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Note

Inhibition of Amyloid β Aggregation by Acteoside, a Phenylethanoid Glycoside

Manami KURISU,[1] Yusaku MIYAMAE,[2] Kazuma MURAKAMI,[3] Junkyu HAN,[1,4] Hiroko ISODA,[1,4] Kazuhiro IRIE,[3] and Hideyuki SHIGEMORI[1,†]

[1]*Graduate School of Life and Environmental Sciences, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8572, Japan*
[2]*Graduate School of Biostudies, Kyoto University, Kitashirakawa Oiwake-cho, Sakyo-ku, Kyoto 606-8502, Japan*
[3]*Graduate School of Agriculture, Kyoto University, Kitashirakawa Oiwake-cho, Sakyo-ku, Kyoto 606-8502, Japan*
[4]*Alliance for Research on North Africa, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8572, Japan*

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We examined the effects of acteoside (1a), which was isolated from *Orobanche minor*, and its derivatives on the aggregation of a 42-mer amyloid β protein (Aβ42) in our search for anti-amyloidogenic compounds for Alzheimer's disease (AD) therapy. Acteoside (1a) strongly inhibited the aggregation of Aβ42 in a dose-dependent manner. The structure-activity relationship for acteoside (1a) and related compounds suggests the catechol moiety of phenylethanoid glycosides to be essential for this inhibitory activity.

Key words: acteoside; amyloid β; aggregation; Alzheimer's disease; phenylethanoid glycoside

Alzheimer's disease (AD) is a neurodegenerative disorder that is considered to be one of the most common types of dementia. It is characterized by deposits of amyloid fibrils that mainly consist of 40- and 42-mer amyloid β-proteins (Aβ40 and Aβ42).[1)] These amyloid β-proteins are produced from the amyloid-β precursor protein (APP) by β- and γ-secretases.[2,3)] Its stronger aggregative ability and neurotoxicity have led to the belief that Aβ42 plays a more important role in the pathogenesis of AD than Aβ40.[1)] The inhibition of Aβ42 aggregation by small molecules is therefore a promising strategy for treating AD.

We have reported in our previous study that such caffeoylquinic acids (CQAs), which are bioactive polyphenols found from natural sources, as coffee beans, sweet potatoes, propolis, and other plants inhibited Aβ42-induced cytotoxicity in human neuroblastoma SH-SY5Y cells by enhancing the expression of mRNA of glycolytic enzymes and intracellular ATP.[4–6)] We have also reported that a CQA treatment improved the spatial learning memory in senescence accelerated-prone mice 8 by increasing the mRNA expression of phosphoglycerate kinase 1.[4)] We have recently reported that CQAs strongly inhibited the aggregation of Aβ42 and suppressed the transformation into β-sheet and cytotoxicity against human neuroblastoma cells of Aβ42.[7)]

Acteoside (**1a**) is an antioxidative phenylethanoid glycoside that has been reported to protect human neuroblastoma SH-SY5Y cells against Aβ cell injury by protecting ROS production and modulating the apoptotic signal pathway.[8)] However, the inhibitory activity of acteoside (**1a**) toward Aβ aggregation has not been proved.

Based on these reports, we investigated in this study the inhibition of the aggregation of Aβ42 by acteoside (**1a**) which had been isolated from *Orobanche minor* and possesses a similar substructure to that of CQAs. To

