Isolation of Tetraprenyltoluquinols from the Brown Alga *Sargassum thunbergii*

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Thunbergols A (4) and B (5), tetraprenyltoluquinols, along with three known compounds (1—3) have been isolated from the brown alga *Sargassum thunbergii*. The structures of these two new compounds were determined to be 9-(3,4-dihydro-2,8-dimethyl-6-hydroxy-2*H*-1-benzopyran-2-yl)-6-methyl-2-(4-methyl-3-pentenyl)-(2*E*,6*E*)-nonadienoic acid (4) and 10-(2,3-dihydro-5-hydroxy-7-methyl-1-benzofuran-2-yl)-10-hydroxy-6-methyl-2-(4-methyl-3-pentenyl)-(2*E*,6*E*)-undecadienoic acid (5), respectively, by combined spectroscopic methods. Both of them exhibited significant scavenging activities on radical and potently inhibited generation of $ONOO^-$ from morpholinosydnonimine (SIN-1).

Key words *Sargassum thunbergii*; tetraprenyltoluquinol; antioxidant activity; peroxynitrite

The brown alga *Sargassum thunbergii* is distributed widely in the coastal area of the Korean peninsula. Few data on secondary metabolites from this brown alga have been reported although it was chemically investigated.[1—3] Recently we reported the isolation and peroxynitrite-scavenging activities of three known compounds (**1**—**3**) from *S. thunbergii*.[4] In our continuous search for bioactive compounds from marine organisms, we re-encountered the brown alga *S. thunbergii* along the shore of Busan. The large-scale extraction of organic materials followed by bioactivity-guided separation using combined chromatographic techniques yielded tetraprenyltoluquinol derivatives, sargahydroquinoic acid (**1**), sargaquinoic acid (**2**), and sargachromenol (**3**), together with two additional metabolites of the same structural class as minor constituents. Herein we report the isolation and structure determination of two novel compounds, thunbergols A, B (**4**, **5**) and their antioxidant activities.

The structures of three known metabolites, sargahydroquinoic acid (**1**),[5] sargaquinoic acid (**2**),[6,7] and sargachromenol (**3**)[6,7] were readily determined by a combination of spectroscopic analysis and comparison with reported data for these compounds.

A closely related metabolite, thunbergol A (**4**) was isolated as a colorless gum. The molecular formula for this compound was deduced as $C_{27}H_{38}O_5$ by HR-FAB-MS and ^{13}C-NMR analyses. Comparison of the spectral data with those obtained for compound **3** showed that **4** was also a tetraprenyltoluquinol analog of the same class as **3**. However, there were significant differences in the ^{13}C-NMR spectrum. Signal of the olefinic carbons at δ 122.8 and 130.5, typical of the C-3 double bond of the chromene ring moiety in **3** was shifted upfield to δ 31.5 and 68.7. Corresponding differences were found in the ^1H-NMR spectrum in which the olefinic protons at δ 6.21 (1H, d, J=9.6 Hz) and 5.54 (1H, d, J=9.6 Hz) were replaced by signals at δ 2.90 (1H, dd, J=16.8, 4.8 Hz), 2.62 (1H, dd, J=16.8, 4.8 Hz), and 3.73 (1H, t, J=4.8 Hz). These differences could be explained by a hydration of the C-3 double bond of sargachromenol. A combination of ^1H-COSY, HMQC, and HMBC experiments fully supported this interpretation. In addition, the downfield shift of proton signal (δ 3.73) at H-3 to δ 5.03 (1H, t, J=5.5 Hz) by acetylation of sodium carboxylate of **4** with acetic anhydride in pyridine which yielded the triacetate derivative (**4a**) also supported this interpretation. Thus, the structure of thunbergol A (**4**) was determined as 9-(3,4-dihydro-2,8-dimethyl-6-hydroxy-2*H*-1-benzopyran-2-yl)-6-methyl-2-(4-methyl-3-pentenyl)-(2*E*,6*E*)-nonadienoic acid.

