Prolyl Endopeptidase Inhibitors from the Underground Part of *Rhodiola sachalinensis*

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The methanolic extract of the underground part of *Rhodiola sachalinensis* was found to show inhibitory activity on prolyl endopeptidase (PEP, EC. 3.4.21.26), an enzyme that plays a role in the metabolism of proline-containing neuropeptidase which is recognized to be involved in learning and memory. From the MeOH extract, five new monoterpenoids named sachalinols A (24), B (25) and C (26) and sachalinosides A (23) and B (27) were isolated, together with twenty-two known compounds, gallic acid (1), *trans-p*-hydroxycinnamic acid (2), *p*-tyrosol (3), salidroside (4), 6″-*O*-galloylsalidroside (5), benzyl β-D-glucopyranoside (6), 2-phenylethyl β-D-glucopyranoside (7), *trans*-cinnamyl β-D-glucopyranoside (8), rosarin (9), rhodiocyanoside A (10), lotaustralin (11), octyl β-D-glucopyranoside (12), 1,2,3,6-tetra-*O*-galloyl-β-D-glucose (13), 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose (14), kaempferol (15), kaempferol 3-*O*-β-D-xylofuranosyl(1→2)-β-D-glucopyranoside (16), kaempferol 3-*O*-β-D-glucopyranosyl(1→2)-β-D-glucopyranoside (17), rhodionin (18), rhodiosin (19), (−)-epigallocatechin (20), 3-*O*-galloylepigallocatechin-(4→8)-epigallocatechin 3-*O*-gallate (21) and rosiridin (22). Among these, nineteen compounds other than 3, 4 and 9 have been isolated for the first time from *R. sachalinensis*, and six (6, 8, 13, 16, 17, 20) are isolated from *Rhodiola* plants for the first time. Among them, six compounds (13, 14, 18, 19, 21, 22) showed noncompetitive inhibition against *Flavobacterium* PEP, with an IC_{50} of 0.025, 0.17, 22, 41, 0.44 and 84 μM, respectively.

Key words *Rhodiola sachalinensis*; Crassulaceae; monoterpenoids; prolyl endopeptidase (PEP) inhibitor; Rhodiola Radix (紅景天)

The Tibetan folk medicine *Rhodiola Radix* (紅景天)[1] originates from several alpine *Rhodiola* (*R.*) plants (Crassulaceae) and is used as a hemostatic, tonic and contusion. Recently it has been reported that *Rhodiola* plants demonstrate anti-fatigue, anti-anoxia and the ability to improve memory ability.[2] In our search for prolyl endopeptidase (PEP, EC 3.4.21.26) inhibitory constituents in natural medicines,[3] we previously reported the inhibitory constituents of *R. sacra* (Sheng-di-hong-jing-tian, 聖地紅景天).[4] In our continuing study, we recently found that the MeOH extract of *R. sachalinensis* also shows potent PEP inhibitory activity. *Rhodiola sachalinensis* A. BOR. (Gao-shan-hong-jing-tian, 高山紅景天) grows in the Changbai Mountain area, and the Tibet and Xinjiang autonomous regions in China. In spite of extensive pharmacological studies,[5] its chemical constituents have been little examined.[6] Thus, we studied the constituents of *R. sachalinensis* and isolated five new monoterpenoids, together with twenty-two known compounds. In this paper, we report the structures of the new monoterpenoids together with their PEP inhibitory activity.

The underground part of *R. sachalinensis* was extracted with MeOH under reflux. The MeOH extract was separated into $CHCl_3$-, EtOAc-, BuOH-, and H_2O-soluble fractions. The EtOAc-soluble (IC_{50}, 0.40 μg/ml) and BuOH-soluble (IC_{50}, 0.56 μg/ml) fractions showed strong inhibitory activity (Chart 1). These fractions were further separated by a combination of silica gel and Sephadex LH-20 column chromatographies and reversed-phase preparative TLC techniques to give five new monoterpenoids, named sachalinols A (**24**), B (**25**) and C (**26**) and sachalinosides A (**23**) and B (**27**), together with twenty-two known compounds. The twenty known compounds other than **12** and **22** were identified by analyses of the spectroscopic data and comparison of their data with those in the literature to be: gallic acid (**1**), *trans-p*-hydroxycinnamic acid (**2**), *p*-tyrosol (**3**),[7] salidroside (**4**),[7] 6″-*O*-galloylslidroside (**5**),[8] benzyl β-D-glucopyranoside (**6**),[7] 2-phenylethyl β-D-glucopyranoside (**7**), *trans*-cinnamyl β-D-glucopyranoside (**8**),[9] rosarin (**9**),[6a] rhodiocyanoside A (**10**),[10] lotaustralin (**11**),[11] 1,2,3,6-tetra-*O*-galloyl-β-D-glucose (**13**),[12] 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose (**14**),[13] kaempferol (**15**), kaempferol 3-*O*-β-D-xylofuranosyl(1→2)-β-D-glucopyranoside (**16**),[14] kaempferol 3-*O*-β-D-glucopyranosyl(1→2)-β-D-glucopyranoside (**17**),[15] rhodionin (**18**),[16] rhodiosin (**19**),[16] (−)-epigallocatechin (**20**) and 3-*O*-galloylepigallocatechin-(4β→8)-epigallocatechin 3-*O*-gallate (**21**) (Chart 2).

