Studies on the Constituents of Fruits of *Helicteres isora* L.[1)]

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Three new compounds; 4′-*O*-*β*-D-glucopyranosyl rosmarinic acid (2), 4,4′-*O*-di-*β*-D-glucopyranosyl rosmarinic acid(3) and 2*R*-*O*-(4′-*O*-*β*-D-glucopyranosyl caffeoyl)-3-(4-hydroxyphenyl) lactic acid named as 4′-*O*-*β*-D-glucopyranosyl isorinic acid (4) were isolated together with rosmarinic acid (1) from the fruit of *Helicteres isora* L. (Sterculiaceae), an Indonesian medicinal plant. The structures of these compounds, including the absolute stereochemistry of 4, were elucidated by spectroscopic analysis and chemical means. Compound 3 had greater scavenging activity against superoxide anion produced with xanthine and xanthine oxidase (XOD) than rosmarinic acid (1).

Key words *Helicteres isora*; isorinic acid; rosmarinic acid glycoside; rosmarinic acid; Sterculiaceae

As a continuation of our studies on Jamu and the medicinal resources in Indonesia, we have isolated three new compounds: 4′-*O*-*β*-D-glucopyranosyl rosmarinic acid (**2**), 4,4′-*O*-di-*β*-D-glucopyranosyl rosmarinic acid (**3**) and 2*R*-*O*-(4′-*O*-*β*-D-glucopyranosyl caffeoyl)-3-(4-hydroxyphenyl) lactic acid (**4**), together with rosmarinic acid (**1**) from the fruit of *Helicteres isora* L. *H. isora* (Sterculiaceae) is one of the Jamu raw materials used in traditional folk medicine in Indonesia; it is called "Buah Kayu Ules or Ulet-Ulet" on Java island and is used for treating of gastrospasm and as an anthelmintic for tapeworm in Indonesia,[2)] and as an antispasmodic, antipyretic, antidiarrhoic, and antidysenteric in Saudi Arabia,[3)] and as a tonic compound after childbirth in the Malay Islands.[4)] In this paper, we report the structural elucidation of **1**—**4** and their superoxide-scavenging activity.

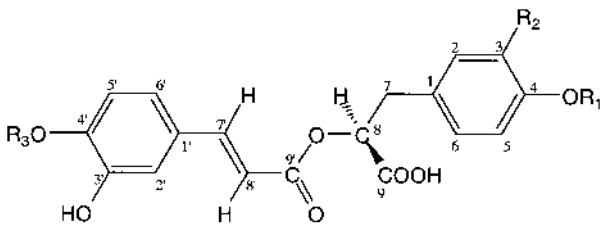
The fruit of *H. isora* (4 kg), purchased in Indonesia, was extracted with MeOH to obtain the MeOH extract. This extract was partitioned into $CHCl_3$–, EtOAc–, *n*-BuOH– and aqueous-soluble fractions, respectively. The *n*-BuOH fraction was separated by a combination of column chromatography on Sephadex LH-20, Rp-8 and silica gel to give compound **1**. The H_2O fraction was subjected to repeated column chromatography on MCI-gel, Sephadex LH-20, Rp-18 and silica gel to afford compounds **2**—**4**.

Compound **1**, a colorless amorphous powder, $[\alpha]_D$ +73.3° (c=0.2, MeOH), showed the molecular formula $C_{18}H_{16}O_8$ on the basis of negative ion HR-FAB-MS.

The ^{1}H-NMR spectrum indicated the presence of two 1,3,4-trisubstituted phenyl groups [δ 7.06 (1H, d, J=2.0 Hz), 7.01 (1H, dd, J=8.2, 2.0 Hz), 6.77 (1H, d, J=8.2 Hz), 6.68 (1H, d, J=2.0 Hz), 6.64 (1H, d, J=8.0 Hz), 6.52 (1H, dd, J=8.0, 2.0 Hz)] and one *trans* double bond [δ 7.46, 6.24 (each 1H, d, J=15.9 Hz)]. The ^{1}H-NMR and ^{13}C-NMR spectra (Tables 1, 2) assigned with the aid of the distortionless enhancement by polarization transfer (DEPT), ^{1}H–^{1}H shift correlation spectroscopy (COSY) and ^{1}H–^{13}C COSY, revealed the signals due to a caffeoyl group and a 3,4-dihydroxyphenyl lactic acid moiety. Based on the hetero nuclear multiple-bond correlation spectroscopy (HMBC) experiment, the structure of **1** was assigned as rosmarinic acid,[5)] including absolute configuration (*R*) at C-8 from optical rotation.

