Revised Absolute Stereochemistry of Rhodiolosides A—D, Rhodiolol A and Sachalinol A from *Rhodiola rosea*

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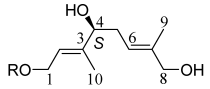
The absolute stereochemistry of rhodiolosides A—D (1—4), four monoterpene glycosides from the roots of *Rhodiola rosea*, and their aglycones, rhodiolol A (5), (−)-rosiridol (6) and sachalinol A (7), were reinvestigated. It was found that the absolute configurations of C-4 in these compounds, previously assigned to be 4-*R*, should be revised to 4-*S*.

Key words *Rhodiola rosea*; Crassulaceae; monoterpene; glycoside; rhodioloside

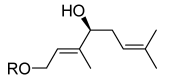
We had reported four monoterpene glycosides, rhodiolosides A—D (**1**—**4**) from the roots of *Rhodiola rosea* L. (Crassulaceae).[1] The absolute these configurations of C-4 in compounds (**1**—**4**), and their aglycones, rhodiolol A (**5**), (−)-rosiridol (**6**) and sachalinol A (**7**) had been assigned to be 4-*R* by application of the modified Mosher's method or direct comparison of the Optical Rotation values with the reported compounds. However, very recently, several glycosides with (−)-rosiridol (**6**) as the aglycone were determined to be 4-*S* configurations.[2] This promoted us to reinvestigate the absolute stereochemistry of rhodiolosides A—D (**1**—**4**) and their aglycones (**5**—**7**).

Results and Discussion

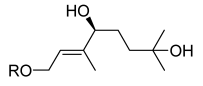
The absolute configurations of C-4 in rhodiolosides A—D (**1**—**4**) were determined by the following strategy. Firstly, the monoterpene glycosides were hydrolyzed enzymatically to obtain the aglycones. Secondly, the primary hydroxyl groups in the aglycones were selectively protected by pivaloylation. Thirdly, the 4-hydroxyl group in the pivaloylated monoterpenes was esterified with (*R*)- and (*S*)-α-methoxy-α-(trifluoromethyl)-phenylacetyl (MTPA) chloride to obtain the 4-(*S*)- and 4-(*R*)-MTPA esters. The absolute configuration of C-4 was determined by observation of the $\Delta\delta$ values (δ_S–δ_R) between the 4-(*S*)- and 4-(*R*)-MTPA esters.



1 R = β-D-Glc
5 R = H



2 R = α-D-Glc-(1→6)-β-D-Glc
3 R = β-D-Glc-(1→3)-β-D-Glc
6 R = H



4 R = β-D-Glc
7 R = H

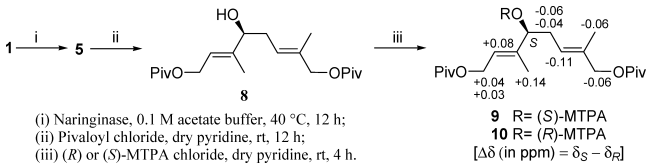
Glc: glucopyranosyl

Chart 1

Enzymatic hydrolysis of rhodioloside A (**1**) by naringinase afforded rhodiolol A (**5**). The 1,8-dipivaloyl ester (**8**) obtained by selective protection of the 1,8-primary alcohol of **5** with pivaloyl chloride, derived the (*S*)- and (*R*)-MTPA esters (**9** and **10**). As shown in Chart 2, the $\Delta\delta$ values (δ_S–δ_R) indicated the 4-*S* configuration. Thus, the structure of rhodioloside A (**1**) was revised to (2*E*,6*E*,4*S*)-4,8-dihydroxy-3,7-dimethyl-2,6-octadienyl β-D-glucopyranoside, and its aglycone, rhodiolol A (**5**) to (2*E*,6*E*,4*S*)-4,8-dihydroxy-3,7-dimethyl-2,6-octadiene.

Enzymatic hydrolysis of rhodioloside B (**2**) and rhodioloside C (**3**) by naringinase afforded (−)-rosiridol (**6**). The 1-pivaloyl ester of (−)-rosiridol (**11**) derived to the (*S*)- and (*R*)-MTPA esters (**12**, **13**). On the basis of the calculate values of $\Delta\delta_{S-R}$, the absolute configuration of C-4 was determined to be *S*. Thus, the structure of rhodioloside B (**2**) was revised to (2*E*,4*S*)-4-hydroxy-3,7-dimethyl-2,6-octadienyl α-D-glucopyranosyl(1→6)-β-D-glucopyranoside, rhodioloside C (**3**) to (2*E*,4*S*)-4-hydroxy-3,7-dimethyl-2,6-octadienyl β-D-glucopyranosyl(1→3)-β-D-glucopyranoside, and (−)-rosiridol (**6**) to (2*E*,4*S*)-4-hydroxy-3,7-dimethyl-2,6-octadiene.

