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Structure Elucidation of Roselipins, Inhibitors of Diacylglycerol Acyltransferase Produced by *Gliocladium roseum* KF-1040

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The structures of roselipins 1A, 1B, 2A and 2B were elucidated by spectroscopic studies including ^{1}H-^{1}H COSY, ^{13}C-^{1}H COSY, ^{13}C-^{1}H HMQC and ^{13}C-^{1}H HMBC NMR experiments, and degradation experiments. They have the common skeleton of 2,4,6,8,10,12,14,16,18-nonamethyl-5,9,13-trihydroxy-2*E*,6*E*,10*E*-icosenoic acid modified with a D-mannose and a D-arabinitol. Roselipin A and B groups were stereoisomers at the arabinitol moiety, which esterified the fatty acid from the different terminal hydroxy residue. Roselipin 2 group was 6″-*O*-acetyl roselipin 1 group.

Roselipins (Fig. 1) were isolated as inhibitors of diacylglycerol acyltransferase from the culture broth of *Gliocladium roseum* KF-1040[1]. The fermentation, isolation and their biological properties are described in the preceding paper[2]. We report herein the structure elucidation of roselipins.

Materials and Methods

General Experimental Procedures

UV spectra were recorded on a Shimadzu UV-200S spectrophotometer. IR spectra were recorded on a Horiba FT-210 infrared spectrometer. Optical rotations were obtained with a JASCO DIP-370 digital polarimeter. Melting points were measured with a Yanaco micro melting point apparatus. EI-MS spectra were recorded on a JEOL JMS-D 100 mass spectrometer at 20 eV. FAB-MS spectra were recorded on a JMS-DX300 mass spectrometer. The various NMR spectra were obtained on a Varian XL-400 spectrometer. HPLC was carried out using the JASCO (TRI ROTAR V) system. A gas chromatography was carried out using the Shimadzu (GC-14A) system with a flame ionization detector (FID).

Acetylation

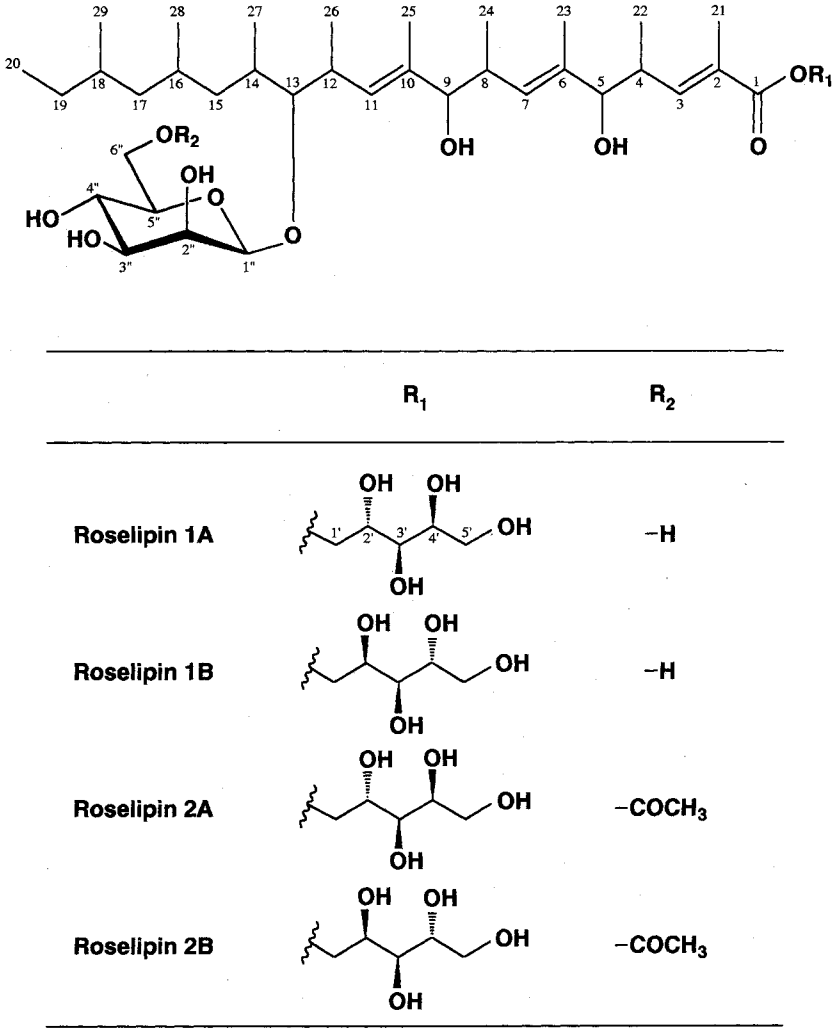
To 1 mg of each roselipin dissolved in pyridine (100 μl) was added acetic anhydride (20 μl). After stirring for 24 hours at room temperature, the reaction mixture was poured into water. The reaction product was extracted with EtOAc, which was concentrated to dryness to give a white powder. All the acetylated roselipins showed the same fragment ion peak of *m/z* 1196 $[M]^+$ and 1219 $[M+Na]^+$ by the FAB-MS spectrum. The Rf values were 0.5 on Kieselgel 60 F_{254} TLC glass plates (E. Merck) with $CHCl_3$/diethylether (6 : 4) as a developing solvent.

Analysis of Hexoses and Alditols by HPLC

One mg of each roselipin was degraded with 2 N trifluoroacetic acid (2 ml) at 120°C for 2 hours. After evaporation, the degradation products were distributed in EtOAc - H_2O (20 ml : 10 ml). The lower layer was concentrated *in vacuo* to dryness, and the resulting material was subjected to an ODS column (Senshu SS 1020T, 250 mg), and was eluted with 30% aq CH_3CN (100 μl×10). The 4th fraction containing the hexose and alditol was analyzed by HPLC.

The analysis of alditols by HPLC was carried out under the following conditions: column, Shodex SUGAR

Fig. 1. Structures of roselipins 1A, 1B, 2A and 2B.



SC 1211 (6.0×250 mm); solvent, 35% aq CH_3CN; column temperature, 70°C; detection, refractive index (Shodex RI-71); flow rate, 0.5 ml/minute; sample, 20 μl of the 4th fraction was injected. Ribitol, arabinitol and xylitol were eluted with retention times of 13.0, 16.8 and 20.8 minutes, respectively (Fig. 7A). The analysis of hexoses by HPLC was done under the following conditions: column, Shodex SUGAR SP 0810 (8.0×300 mm); solvent, H_2O; column temperature, 80°C; detection, refractive index (Shodex RI-71); flow rate, 0.5 ml/minute; sample, 20 μl of the 4th fraction was injected. Glucose, galactose, and mannose were eluted with retention times of 17.6, 20.0, and 22.0 minutes, respectively (Fig. 8A).

Determination of the Absolute Configuration of Arabinitol and Mannose

The absolute configuration of arabinitol was determined using gas chromatography according to the method of LARSSON *et al.*[3]. One mg of each roselipin was degraded with 2 N trifluoroacetic acid (2 ml) at 120°C for 2 hours. After evaporation, the degradation products were treated with trifluoroacetic anhydride (200 μl) in dichloromethane (200 μl). Trifluoroacetylation of arabinitol was carried out by heating at 80°C for 10 minutes and the product was subsequently analyzed by gas chromatography under the following conditions. The fused-silica column (30 m by 0.25 mm [inner diameter]) was coated with 0.25-μm (film thickness) cyclodextrin (Beta Dex-120; Supelco Inc.). The column temperature was programmed to rise 4°C/minute from 70 to 120°C and then to rise 8°C/minute to 190°C.