Compound **2** was isolated as a colorless amorphous powder, $[\alpha]_D$ +9.0° (c=0.9, MeOH), molecular formula $C_{24}H_{26}O_{13}$, from negative ion HR-FAB-MS. The anthrone reaction was positive, suggesting for **2** to be a glycoside. The ^{1}H- and ^{13}C-NMR spectra of **2** were similar to those of **1**, besides signals corresponding to the sugar moiety which included an anomeric proton signal [δ 4.77 (1H, d, J=7.3 Hz)] and an anomeric carbon signal (δ 101.65). Enzymic hydrolysis (emulsin) of **2** yielded rosmarinic acid (**1**), $[\alpha]_D$ +74.4° (c=0.5, MeOH) and D-glucose. The position of the hydroxyl group which was attached to the glucosyl unit, was concluded to be at C-4′ by the observed nuclear Overhauser effect (NOE) interactions between the anomeric proton at δ 4.77 and H-5′ at δ 7.10 (1H, d, J=8.5 Hz) in the nuclear Overhauser enhancement spectroscopy (NOESY) spectrum. Furthermore, its linkage was *β*-configuration from the coupling constant of the anomeric proton. Thus, **2** was determined to be 4′-*O*-*β*-D-glucopyranosyl rosmarinic acid.[6)]

Compound **3** was obtained as a colorless amorphous powder, $[\alpha]_D$ −17.9° (c=1.0, H_2O) and had a molecular formula $C_{30}H_{36}O_{18}$ from negative ion HR-FAB-MS. The ^{1}H- and ^{13}C-NMR spectra showed two anomeric proton signals [δ 4.77 (1H, d, J=7.1 Hz), 4.61 (1H, d, J=7.1 Hz)], two anomeric



	R1	R2	R3
Compound 1:	H	OH	H
2:	H	OH	β-D-glc
3:	β-D-glc	OH	β-D-glc
4:	H	H	β-D-glc
5:	H	H	H

Fig. 1. Chemical Structures of Compounds **1**—**5**

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Table 1. ^{1}H-NMR Spectral Data (δ) for Compounds **1**—**5** (DMSO-d_6, 400 MHz)

H	1	2	3	4	5
2-H	6.68 (d, 2.0)	6.68 (d, 1.6)	6.74 (d, 1.8)	7.08 (d, 8.5)	7.08 (d, 8.5)
3-H				6.67 (d, 8.5)	6.68 (d, 8.5)
5-H	6.64 (d, 8.0)	6.60 (d, 8.0)	6.98 (d, 8.3)	6.67 (d, 8.5)	6.68 (d, 8.5)
6-H	6.52 (dd, 8.0, 2.0)	6.50 (dd, 8.0, 1.6)	6.63 (dd, 8.3, 1.8)	7.08 (d, 8.5)	7.08 (d, 8.5)
7-H	2.89 (dd, 14.3, 8.6)	2.78 (dd, 13.9, 9.7)	2.84 (dd, 13.8, 9.6)	2.94 (dd, 14.3, 9.0)	2.94 (dd, 14.3, 9.0)
	2.99 (dd, 14.3, 4.1)	3.04 (dd, 13.9, 3.1)	3.06 (dd, 13.8, 3.2)	3.07 (dd, 14.3, 4.1)	3.08 (dd, 14.3, 3.7)
8-H	5.02 (dd, 8.6, 4.1)	5.02 (dd, 9.7, 3.1)	4.93 (dd, 9.6, 3.2)	5.02 (dd, 9.0, 4.1)	5.03 (dd, 9.0, 3.7)
2′-H	7.06 (d, 2.0)	7.14 (d, 1.6)	7.14 (d, 1.6)	7.16 (d, 2.0)	7.05 (d, 1.9)
5′-H	6.77 (d, 8.2)	7.10 (d, 8.5)	7.10 (d, 8.5)	7.12 (d, 8.5)	6.77 (d, 8.2)
6′-H	7.01 (dd, 8.2, 2.0)	7.05 (dd, 8.5, 1.6)	7.06 (dd, 8.5, 1.6)	7.10 (dd, 8.5, 2.0)	7.98 (dd, 8.2, 1.9)
7′-H	7.46 (d, 15.9)	7.42 (d, 16.0)	7.43 (d, 15.8)	7.48 (d, 15.9)	7.43 (d, 15.9)
8′-H	6.24 (d, 15.9)	6.24 (d, 16.0)	6.36 (d, 15.8)	6.40 (d, 15.9)	6.23 (d, 15.9)
Glc-1″-H		4.77 (d, 7.3)	4.77 (d, 7.1)	4.78 (d, 7.3)	
Glc-1‴-H			4.61 (d, 7.1)		