Compound **12** was obtained as a colorless amorphous powder with $[\alpha]_D^{25}$ of −5.96° (MeOH). The negative ion FAB-MS and high resolution (HR)-FAB-MS of **12** indicated the molecular formula $C_{14}H_{28}O_6$ (M.W. 292). The ^{1}H- and ^{13}C-NMR spectra (Table 1) of **12** were similar to that of rhodiooctanoside previously isolated from *R. sacra*,[4,9] and indicated the presence of an octanol moiety and a β-glucopyranosyl part. Furthermore, a long-range correlation was observed between the anomeric proton (δ 4.55) and C-8 of the octanol moiety (δ 70.9) in the heteronuclear multiple bond correlation (HMBC) spectrum. From these data and the negative $[\alpha]_D$ value, **12** was determined to be octyl β-D-glucopyranoside. Previously, this compound was reported as a synthetic lipid activator, although the spectral data could not be compared because the data were not reported in the literature.[17] Thus, this is the first report of the isolation from a natural source.

Compound **22** was isolated as a colorless viscous oil of negative optical rotation ($[\alpha]_D^{25}$ −16.9°, MeOH). The ^{1}H- and ^{13}C-NMR spectra of **22** (Table 1) indicated the presence of

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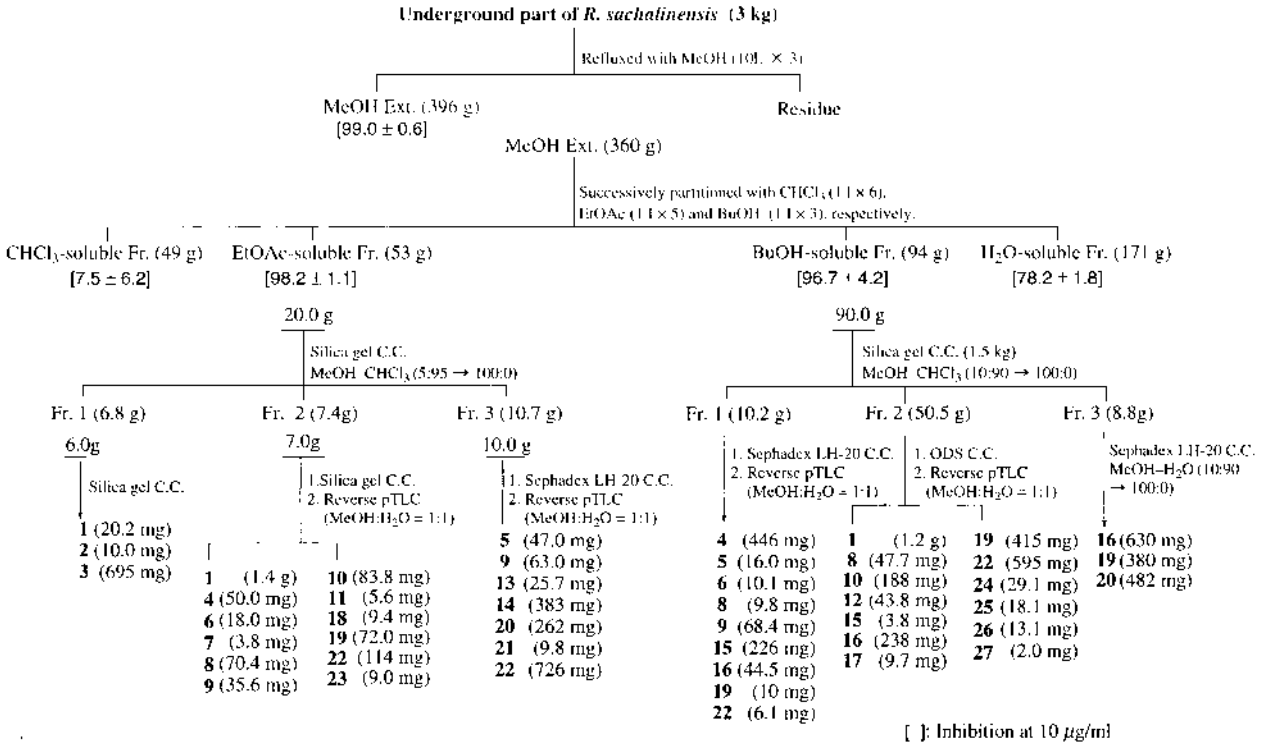