The flow rate of the nitrogen carrier gas through the column was 2 ml/minute. The temperature of the injector was 170°C, and that of the detector was 260°C. Authentic pertrifluoroacetyl-D-, and -L-arabinitols were eluted as peaks with retention times of 11.85 and 12.12 minutes, respectively (Fig. 9A).

The absolute configuration of mannose was determined using gas chromatography according to the method of GERWING *et al.*[4] with some modifications. Methanolysis of each roselipin (1 mg) was carried out by treatment with 2 N HCl-MeOH (500 μl). After heating at 80°C for 17 hours, the solution, divided into two ampoules (250 μl each), was evaporated to dryness. Nitrogen was bubbled through a solution of the dry sample in (+)- or (±)-2-butanol (300 μl) and acetyl chloride (50 μl), and the ampoule was then sealed. After butanolysis at 80°C for 8 hours, the solution was neutralized with Ag_2CO_3. After centrifugation at 2000 rpm for 10 minutes the supernatant solution was concentrated under reduced pressure at 45°C. The residue was treated with acetic anhydride (100 μl) in pyridine (200 μl). Acetylation was carried out by heating at 70°C for 30 minutes. The resultants were subsequently analyzed by gas chromatography using a fused silica column (25 m by 0.25 mm, SHIMADZU CBP1). The column temperature was programmed to rise 5°C/minute from 170 to 260°C. The flow rate of the nitrogen carrier gas through the column was 2 ml/minute. The temperature of the injector and the detector was 300°C. Authentic 1-((+)-2-butyl)-2,3,4,6-tetraacetyl-D- and -L-mannose, and 1-methyl-2,3,4,6-tetraacetyl-D-mannose were eluted as peaks with retention times of 10.45, 10.56 and 8.28 minutes, respectively (Fig. 10A).

Table 1. Physico-chemical properties of roselipins 1A, 1B, 2A and 2B.

	Roselipin 1A	Roselipin 1B	Roselipin 2A	Roselipin 2B
Appearance	white powder	white powder	colorless oil	colorless oil
Molecular formula	$C_{40}H_{72}O_{14}$	$C_{40}H_{72}O_{14}$	$C_{42}H_{74}O_{15}$	$C_{42}H_{74}O_{15}$
Molecular weight	776	776	818	818
FAB-MS (*m/z*)				
Positive	777 $[M+H]^+$ 799 $[M+Na]^+$	777 $[M+H]^+$ 799 $[M+Na]^+$	819 $[M+H]^+$ 841 $[M+Na]^+$	819 $[M+H]^+$ 841 $[M+Na]^+$
Negative	775 $[M-H]^-$	775 $[M-H]^-$	817 $[M-H]^-$	817 $[M-H]^-$
HRFAB-MS (*m/z*)				
	$C_{40}H_{72}O_{14}Na$ $[M+Na]^+$	$C_{40}H_{72}O_{14}Na$ $[M+Na]^+$	$C_{42}H_{74}O_{15}Na$ $[M+Na]^+$	$C_{42}H_{74}O_{15}Na$ $[M+Na]^+$
Calcd:	799.4820	799.4820	841.4925	841.4925
Found:	799.4821	799.4822	841.4929	841.4918
	$C_{40}H_{71}O_{14}$ $[M-H]^-$	$C_{40}H_{71}O_{14}$ $[M-H]^-$		$C_{42}H_{75}O_{15}$ $[M+H]^+$
Calcd:	775.4843	775.4843		819.5106
Found:	775.4835	775.4825		819.5047
$[\alpha]_D^{24}$ (*c* 0.1, MeOH)	+ 12 °	+ 8.0 °	+ 22 °	+ 10 °
UV $\lambda_{max}^{CH_3OH}$ nm (ε)	203 (45,800)	203 (25,800)	203 (27,000)	203 (41,600)
	222 (33,100)	222 (17,800)	222 (20,900)	222 (32,000)
IR υ_{max}^{KBr} (cm^{-1})	3437, 2962, 2927, 2875, 1707, 1641, 1630, 1458, 1375, 1275, 1228, 1074, 1026	3437, 2960, 2926, 2873, 2854, 1701, 1653, 1637, 1458, 1375, 1269, 1230, 1070, 1024	3434, 2962, 2927, 2873, 1741, 1701, 1655, 1637, 1458, 1375, 1273, 1232, 1128, 1078, 1036	3434, 2960, 2926, 2873, 2854, 1743, 1707, 1655, 1637, 1458 1375, 1269, 1238, 1124, 1078, 1034
Melting point	36.7 °C	35.6 °C	oily	oily
Solubility				
Soluble:	CH_3OH, $CHCl_3$, CH_3CN, Acetone, C_2H_5OH, Ethyl acetate	CH_3OH, $CHCl_3$, CH_3CN, Acetone, C_2H_5OH, Ethyl acetate	CH_3OH, $CHCl_3$, CH_3CN, Acetone, C_2H_5OH, Ethyl acetate	CH_3OH, $CHCl_3$, CH_3CN, Acetone, C_2H_5OH, Ethyl acetate
Insoluble:	H_2O, *n*-Hexane	H_2O, *n*-Hexane	H_2O, *n*-Hexane	H_2O, *n*-Hexane
Color reaction				
Positive:	50% H_2SO_4	50% H_2SO_4	50% H_2SO_4	50% H_2SO_4
Negative:	Ninhydrin reagent	Ninhydrin reagent	Ninhydrin reagent	Ninhydrin reagent

Results

Physico-chemical Properties of Roselipins

Physico-chemical properties of roselipins are summarized in Table 1. They all showed the same UV absorption maxima at 203 and 222 nm. The IR absorption at 3437~3434 cm^{-1} suggested the presence of hydroxyl groups in the structure[5].

Structure of Roselipin 1A

The molecular formula of roselipin 1A was determined to be $C_{40}H_{72}O_{14}$ on the basis of HRFAB-MS measurement.

Table 2-1. ^{1}H and ^{13}C NMR chemical shifts of roselipins 1A and 1B.