Coupling patterns and coupling constants (J) in Hz are given in parentheses.

Table 2. ^{13}C-NMR Data (δ) for Compounds **1**—**5** (DMSO-d_6, 400 MHz)

C	1	2	3	4	5
1	128.71	129.45	133.84	127.32	127.41
2	116.90	116.65	116.90	130.25	130.24
3	144.13	144.97	143.85	115.07	115.08
4	144.00	143.67	146.56	156.03	156.02
5	115.63	115.45	116.81	115.07	115.08
6	120.02	119.80	120.54	130.25	130.24
7	36.85	36.94	36.85	36.20	36.25
8	75.54	75.53	75.31	73.85	73.73
9	172.05	173.16	172.11	171.40	171.69
1′	125.66	128.78	128.83	128.56	125.45
2′	115.18	115.00	115.04	115.07	115.08
3′	145.18	147.03	143.85	147.62	148.67
4′	148.81	147.44	147.47	146.96	145.73
5′	116.13	116.16	116.20	116.68	115.94
6′	121.49	120.64	119.82	120.95	121.45
7′	145.35	143.91	147.14	144.88	145.51
8′	116.13	116.75	116.68	115.81	114.98
9′	166.29	166.02	166.06	165.87	166.05
1″		101.65	101.68	101.58	
2″		73.27	73.38	73.25	
3″		75.87	75.88	75.85	
4″		69.82	69.81	69.82	
5″		77.29	77.27	77.28	
6″		60.77	60.75	60.76	
1‴			102.56		
2‴			73.25		
3‴			75.88		
4‴			69.81		
5‴			77.14		
6‴			60.75		

carbon signals (δ 101.68, 102.56) and characteristic carbon signals due to two glucopyranosyl moieties together with proton and carbon signals corresponding to rosmarinic acid. The enzymic hydrolysis (emulsin) of **3** yielded rosmarinic acid (**1**), $[\alpha]_D$ +74.8° (c=1.0, MeOH) and only D-glucose as the sugar moiety. In the HMBC spectrum of **3**, the anomeric proton signals at δ 4.77 and δ 4.61 exhibited cross peaks due to long-range coupling with the carbon signals at δ 146.56 (C-4) and δ 147.47 (C-4′), respectively. Furthermore, in a NOESY experiment, NOE correlations were observed between the anomeric proton at δ 4.77 and an aromatic proton at δ 6.98 (1H, d, J=8.3 Hz, H-5), and the anomeric proton at δ 4.61 and an aromatic proton at δ 7.10 (1H, d, J=8.5 Hz, H-5′). These findings and the coupling constants of each anomeric proton signal indicated that each D-glucose was linked to hydroxyl groups at C-4 and C-4′ in a β-configuration. Consequently, compound **3** was determined to be 4,4′-*O*-di-β-D-glucopyranosyl rosmarinic acid.