Another closely related metabolite, thunbergol B (**5**) was isolated as a colorless gum that was determined to have the same composition $C_{27}H_{38}O_5$ as **4** by HR-FAB-MS and ^{13}C-NMR spectrometry. The NMR spectral data for **5** were almost identical with those derived from **4**. Careful examina-

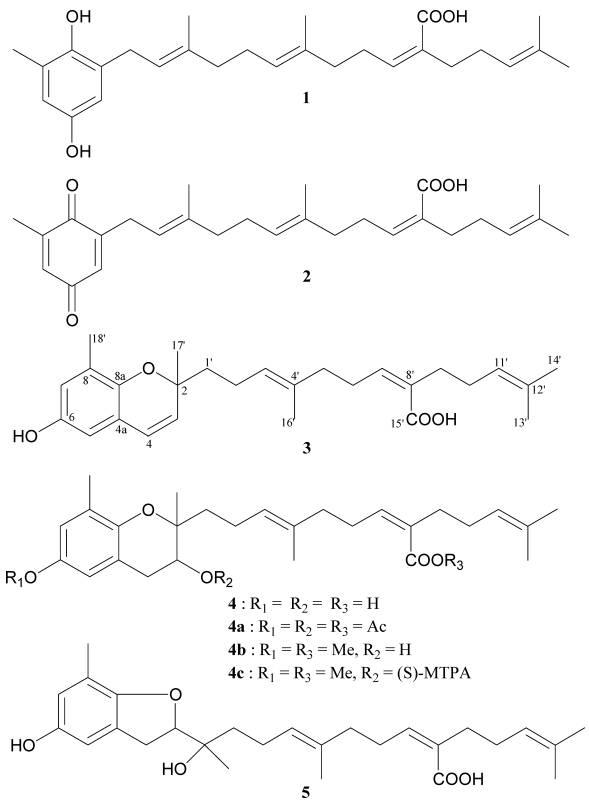

Fig. 1. Chemical Structure of Compounds **1**—**5**

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tion of the ^{13}C-NMR data revealed that the signal from the methine carbon at δ 68.7 of **4** was shifted downfield in **5** to δ 88.3, while that of the quaternary carbon at δ 78.0 of **4** was shifted upfield in **5** to δ 73.7. In addition, ^{13}C-NMR signals in the aromatic ring moiety of **5** exhibited considerable discrepancies compared with those of **4**. A corresponding change was observed in the ^{1}H-NMR spectrum in which a signal of the H-3 proton of **4** at δ 3.73 (1H, t, J=4.8 Hz) was replaced by a signal at δ 4.48 (1H, dd, J=9.3, 8.8 Hz). This difference could be explained by formation of a 2,3-dihydrobenzofuran ring by the oxygen atom between C-3 and C-8a instead of the 3,4-dihydro-2*H*-1-benzopyran ring of **4**, together with consideration of the molecular formula. This interpretation was also supported by HMBC correlation between the key carbons and adjacent protons, particularly, between H-3 at δ 4.48 and C-8a at δ 151.8. Thus, the structure of thunbergol B was unequivocally defined as 10-(2,3-dihydro-5-hydroxy-7-methyl-1-benzofuran-2-yl)-10-hydroxy-6-methyl-2-(4-methyl-3-pentenyl)-(2*E*,6*E*)-undecadienoic acid.

Thunbergols A (**4**) and B (**5**) possess two asymmetric carbon centers at C-2 and C-3. In the case of **4**, analysis of the vicinal coupling constants between the H-1′ methylene protons and the H-3 methine proton ($J_{1,2}$=4.8, 4.8 Hz) revealed that the H-3 proton is pseudoequatorial on the cyclohexene. Assignments of the stereochemistry of asymmetric centers (C-2, C-3) were done by a NOESY experiment (Fig. 2). The H-3 proton was correlated with H-4 (α, β), H-1′, and H-17′ protons while the H-4 (α, β) protons were correlated with the H-17′ proton. Therefore, the relative configurations of asymmetric centers of **4** were defined as 2*S**,3*S**. In an attempt to determine the absolute configurations of the asymmetric centers at C-2 and C-3, treatment of **4** with diazomethane in dry ether gave a methoxy derivative (**4b**). The structure of **4b** and full assignments of its carbons and protons were made with a combination of the ^{1}H- and ^{13}C-NMR, ^{1}H-COSY, HMQC, and HMBC experiments. And **4b** was then esterified with (+)-MTPA chloride. Surprisingly signals from the H-4 methylene protons and the H-17′ methyl protons in the ^{1}H-NMR spectrum of the MTPA-ester (**4c**) were clearly resolved into two areas containing twin peaks of equal ratio suggesting that **4** was indeed a racemic mixture of enantiomers at C-2 and C-3: δ 3.23 (1H, dd, J=16.6, 7.0 Hz, H-4) and 2.79 (1H, dd, J=16.6, 7.0 Hz, H-4), and 3.25 (1H, dd, J=17.2, 7.0 Hz, H-4) and 2.90 (1H, dd, J=17.2, 7.0 Hz, H-4); 1.21 (3H, s, H-17′) and 1.24 (3H, s, H-17′). On the other hand, the NOESY experiment for **5** failed to provide any valuable information due to free rotation of substituents located at C-2 of the open chain. However, the structural similarity of **4** with **5** suggested the same configuration for asymmetric centers but the