Carbon No.	Roselipin 1A			Roselipin 1B	
	^{13}C chemical shifts (ppm)[a]	^{1}H chemical shifts (ppm)[b]	$^{1}J_{CH}$ (Hz)	^{13}C chemical shifts (ppm)[a]	^{1}H chemical shifts (ppm)[b]
C-1	170.05			169.82	
C-2	128.81			128.73	
C-3	147.57	6.80 (1H, dd, *J*=10.0, 1.5 Hz)	146.2 (d)	147.71	6.78 (1H, dd, *J*=10.0, 1.5 Hz)
C-4	38.02	2.73 (1H, m)	128.0 (d)	38.07	2.75 (1H, m)
C-5	83.71	3.82 (1H, d, *J*=8.5 Hz)	143.8 (d)	83.72	3.82 (1H, d, *J*=9.0 Hz)
C-6	137.14			137.20	
C-7	134.30	5.33 (1H, dd, *J*=9.0, 1.0 Hz)	150.6 (d)	134.30	5.33 (1H, dd, *J*=9.0, 1.0 Hz)
C-8	37.08	2.62 (1H, m)	128.0 (d)	37.12	2.63 (1H, m)
C-9	84.33	3.72 (1H, d, *J*=9.5 Hz)	143.8 (d)	84.31	3.72 (1H, d, *J*=9.5 Hz)
C-10	134.79			134.84	
C-11	134.69	5.56 (1H, dd, *J*=9.5, 1.0 Hz)	154.6 (d)	134.67	5.57 (1H, dd, *J*=9.5, 1.5 Hz)
C-12	36.20	2.75 (1H, m)	125.7 (d)	36.24	2.76 (1H, m)
C-13	87.31	3.49 (1H, dd, *J*=7.0, 3.5 Hz)	139.3 (d)	87.33	3.49 (1H, dd, *J*=7.0, 3.5 Hz)
C-14	34.27	1.87 (1H, m)	124.6 (d)	34.29	1.87 (1H, m)
C-15	43.93	0.95 (1H, m)	124.3 (t)	43.97	1.02 (1H, m)
		1.37 (1H, m)			1.42 (1H, m)
C-16	28.85	1.62 (1H, m)	123.4 (d)	28.90	1.62 (1H, m)
C-17	46.04	0.88 (1H, m)	126.3 (t)	46.07	0.94 (1H, m)
		1.24 (1H, m)			1.27 (1H, m)
C-18	32.91	1.45 (1H, m)	125.8 (d)	32.94	1.45 (1H, m)
C-19	29.86	1.08 (1H, m)	125.1 (t)	29.89	1.22 (1H, m)
		1.42 (1H, m)			1.45 (1H, m)
C-20	11.53	0.89 (3H, t, *J*=7.0 Hz)	122.9 (q)	11.52	0.89 (3H, t, *J*=6.5 Hz)
C-21	12.88	1.90 (3H, d, *J*=1.5 Hz)	128.0 (q)	12.87	1.89 (3H, d, *J*=1.5 Hz)
C-22	16.81	0.86 (3H, d, *J*=7.0 Hz)	126.8 (q)	16.82	0.85 (3H, d, *J*=7.0 Hz)
C-23	11.36	1.68 (3H, d, *J*=1.0 Hz)	126.8 (q)	11.36[d]	1.67 (3H, d, *J*=1.0 Hz)
C-24	17.82	0.78 (3H, d, *J*=7.0 Hz)	126.8 (q)	17.82	0.78 (3H, d, *J*=7.0 Hz)
C-25	11.32	1.64 (3H, d, *J*=1.0 Hz)	126.8 (q)	11.36[d]	1.64 (3H, d, *J*=1.5 Hz)
C-26	18.63	0.99 (3H, d, *J*=7.0 Hz)	125.7 (q)	18.63	0.99 (3H, d, *J*=7.0 Hz)
C-27	15.56	0.94 (3H, d, *J*=7.0 Hz)	125.7 (q)	15.58	0.95 (3H, d, *J*=7.0 Hz)
C-28	21.34	0.90 (3H, d, *J*=6.5 Hz)	121.2 (q)	21.35	0.90 (3H, d, *J*=7.0 Hz)
C-29	20.73	0.90 (3H, d, *J*=6.5 Hz)	126.8 (q)	20.73	0.90 (3H, d, *J*=7.0 Hz)
C-1'	67.88	4.25 (1H, dd, *J*=12.0, 6.5 Hz)	146.7 (t)	67.16	4.20 (1H, dd, *J*=11.0, 5.5 Hz)
		4.41 (1H, dd, *J*=12.0, 3.0 Hz)			4.27 (1H, dd, *J*=11.0, 7.0 Hz)
C-2'	70.63	3.94 (1H, ddd, *J*=9.0, 6.5, 3.0 Hz)	141.6 (d)	69.38	4.14 (1H, ddd, *J*=7.0, 5.5, 2.0 Hz)
C-3'	71.93	3.58 (1H, dd, *J*=9.0, 2.0 Hz)	139.3 (d)	72.30	3.53 (1H, dd, *J*=8.0, 2.0 Hz)
C-4'	71.64	3.92 (1H, ddd, *J*=6.5, 6.5, 2.0 Hz)	142.7 (d)	72.71	3.73 (1H, ddd, *J*=8.0, 6.0, 3.5 Hz)
C-5'	64.8	3.65 (2H, ddd, *J*=7.0, 6.5, 6.5 Hz)	141.6 (t)	65.03	3.64 (1H, dd, *J*=11.0, 6.0 Hz)
					3.81 (1H, dd, *J*=11.0, 3.5 Hz)
C-1"	102.64	4.49 (1H, bs)	155.1 (dd)[c]	102.65	4.49 (1H, bs)
C-2"	72.68	3.90 (1H, d, *J*=3.0 Hz)	146.1 (dd)[c]	72.74	3.90 (1H, d, *J*=3.0 Hz)
C-3"	75.65	3.38 (1H, dd, *J*=9.0, 3.0 Hz)	135.9 (d)	75.68	3.38 (1H, dd, *J*=9.0, 3.0 Hz)
C-4"	68.54	3.57 (1H, dd, *J*=9.5, 9.0 Hz)	142.5 (d)	68.57	3.57 (1H, dd, *J*=9.5, 9.0 Hz)
C-5"	78.26	3.16 (1H, ddd, *J*=9.5, 5.0, 2.0 Hz)	139.3 (d)	78.28	3.16 (1H, ddd, *J*=9.5, 5.0, 2.0 Hz)
C-6"	62.94	3.76 (1H, dd, *J*=11.5, 5.0 Hz)	143.3 (t)	62.96	3.76 (1H, dd, *J*=11.5, 5.0 Hz)
		3.89 (1H, dd, *J*=11.5, 2.0 Hz)			3.89 (1H, dd, *J*=11.5, 2.0 Hz)

[a] Chemical shifts are shown with reference to CD_3OD as 49.8 ppm. [b] Chemical shifts are shown with reference to CD_3OD as 3.30 ppm. [c] Signal was observed as a double doublet with $^{2}J_{CH}$=6.8 Hz. [d] The signals were observed as the same chemical shifts.

The ^{13}C NMR spectrum (CD_3OD) showed 40 resolved peaks (Table 2-1), which were classified into ten methyl, three methylene, three *O*-methylene, six methine, eleven *O*-methine, three sp^2 methine, three sp^2 quaternary and one carbonyl carbons by analysis of the DEPT spectra. The ^1H NMR spectrum displayed 62 proton signals (Table 2-1). To fulfill the molecular formula of roselipin 1A, the presence of ten hydroxyl groups was suggested. Acetylation of roselipin 1A with acetic anhydride in pyridine gave the fragment ion peak of *m/z* 1196 $[M]^+$ and 1219 $[M+Na]^+$ in the FAB-MS spectrum, supporting the presence of ten hydroxy groups in the structure. The connectivity of proton

Table 2-2. ^1H and ^{13}C NMR chemical shifts of roselipins 2A and 2B.