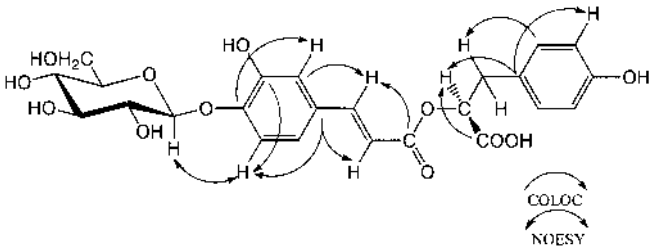

Fig. 2. COLOC and NOESY Spectral Data of **4**

Compound **4**, a colorless amorphous powder, $[\alpha]_D$ −16.0° (c=0.3, H_2O), had a molecular formula $C_{24}H_{26}O_{12}$ from negative ion HR-FAB-MS. In the comparison of the ^{1}H- and ^{13}C-NMR spectra of **4** with those of **2**, compound **4** showed NMR signals similar to those of **2**, except for the proton and carbon signals corresponding to the 4-hydroxyphenyl group instead of those due to the 3,4-dihydroxyphenyl group in **2**. In the correlation spectroscopy *via* long range coupling (COLOC) experiments of **4** described in Fig. 2, long range correlations were observed between the aromatic carbon (C-1) at δ 127.32 and both the oxygen-bearing methine proton (H-8) at δ 5.02 and the aromatic protons (H-3, H-5) at δ 6.67, and the aromatic carbon (C-1′) at δ 128.56 and both the olefinic proton (H-8′) at δ 6.40 and the aromatic proton (H-5′) at δ 7.12. The enzymic hydrolysis (emulsin) of **4** yielded D-glucose and compound **5** ($C_{18}H_{16}O_7$). On the base of these findings, the planar structure of **5** was elucidated as 2-*O*-caffeoyl-3-(4-hydroxyphenyl) lactic acid. The absolute configuration of **5** was deduced by the following manner. The saponification of compound **5** followed by acidification furnished caffeoic acid and 2*R*-3-(*p*-hydroxyphenyl)-lactic acid {**6**, $[\alpha]_D$ +13.9° (c=0.6, MeOH)}.[7] The stereochemistry of **6** was supported by the phenylglycine methyl ester (PGME) method.[8] The values of $\Delta\delta$ [δ(*S*)-PGME amide−δ(*R*)-PGME amide] in the ^{1}H-NMR spectra (Fig. 3) suggested that the absolute configuration at C-2 of **6** was (*R*). The D-glucose was indicated to be linked to the hydroxyl groups at C-4′ from

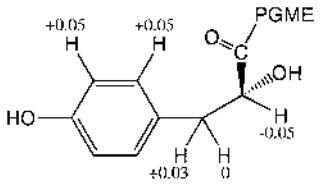

Fig. 3. ^{1}H-NMR Chemical Shift Differences ($\Delta\delta$) for PGME Amide of Compound **6**

$\Delta\delta$(ppm)=δ[(*S*)-PGME amide]−δ[(*R*)-PGME amide].

the NOE observed between the anomeric proton (1″-H) and the aromatic proton (5′-H) and the glycosidic linkage was β-configuration from the coupling constant (J=7.3 Hz). Accordingly, compound **4** was established as 2*R*-*O*-(4′-*O*-β-D-glucopyranosyl caffeoyl)-3-(4-hydroxyphenyl) lactic acid. We named compound **5** as isorinic acid. Diglucosyl rosmarinic acid (**3**, 51.5%, 0.1 mM) exhibited greater scavenging activity against superoxide anion produced with xanthine and xanthine oxidase (XOD) than rosmarinic acid (**1**, 35.5%, 0.1 mM), when superoxide dismutase (SOD) was used as a positive control (48.0%).