Chart 1

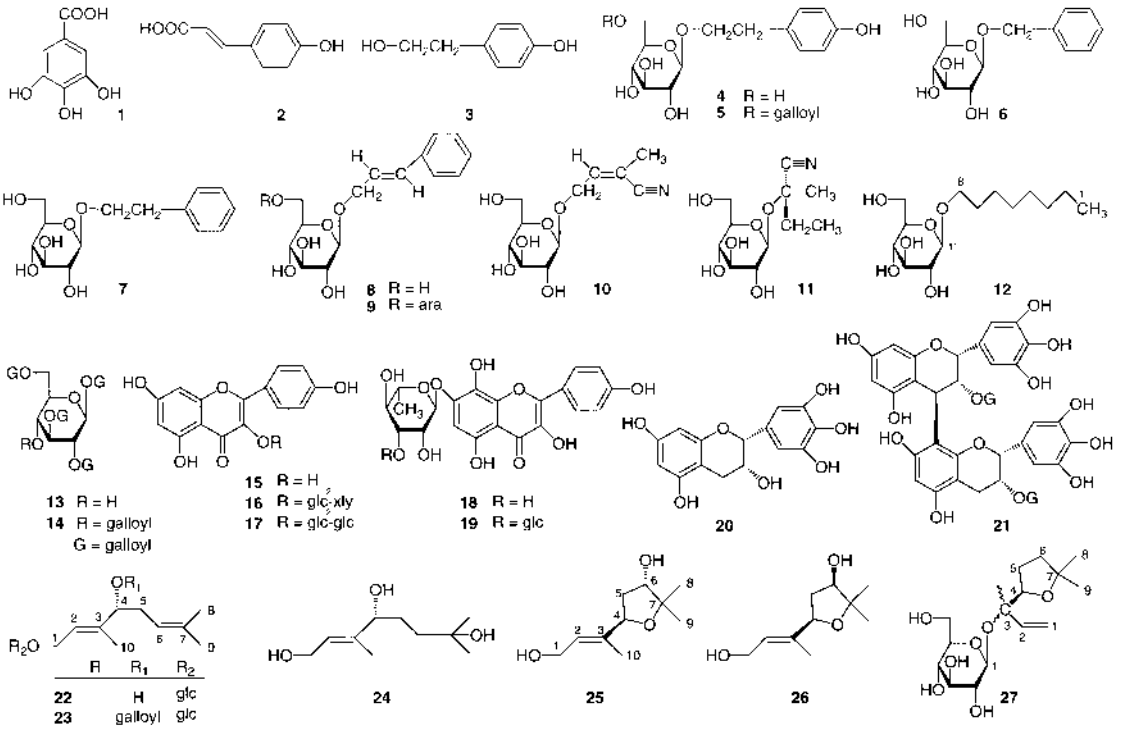


Chart 2

three methyls, two olefins, two (one is oxygen-substituted) methylenes, an oxymethine and a β-glucopyranosyl group. They were analyzed by the ^1H–^1H shift correlation spectroscopy (COSY), heteronuclear multiple quantum coherence (HMQC) and HMBC spectra, and **22** was identified as rosiridin, previously isolated from *R. rosea*.[18] However, the configuration at C-4 of rosiridin was not determined, and thus we determined the absolute configuration of C-4 by applying a modified Mosher's method.[19] An excess amount of (*R*)-α-methoxy-α-trifluoromethylphenylacetic acid (MTPA) chlorides gave per-MTPA esters, which failed to give definitive results, perhaps due to the effect of the MTPA moieties on the glucose unit. On the other hand, limited amounts of the chloride gave 4,6′-di-MTPA esters together with a com-

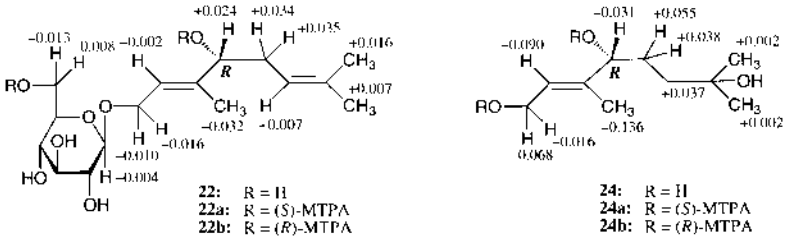


Fig. 1. $\Delta\delta$ ($=\delta_S-\delta_R$) Values Obtained from the MTPA Esters of **22** (**22a**, **b**) and **24** (**24a**, **b**) in $CDCl_3$

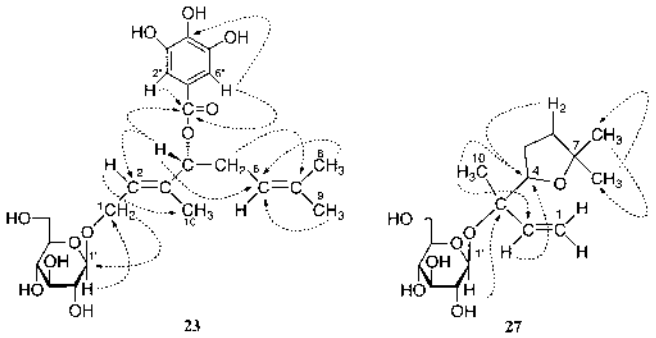


Fig. 2. Long-Range Correlations Observed in the FG-Pulsed HMBC Spectrum of **23** and **27**

The same numbering system as for **22** is used.

plex mixture of other esters. As can be seen in Fig. 1, H_2-5, H-6, H_3-8 and H_3-9 of (*R*)-MTPA ester (**22a**) appeared at higher fields than those of the (*S*)-MTPA ester (**22b**), whereas H_2-1, H-2 and H-3 of **22a** were observed at lower fields as compared to those of **22b**. Thus, the absolute configuration at C-4 of **22** was concluded to be *R*.

Sachalinoside A (**23**) was isolated as a colorless powder of negative optical rotation ($[\alpha]_D^{25}$ −105.7°, MeOH). The negative ion FAB-MS of **23** showed a quasimolecular ion at *m/z* 483 $(M-H)^-$ and HR-FAB-MS analysis of the a quasimolecular ion revealed the molecular formula to be $C_{23}H_{32}O_{11}$. The ^{1}H- and ^{13}C-NMR spectra of **23** (Table 1) were similar to those of **22**, except for the presence of the signals ascribable to a galloyl group [δ_H 7.06 (2H, s); δ_C 121.8, 110.6, 140.6, 146.5, 167.6]. Thus, sachalinoside A was considered to be a mono gallate derivative of **22**, which was confirmed by the COSY, HMQC and HMBC spectra. Furthermore, the oxymethine proton (H-4) appeared at lower field (δ 5.23) compared with that of **22** (δ 3.98), indicating the galloyl group to be located at C-4. This was further confirmed by the HMBC spectrum (Fig. 2). The configuration at C-4 of **23** was determined to be the same as that of **22**, because **23** gave **22** after alkaline hydrolysis and they were isolated from the same extract. Thus, sachalinoside A was concluded to be 4-*O*-galloylrosiridin (**23**).

