 136.9 (CH_2=$\underline{C}$H), 143.6 (C-3), 178.4 [(CH_3)$_3\underline{C}\underline{C}$OO]; EI-MS *m/z*: 404 $[M]^+$; HR-EI-MS *m/z*: 404.2932 (Calcd for $C_{25}H_{40}O_4$ $[M]^+$, 404.2926).

Compound **10**: Obtained as colorless viscous oil; $[\alpha]_D^{25}$ +15.0° (*c*=0.07, acetone); IR (Film) ν_{max} 1732, 1653, 1048 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 1.19 (9H, s, ($C\underline{H}_3$)$_3$CCOO), 1.47—2.06 (6H, m, $CH_2CH_2CH_2$), 1.59, 1.69, 1.69, (3H each, both s, H_3-8, 10, 9), 1.66 (1H, m, $OCH_2CH_a\underline{H}_b$), 1.90 (1H, m, $OCH_2C\underline{H}_aH_b$), 2.10 (1H, dd, *J*=7.6, 13.7 Hz, CH_2=CH–$CH_a\underline{H}_b$), 2.10 (2H, m, H-5a), 2.22 (1H, dd, *J*=6.9, 14.5 Hz, H-5b), 2.27 (1H, dd, *J*=7.6, 13.7 Hz, CH_2=CH–$C\underline{H}_aH_b$), 3.65 (1H, dd, *J*=7.6, 15.1 Hz, $OCH_a\underline{H}_bCH_2$), 3.74 (1H, m, $OC\underline{H}_aH_bCH_2$), 3.98 (1H, t, *J*=6.9 Hz, H-4), 4.59 (2H, m, H_2-1), 5.05 (2H, m, $C\underline{H}_2$=$CHCH_2$), 5.05 (1H, m, H-6), 5.49 (1H, t, *J*=6.9 Hz, H-2), 5.85 (1H, m, CH_2=$C\underline{H}CH_2$); ^{13}C-NMR ($CDCl_3$, 150 MHz) δ: 12.4 (C-10), 17.9 (C-8), 25.8 (C-9), 27.2 [($\underline{C}H_3$)$_3$CCOO], 33.7 (C-5), 36.3, 34.7, 21.4 ($C\underline{C}H_2\underline{C}H_2\underline{C}H_2$), 38.3 ($OCH_2\underline{C}H_2$), 38.7 [($CH_3$)$_3\underline{C}$COO], 40.4 ($CH_2$=$CH\underline{C}H_2$), 54.7 ($OCH_2CH_2\underline{C}$), 61.0 (C-1), 66.1 ($O\underline{C}H_2CH_2$), 78.7 (C-4), 116.6 ($\underline{C}H_2$=CH), 117.9 [$O\underline{C}O$(C)($CH_2$)], 118.5 (C-2), 120.8 (C-6), 132.8 (C-7), 136.9 (CH_2=$\underline{C}$H), 143.8 (C-3), 178.5 [(CH_3)$_3\underline{C}\underline{C}$OO]; EI-MS *m/z*: 404 $[M]^+$; HR-EI-MS *m/z*: 404.2917 (Calcd for $C_{25}H_{40}O_4$ $[M]^+$, 404.2926).

Treatment of 9 and 10 with *p*-TsOH A solution of **9** (31.0 mg, 0.077 mmol) in MeOH (1 ml) was treated with *p*-TsOH (1 mg), and the mixture was stirred at rt for 2 h. The reaction mixture was poured into saturated aqueous solution of sodium hydrogen carbonate and extracted with EtOAc (10 ml). The EtOAc extract was washed with H_2O and brine, then dried over Na_2SO_4 powder and filtered. Removal of the solvent under reduced pressure gave a residue, which was purified by normal-phase silica gel column chromatography [*n*-hexane–EtOAc (2 : 1, v/v)] to give synthetic **6** (18.8 mg, 97%). Through a similar procedure, **11** (20.4 mg, 87%) was obtained from **10** (37.2 mg, 0.092 mmol).