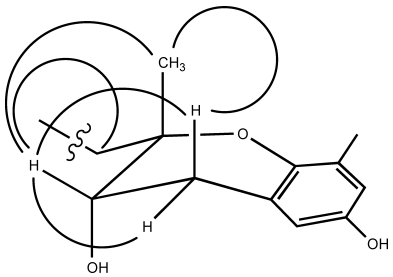

Fig. 2. Key NOESY Correlation for **4**

Table 1. NMR Spectral Assignments for Compounds **4** and **5**

No.	**4**		**5**	
	H	C	H	C
2		78.0 s		73.7 s
3	3.73 (1H, dd, 4.8, 4.8)	68.7 d	4.48 (1H, dd, 9.3, 8.8)	88.3 d
4	2.90 (1H, dd, 16.8, 4.8)	31.5 t	3.08 (1H, dd, 16.0, 8.8)	31.0 t
	2.62 (1H, dd, 16.8, 4.8)		2.95 (1H, dd, 16.0, 9.3)	
4a		119.1 s		119.6 s
5	6.40 (1H, d, 2.2)	116.1 d	6.43 (1H, d, 2.1)	115.5 d
6		148.5 s		149.4 s
7	6.27 (1H, d, 2.2)	113.1 d	6.32 (1H, d, 2.1)	109.3 d
8		127.4 s		127.2 s
8a		144.4 s		151.8 s
1′	1.46 (2H, m)	36.8 t	1.44 (2H, t, 6.6)	36.9 t
2′	2.03 (2H, m)	21.7 t	2.03 (2H, m)	22.0 t
3′	5.03 (1H, t, 7.4)	124.5 d	5.06 (1H, t, 6.6)	124.7 d
4′		134.6 s		134.6 s
5′	1.97 (2H, t, 7.3)	39.1 t	2.00 (2H, t, 7.3)	39.1 t
6′	2.49 (2H, q, 7.3)	28.2 t	2.52 (2H, q, 7.3)	28.3 t
7′	5.87 (1H, t, 7.3)	145.0 d	5.90 (1H, t, 7.3)	145.2 d
8′		130.5 s		130.5 s
9′	2.18 (2H, t, 7.0)	34.6 t	2.17 (2H, t, 8.0)	34.6 t
10′	2.02 (2H, m)	27.9 t	2.05 (2H, m)	27.9 t
11′	5.00 (1H, m)	123.3 d	5.00 (1H, t, 9.9)	123.3 d
12′		132.2 s		132.2 s
13′	1.60 (3H, s)	25.7 q	1.60 (3H, s)	25.8 q
14′	1.50 (3H, s)	17.8 q	1.51 (3H, s)	17.8 q
15′		172.1 s		172.3 s
16′	1.48 (3H, s)	15.9 q	1.54 (3H, s)	16.0 q
17′	1.23 (3H, s)	19.2 q	1.23 (3H, s)	23.1 q
18′	2.06 (3H, s)	16.2 q	2.07 (3H, s)	15.4 q

All assignments were based on DEPT, COSY, TOCSY, HMQC, HMBC, and NOESY experiments.

stereochemistry of thunbergol B (**5**) remained to be determined. In our measurement for optical rotation of compounds (**3**—**5**), they were optically inactive.