	Roselipin 2A		Roselipin 2B	
Carbon No.	^{13}C chemical shifts (ppm)[a]	^1H chemical shifts (ppm)[b]	^{13}C chemical shifts (ppm)[a]	^1H chemical shifts (ppm)[b]
C-1	170.06		169.81	
C-2	128.82		128.73	
C-3	147.59	6.81 (1H, dd, *J*=9.5, 1.5 Hz)	147.71	6.78 (1H, dd, *J*=10.0, 1.5 Hz)
C-4	38.04	2.75 (1H, m)	38.07	2.73 (1H, m)
C-5	83.76	3.82 (1H, d, *J*=8.5 Hz)	83.77	3.80 (1H, d, *J*=9.0 Hz)
C-6	137.15		137.16	
C-7	134.44	5.33 (1H, dd, *J*=9.0, 1.0 Hz)	134.46	5.30 (1H, dd, *J*=9.0, 1.0 Hz)
C-8	36.99	2.61 (1H, m)	37.01	2.60 (1H, m)
C-9	84.49	3.66 (1H, d, *J*=9.5 Hz)	84.47	3.65 (1H, d, *J*=9.5 Hz)
C-10	134.87		134.81	
C-11	134.79	5.53 (1H, dd, *J*=9.0, 1.0 Hz)	134.86	5.52 (1H, dd, *J*=9.0, 1.0 Hz)
C-12	36.10	2.73 (1H, m)	36.11	2.73 (1H, m)
C-13	87.46	3.43 (1H, dd, *J*=7.0, 3.5 Hz)	87.43	3.42 (1H, dd, *J*=7.0, 3.5 Hz)
C-14	34.35	1.86 (1H, m)	34.35	1.83 (1H, m)
C-15	43.96	1.01 (1H, m)	43.97	0.99 (1H, m)
		1.37 (1H, m)		1.38 (1H, m)
C-16	28.84	1.61 (1H, m)	28.86	1.60 (1H, m)
C-17	46.06	0.88 (1H, m)	46.07	0.91 (1H, m)
		1.24 (1H, m)		1.23 (1H, m)
C-18	32.93	1.44 (1H, m)	32.93	1.43 (1H, m)
C-19	29.89	1.09 (1H, m)	29.89	1.20 (1H, m)
		1.40 (1H, m)		1.40 (1H, m)
C-20	11.53	0.89 (3H, t, *J*=6.5 Hz)	11.53	0.87 (3H, t, *J*=6.5 Hz)
C-21	12.87	1.90 (3H, d, *J*=1.5 Hz)	12.87	1.88 (3H, d, *J*=1.5 Hz)
C-22	16.80	0.86 (3H, d, *J*=7.0 Hz)	16.79	0.83 (3H, d, *J*=7.0 Hz)
C-23	11.37	1.68 (3H, d, *J*=1.0 Hz)	11.34	1.66 (3H, d, *J*=1.0 Hz)
C-24	17.79	0.76 (3H, d, *J*=7.0 Hz)	17.79	0.75 (3H, d, *J*=7.0 Hz)
C-25	11.15	1.63 (3H, d, *J*=1.0 Hz)	11.15	1.62 (3H, d, *J*=1.0 Hz)
C-26	18.70	0.98 (3H, d, *J*=7.0 Hz)	18.69	0.95 (3H, d, *J*=7.0 Hz)
C-27	15.52	0.95 (3H, d, *J*=7.0 Hz)	15.52	0.93 (3H, d, *J*=7.0 Hz)
C-28	21.27	0.90 (3H, d, *J*=7.0 Hz)	21.26	0.88 (3H, d, *J*=7.0 Hz)
C-29	20.72	0.90 (3H, d, *J*=7.0 Hz)	20.72	0.88 (3H, d, *J*=7.0 Hz)
C-1'	67.90	4.25 (1H, dd, *J*=12.0, 6.5 Hz)	67.15	4.18 (1H, dd, *J*=11.0, 5.5 Hz)
		4.41 (1H, dd, *J*=12.0, 3.0 Hz)		4.25 (1H, dd, *J*=11.0, 7.0 Hz)
C-2'	70.68	3.94 (1H, ddd, *J*=9.0, 6.5, 3.0 Hz)	69.38	4.14 (1H, ddd, *J*=7.0, 5.5, 2.0 Hz)
C-3'	71.97	3.58 (1H, dd, *J*=9.0, 2.0 Hz)	72.30	3.52 (1H, dd, *J*=8.0, 2.0 Hz)
C-4'	71.67	3.93 (1H, ddd, *J*=6.5, 6.5, 2.0 Hz)	72.74	3.71 (1H, ddd, *J*=8.0, 6.0, 3.5 Hz)
C-5'	64.82	3.65 (2H, ddd, *J*=7.0, 6.5, 6.5 Hz)	65.03	3.63 (1H, dd, *J*=11.0, 6.0 Hz)
				3.80 (1H, dd, *J*=11.0, 3.5 Hz)
C-1"	102.74	4.46 (1H, bs)	102.73	4.45 (1H, bs)
C-2"	72.46	3.91 (1H, d, *J*=3.0 Hz)	72.74	3.90 (1H, d, *J*=3.0 Hz)
C-3"	75.46	3.38 (1H, dd, *J*=9.0, 3.0 Hz)	75.48	3.37 (1H, dd, *J*=9.0, 3.0 Hz)
C-4"	68.92	3.51 (1H, dd, *J*=9.5, 9.0 Hz)	68.93	3.50 (1H, dd, *J*=9.5, 9.0 Hz)
C-5"	75.76	3.33 (1H, ddd, *J*=9.5, 7.0, 2.0 Hz)	75.76	3.33 (1H, ddd, *J*=9.5, 7.0, 2.0 Hz)
C-6"	65.44	4.26 (1H, dd, *J*=11.5, 7.0 Hz)	65.44	4.26 (1H, dd, *J*=11.5, 7.0 Hz)
		4.42 (1H, dd, *J*=11.5, 2.0 Hz)		4.41 (1H, dd, *J*=11.5, 2.0 Hz)
C-6"CO	172.70		172.70	
C-6"$COCH_3$	21.01	2.09 (3H, s)	21.01	2.08 (3H, s)

[a] Chemical shifts are shown with reference to CD_3OD as 49.8 ppm. [b] Chemical shifts are shown with reference to CD_3OD as 3.30 ppm.

and carbon atoms was established by the ^{13}C-^{1}H HMQC spectrum (Table 2-1). Analysis of the ^{1}H-^{1}H COSY spectrum revealed the six partial structures I to VI (Fig. 2). ^{13}C-^{1}H long range couplings of ^{2}J and ^{3}J observed in the ^{13}C-^{1}H HMBC experiment (Fig. 3) confirmed the partial structures I to VI and gave the following results:

1) The long range couplings from H_2-15 (δ 0.95, 1.37) to C-27 (δ 15.56), from H_2-17 (δ 0.88, 1.24) to C-18 (δ 32.91), and C-29 (δ 20.73), from H-18 (δ 1.45) to C-17 (δ 46.04) and C-29, from H_2-19 (δ 1.08, 1.42) to C-18, from H_3-20 (δ 0.89) to C-18, from H_3-27 (δ 0.94) to C-14 (δ 34.27) and C-15 (δ 43.93), and from H_3-29 (δ 0.90) to C-17 showed the bigger partial structure VII including the partial structure I.

2) The cross peaks from H-13 (δ 3.49) to C-15 and C-27, from H-15 to C-13 (δ 87.31), and from H_3-27 to C-13 and C-14 showed the structure VIII containing the partial structures I and II.

Fig. 2. Partial structures I to VI of roselipin 1A.