Sachalinol A (**24**) was isolated as a colorless viscous oil of negative optical rotation ($[\alpha]_D^{25}$ −17.1°, MeOH). The positive ion FAB-MS and HR-FAB-MS of **24** showed the molecular formula $C_{10}H_{20}O_3$. The ^{1}H-NMR spectrum of **24** displayed signals of three singlet methyls (δ 1.16, 1.17, 1.65), an oxymethine [δ 3.81 (t, J=6.6 Hz)], an oxymethylene [δ 4.13 (d, J=6.3 Hz)], two methylenes [δ 1.50 (dd, J=6.8, 6.6 Hz), 1.56 (dd, J=6.8, 6.6 Hz)] and an olefin (δ 5.54, t, J=6.3 Hz). The ^{13}C-NMR spectrum of **24** revealed the presence of ten carbons including three methyls (δ 11.6, 29.2, 29.3), three methylenes (δ 30.6, 40.8, 59.2), a methine (δ 78.6), a quaternary carbon (δ 71.1) and an olefinic group (δ 126.2, 140.8). These spectral data were analyzed by COSY, HMQC and HMBC spectra, and the absolute configuration at C-4 was determined to be *R* by the modified Mosher's method (Fig. 1). Thus, sachalinol A was determined to be (4*R*)-3,7-dimethyl-2*E*-octene-1,4,7-triol (**24**).

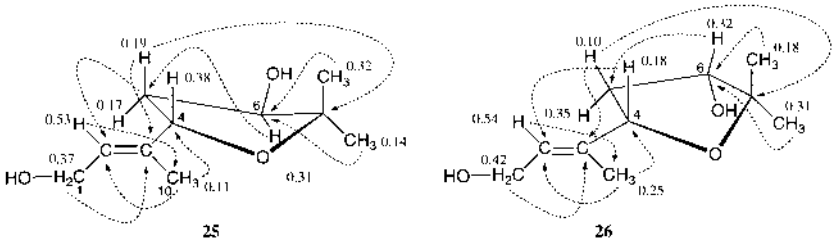
Sachalinols B (**25**) and C (**26**) were isolated as colorless viscous oils of positive optical rotation $[\alpha]_D^{25}$ +60.3° (MeOH) and +40.2° (MeOH), respectively]. They showed the same molecular weight (186 amu) and molecular formula ($C_{10}H_{18}O_3$), suggesting they are stereoisomers. In the ^{1}H- and ^{13}C-NMR spectra (Table 1), **25** and **26** showed signals of an olefin, two oxymethines, two methylenes, three methyls and a quaternary carbon. These were identical with those of **24**, but they were characterized by the presence of an oxymethine instead of a methylene in **24**. This and the fact that the molecular formula is two hydrogens less than that of **24** suggested that **25** and **26** would have an ether bond in the molecules. This was confirmed by the COSY, HMQC and HMBC spectra (Fig. 3). Because the ROESY and NOESY experiments on **25** and **26** did not give enough data to determine the stereochemistry at C-4, the relative configuration between C-4 and C-6 was determined based on the pyridine-induced solvent shift in the ^{1}H-NMR spectra.[20] By changing the solvent from methanol-d_4 to pyridine-d_5, H-4 of **25** showed a larger lowfield shift ($\Delta\delta$ 0.38) than that of **26** ($\Delta\delta$ 0.18), while H_3-10 of **26** showed a larger lowfield shift ($\Delta\delta$ 0.25) than that of **25** ($\Delta\delta$ 0.11). Thus, H-4 in **25** and H_3-10 in **26** should be *cis* to the 6-hydroxyl group. On the other hand, the absolute configuration at C-4 was assumed to be the same as that of **22**, because **25** and **26** were considered to be biosynthesized from **22** through an epoxidation of the olefin, followed by the ether-bond formation. From these data, sachalinols B and C were determined to be 3-(2,4-*trans*-4-hydroxy-5,5-dimethyltetrahydrofuranyl)-2*E*-buten-1-ol (**25**) and 3-(2,4-*cis*-4-hydroxy-5,5-dimethyltetrahydrofuranyl)-2*E*-buten-1-ol (**26**), respectively.

Sachalinoside B (**27**) was also isolated as a colorless powder of negative optical rotation ($[\alpha]_D^{25}$ −130.0°, MeOH). The negative ion FAB-MS of **27** showed a quasimolecular ion at *m/z* 331 $(M-H)^-$ and HR-FAB-MS of the quasimolecular ion revealed the molecular formula to be $C_{16}H_{28}O_7$. The ^{1}H-NMR spectrum of **27** displayed the presence of three tertiary methyls (δ 1.21, 1.25, 1.31), two methylenes [δ 1.87 (m), 1.90 (m)], a monosubstituted double bond [δ 4.98 (dd, J=10.7, 1.5 Hz; 5.20 (dd, J=17.3, 1.5 Hz), 6.00 (dd, J=17.3, 10.7 Hz)], a methine [δ 4.07 (t, J=7.8 Hz)] and a β-glucopyranosyl group (Table 1). The analyses of the COSY, HMQC