Experimental

General Procedures The following instruments were used to obtain physical data: melting point, Yanagimoto micro-melting point apparatus (values are uncorrected); optical rotation, JASCO DIP-1000 Digital polarimeter; IR spectra, Shimazu FT-IR 8300 infrared spectrometer; UV spectra, Hitachi U-3000 spectrophotometer; HR-FAB-MS, JEOL JMS-BU20 spectrometer using diethanolamine as a matrix. ^{1}H-NMR spectra, Bruker DPX-400 FT-NMR spectrometer (400 MHz) with tetramethylsilane as an internal standard. ^{13}C-NMR spectra, Bruker DPX-400 FT-NMR spectrometer and Bruker ARX-500 FT-NMR spectrometer with tetramethylsilane as an internal standard. Gas-liquid chromatography (GLC) used a Shimazu gas chromatograph model GC-17A with a capillary column (30 m×0.32 mm i.d., DB-17); thin layer chromatography (TLC) was run on precoated TLC plates (Merck, Kieselgel 60F_{254}, RP-18F_{254}); for column chromatography, Kieselgel 60 (70—230 Mesh, Merck), Sephadex LH-20 (Pharmacia), and MCI-CHP20P (Mitsubishi Chemical Ltd.), were used. Medium pressure liquid chromatography (MPLC, micro pump KP-7, Kusano Scientific Co., Tokyo) was carried out on a CIG column [SiO_2, ODS (C-18)]. Silylating Reagent, TMS-HT Kit [hexamethyldisilazane and trimethylchlorosilane in pyridine (Tokyo Kasei Kogyo Co., Ltd.)] was used for silylation.

Plant Material The fruit of *Helicteres isora* was purchased from a Jamu factory in Jakarta and the botanical identification was made by Dr. Asmanizar, Indonesian University. A voucher specimen has been deposited at the Botanical Museum of Kobe Gakuin University.

Extraction and Separation of *Helicteres isora* The fruit of *H. isora* (4 kg) purchased in Indonesia was extracted with methanol (7 h, 12 l×5) at 60 °C. The combined extracts (60 l) were evaporated off under reduced pressure to give the MeOH extract, which was partitioned into $CHCl_3$: MeOH : H_2O (5 : 3 : 8), and the H_2O layer was extracted with EtOAc and then with *n*-BuOH successively. Each solvent was removed under reduced pressure to yield the $CHCl_3$ extract (51.4 g), the EtOAc extract (3.93 g), the *n*-BuOH extract (25.9 g) and the H_2O extract (28.5 g). The *n*-BuOH extract was subjected to column chromatography on Sephadex LH-20 (MeOH : H_2O 2 : 1) to give fractions 1—5. Fraction 4 was subjected to MPLC [Rp-18 (MeOH : H_2O 1 : 4→1 : 2) and silica gel (EtOAc : MeOH : H_2O : CH_3CN 15 : 2 : 1 : 2)] to furnish compound **1** (310 mg). The H_2O extract was column chromatographed over MCI-CHP20P (H_2O : MeOH 5 : 1→H_2O : MeOH 3 : 1→H_2O : MeOH 2 : 1→MeOH) to afford fractions 1—4. Fraction 3 was subjected to column chromatography on Sephadex LH-20 (MeOH : H_2O 1 : 1) followed by MPLC [Rp-8 (MeOH : H_2O 1 : 3) and silica gel (AcOEt : MeOH : H_2O : CH_3CN 7 : 2 : 1 : 1)] to give compound **3** (50 mg). Fraction 4 was chromatographed on Sephadex LH-20 (MeOH : H_2O 2 : 1) followed by MPLC [silica gel (EtOAc : MeOH : H_2O 60 : 25 : 15) and Rp-18 (H_2O : MeOH 3 : 1) to yield compound **2** (310 mg) and **4** (40 mg).

Compound **1**: A colorless amorphous powder, $[\alpha]_D$ +73.3° (c=0.2, MeOH), negative ion HR-FAB-MS *m/z*: Calcd for $C_{18}H_{16}O_8$−H: 359.0797, Found: 359.0767 (M−H)$^-$. UV λ_{max} (MeOH) nm (log ε): 216.7 (4.25), 291.0 (4.08), 329.3 (4.19). IR (KBr) cm^{-1}: 3360 (OH), 1700 (C=O), 1650, 978 (*trans* C=C), 1606, 1524, 1448 (aromatic ring). The ^{1}H- and ^{13}C-NMR spectra: See Tables 1, 2. This product was identical with an authentic sample (rosmarinic acid) on direct comparison (TLC, IR, ^{1}H-NMR, ^{13}C-NMR, $[\alpha]_D$)

Compound **2**: A colorless amorphous powder, $[\alpha]_D$ +9.0° (c=0.9, MeOH), negative ion HR-FAB-MS *m/z*: Calcd for $C_{24}H_{26}O_{13}$−H: 521.1295, Found: 521.1303 (M−H)$^-$. The anthrone reaction was positive. UV λ_{max} (MeOH) nm (log ε): 204.5 (4.52), 287.0 (4.22), 319.0 (4.11). IR (KBr) cm^{-1}: 3337 (OH), 1687 (C=O), 1630, 980 (*trans* C=C), 1600, 1589, 1515, 1508 (aromatic ring). The ^{1}H- and ^{13}C-NMR spectra: See Tables 1, 2.