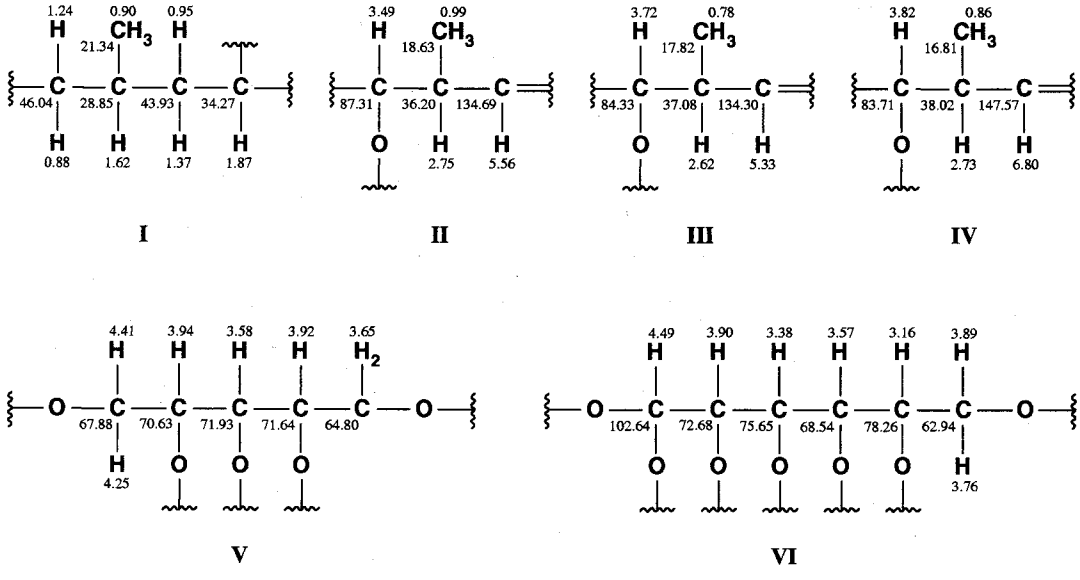
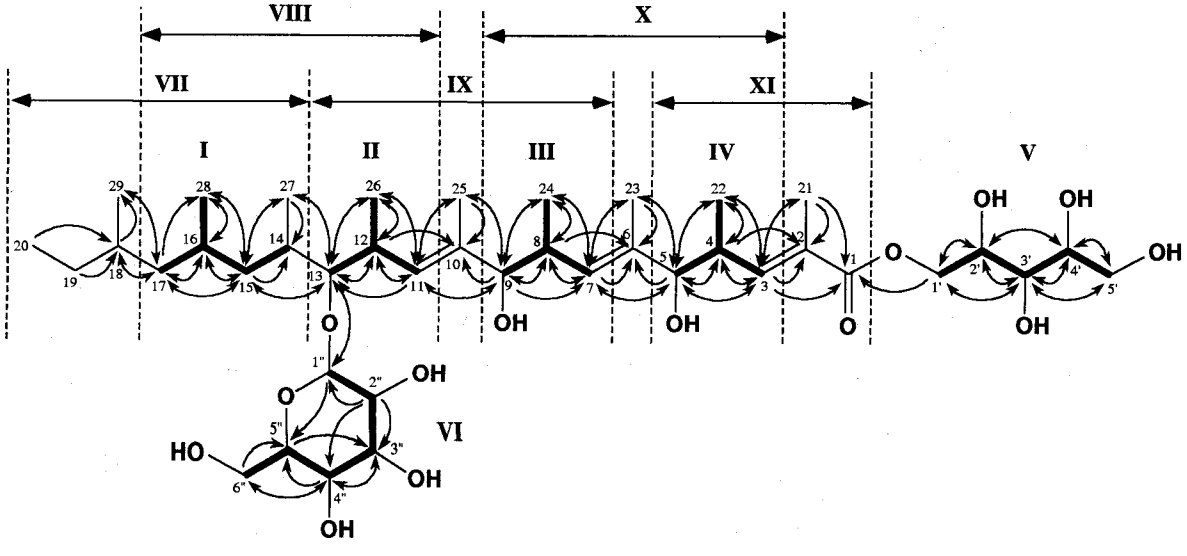


Fig. 3. ^{1}H-^{1}H COSY, ^{13}C-^{1}H HMQC and ^{13}C-^{1}H HMBC experiments of roselipin 1A.

^{1}H-^{1}H COSY: ▬, ^{13}C-^{1}H HMBC: H ⟶ C.



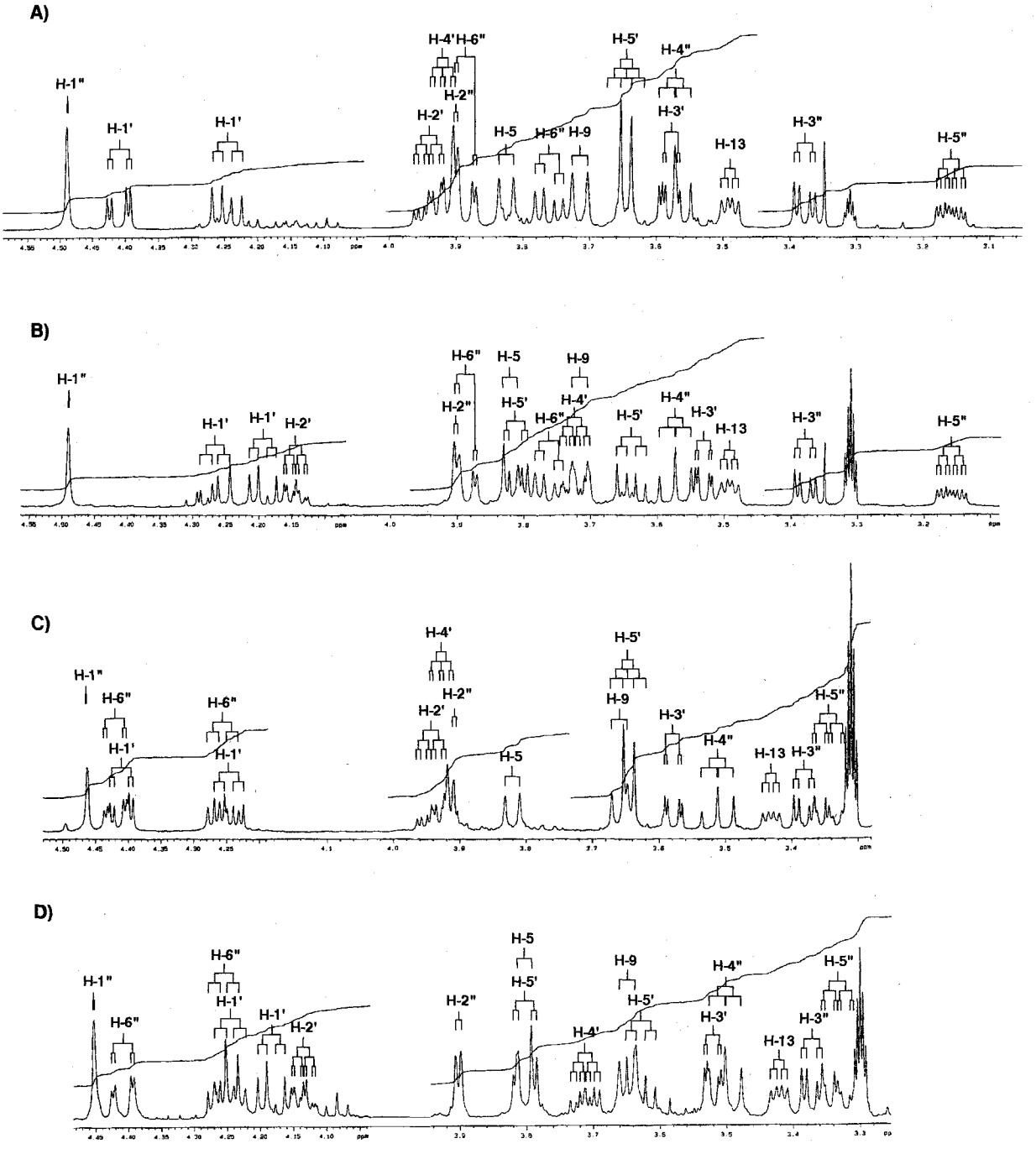
3) The long range couplings from H-9 (δ 3.72) to C-10 (δ 134.79), C-11 (δ 134.69), and C-25 (δ 11.32), from H-11 (δ 5.56) to C-9 (δ 84.33) and C-25, from H-12 (δ 2.75) to C-10, and from H_3-25 (δ 1.64) to C-9, C-10 and C-11 showed the structure IX including the partial structures II and III.