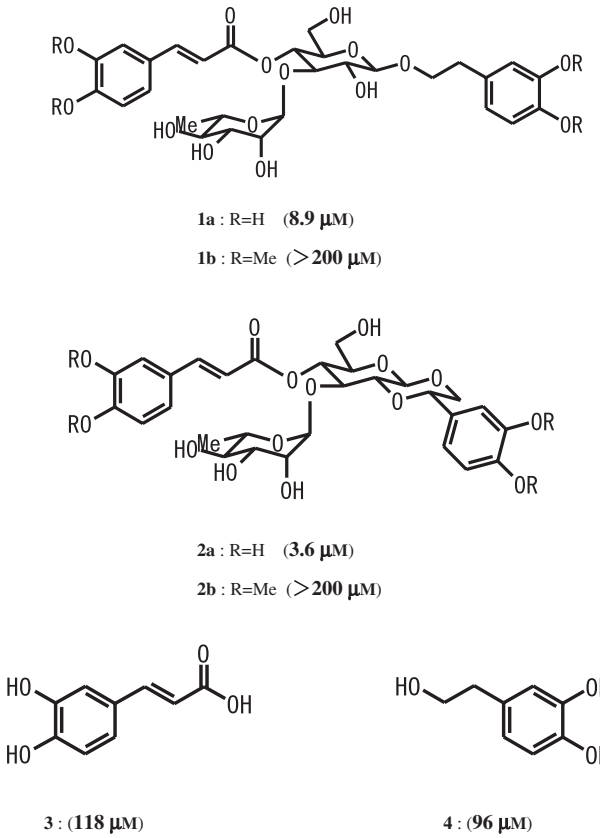

Fig. 1. Structures and IC_{50} Values of Acteoside (**1a**) and Its Derivatives for the Inhibitory Eeffects of Aβ42 Aggregation.

IC_{50} values were calculated from the inhibitory rate (%) of each concentration of the derivatives toward Aβ42 aggregation that was estimated by using the Th-T assay.

† To whom correspondence should be addressed. Tel/Fax: +81-29-853-4603; E-mail: shigemori.hideyuk.fn@u.tsukuba.ac.jp