Synthetic **6**: Obtained as colorless viscous oil; IR (Film) ν_{max} 3502, 2972, 1730, 1655, 1049 cm^{-1}; $[\alpha]_D^{25}$ −5.9° (*c*=0.45, acetone); The proton and carbon signals of ^{1}H- and ^{13}C-NMR were completely the same values as those of **6**; positive-ion FAB-MS *m/z*: 277 $[M+Na]^+$; HR-FAB-MS *m/z*: 277.1784 (Calcd for $C_{15}H_{26}O_3Na$ $[M+Na]^+$, 277.1780).

Compound **11**: Obtained as colorless viscous oil; $[\alpha]_D^{25}$ +4.4° (*c*=0.45, acetone); IR (Film) ν_{max} 3503, 2973, 1730, 1655, 1049 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 1.20 (9H, s, ($C\underline{H}_3$)$_3$CCOO), 1.65, 1.72, 1.73, (3H each, both s, H_3-8, 10, 9), 2.27 (2H, m, H_2-5), 4.04 (1H, dd, *J*=6.4, 6.8 Hz, H-4), 4.62 (2H, d, *J*=6.4 Hz, H_2-1), 5.09 (1H, t, *J*=7.3 Hz, H-6), 5.59 (1H, t, *J*=6.4 Hz, H-2); ^{13}C-NMR ($CDCl_3$, 150 MHz) δ: 12.4 (C-10), 18.0 (C-8), 25.9 (C-9), 27.2 [($\underline{C}H_3$)$_3$CCOO], 34.1 (C-5), 38.7 [(CH_3)$_3\underline{C}$COO], 60.9 (C-1), 76.1 (C-4), 119.5 (C-2), 119.8 (C-6), 135.5 (C-7), 142.3 (C-3), 178.5 [(CH_3)$_3\underline{C}\underline{C}$OO]; positive-ion FAB-MS *m/z*: 277 $[M+Na]^+$; HR-FAB-MS *m/z*: 277.1776 (Calcd for $C_{15}H_{26}O_3Na$ $[M+Na]^+$, 277.1780).

Deacylation of 11 A solution of **11** (15.0 mg, 0.059 mmol) in 1% NaOMe–MeOH (1.0 ml) was stirred at rt for 12 h. The reaction mixture was neutralized with Dowex HCR-W2 (H^+ form) and the resin was removed by filtration. Evaporation of the solvent from the filtrate under reduced pressure gave a residue, which was purified by normal-phase silica gel column chromatography [$CHCl_3$→$CHCl_3$–MeOH (10 : 1, v/v)] to give (+)-rosiridol (**12**, 3.2 mg, 32%).

(+)-Rosiridol (**12**): Obtained as colorless viscous oil; $[\alpha]_D^{25}$ +6.1° (*c*=0.32, acetone); $[\alpha]_D^{22}$ +14.1° (*c*=0.32, $CHCl_3$); IR (Film) ν_{max} 3500, 2975, 1655 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 1.65, 1.69, 1.74 (3H each, both s, H_3-8, 10, 9), 2.27 (2H, m, H_2-5), 4.03 (1H, dd, *J*=5.5, 7.6 Hz, H-4), 4.23 (2H, dd, *J*=3.4, 6.9 Hz, H_2-1), 5.12 (1H, t-like, *J*=*ca*. 7 Hz, H-6), 5.67 (1H, t-like, *J*=*ca*. 7 Hz, H-2); ^{13}C-NMR ($CDCl_3$, 150 MHz) δ: 12.2 (C-10), 18.0 (C-8), 25.9 (C-9), 34.2 (C-5), 59.2 (C-1), 76.3 (C-4), 124.5 (C-2), 119.7 (C-6), 135.4 (C-7), 140.4 (C-3); EI-MS *m/z*: 170 $[M]^+$, HR-EI-MS *m/z*: 170.1300 (Calcd for $C_{10}H_{18}O_2$ $[M]^+$, 170.1307).