The literature survery revealed that tetraprenyltoluquinol derivatives, which were structurally closely related to thunbergols but including chromane ring instead of 3-hydroxychromane ring moiety, were previously isolated from the terrestrial plant *Iryanthera grandis*[8] and the brown alga *Sargassum siliquastrum*.[9] For compounds isolated from *I. grandis*, there was no mention for configurations of their asymmetric carbons at C-2. On the other hand, for those isolated from *S. siliquastrum*, configurations of their asymmetric carbons at C-2 were reported to be *R* on the basis of CD measurement. However, configuration of tetraprenyltoluquinol derivatives including 3-hydroxychromane ring moiety has never been reported until now.

The structure of thunbergol A (**4**) was very closely related with that of sargachromenol (**3**) which was isolated as the epimeric mixture of C-3 and was previously considered both as an artifact from sargaquinoic acid (**2**)[5,6] and as a natural product.[10] Also there can be suggested a possibility that thunbergols might be chemical artifacts. However, any efforts to separate from *S. thunbergii*, any likely precursors from which the thunbergols were derived, didn't succeed. In addition, isolation of 1,2-dihydrosargachromenol and related derivatives as natural products, suggests that 2*H*-1-benzopyran ring can be formed by the biosynthetic pathway.[7,8] Nevertheless, there can be a question whether or not sargachromenol (**3**) might be a possible precursor to thunbergol A (**1**) formed by hydration during the process of isolation. Usually hydration reaction requires an acid catalyst. However, it is very difficult that hydration reaction for sargachromenol (**3**) under acidic condition occurs since dehydration of thunbergol A (**1**) is a more favorable process due to stabilization by conjugation than hydration of **3**. Based on all these things, thunbergols could be considered as natural products.

Although tetraprenyltoluquinols have been well known as secondary metabolites of the brown algae,[5,6,11—15] thunbergol can have two unusual structural features compared with previous isolates. In the first place, thunbergol A existed as racemic mixtures at two asymmetric carbon centers. To the best of our knowledge, this is the first example of a tetraprenyltoluquinol isolated as the racemic mixture at two asymmetric carbon centers. A second unusual feature is the presence of 3-hydroxyhydrobenzopyran or 2,3-dihydrobenzofuran rings. These kinds of functionalities appear to have been reported only once previously as reaction products of photochemical rearrangement of plastoquinone-9.[16,17]

Polyprenylated quinols (hydroquinones) are widely recognized to show a potent antioxidant effect.[7,10,18] Compounds **1**—**3**, structurally related to the natural antioxidant vitamin E, were reported to be antioxidants.[4,10] In our measurement for evaluating the capacity of compounds **4** and **5** to scavenge DPPH radical, they exhibited a EC_{50} value of 30 and 31 μg/ml, respectively, compared with Butylated hydroxytoluene (BHT) (EC_{50}, 32 μg/ml) and α-tocopherol (EC_{50}, 18 μg/ml). Also, in our measurement for the scavenging activity of compounds **4** and **5** on authentic $ONOO^-$/induced $ONOO^-$ from morpholinosydnonimine (SIN-1), their scavenging ratios on authentic $ONOO^-$ were 60.0 and 57.1% at 5 μg/ml, respectively, while their inhibition ratios against the generation of $ONOO^-$ from SIN-1, which simultaneously generates $NO\cdot$ and $O_2\cdot^-$, were 98.6 and 90.6% at the same concentration, respectively. Scavenging activities of L-ascorbic acid and penicillamine, positive controls, on authentic/induced $ONOO^-$ were 98.1 and 90.4%, and 93.5 and 88.2%, respectively.

Experimental

General NMR spectra were recorded in $CDCl_3$ on a Varian Mercury 300 spectrometer using standard pulse sequence programs. Proton and carbon NMR spectra were measured at 300 and 75 MHz, respectively. All chemical shifts were recorded with respect to TMS as an internal standard. Mass spectral data were obtained at the Korean Basic Science Institute, Taejeon, Korea. UV spectra were measured on a Shimadzu UV1201 spectrophotometric instrument. IR spectra were recorded on a Shimadzu 8700 spectrophotometer. High performance liquid chromatography (HPLC) was performed with a Dionex P580 with Varian 350 RI detector. All solvents used were spectral grade or were distilled from glass prior to use.