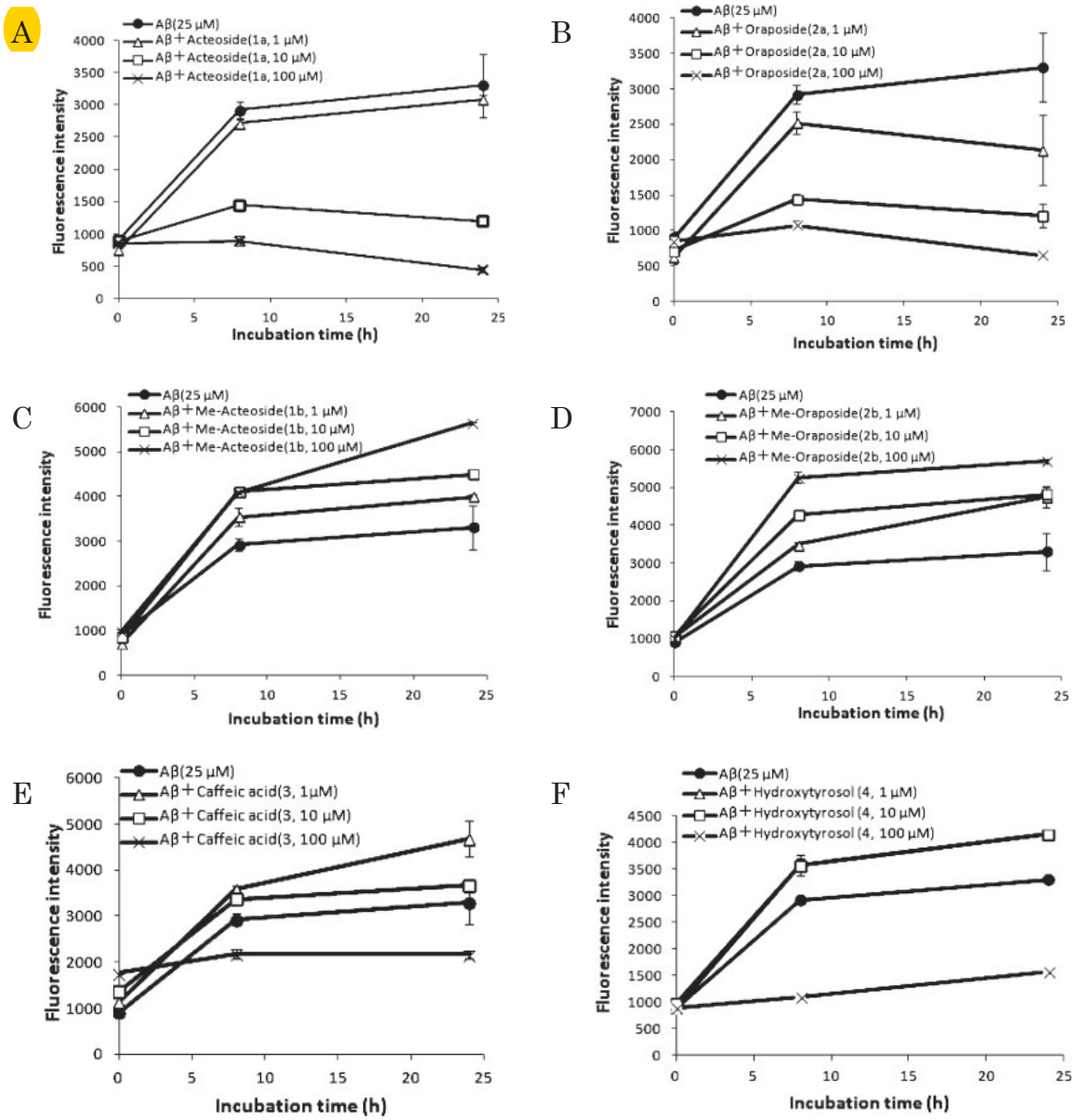

Fig. 2. Effect of Acteoside (**1a**) and Its Derivatives on the Aaggregation of Aβ42 in the Th-T Assay.

Aβ42 (25 μM) was monitored by Th-T fluorescence in the presence of 1, 10, and 100 μM (A) acteoside (**1a**), (B) oraposide (**2a**), (C) methyl-acteoside (**1b**), (D) methyl-oraposide (**2b**), (E) caffeic acid (**3**), and (F) hydroxytyrosol (**4**). The fluorescence intensity was measured at an excitation wavelength of 420 nm and emission wavelength of 480 nm. Each value is presented as the mean ± SD (n = 6).

evaluate the potential role of acteoside (**1a**) in the treatment of AD, we examined the effects of acteoside (**1a**) on Aβ42 aggregation by using the thioflavin-T (Th-T) assay, transmission electron microscopy (TEM), and circular dichroism (CD) spectroscopy.

Acteoside (**1a**) and oraposide (**2a**) were isolated from the whole plant of *O. minor* (1 kg fresh weight). The plant was extracted with MeOH (1 L × 3), and the MeOH extract (63.5 g) was partitioned between EtOAc and H_2O. The water-soluble portion was then partitioned between BuOH and H_2O. The BuOH-soluble portion (492 mg from 21.1 g) was separated by using a C_{18} Sep-Pak cartridge (Waters; MeOH/H_2O, 3:7 → 1:0) to give acteoside (**1a**, 167 mg) and oraposide (**2a**, 46 mg). The phenolic hydroxyl group of acteoside (**1a**) was partially methylated by using trimethylsilyldiazomethane ($TMSCHN_2$). Acteoside (**1a**, 30 mg) was dissolved in MeOH, $TMSCHN_2$ in hexane (2 M, 1 mL) was added to the solution, and the mixture was kept at room temperature for 3 h while stirring. The solvents and $TMSCHN_2$ were next removed by evaporation under N_2. Acteoside-tetramethylether (**1b**, 34.5 mg) was purified from the reaction products by using SiO_2 TLC. Oraposide-tetramethylether (**2b**) was prepared by using the same method. The structures of these compounds were confirmed by NMR (Avance 500, Bruker, Germany) spectral analyses (^1H, ^{13}C, COSY, HMQC, HMBC, and NOESY) and HR-ESI-MS (Synapt G2, Waters, USA) data analyses.[9,10] Caffeic acid (**3**) and hydroxytyrosol (**4**) were purchased from Sigma (St. Louis, MO, USA).

Th-T fluorescence assays were performed (Fig. 2) to examine the inhibitory effects on Aβ aggregation of acteoside (**1a**) and its derivatives **1b**, **2a**, **2b**, **3**, and **4** (Fig. 1).[7,11] Briefly, Aβ42 was dissolved in 0.1% NH_4OH at 250 μM. The Aβ42 solution was diluted 10-fold with 50 mM sodium phosphate (pH 7.4), and the solution incubated at 37 °C with or without acteoside