Table 1. ^{1}H- and ^{13}C-NMR Data of **22**—**27** in CD_3OD

	22		**23**		**24**		**25***		**26***		**27***	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	4.38 dd (11.7, 6.3) 4.32 dd (11.7, 6.3)	66.1	4.39 dd (11.7, 6.3) 4.28 dd (11.7, 6.3)	66.0	4.13 d (6.3) (2H)	59.2	4.15 d (6.6) (2H)	59.2	4.12 d (6.6) (2H)	59.2	5.20 dd (17.3, 1.5) 4.98 dd (10.7, 1.5)	111.9
2	5.58 t (6.3)	122.9	5.68 t (6.3)	124.5	5.54 t (6.3)	126.2	5.65 t (6.6)	125.9	5.65 t (6.6)	126.0	6.00 dd (17.3, 10.7)	145.3
3		142.9		139.0		140.8		139.0		138.8		79.9
4	3.98 t (6.8)	78.0	5.23 t (6.6)	79.6	3.81 t (6.6)	78.6	4.50 t (8.1)	82.2	4.30 dd (9.3, 6.6)	80.7	4.07 t (7.8)	85.2
5	2.22 t (6.8) (2H)	34.8	2.50 ddd (11.0, 6.8, 6.6) 2.42 ddd (11.0, 6.8, 6.6)	32.8	1.50 dd (6.8, 6.6) (2H)	30.6	2.06 ddd (12.9, 8.1, 6.1) 1.99 ddd (12.9, 8.1, 3.9)	39.8	2.35 ddd (12.7, 7.1, 6.6) 1.75 ddd (12.7, 9.3, 7.1)	39.8	1.90 m (2H)	38.7
6	5.12 t (6.8)	121.6	5.12 t (6.8)	120.2	1.56 dd (6.8, 6.6)	40.8	3.95 dd (6.1, 3.9)	78.5	4.00 t (7.1)	78.7	1.87 m	28.1
7		134.0		135.5		71.1		84.4		83.6		84.4
8	1.63 s	19.0	1.65 s	19.1	1.17 s	29.2	1.20 s	22.0	1.18 s	23.2	1.21 s	22.7
9	1.68 s	26.3	1.67 s	26.0	1.16 s	29.3	1.22 s	28.1	1.22 s	28.0	1.25 s	25.9
10	1.70 s	12.0	1.76 s	13.0	1.65 s	11.6	1.61 s	12.0	1.65 s	12.0	1.31 s	23.4
1′	4.29 d (7.8)	102.8	4.28 d (7.8)	103.1							4.55 d (7.6)	98.4
2′	3.17 t (7.8)	75.0	3.16 t (7.8)	75.0							3.14 t (7.6)	75.3
3′		77.9		78.4								77.5
4′	3.20—3.36 (m, 3H)	71.6	3.23—3.38 (m, 3H)	71.6							3.34—3.50 (m, 3H)	71.6
5′		78.1		78.1								77.9
6′	3.88 dd (12.0, 2.0) 3.67 dd (12.0, 5.0)	62.8	3.85 dd (12.0, 2.0) 3.65 dd (12.0, 5.0)	62.7							3.82 dd (12.0, 2.0) 3.62 dd (12.0, 5.0)	62.8
1				121.8								
2, **6**			7.06 s	110.6								
3, **5**				140.6								
4				146.5								
C=O				167.6								

* The same numbering system as for **22** is used.



25 **26**

Fig. 3. Long-Range Correlations Observed in the FG-Pulsed HMBC Spectra and the Solvent Shift ($\Delta = \delta_{\text{Pyridine-}d_5} - \delta_{CD_3OD}$) of **25** and **26**

The same numbering system as for **22** is used.

and HMBC spectra (Fig. 2) indicated the aglycone to be 3-(5,5-dimethyltetrahydrofuranyl)-1-buten-3-ol. The long-range correlation between the anomeric proton (δ 4.55) and C-3 (δ 79.9) of the aglycone moiety in the HMBC spectrum revealed the location of the glucose at C-3. Although the stereochemistry could not be determined due to the small amount obtained, the absolute configuration at C-4 was assumed to be the same as those of **22**—**26** based on the same consideration as in the case of **25** and **26**. From these data, sachalinoside B was determined to be 3-(5,5-dimethyltetrahydrofuranyl)-1-buten-3-ol 3-*O*-β-D-glucopyranoside.

Among the isolated compounds, eight (**1**—**4**, **7**, **10**, **11**, **21**) were common with *R. sacra*[4,9]; six (**1**, **3**, **5**, **14**, **18**, **19**) were isolated from *R. crenulata*[16]; one (**15**) from *R. quadrifida*[21]; and two (**3**, **4**) were reported as constituents of *R. sachalinensis*.[6a] However, compounds **6**, **8**, **13**, **16**, **17** and **20** have been isolated from *Rhodiola* plants for the first time.[22]

PEP inhibitory activities of the twenty-seven compounds were examined against *Flavobacterium* PEP and are listed in Table 2, together with those of positive controls, *Z*–Pro-prolinol and *Z*–Pro-prolinal. Among the twenty-seven compounds, six (**13**, **14**, **18**, **19**, **21**, **22**) showed inhibitory activities concentration-dependently with an IC_{50} of 0.025, 0.17, 22, 41, 0.44 and 84 μM, respectively. The inhibition mode of these compounds was determined to be non-competitive by the analysis of Lineweaver–Burk plot.

Experimental

Optical rotations were measured with a JASCO DIP-4 digital polarimeter and UV spectra were obtained with a Shimadzu UV 160A spectrometer. FAB-MS and HR-FAB-MS were measured with JEOL JMS-700T and NMR spectra were recorded on a JEOL JNM-GX400 spectrometer with tetramethylsilane (TMS) as an internal standard. PEP inhibitory activity was measured with a Perkin-Elmer HTS7000 bioassay reader. Column chromatography was performed over normal-phase (Fuji Silysia BW-820MH) or reversed-phase (Cosmosil 75C_{18}-OPN, Nacalai Tesque Inc., Kyoto) silica gel or Sephadex LH-20 (Pharmacia), and TLC was conducted on precoated Merck Kieselgel 60F_{254} or RP-18F_{254} plates. Dried underground part of *R. sachalinensis* A. Bor. was collected in Changbai mountain areas of Yanbian Autonomous Region in China. The plant material was identified by Prof. W. Wu of Shenyang Pharmaceutical University, People's Republic of China.