Preparation of the (*S*)- and (*R*)-MTPA Esters (6a, 6b) with MTPA-Cl A Solution of **6** and synthetic **6** (each 2.0 mg, 0.008 mmol) in pyridine (1 ml) was each treated with (−)-MTPA-Cl (both 0.01 ml, 0.066 mmol), and the mixture was stirred at rt for 6 h. The reaction mixture was poured into water (1 ml) and the whole was extracted with EtOAc (6 ml). The EtOAc extract was washed with H_2O and brine, then dried over Na_2SO_4 powder and filtered. Removal of the solvent from the filtrate under reduced pressure furnished a residue, which was purified by normal-phase silica gel column chromatography [*n*-hexane–EtOAc (30 : 1, v/v)] to give (*S*)-MTPA ester derivative (**6a**, 4.1 mg, 79% from **6**; 2.4 mg, 65% from synthetic **6**). Through a similar procedure, the (*R*)-MTPA ester derivative (**6b**, 3.1 mg, 69% from **6**; 2.7 mg, 73% from synthetic **6**) was obtained from **6** (2.5 mg, 0.010 mmol) and synthetic **6** (2.1 mg, 0.008 mmol), respectively, using (+)-MTPA-Cl.

(*S*)-MTPA Ester Derivative (**6a**): ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 1.54 (3H, s, H_3-8), 1.63 (3H, s, H_3-9), 1.72 (3H, s, H_3-10), 2.28 (1H, m, H-5a), 2.42 (1H, m, H-5b), 4.61 (2H, dd-like, H_2-1), 4.92 (1H, m, H-6), 5.38 (1H, dd-like, H-4), 5.67 (1H, t-like, H-2); ^{13}C-NMR ($CDCl_3$, 150 MHz) δ: 12.6 (C-10), 17.8 (C-8), 25.7 (C-9), 31.4 (C-5), 60.4 (C-1), 80.7 (C-4), 118.1 (C-6), 123.8 (C-2), 135.2 (C-7), 137.4 (C-3).

(*R*)-MTPA Ester Derivative (**6b**): ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 1.59 (3H, s, H_3-8), 1.60 (3H, s, H_3-10), 1.70 (3H, s, H_3-9), 2.32 (1H, m, H-5a), 2.49 (1H, m, H-5b), 4.58 (2H, dd-like, H_2-1),5.04 (1H, t-like, H-6), 5.34 (1H, dd-like, H-4), 5.60 (1H, t-like, H-2); ^{13}C-NMR ($CDCl_3$, 150 MHz) δ: 12.3 (C-10), 17.9 (C-8), 25.8 (C-9), 31.5 (C-5), 60.4 (C-1), 80.9 (C-4), 118.5 (C-6), 123.5 (C-2), 135.3 (C-7), 137.3 (C-3).

Preparation of the (*S*)- and (*R*)-MTPA Esters (6a, 6b) with MTPA A Solution of **6** (3.0 mg, 0.012 mmol) in CH_2Cl_2 (1 ml) was added (*S*)-MTPA (5.5 mg, 0.025 mmol), EDC (9.0 mg, 0.047 mmol), and 4-DMAP (3.5 mg, 0.028 mmol). The mixture was stirred at rt for 12 h. The reaction mixture was poured into water (1 ml) and the whole was extracted with EtOAc (6 ml). The EtOAc extract was successively washed with H_2O and brine, then dried over Na_2SO_4 powder and filtered. Removal of the solvent from the filtrate under reduced pressure furnished a resudue, which was purified by normal-phase silica gel column chromatography [*n*-hexane–EtOAc (30 : 1, v/v)] to give (*S*)-MTPA ester derivative **6a** (0.9 mg, 16%). Through a similar procedure, the (*R*)-MTPA ester derivative (**6b**, 1.0 mg, 18%) was obtained from **6** (3.0 mg, 0.012 mmol) using (*R*)-MTPA.

Preparation of the (*S*)- and (*R*)-MTPA Esters (11a, 11b) with MTPA-Cl (*S*)- and (*R*)-MTPA esters, **11a** (2.8 mg, 54%) and **11b** (3.9 mg, 72%), were each obtained from **11** (2.5 mg, 0.010 mmol for **11a**, 2.9 mg, 0.011 mmol for **11b**) by using the similar procedure when **6a** and **6b** were given.