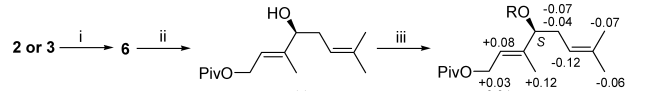
As for rhodioloside D (**4**), its aglycone, sachalinol A (**7**) obtained by enzymatic hydrolysis, was selectively converted into its 1-pivaloyl ester (**14**) with pivaloyl chloride, following derived to the (*S*)- and (*R*)-MTPA esters (**15**, **16**). The $\Delta\delta$ values (δ_S–δ_R) shown in Chart 4, indicated the 4-*S* configuration. Thus, the structure of rhodioloside D was revised to



(i) Naringinase, 0.1 M acetate buffer, 40 °C, 12 h;
(ii) Pivaloyl chloride, dry pyridine, rt, 12 h;
(iii) (*R*) or (*S*)-MTPA chloride, dry pyridine, rt, 4 h.

9 R= (*S*)-MTPA
10 R= (*R*)-MTPA
[$\Delta\delta$ (in ppm) = $\delta_S - \delta_R$]

Chart 2



(i) Naringinase, 0.1 M acetate buffer, 40 °C, 12 h;
(ii) Pivaloyl chloride, dry pyridine, rt, 12 h;
(iii) (*R*) or (*S*)-MTPA chloride, dry pyridine, rt, 4 h.

12 R= (*S*)-MTPA
13 R= (*R*)-MTPA
[$\Delta\delta$ (in ppm) = $\delta_S - \delta_R$]

Chart 3

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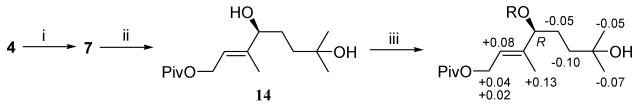

(i) Naringinase, 0.1 M acetate buffer, 40 °C, 12 h;
(ii) Pivaloyl chloride, dry pyridine, rt, 12 h;
(iii) (*R*) or (*S*)-MTPA chloride, dry pyridine, rt, 4 h.

Chart 4

(2*E*,4*S*)-4,7-dihydroxy-3,7-dimethyl-2-octenyl β-D-glucopyranoside, and sachalinol A to (2*E*,4*S*)-4,7-dihydroxy-3,7-dimethyl-2-octene.

As a conclusion, the absolute configurations of C-4 in rhodiolosides A—D (**1**—**4**), rhodiolol A (**5**), (−)-rosiridol (**6**) and sachalinol A (**7**) were all revised to 4-*S*.

Experimental

General Experimental Procedures Optical rotations were measured with a JASCO DIP-370 digital polarimeter in a 0.5-dm length cell. The ^{1}H-NMR spectra were measured with a JEOL ECP-500 spectrometer or JEOL AL-400 spectrometer with TMS as the internal reference, and chemical shifts are expressed in δ (ppm). Diaion HP-20 resin (Mitsubishi Chemical Corporation, Tokyo, Japan) and silica gel (silica Gel 60N, Kanto Chemical Co., Inc., Tokyo, Japan) were used for column chromatography. Preparative HPLC was performed using an ODS column (YMC-Pack Pro C18, 10 mm i.d.×250 mm, YMC Co., Ltd., Kyoto, Japan).

Enzymatic Hydrolysis of 1, 2, 3 and 4 A solution of **1** (7.0 mg) in 0.1 M acetate buffer (pH 4.0, 1.0 ml) was treated with naringinase (Sigma Chemical Co., 2 units), and then the reaction mixture was stirred at 40 °C for 12 h. The reaction mixture was passed through a Diaion HP-20 column, and washed with H_2O and MeOH. The MeOH fraction was chromatographed over a silica gel column to give rhodiolol A (**5**, 2.8 mg, eluted with $CHCl_3$–MeOH, 92 : 8). Through a similar procedure, enzymatic hydrolysis of **2** (8.0 mg), **3** (7.1 mg) and **4** (9.9 mg) was carried out to afford (−)-rosilidol (**6**, 1.9 mg from **2**; 0.5 mg from **3**, eluted with $CHCl_3$–MeOH, 94 : 6) and sachalinol A (**7**, 3.7 mg, eluted with $CHCl_3$–MeOH, 9 : 1). The Optical Rotation values and the ^{1}H-NMR data of **5**, **6** and **7** were identical with those in literature.[1)]

Pivaloylation of 5, 6 and 7 A solution of **5** (2.1 mg) in dry pyridine (1.0 ml) was treated with pivaloyl chloride (13 μl), and the mixture was stirred at room temperature for 12 h. After addition of H_2O (1 ml), the mixture was extracted with EtOAc (2 ml×3), dried over Na_2SO_4, and purified with silica gel column chromatography to give the pivaloyl ester **8** (3.7 mg, eluted with $CHCl_3$). Through a similar procedure, pivaloylation of **6** (1.9 mg) afforded the pivaloyl ester **11** (1.3 mg, eluted with $CHCl_3$), and pivaloylation of **7** (3.7 mg) afforded the pivaloyl ester **14** (2.9 mg, eluted with $CHCl_3$–MeOH, 96 : 4). The Optical Rotation values and the ^{1}H-NMR data of **8** and **11** were identical with those in literature.[1)]

Compound **14**: $[\alpha]_D^{20}$ +5.5° (c=0.29, MeOH). ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 5.54 (1H, tt, J=6.9, 1.0 Hz, H-2), 4.63 (2H, d, J=6.8 Hz, H_2-1), 3.94 (1H, t, J=6.5 Hz, H-4), 1.70 (3H, br s, H_3-10), 1.61 (2H, m, H_2-5), 1.51 (1H, m, H_a-6), 1.36 (1H, m, H_b-6), 1.18 (9H, s, H_3-pivaloyl×3), 1.17 (3H, s, H_3-8 or H_3-9), 1.17 (3H, s, H_3-8 or H_3-9).