Hydrolysis of Compound 2 with β-Glucosidase (Emulusin) A mixture of compound **2** (30 mg) and β-glucosidase (40 mg) in AcOH–AcONa buffer (pH 4.7, 5 ml) was incubated with gentle stirring at 37 °C for 20 h. The reaction mixture was diluted with H_2O (20 ml), and extracted with EtOAc (50 ml×3). The organic layer was washed with aq. sat. NaCl, then dried, and evaporated to dryness, which was chromatographed on sephadex LH-20 (MeOH) to give rosmarinic acid {**1**, 9.2 mg, $[\alpha]_D$ +74.4° (c=0.5, MeOH)}. The aqueous layer was evaporated to dryness and the residue was chromatographed (MPLC) on silica gel (EtOAC : MeOH : H_2O 85 : 10 : 5) to give a syrup {9.3 mg, $[\alpha]_D$ +47.1° (c=0.5, H_2O)}, which was trimethylsilylated with the TMS-HT Kit, then subjected to GLC: column temp. 170 °C, carrier gas He (1.73 ml/min), t_R (min) 5.84 and 7.57, in agreement with those of an authentic sample (glucose) .

Compound **3**: A colorless amorphous powder, $[\alpha]_D$ −17.9° (c=1.0, H_2O), negative ion HR-FAB-MS *m/z*: Calcd for $C_{30}H_{36}O_{18}$−H: 683.1823, Found: 683.1807 (M−H)$^-$. The anthrone reaction was positive. UV λ_{max} (MeOH) nm (log ε): 216.4 (4.34), 284.0 (4.24), 317.5 (4.12). IR (KBr) cm^{-1}: 3392 (OH), 1698 (C=O), 1640, 984 (*trans* C=C), 1600, 1512, 1448 (aromatic ring). The ^{1}H- and ^{13}C-NMR spectra: See Tables 1, 2.

Hydrolysis of Compound 3 with β-Glucosidase (Emulusin) Compound **3** (60 mg) was hydrolyzed in the same way as for compound **2** to yield rosmarinic acid {27 mg, $[\alpha]_D$ +74.8° (c=1.0, MeOH)} and only D-glucose as the sugar moiety.

Compound **4**: A colorless amorphous powder, $[\alpha]_D$ −16.0° (c=0.3, H_2O), negative ion HR-FAB-MS *m/z*: Calcd for $C_{24}H_{26}O_{12}$−H: 505.1345, Found: 505.1353 (M−H)$^-$. The anthrone reaction was positive. UV λ_{max} (MeOH) nm (log ε): 219.0 (4.26), 286.2 (4.16), 318.6 (4.07). IR (KBr) cm^{-1}: 3392 (OH), 1696 (C=O), 1634, 986 (*trans* C=C), 1608, 1589, 1514, 1444 (aromatic ring). The ^{1}H- and ^{13}C-NMR spectra: See Tables 1, 2.

Hydrolysis of Compound 4 with β-Glucosidase (Emulusin) Compound **4** (60 mg) was hydrolyzed in the same way as for compound **2** to yield compound **5** (27 mg) and D-glucose as the sugar moiety.

Compound **5**: A colorless amorphous powder, $[\alpha]_D$ +82.5° (c=1.0, MeOH), negative ion HR-FAB-MS *m/z*: Calcd for $C_{18}H_{16}O_7$−H: 343.0817, Found: 343.0828 (M−H)$^-$. UV λ_{max} (MeOH) nm (log ε): 221.0 (4.26), 244.5 (3.97), 329.5 (4.18). IR (KBr) cm^{-1}: 3400 (OH), 1700 (C=O), 1650, 978 (*trans* C=C), 1601, 1589, 1516, 1450 (aromatic ring). The ^{1}H- and ^{13}C-NMR spectra: See Tables 1, 2.