4) The long range couplings from H-5 (δ 3.82) to C-6 (δ 137.14), C-7 (δ 134.30) and C-23 (δ 11.36), from H-7 (δ 5.33) to C-5 (δ 83.71), C-6 and C-23, from H-8 (δ 2.62) to C-6, and from H_3-23 (δ 1.68) to C-5, C-6 and C-7 showed the structure X including the partial structures III and IV.

5) The long range couplings from H-3 (δ 6.80) to C-1 (δ 170.05), C-2 (δ 128.81) and C-21 (δ 12.88), from H-4 (δ 2.73) to C-2, from H_3-21 (δ 1.90) to C-1, C-2 and C-3 (δ 147.57) showed the structure XI containing the partial

Fig. 4. Comparison of coupling constants among roselipins.

(A) roselipin 1A, (B) roselipin 1B, (C) roselipin 2A and (D) roselipin 2B.



structure IV. Thus, the skeleton of nonamethyl icosenoic acid (highly methylated C20 fatty acid) was suggested from the alignment of the structures VII to XI.

6) The presence of an alditol moiety containing the partial structure V was established by the long range couplings as shown in Fig. 3. The long range coupling from H_2-1′ (δ 4.25, 4.41) to C-1 indicated that the alditol moiety is attached to the C-1 of the fatty acid skeleton *via* the ester bond.

7) The presence of a hexose moiety containing the partial structure VI was also established by the long range couplings as shown in Fig. 3. The long range couplings observed from H-13 to C-1″ (δ 102.64), from H-1″ (δ 4.49) to C-13 and C-5″ (δ 78.26) indicated that the hexapyranoside is attached to the 13-O of the fatty acid skeleton *via* the glycoside linkage. Therefore, the structure of roselipin 1A was elucidated, comprising of highly

Fig. 5. ^{1}H NMR and NOE experiments of mannose moiety of roselipin 1A.

NOE: ⟷, proton coupling: ------.

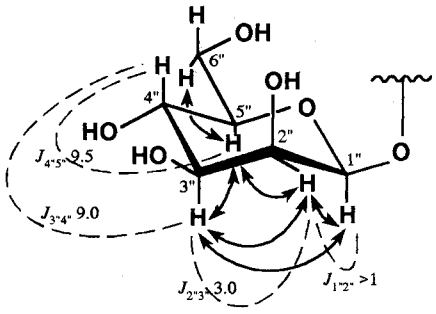


Fig. 6. ^{1}H NMR and NOE experiments of arabinitol moiety.

(A) roselipin 1A. (B) roselipin 1B.
NOE: ⟷, proton coupling: ------.

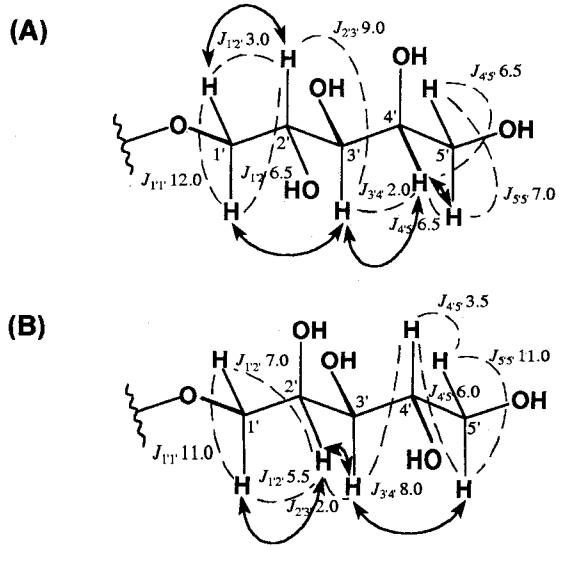
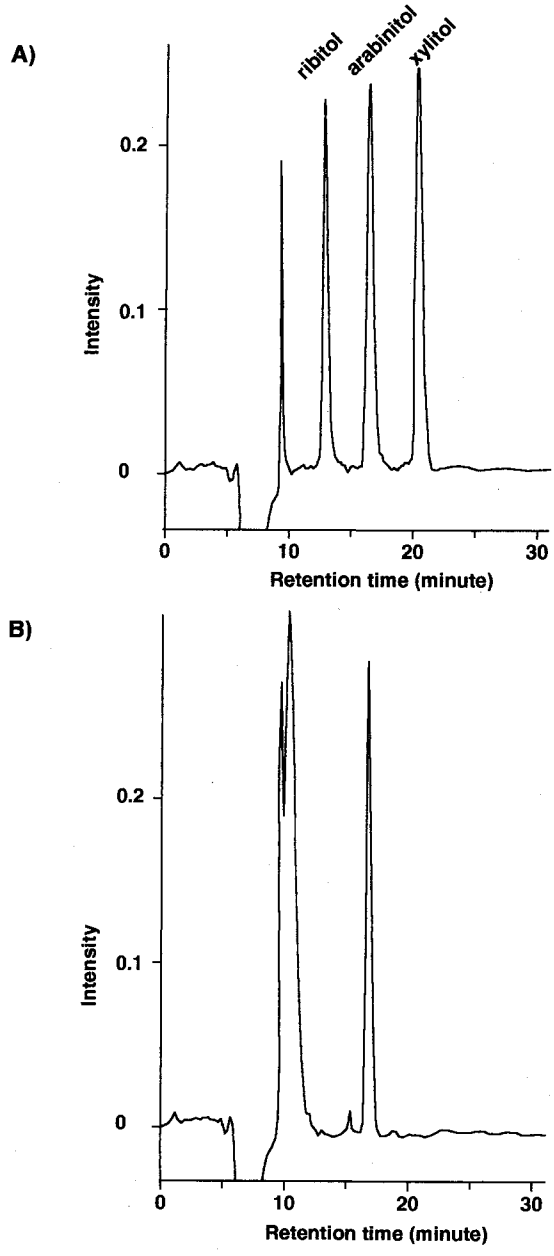


Fig. 7. Analysis of alditols by HPLC.

(A) Authentic ribitol, arabinitol and xylitol.
(B) Hydrolysate prepared from roselipin 1A.



Column, Shodex SUGAR SC 1211 (6.0×250 mm); solvent, 35% CH_3CN aq; column temperature, 70°C; detection, refractive index (Shodex RI-71); flow rate, 0.5 ml/minute.

methylated C20 fatty acid, hexose and alditol.

The configurations of roselipin 1A were studied.