(*S*)- and (*R*)-MTPA Derivatives of 8, 11 and 14 A solution of **8** (1.0 mg) in dry pyridine (0.1 ml) was added (−)-MTPA chloride (10 μl) at room temperature. After being stirred at room temperature for 4 h, the mixture was evaporated to dryness and purified by RP-HPLC with 90% MeOH to give (*S*)-MTPA ester **9** (1.2 mg). Using a similar procedure, treatment of **8** (1.0 mg) with (+)-MTPA chloride afforded (*R*)-MTPA ester **10** (1.3 mg). (*S*)-MTPA ester **12** (0.6 mg) and (*R*)-MTPA ester **13** (0.9 mg) were obtained from **11** (each 0.6 mg). (*S*)-MTPA ester **15** (1.2 mg) and (*R*)-MTPA ester **16** (1.1 mg) were obtained from **14** (each 1.0 mg).

(*S*)-MTPA Ester of **8** (**9**): ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 5.68 (1H, t, J=6.7 Hz, H-2), 5.40 (1H, dd, J=7.7, 6.0 Hz, H-4), 5.25 (1H, d, J=6.7 Hz, H-6), 4.63 (1H, dd, J=13.0, 6.7 Hz, H-1), 4.57 (1H, dd, J=13.0, 6.7 Hz, H-1), 4.36 (2H, br s, H_2-8), 2.50 (1H, m, H-5), 2.36 (1H, m, H-5), 1.72 (3H, s, H_3-10), 1.58 (3H, s, H_3-9).

(*R*)-MTPA Ester of **8** (**10**): ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 5.60 (1H, t, J=6.4 Hz, H-2), 5.38 (1H, t, J=5.5 Hz, H-4), 5.36 (1H, t, J=6.4 Hz, H-6), 4.59 (1H, dd, J=13.0, 6.4 Hz, H-1), 4.54 (1H, dd, J=13.0, 6.4 Hz, H-1), 4.42 (2H, br s, H_2-8), 2.56 (1H, m, H-5), 2.40 (1H, m, H-5), 1.64 (3H, s, H_3-9), 1.58 (3H, s, H_3-10).

(*S*)-MTPA Ester of **11** (**12**): ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 5.67 (1H, t, J=6.5 Hz, H-2), 5.37 (1H, dd, J=7.6, 6.2 Hz, H-4), 4.92 (1H, t, J=7.3 Hz, H-6), 4.63 (1H, dd, J=11.1, 6.5 Hz, H-1), 4.59 (1H, dd, J=11.1, 6.5 Hz, H-1), 2.42 (1H, m, H-5), 2.28 (1H, m, H-5), 1.72 (3H, s, H_3-10), 1.63 (3H, s, H_3-9), 1.54 (3H, s, H_3-8).

(*R*)-MTPA Ester of **11** (**13**): ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 5.59 (1H, t, J=6.6 Hz, H-2), 5.33 (1H, dd, J=7.9, 5.7 Hz, H-4), 5.04 (1H, t, J=7.2 Hz, H-6), 4.60 (1H, dd, J=11.0, 5.5 Hz, H-1), 4.55 (1H, dd, J=11.0, 5.5 Hz, H-1), 2.49 (1H, m, H-5), 2.32 (1H, m, H-5), 1.70 (3H, s, H_3-9), 1.60 (3H, s, H_3-8), 1.60 (3H, s, H_3-10).

(*S*)-MTPA Ester of **14** (**15**): ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 5.67 (1H, t, J=6.4 Hz, H-2), 5.37 (1H, dd, J=7.2, 6.2 Hz, H-4), 4.63 (1H, dd, J=13.0, 6.4 Hz, H-1), 4.57 (1H, dd, J=13.0, 6.4 Hz, H-1), 1.75 (2H, m, H_2-5), 1.70 (3H, br s, H_3-10), 1.28 (2H, m, H_2-6), 1.14 (3H, s, H_3-8 or H_3-9), 1.12 (3H, s, H_3-8 or H_3-9).

(*R*)-MTPA Ester of **14** (**16**): ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 5.59 (1H, t, J=6.9 Hz, H-2), 5.34 (1H, t, J=6.9 Hz, H-4), 4.59 (1H, dd, J=11.5, 6.2 Hz, H-1), 4.55 (1H, dd, J=11.5, 6.2 Hz, H-1), 1.80 (2H, m, H_2-5), 1.57 (3H, br s, H_3-10), 1.38 (2H, m, H_2-6), 1.19 (3H, s, H_3-8 or H_3-9), 1.19 (3H, s, H_3-8 or H_3-9).

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References

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