Plant Material The specimens of brown alga *Sargassum thunbergii* (Mertens ex Roth) Kuntze were collected by hand at the coast of Busan in November 2003, South Sea, Korea. A voucher for the specimens (sample number 02PS-15) is deposited at the Herbarium of the Division of Marine Environment and Bioscience, Korea Maritime University, Busan, Korea under curatorship of Dr. Jong-Su Yoo.

Extraction and Isolation The collected samples were briefly dried under shade and kept at −25 °C until chemically investigated. Samples (0.5 kg) of *Sargassum thunbergii* were ground to a powder and extracted for 2 d with MeOH (3 l×2) and CH_2Cl_2 (3 l×2). The combined crude extracts (29.8 g) were evaporated under reduced pressure and partitioned between CH_2Cl_2 and water. The organic layer was further partitioned between 15% aq. MeOH and *n*-hexane. An aliquot of (5.2 g) of the lower layer was subjected to C_{18} reversed-phase vacuum flash chromatography using stepwise gradient mixtures of MeOH and water (50%, 60%, 70%, 80%, 90% aq. MeOH, and 100% MeOH) as eluents. Purification of fraction 5 by semi-preparative C_{18} HPLC (YMC ODS-A column, 87% aq. MeOH, 1 cm×25 cm, 2 ml/min) gave compounds **1** (13.7 mg) and a mixture (12.6 mg) of **4** and **5** which was further separated by Prep. TLC on a Si gel plate with MeOH/$CHCl_3$ (1 : 19) as a solvent system to afford 5.2 mg and 4.9 mg of **4** and **5**, respectively. Fraction 6 was subjected to reversed-phase HPLC (YMC ODS-A, 80% aq. MeCN, 1 cm×25 cm, 2 ml/min) to give 18.2 mg and 20.2 mg of **2** and **3**, respectively.

Sargahydroquinoic Acid (**1**): Colorless gum. Positive HR-FAB-MS *m/z*: 449.2670 $(M+Na)^+$ (Calcd for $C_{27}H_{38}O_4$+Na: 449.2668).

Sargaquinoic Acid (**2**): Colorless gum. Positive HR-FAB-MS *m/z*: 447.2510 $(M+Na)^+$ (Calcd for $C_{27}H_{36}O_4$+Na: 447.2512).

Sargachromenol (**3**): Colorless gum. Positive HR-FAB-MS *m/z*: 447.2514 $(M+Na)^+$ (Calcd for $C_{27}H_{36}O_4$+Na: 447.2512).

Thunbergol A (**4**): Colorless gum. UV λ_{max} (MeOH) nm (log ε): 297 (3.28). IR (KBr) ν_{max} cm^{-1}: 3550, 2970, 1680, 1630, 1430, 1380, 1230, 1085, 930 cm^{-1}. ^{1}H- and ^{13}C-NMR spectral data, see Table 1. HMBC correlations: H-3/C-8, C-17′; H-4/C-2, C-3, C-7, C-8, C-8a; H-5/C-7, C-8a; H-7/C-5, C-6, C-8a; H-1′/C-2′; H-2′/C-2, C-4′; H-3′/C-2′, C-5′, C-16′; H-5′/C-3′, C-4′, C-16′; H-6′/C-4′, C-5′, C-7′, C-8′; H-7′/C-5′, C-6′, C-9′, C-15′; H-9′/C-7′, C-8′, C-10′, C-11′, C-15′; H-10′/C-9′, C-12′; H-11′/C-13′; H-13′/C-11′, C-12′, C-14′; H-14′/C-11′, C-12′, C-13′; H-16′/C-3′, C-4′, C-5′; H-17′/C-2, C-3, C-1′; H-18′/C-5, C-4a, C-8a. Positive HR-FAB-MS *m/z*: 465.2620 $(M+Na)^+$ (Calcd for $C_{27}H_{38}O_5$+Na: 465.2617).