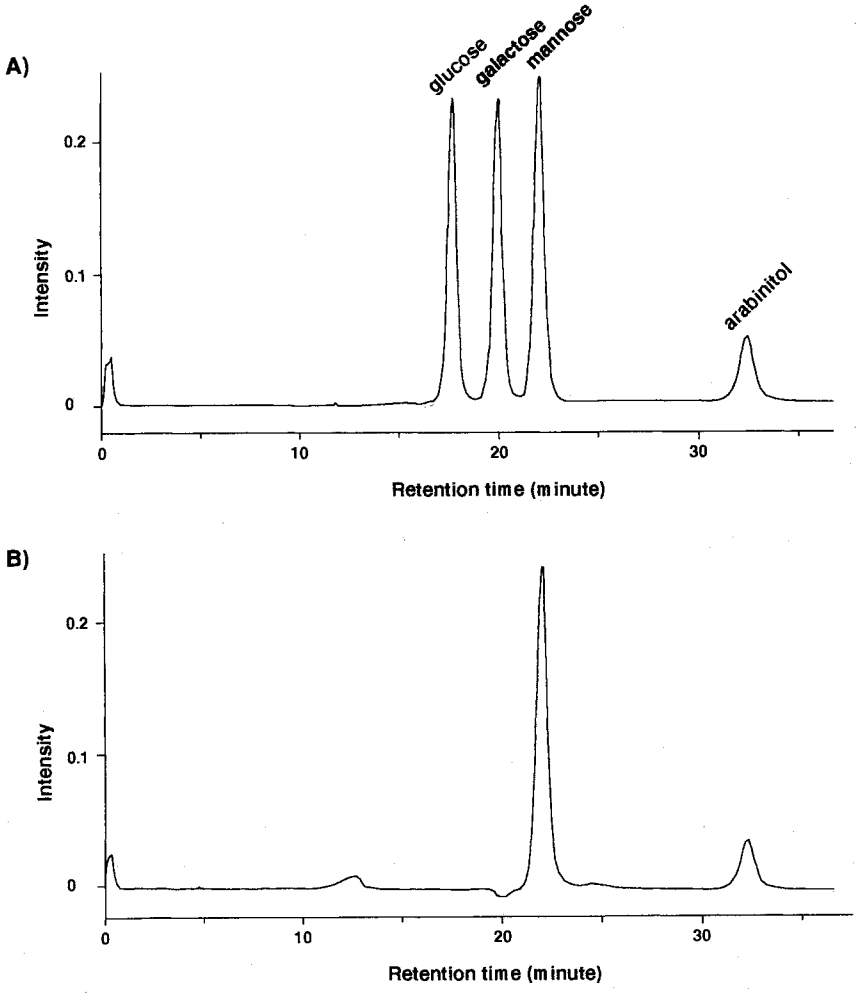
1) Regarding the stereochemistry of the three olefins, the NOEs were observed between H_3-26 and H-11, H_3-26 and H-13, H_3-25 and H-8, H_3-25 and H-12, and H-9 and H-11, suggesting the *E* stereochemistry of the C-10-C-11 olefin. Similarly, the stereochemistries of the C-6-C-7 and C-2-C-3 olefins were both *E* from the NOE experiments.

2) Regarding the stereochemistry of the hexose and alditol, the proton coupling constants were measured as summarized in Fig. 4. In order to unambiguously determine coupling constants, the crowded signals were analyzed by differential selective proton decoupling experiments. The irradiation at H-3″ (δ 3.38), H-5′ (δ 3.65), H-2′ (δ 3.94) and H-1″ (δ 4.49) simplified the two signals of H-4″ (δ 3.57) and H-2″ (δ 3.90), the one signal of H-4′ (δ 3.92), the three signals of H-3′ (δ 3.58), H-1′ (δ 4.25) and H-1′ (δ 4.41) and the one signals of H-2″ (δ 3.90), respectively. The proton coupling constants were 9.0 Hz between the vicinal H-3″ and H-4″ and 9.5 Hz between the vicinal H-4″ and H-5″, indicating that the hexose was mannose (Fig. 5). The ^{13}C NMR chemical shifts also supported mannopyranoside[6,7]. The proton coupling constants were 9.0 Hz between vicinal H-2′ and H-3′ and 2.0 Hz between vicinal H-3′ and H-4′, indicating that the alditol was arabinitol (Fig. 6).

3) Regarding the configuration of the glycoside linkage, the direct coupling constant was measured as 155.1 Hz between the anomeric carbon atom and proton [J_{CH}] in the gated decoupling experiment. Furthermore, the

Fig. 8. Analysis of hexoses by HPLC.

(A) Authentic glucose, galactose and mannose. (B) Hydrolysate prepared from roselipin 1A.



Column, Shodex SUGAR SP 0810 (8.0×300 mm); solvent, 100% H_2O; column temperature, 80°C; detection, refractive index (Shodex RI-71); flow rate, 0.5 ml/minutre.

Fig. 9. Analysis of trifluoroacetyl D- and L-arabinitol by gas chromatography.

(A) Authentic D-arabinitol and L-arabinitol,
(B) Hydrolysate prepared from roselipin 1A.

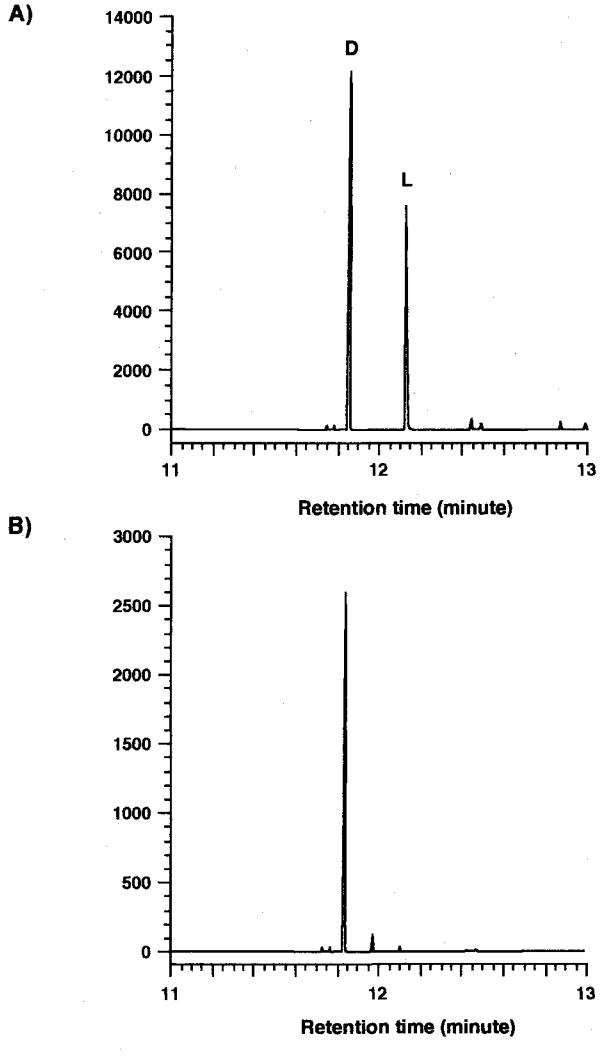
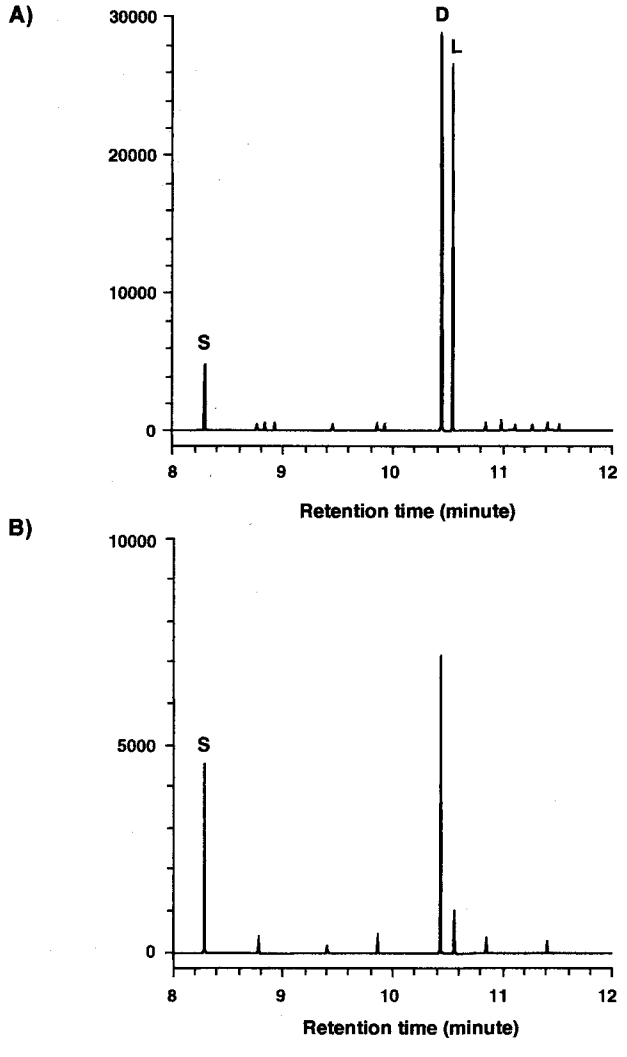


Fig. 10. Analysis of acetyl (+)- or (±)-2-butyl D- and L- mannoses by gas chromatography.