Extraction and Isolation The underground part of *R. sachalinensis* (3 kg) was pulverized and extracted three times with MeOH under reflux (each 4 l) to give a MeOH extract (396 g). The MeOH extract (360 g) was suspended in water and partitioned successively with $CHCl_3$ (1 l×6), EtOAc

Table 2. PEP Inhibitory Activity of Isolated Compounds

Compounds	Concentration (μM)	Inhibition (%)[a]	IC_{50} (μM)	K_i (μM)[b]
1	100	33.0±5.2		
2	100	3.6±2.8		
3	100	17.2±3.4		
4	100	10.6±1.9		
5	100	24.0±2.7		
6	100	14.7±3.3		
7	100	7.6±4.5		
8	100	12.5±4.0		
9	100	16.9±2.5		
10	100	11.3±2.9		
11	100	8.3±4.6		
12	100	38.7±3.1		
13	0.1	99.1±0.3	25.0 nM	15±2 nM
	0.01	16.4±2.6		
	0.001	12.5±2.5		
14	1	100.0±0.0	0.17	0.15±0.03
	0.1	34.4±3.8		
	0.01	13.3±2.8		
15	100	32.5±16.4		
16	100	26.4±5.0		
17	100	20.2±3.1		
18	100	72.1±3.6	22	23±1
	10	36.1±8.3		
	1	9.9±2.5		
19	100	63.7±2.8	41	28±7
	10	28.7±4.0		
	1	19.4±3.2		
20	100	18.1±4.8		
21[c]			0.44	0.17±0.03
22	100	52.4±7.2	84	56±0
	10	20.0±4.9		
	1	13.5±6.1		
23	100	31.0±3.6		
24	100	20.0±1.6		
25	100	4.2±2.2		
26	100	6.2±4.6		
27	100	8.2±3.1		
Z–Pro-prolinol[c]			641	283±5
Z–Pro-prolinal[c]			2.6 nM	0.42±0.17 nM

a) The values are the means±S.D. of triplicate experiments. *b*) K_i values were obtained from Dixon plot. *c*) Data from ref. 3.

(11×5) and BuOH (11×3) (Chart 1). Each extract was evaporated to dryness *in vacuo* to give $CHCl_3$-soluble (49 g), EtOAc-soluble (53 g) and BuOH-soluble (94 g) fractions, while the remaining water layer was freeze-dried to give an H_2O-soluble (171 g) fraction.

The EtOAc-soluble fraction (20 g) was subjected to silica gel (1.3 kg) column chromatography with a MeOH–$CHCl_3$ solvent system (5 : 95→100 : 0) and eluates were separated into three fractions by their behavior on TLC (fr. 1, 6.8 g; fr. 2, 7.4 g; fr. 3, 10.7 g). Fraction 1 (6.0 g) was further separated by silica gel (500 g) column chromatography with MeOH–$CHCl_3$ (5 : 95→30 : 70) to furnish gallic acid (**1**, 20.2 mg), *trans-p*-hydroxycinnamic acid (**2**, 10.0 mg) and *p*-tyrosol (**3**, 695 mg). Fraction 2 (7.0 g) was separated by a combination of silica gel (500 g) column chromatography with (MeOH–$CHCl_3$, 5 : 95→45 : 55) and reversed-phase preparative TLC (H_2O–MeOH, 3 : 2) to furnish 1 (1.4 g), salidroside (**4**, 50.5 mg), benzyl β-D-glucopyranoside (**6**, 18.0 mg), 2-phenylethyl β-D-glucopyranoside (**7**, 3.8 mg), *trans*-cinnamyl β-D-glucopyranoside (**8**, 70.4 mg), rosarin (**9**, 35.6 mg), rhodiocyanoside A (**10**, 83.8 mg), lotaustralin (**11**, 5.6 mg), rhodionin (**18**, 9.4 mg), rhodiosin (**19**, 72.0 mg), rosiridin (**22**, 114 mg) and sachalinoside A (**23**, 9.0 mg). Fraction 3 (10.0 g) was separated by a combination of Sephadex LH-20 (500 g) column chromatography with (H_2O–MeOH, 100 : 0→0 : 100) and reversed-phase preparative TLC (H_2O–MeOH, 1 : 1) to furnish 6″-*O*-galloylsalidroside (**5**, 47.0 mg), **9** (63.0 mg), 1,2,3,6-tetra-*O*-galloyl-β-D-glucose (13, 25.7 mg), 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose (**14**, 383 mg), (−)-epigallocatechin (**20**, 262 mg), 3-*O*-galloylepigallocatechin-(4→8)-epigallocatechin 3-*O*-gallate (**21**, 9.8 mg) and **22** (726 mg).