Thunbergol B (**5**): Colorless gum. UV λ_{max} (MEOH) nm (log ε): 300 (3.31). IR (KBr) ν_{max} cm^{-1} 3570, 2980, 1690, 1650, 1430, 1380, 1230, 1100, 920 cm^{-1}. ^{1}H- and ^{13}C-NMR spectral data, see Table 1. HMBC correlations: H-4/C-3, C-2, C-8a, C-8; H-3/C-17′, C-8a, C-8; H-1′/C-2′; H-2′/C-2, C-4′; H-3′/C-2′, C-5′, C-16′; H-5′/C-3′, C-4′, C-16′; H-6′/C-4′, C-5′, C-7′, C-8′; H-7′/C-5′, C-6′, C-9′, C-15′; H-9′/C-7′, C-8′, C-10′, C-11′, C-15′; H-10′/C-9′, C-12′; H-11′/C-13′; H-13′/C-11′, C-12′, C-14′; H-14′/C-11′, C-12′, C-13′; H-16′/C-3′, C-4′, C-5′; H-17′/C-3, C-2, C-1′; H-7/C-8a, C-6, C-5; H-5/C-8a, C-7; Ar-Me/C-8a. C-5, C-4a. Positive HR-FAB-MS *m/z*: 465.2619 $(M+Na)^+$ (Calcd for $C_{27}H_{38}O_5$+Na: 465.2617).

Thunbergol A Triacetate (**4a**): To a stirred solution of sodium carboxylate (2.0 mg) of **4** in 0.3 ml of dry pyridine was added 0.2 ml of acetic anhydride. The mixture was stirred at room temperature for 1 h. After removing the pyridine and excess acetic anhydride under vacuum, the residue was purified

by reversed-phase HPLC (100% MeOH) to yield 1.8 mg of **4a**: a colorless gum. ^{1}H-NMR ($CDCl_3$) δ: 6.69 (1H, d, J=2.4 Hz, H-5), 6.58 (1H, d, J=2.4 Hz, H-7), 5.90 (1H, t, J=6.9 Hz, H-7′), 5.08 (2H, t, J=7.2 Hz, H-3′, H-11′), 5.03 (1H, t, J=5.5 Hz, H-3), 3.11 (1H, dd, J=17.4, 5.5 Hz, H-4), 2.74 (1H, dd, J=17.4, 5.5 Hz, H-4), 2.56 (2H, m, H-6′), 2.19—2.01 (8H, m, H-2′, H-5′, H-9′, H-10′), 2.26 (3H, s, 3-OAc/15′-OAc/6-OAc), 2.18 (3H, s, 3′-Me), 2.08 (6H, s, 3-OAc/15′-OAc/6-OAc), 1.68 (3H, s, H-13′), 1.58 (3H, s, H-14′), 1.57 (2H, m, H-1′), 1.55 (3H, s, H-16′), 1.29 (3H, s, H-17′). Positive HR-FAB-MS m/z: 591.2931 $(M+Na)^+$ (Calcd for $C_{33}H_{44}O_8$+Na: 591.2934).

Thunbergol A Methyl Ether (**4b**): To a solution of thunbergol A (**4**) (4.3 mg) in dry Et_2O (2 ml) was added an ethereal solution of diazomethane that was generated from a reaction of MNNG (Aldrich Chemical Co.) with KOH. The reaction mixture was stirred at room temperature for 1 h and evaporated to dryness by blowing N_2 over the mixture. Si gel preparative TLC with the solvent EtOAc gave 3.5 mg of thunbergol A methy ether (**4b**): ^{1}H-NMR ($CDCl_3$) δ: 6.51 (1H, br s, H-5), 6.38 (1H, br s, H-7), 5.83 (1H, t, J=7.4 Hz, H-7′), 5.13 (1H, t, J=6.9 Hz, H-3′), 5.08 (1H, t, J=7.2 Hz, J-11′), 3.82 (1H, dd, J=6.0, 5.0 Hz, H-3), 3.73 (6H, s, 6-OMe, 15′-OMe), 3.00 (1H, dd, J=16.5, 5.0 Hz, H-4), 2.73 (1H, dd, J=16.5, 6.0 Hz, H-4), 2.50 (2H, q, J=7.4 Hz, H-6′), 2.30—2.18 (4H, m, H-2′, H-9′), 2.17 (3H, s, H-18′), 2.12—2.02 (4H, m, H-5′, H-10′), 1.69 (3H, s, H-13′), 1.61 (2H, m, H-1′), 1.59 (6H, s, H-14′, H-16′), 1.32 (3H, s, H-17′). Positive HR-FAB-MS m/z: 493.2928 $[M+Na]^+$ (Calcd for $C_{29}H_{42}O_5$+Na: 493.2930).