(A) Authentic acetyl (+)-2-butyl-D-mannose and -L-mannose; acetylated methyl-D-mannose.
(B) Hydrolysate prepared from roselipin 1A.



NOEs were observed between H-1″ and H-2″, H-1″ and H-3″, H-2″ and H-3″, H-2″ and H-5″, H-5″ and H-3″, and H-5″ and H-6″ (d 3.76) (Fig. 5). These observations indicate that the glycoside linkage has a β configuration[8].

To confirm the presence of mannose and arabinitol moieties, the hydrolysate prepared from roselipin 1A was analyzed by HPLC using Shodex SUGAR SC1211 and Shodex SUGAR SP0810 columns. In comparison with peaks of the authentic alditols and hexoses (Figs. 7 and 8), arabinitol and mannose were detected in almost equimolar amount. To determine the absolute configuration of arabinitol in roselipin 1A, the trifluoroacetylated derivative prepared from roselipin 1A was analyzed by gas chromatography using a chiral column (Supelco Inc., Beta Dex-120)[3]. The derivative was eluted as a peak with a retention time of 11.83 minutes, which was identical with that of pertrifluoroacetyl-D-arabinitol (Fig. 9). To determine the absolute configuration of mannose in roselipin 1A, the acetylated-2-butyl derivatives prepared from roselipin 1A were analyzed by gas chromatography using a glass-capillary column (SHIMADZU CBP1)[4]. The (acetylated (+)-2-butyl) derivatives were eluted as a peak with retention time of 10.45 minutes, which was identical with that of tetraacetyl (+)-2-butyl-D-mannose (Fig. 10B). These results revealed that roselipin 1A contains D-mannose and D-arabinitol.

Taken together, the structure of roselipin 1A was elucidated as shown in Fig. 1.

Structure of Roselipin 1B

The molecular formula $C_{40}H_{72}O_{14}$ of roselipin 1B was the same as that of roselipin 1A. Various spectral data of roselipin 1B were very similar to those of roselipin 1A (Tables 1 and 2-1). The general structure of roselipin 1B was the same as roselipin 1A, suggesting they are stereoisomers. In fact, the ^{13}C NMR chemical shifts and ^{1}H coupling constants of the arabinitol moiety were different between roselipins 1A and 1B (Table 2-1). The coupling constants of roselipins 1A and 1B were 9.0 and 2.0 Hz between the vicinal H-2′ and H-3′ protons, and 2.0 and 8.0 Hz between the vicinal H-3′ and H-4′ protons, respectively, suggesting that the different terminal hydroxy moiety of the D-arabinitol is bound to the fatty acid as shown in Fig. 1.

Structures of Roselipins 2A and 2B

The same molecular formulas $C_{42}H_{74}O_{15}$ were obtained for roselipins 2A and 2B, which are a C_2OH_2 unit larger than those of roselipins 1A and 1B. The NMR data (Table 2-2) suggested the presence of an acetoxy residue at C-6″ of the mannose. Other spectral data were very similar to those of roselipins 1A and 1B. Roselipins 2A and 2B were formulated as 6″-*O*-acetyl roselipins 1A and 1B, respectively, as shown in Fig. 1.

Discussion

The general structures of roselipins were elucidated. They have a unique structure composed of three parts, a highly methylated fatty acid, a hexose and an alditol. As demonstrated for roselipin 1A in this paper, the structures and stereochemistry of the hexose and the alditol were defined as D-mannose and D-arabinitol by analyses of the acid hydrolysates using HPLC and gas chromatography. The same results were obtained for roselipins 2A, 1B and 2B (data not shown). It might be that the four roselipins possess the same stereochemistry of the fatty acid moiety, because the chemical shifts corresponding to the moiety showed a good agreement. However, the stereochemistry of the fatty acid has not yet been elucidated. The carbon skeleton of the fatty acid is very rare in that the even numbered carbons of icosanoic acid were all methylated to form 2,4,6,8,10,12,14,16,18-nonamethyl icosanoic acid. Similar substitution pattern was reported for fungal radiclonic acid[9] with a carbon skeleton of 2,4,6,8,10,12,14-heptamethyl palmitic acid. Such methyl residues in fungal polyketide metabolites, are usually biosynthesized from methionine. In fact, SETO *et al.*[9], demonstrated that all the methyl residues of radiclonic acid are derived from the *S*-methyl of methionine. Therefore, roselipin seems to be the case. Furthermore, from the structural analysis of the arabinitol moiety, it was demonstrated that the stereoisomers between roselipin A and B groups are derived from esterification of the fatty acid with the different terminal hydroxy residue of arabinitol.

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References

1) ŌMURA, S.; H. TOMODA, N. TABATA, Y. OHYAMA, T. ABE & M. NAMIKOSHI: Roselipins, novel fungal metabolites having a highly methylated fatty acid modified with a mannose and an arabinitol. J. Antibiotics 52: 586~589, 1999
2) TOMODA, H.; Y. OHYAMA, T. ABE, N. TABATA, M. NAMIKOSHI, Y. YAMAGUCHI, R. MASUMA & S. ŌMURA: Roselipins, Inhibitors of diacylglycerol acyltransferase produced by *Gliocladium roseum* KF-1040. J. Antibiotics 52: 689~694, 1999
3) LARSSON, L.; C. PEHRSON, T. WIEBE & B. CHRISTENSSON: Gas chromatographic determination of D-arabinitol/L-arabinitol ratios in urine: a potential method for diagnosis of disseminated candidiasis. J. Clin. Microbiol. 32: 1855~1859, 1994
4) GERWIG, G. J.; J. P. KAMERLING & J. F. G. VLIEGENTHART: Determination of the D and L configuration of neutral monosaccharides by high-resolution capillary G.L.C. Carbohydr. Res. 62: 349~357, 1978
5) FRESENIUS W.; J. F. K. HUBER, E. PUNGOR, G. A. RECHNITZ, W. SIMON & TH. S. WEST: *In* Tables of spectral data for structure determination of organic compounds. 2nd English ed. I185, Springer-Verlag, Berlin Heidelberg, 1989
6) WALKER, T. E.; R. E. LONDON, T. W. WHALEY, R. BARKER & N. A. NATWIYOFF: Carbon-13 nuclear magnetic resonance spectroscopy of [1-^{13}C] enriched monosaccharides. Signal assignments and orientational dependence of geminal and vicinal carbon-carbon and

carbon-hydrogen spin-spin coupling constants. J. Am. Chem. Soc. 98: 5807~5813, 1976

7) GORIN, P. A. J. & M. MAZUREK: Further studies on the assignment of signals in ^{13}C magnetic resonance spectra of aldoses and derived methyl glycosides. Can. J. Chem. 53: 1212~1223, 1975

8) Bock, K. & C. Pedersen: A study of ^{13}CH coupling constants in hexopyranoses. J. Chem. Soc., Perkin II. 293~297, 1974

9) SETO, H.; T. SASAKI & H. YONEHARA: Studies on the biosynthesis of radiclonic acid. Tetrahedron Lett. 4083~4084, 1977