The BuOH-soluble fraction (90 g) was chromatographed over silica gel (1.5 kg) with a MeOH–$CHCl_3$ solvent system (10 : 90→100 : 0) to afford three fractions. (fr. 1, 10.2 g; fr. 2, 50.5 g; fr. 3, 8.8 g). Fraction 1 was further subjected to Sephadex LH-20 (300 g) column chromatography with H_2O–MeOH (100 : 0→0 : 100) and the eluates were separated by reversed-phase preparative TLC (H_2O–MeOH, 1 : 1) to furnish **4** (446 mg), **5** (16.0 mg), **6** (10.1 mg), **8** (9.8 mg), **9** (68.4 mg), kaempferol (**15**, 226 mg), kaempferol 3-*O*-β-D-xylofuranosyl(1→2)-β-D-glucopyranoside (**16**, 44.5 mg), **19** (10.0 mg) and **22** (6.1 mg). Fraction 2 was separated by a Cosmosil 75C_{18}-OPN column chromatography with H_2O–MeOH (10 : 90→100 : 0) and the eluates were further purified by reversed-phase preparative TLC (H_2O–MeOH, 1 : 1) to furnish **1** (1.2 g), **8** (47.7 mg), **10** (187.8 mg), octyl β-D-glucopyranoside (**12**, 43.8 mg), **15** (3.8 mg), **16** (238 mg), kaempferol 3-*O*-β-D-glucopyranosyl(1→2)-β-D-glucopyranoside (**17**, 9.7 mg), **19** (415 mg), **22** (595 mg), sachalinols A (**24**, 29.1 mg), B (**25**, 18.1 mg) and C (**26**, 13.1 mg) and sachalinoside B (**27**, 2.0 mg). Fraction 3 was separated by Sephadex LH-20 (500 g) column chromatography with H_2O–MeOH (100 : 0→0 : 100) to furnish **16** (630 mg), **19** (380 mg) and **20** (482 mg).

Octyl β-D-Glucopyranoside (**12**): Colorless amorphous powder, $[\alpha]_D^{25}$ −5.96° (*c*=0.10, MeOH). Negative ion HR-FAB-MS *m/z*: 291.3656 [Calcd for $C_{14}H_{27}O_6$ $(M-H)^-$ 291.3685]. ^{1}H-NMR (CD_3OD) δ: 0.92 (3H, t, *J*=6.5 Hz, H_3-1), 1.23—1.48 (10H, m, H_2-2, H_2-3, H_2-4, H_2-5, H_2-6), 1.63 (2H, quintet, *J*=6.9 Hz, H_2-7), 3.52 (1H, m, H-8), 3.85 (1H, m, 8-H), 3.14 (1H, t, *J*=7.8 Hz, H-2′), 3.34—3.50 (3H, m, H-3′, H-4′, H-5′), 3.62 (1H, dd, *J*=12.0, 5.0 Hz, H-6′), 3.82 (1H, dd, *J*=12.0, 2.0 Hz, H-6′), 4.55 (1H, d, *J*=7.8 Hz, H-1′). ^{13}C-NMR (CD_3OD) δ: 14.4 (C-1), 23.7 (C-2), 27.7 (C-3), 30.4, 30.6, 30.8 (C-4, 5, 6), 33.0 (C-7), 70.9 (C-8), 62.7 (C-6′), 71.5 (C-4′), 75.1 (C-2′), 77.7 (C-5′), 78.0 (C-3′), 104.3 (C-1′).

Rosiridin (**22**): A colorless viscous oil, $[\alpha]_D^{25}$ −16.9° (*c*=0.087, MeOH). Negative ion HR-FAB-MS *m/z*: 331.1753 [Calcd for $C_{16}H_{27}O_7$ $(M-H)^-$ 331.1757]. ^{1}H- and ^{13}C-NMR: Table 1.

Sachalinoside A (**23**): Colorless powder, $[\alpha]_D^{25}$ −105.7° (*c*=0.027, MeOH). UV λ_{max} (MeOH) nm (log ε): 216 (4.3), 269 (4.1). Negative ion HR-FAB-MS *m/z*: 483.1825 [Calcd for $C_{23}H_{31}O_{11}$ $(M-H)^-$ 483.1866]. ^{1}H- and ^{13}C-NMR: Table 1.

Sachalinol A (**24**): A colorless viscous oil, $[\alpha]_D^{25}$ −17.1° (*c*=0.17, MeOH). Positive ion HR-FAB-MS *m/z*: 189.1499 [Calcd for $C_{10}H_{21}O_3$ $(M+H)^+$ 189.1491]. ^{1}H- and ^{13}C-NMR: Table 1.

Sachalinol B (**25**): A colorless viscous oil, $[\alpha]_D^{25}$ +60.3° (*c*=0.07, MeOH). Negative ion HR-FAB-MS *m/z*: 185.1167 [Calcd for $C_{10}H_{17}O_3$ $(M-H)^-$ 185.1178]. ^{1}H- and ^{13}C-NMR: Table 1.

Sachalinol C (**26**): A colorless viscous oil, $[\alpha]_D^{25}$ +40.2° (*c*=0.05, MeOH). Negative ion HR-FAB-MS *m/z*: 185.1159 [Calcd for $C_{10}H_{17}O_3$ $(M-H)^-$ 185.1178]. ^{1}H- and ^{13}C-NMR: Table 1.

Sachalinoside B (**27**): Colorless powder, $[\alpha]_D^{25}$ −130.0° (*c*=0.13, MeOH). Negative ion HR-FAB-MS *m/z*: 331.1749 [Calcd for $C_{16}H_{27}O_7$ $(M-H)^-$ 331.1757]. ^{1}H- and ^{13}C-NMR: Table 1.

MTPA Esters of Rosiridin (22) and Sachalinol (24) To a solution of **22** (15 mg, 45 μmol) in a mixture of $CHCl_3$ (0.5 ml) and pyridine (0.5 ml), (*S*)-(+)- or (*R*)-(−)-MTPA chloride (50 μl, 266 μmol) was added. The reaction mixture was stirred at room temperature overnight and was submitted to preparative TLC ($CHCl_3$–MeOH=9 : 1 and then hexane–EtOAc=4 : 1) to give 4,6′-di-(*R*)-MTPA ester (**22a**, 1.5 mg) or 4,6′-di-(*S*)-MTPA ester (**22b**, 3.0 mg). In the same way, **24** (10 mg, 53 μmol) reacted with (*S*)-(+)- or (*R*)-(−)-MTPA chloride (100 μl, 532 μmol) to give 1,4-di-(*R*)-MTPA ester (**24a**, 15.6 mg) or 1,4-di-(*S*)-MTPA ester (**24b**, 20.1 mg).