Determination of Absolute Configuration of Compound 5 Compound **5** (17 mg) was saponificated at 60 °C under a N_2 atmosphere for 2 h with KOH (140 mg) in 95% EtOH (2 ml). After cooling, the reaction mixture was acidified with 5% HCl solution and was evaporated off under reduced pressure to give a syrup which was chromatographed on Sephadex LH-20 (MeOH : H_2O 1 : 1) to give compound **6** {5.8 mg, $[\alpha]_D$ +13.9° (c=0.6, MeOH)} ^{1}H-NMR (δ, CD_3OD): 7.17 (2H, d, J=8.5 Hz), 6.69 (2H, d, J=8.5 Hz), 4.26 (1H, dd, J=4.4, 7.7 Hz), 2.99 (1H, dd, J=4.4, 13.8 Hz), 2.80 (1H, dd, J=7.7, 13.8 Hz).

Condensation of Compound 6 with (*S*)-PGME To a stirred solution of a mixture of compound **6** (2.5 mg) and (*S*)-PGME (5 mg) in *N*,*N*-dimethylformamide (DMF, 1 ml) were successively added benzotriazole-1-yl-oxy-tris-pyrrolidinophosphonium hexafluorophosphate (PyBOP, 5.2 mg), 1-hydroxy-benzotriazole (HOBT, 3.3 mg) and *N*-methyl-morphorine (26 μl) at 0 °C. After the mixture was stirred at room temperature for 1.5 h, benzene (10 ml) and ethyl acetate (20 ml) were added and resulting diluted solution was successively washed with 5% HCl, saturated sodium bicarbonate and saturated NaCl solution. The organic layer was dried and concentrated to give a residue which was purified by preparative TLC (EtOAc : MeOH : H_2O 65 : 25 : 15) to (*S*)-PGME amide. ^{1}H-NMR (δ, CD_3OD): 7.04 (2H, d, J=8.6 Hz), 6.67 (2H, d, J=8.6 Hz), 4.22 (1H, dd, J=4.3, 7.6 Hz), 2.97 (1H, dd, J=4.3, 15.0 Hz), 2.77 (1H, dd, J=7.6, 15.0 Hz).

Condensation of Compound 6 with (*R*)-PGME Compound **6** (2.5 mg) was treated with (*R*)-PGME (4.8 mg) by the same procedure as described

above to afford the (*R*)-PGME amide. ^{1}H-NMR (δ, CD_3OD): 6.99 (2H, d, J=8.6 Hz), 6.62 (2H, d, J=8.6 Hz), 4.27 (1H, dd, J=4.1, 6.8 Hz), 2.94 (1H, dd, J=4.1, 14.3 Hz), 2.77 (1H, dd, J=6.8, 14.3 Hz).

Assay for Superoxide Scavenging Activity Superoxide scavenging activity was made by the reported method[9] modified as follows: A mixture (0.5 ml) consisting of an aqueous solution of xanthine (0.5 mM, 200 μl), hydroxylamine *O*-sulfonic acid (1 mg/ml, 100 μl) and buffer solution (final concentration of 13 mM KH_2PO_4, 7 mM $Na_2B_4O_7$ and 0.1 mM EDTA-2Na) was added to the sample solution or H_2O (100 μl). Then, XOD solution (6.2×10^{-2} U/ml, 200 μl) or H_2O was added to the mixture, and the solution was incubated for 30 min at 37 °C. A solution (2.0 ml) of sulfanilic acid (3 mM), *N*-1-naphthylethylenediamine (30 mM), acetic acid (25%) was added to the reaction mixture. The final mixture was allowed to stand for 30 min at room temperature, and the absorption of the reaction solution at 550 nm was measured. SOD from bovine erythrocytes was used as a positive control (final concentration 0.01 μg/ml, 4.4×10^{-2} U/ml). The percentage inhibition of superoxide anion is 35.5% for rosmarinic acid (**1**, 0.1 mM), 51.5% for compound **3** (0.1 mM) and 48.0% for SOD as a positive control, respectively.

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