(*S*)-MTPA Ester Derivative (**11a**): ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 1.60 (3H, s, H_3-8), 1.62 (3H, s, H_3-10), 1.70 (3H, s, H_3-9), 2.32 (1H, m, H-5a), 2.49 (1H, m, H-5b), 4.57 (2H, dd, J=6.9, 9.7 Hz, H_2-1), 5.04 (1H, t-like, H-6), 5.33 (1H, dd, J=5.5, 8.3 Hz, H-4), 5.60 (1H, t, J=6.9 Hz, H-2); ^{13}C-NMR ($CDCl_3$, 150 MHz) δ: 12.3 (C-10), 17.9 (C-8), 25.8 (C-9), 31.5 (C-5), 60.4 (C-1), 80.9 (C-4), 118.5 (C-6), 123.5 (C-2), 135.3 (C-7), 137.4 (C-3).

(*R*)-MTPA Ester Derivative (**11b**): ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 1.54 (3H, s, H_3-8), 1.63 (3H, s, H_3-9), 1.72 (3H, s, H_3-10), 2.28 (1H, m, H-5a), 2.42 (1H, m, H-5b), 4.61 (2H, dd-like, H_2-1), 4.92 (1H, t-like, H-6), 5.38 (1H, dd, J=6.2, 7.6 Hz, H-4), 5.67 (1H, t-like, H-2); ^{13}C-NMR ($CDCl_3$, 150 MHz) δ: 12.6 (C-10), 17.8 (C-8), 25.7 (C-9), 31.4 (C-5), 60.4 (C-1), 80.7 (C-4), 118.1 (C-6), 123.8 (C-2), 135.2 (C-7), 137.4 (C-3).

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References and Notes

1) This paper is number 30 in the series "Bioactive Constituents from Chinese Natural Medicines". For paper number 29, see: Li X., Nakamura S., Matsuda H., Yoshikawa M., *Chem. Pharm. Bull.*, **56**, 612—615 (2008).
2) Morikawa T., Xie H., Wang T., Matsuda H., Yoshikawa M., *Helv. Chim. Acta*, in press (2008).
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., Shimoda H., Matsuda H., Yamahara J., Murakami N., *Chem. Pharm. Bull.*, **43**, 1245—1247 (1995).
14) Yoshikawa M., Shimada H., Horikawa S., Murakami T., Shimoda H., Yamahara J., Matsuda H., *Chem. Pharm. Bull.*, **45**, 1498—1503 (1997).
15) Nakamura S., Li X., Matsuda H., Yoshikawa M., *Chem. Pharm. Bull.*, **56**, 536—540 (2008).
16) Nakamura S., Li X., Matsuda H., Ninomiya K., Morikawa T., Yamaguti K., Yoshikawa M., *Chem. Pharm. Bull.*, **55**, 1505—1511 (2007).
17) Hong B. C., Hong J. H., *Angew. Chem. Int. Ed.*, **37**, 468—470 (1998).
18) Fan W., Tezuka Y., Ni K. M., Kadota S., *Chem. Pharm. Bull.*, **49**, 396—401 (2001).
19) 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).
20) Kurkin V. A., Zapesochnaya G. G., Shchavinskii A. N., *Khim. Prir. Soedin.*, **5**, 632—636 (1985).
21) The ^{1}H- and ^{13}C-NMR spectra of **1**—**5** 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.
22) Nemoto H., Tsutsumi H., Yuzawa S., Peng X., Zhong W., Xie J., Miyoshi N., Suzuki I., Shibuya M., *Tetrahedron Lett.*, **45**, 1667—1670 (2004).
23) Murakami T., Nakamura J., Matsuda H., Yoshikawa M., *Chem. Pharm. Bull.*, **47**, 1759—1764 (1999).
24) Murakami T., Nakamura J., Kageura T., Matsuda H., Yoshikawa M., *Chem. Pharm. Bull.*, **48**, 1720—1725 (2000).