(*R*)-MTPA Ester of Rosiridin (**22a**): A colorless amorphous solid. ^{1}H-NMR ($CDCl_3$) aglycone part: δ: 1.53 (3H, s, H_3-9), 1.58 (3H, s, H_3-8), 1.72 (3H, s, H_3-10), 2.13, 2.24 (each 1H, dt, *J*=12.0, 6.6 Hz, H_2-5), 3.40 (1H, t, *J*=6.6 Hz, H-4), 4.18, 4.31 (each 1H, dd, *J*=11.7, 6.3 Hz, H_2-1), 5.04 (1H, t, *J*=6.6 Hz, H-6), 5.49 (1H, t, *J*=6.3 Hz, H-2); sugar part: δ: 3.27—3.33 (2H, m, H-3′, H-4′), 3.45 (1H, t, *J*=7.8 Hz, H-2′), 3.63 (1H, m, H-5′), 4.38 (1H, d, *J*=7.8 Hz, H-1′), 4.51 (1H, dd, *J*=12.0, 5.0 Hz, H-6′), 4.80 (1H, dd, *J*=12.0, 2.0 Hz, H-6′). Negative ion HR-FAB-MS *m/z*: 763.2581 [Calcd for $C_{36}H_{41}F_6O_{11}$ $(M-H)^-$ 763.2553].

(*S*)-MTPA Ester of Rosiridin (**22b**): A colorless amorphous solid. ^{1}H-NMR ($CDCl_3$): aglycone part: δ: 1.54 (3H, s, H_3-9), 1.60 (3H, s, H_3-8), 1.69 (3H, s, H_3-10), 2.17, 2.28 (each 1H, dt, *J*=12.0, 6.6 Hz, H_2-5), 3.48 (1H, t, *J*=6.6 Hz, H-4), 4.19, 4.30 (each 1H, dd, *J*=11.7, 6.3 Hz, H_2-1), 5.04 (1H, t, *J*=6.6 Hz, H-6), 5.49 (1H, t, *J*=6.3 Hz, H-2); sugar part: 3.26—3.35 (2H, m, H-3′, H-4′), 3.48 (1H, t, *J*=7.8 Hz, H-2′), 3.56 (1H, m, H-5′), 4.38 (1H, d, *J*=7.8 Hz, H-1′), 4.49 (1H, dd, *J*=12.0, 5.0 Hz, H-6′), 4.78 (1H, dd, *J*=12.0, 2.0 Hz, H-6′). Negative ion HR-FAB-MS *m/z*: 763.2569 [Calcd for $C_{36}H_{41}F_6O_{11}$ $(M-H)^-$ 763.2553].

(*R*)-MTPA Ester of Sachalinol A (**24a**): A colorless amorphous solid. ^{1}H-NMR ($CDCl_3$) δ: 1.32 (3H, s, H_3-8), 1.33 (3H, s, H_3-9), 1.64 (3H, s, H_3-10), 1.69 (2H, t, J=6.8 Hz, H-6), 2.31, 2.46 (each 1H, ddd, J=12.0, 6.8, 6.6 Hz, H_2-5), 4.76, 4.89 (each 1H, dd, J=12.0, 6.3 Hz, H_2-1), 5.28 (1H, t, J=6.6 Hz, H-4), 5.67 (1H, t, J=6.3 Hz, H-2). Positive ion HR-FAB-MS *m*/*z*: 643.2062 [Calcd for $C_{30}H_{34}F_6NaO_7$ $(M+Na)^+$ 643.2106].

(*S*)-MTPA Ester of Sachalinol A (**24b**): A colorless amorphous solid. ^{1}H-NMR ($CDCl_3$) δ: 1.32 (3H, s, H_3-8), 1.34 (3H, s, H_3-9), 1.50 (3H, s, H_3-10), 1.72 (2H, t, J=6.8 Hz, H-6), 2.35, 2.51 (each 1H, ddd, J=12.0, 6.8, 6.6 Hz, H_2-5), 4.74, 4.82 (each 1H, dd, J=12.0, 6.3 Hz, H_2-1), 5.24 (1H, t, J=6.6 Hz, H-4), 5.58 (1H, t, J=6.3 Hz, H-2). Positive ion HR-FAB-MS *m*/*z*: 643.2079 [Calcd for $C_{30}H_{34}F_6NaO_7$ $(M+Na)^+$ 643.2106].

Alkaline Hydrolysis of 23 A solution of **23** (76 μg) in MeOH (50 μl) was stirred with 1 M NaOH (50 μl) overnight at room temperature. The reaction products were identified by reversed-phase TLC (MeOH–H_2O, 1 : 1) to be rosiridin (**22**, *Rf* 0.32) and gallic acid (**1**, *Rf* 0.76).

PEP Inhibitory Activity PEP (*Flavobacterium meningosepticum* origin) was purchased from Seikagaku Corporation (Tokyo, Japan), and *Z*–Gly–Pro–*p*NA from Bachem Fine Chemical Co. (Switzerland). Two positive controls, *Z*–Pro-prolinal and *Z*–Pro-prolinol, were synthesized by the method in the literature.[23] PEP inhibitory activities were measured by the method of Yoshimoto *et al*.,[24] as described in the previous papers.[3]

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