(*S*)-MTPA Ester (**4c**) of **4**: To a stirred solution of **4b** (5.3 mg) in dry pyridine (0.3 ml) was added (*S*)-MTPA chloride (50 μl). After stirring of the mixture under N_2 at room temperature for 1 h. The solvent was removed by blowing N_2 over the mixture. The residue was redissolved in 40% EtOAc/*n*-hexane (2 ml) and filtered through a Sepak silica column. After removing the solvent under vacuum, the residue was separated by reversed-phase HPLC (YMC ODS-A column, 95% aq. MeOH) to afford 2.8 mg of (*S*)-MTPA ester (**4c**): ^{1}H-NMR ($CDCl_3$) δ: 7.63 (2H, m, Ar), 7.50—7.40 (4H, m, Ar), 3.50 (3H, s, Ar-OMe), 6.77 (1H, d, J=2.5 Hz, H-5), 6.75 (1H, d, J=2.5 Hz, H-5), 6.67 (1H, d, J=2.5 Hz, H-7), 6.64 (1H, d, J=2.5 Hz, H-7), 5.84 (1H, t, J=7.3 Hz, H-7′), 5.22 (1H, t, J=7.0 Hz, H-3), 5.10 (1H, t, J=6.7 Hz, H-3′), 5.08 (1H, t, J=5.6 Hz, H-11′), 3.74 (6H, s, 6-OMe, 15′-OMe), 3.25 (1H, dd, J=17.2, 7.0 Hz, H-4), 3.23 (1H, dd, J=16.6, 7.0 Hz, H-4), 2.90 (1H, dd, J=17.2, 7.0 Hz, H-4), 2.79 (1H, dd, J=16.6, 7.0 Hz, H-4), 2.52 (2H, t, J=7.3 Hz, H-6′), 2.26 (2H, t, J=7.3 Hz, H-9′), 2.19 (3H, s, H-18′), 2.18 (3H, s, H-18′), 2.14—2.03 (6H, m, H-2′, H-5′, H-10′), 1.68 (3H, s, H-13′), 1.62 (2H, m, H-1′), 1.59 (6H, s, H-14′, H-16′), 1.24 (3H, s, H-17′), 1.21 (3H, s, H-17′). Positive HR-FAB-MS m/z 709.3328 $[M+Na]^+$ (Calcd for $C_{39}H_{49}F_3O_7$+Na: 709.3328).

Determination of the Scavenging Effect on Diphenylpicrylhydrazine (DPPH) Radical The DPPH radical scavenging effect was evaluated according to the method employed by Blois.[19] To 1.0 ml of DPPH methanol solution (1.5×10^{-1} M), 4 ml of MeOH solution of various sample concentrations was added. After mixing gently and leaving for 30 min at room temperature, the optical density was measured at 520 nm using a spectrophotometer. The scavenging activity was determined by comparing the absorbance with that of the blank (100%) containing only DPPH and solvent.

Measurement of $ONOO^-$ Scavenging Activity $ONOO^-$ scavenging ability was measured by monitoring the oxidation of dihydrorhodamine 123 with a modified version of the method of Kooy *et al.*[20] A stock solution of DHR 123 (5 mM) purged with nitrogen and was prepared in advance and stored at −80 °C. A working solution of DHR 123 (final concentration, 5 M) diluted from the stock solution was placed on ice in the dark immediately prior to the study. Buffer containing 90 mM sodium chloride, 50 mM sodium phosphate (pH 7.4) and 5 mM potassium chloride with 100 μM (final conc.) diethylenetriaminepentaacetic acid (DTPA) was purged with nitrogen and placed on ice before use. The $ONOO^-$ scavenging ability, based on the oxidation of DHR 123, was determined at room temperature with a microplate fluorescence spectrophotometer, FL 500 (Bio-Tek instruments, U.S.A.) using excitation and emission wavelengths of 485 and 530 nm. The background and final fluorescent intensities were measured 5 min after treatment with or without SIN-1 (f.c. 10 μM) or authentic $ONOO^-$ (f.c. 10 μM) in 0.3 M sodium hydroxide. Oxidation of DHR 123 by decomposition of SIN-1 gradually increased whereas authentic $ONOO^-$ rapidly oxidized DHR 123 with its final fluorescent intensity being stable over time. Penicillamine was used as a positive control.

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