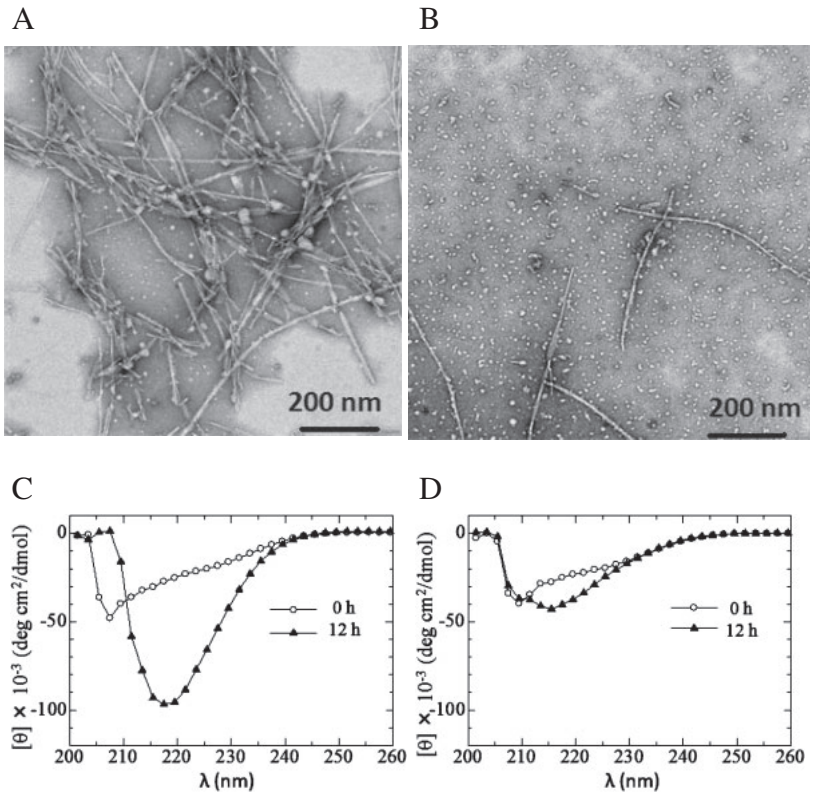

Fig. 3. Effects of Acteoside (**1a**) on Aβ Aggregation by TEM (A and B) and CD Spectra (C and D).
(A) Micrographs of 25 μM Aβ42 alone and (B) 25 μM Aβ42 with 50 μM acteoside (**1a**) (24 h) and (C) CD spectra of 25 μM Aβ42 alone and (D) 25 μM Aβ42 with 50 μM acteoside (**1a**) (12 h).

(**1a**). A 2.5-μL volume of a peptide solution was added to 250 μL of 1 mM Th-T in 50 mM Gly-NaOH (pH 8.5). The fluorescence intensity was measured at an excitation wavelength of 420 nm and an emission wavelength of 485 nm by a Multidetection Microplate Reader Powerscan HT instrument (Dainippon Sumitomo Pharma). Incubating Aβ42 alone for 8 h and 24 h resulted in an increased fluorescence intensity (Fig. 2).

The inhibitory effects on Aβ aggregation by acteoside (**1a**) and its derivatives are shown in Figs. 1 and 2. The IC_{50} values were calculated from the inhibitory rate (%) of each compound toward Aβ42 aggregation after 24 h by using the Th-T assay. Acteoside (**1a**, IC_{50} 8.9 μM) and oraposide (**2a**, IC_{50} 3.6 μM) exhibited potent inhibitory effects on the aggregation of Aβ42 (Fig. 2A and B), while acteoside-tetramethylether (**1b**, $IC_{50} > 200$ μM) and oraposide-tetramethylether (**2b**, $IC_{50} > 200$ μM) did not show inhibitory activity toward Aβ aggregation (Fig. 2C and D), and caffeic acid (**3**, IC_{50} 117.6 μM) and hydroxytyrosol (**4**, IC_{50} 117.6 μM) showed very weak inhibitory activities (Fig. 2E and F).

These results indicated that the compounds with more than two catechol moieties such as acteoside (**1a**) and oraposide (**2a**) exhibited greater inhibitory activity than the compounds with more than one catechol moiety such as caffeic acid (**3**) and hydroxytyrosol (**4**). We therefore focused on acteoside (**1a**) in the subsequent experiments.

We investigated the effects of acteoside (**1a**) on Aβ42 fibrillogenesis by using TEM. The incubating solution was the same as that used for preparing the samples for Th-T assay. A 5-μL volume of each sample was spotted on to a glow-discharged, Formvar-carbon-coated grid and was incubated for 2 min and then washed twice with 5 μL of pure water. The grid was finally negatively stained twice for 1 min each with 5 μL of 0.4% silicotungstic acid, and the solution was removed. After air drying for 5 min, each sample was examined with a Jeol JEM-1400 electron microscope.

The formation of Aβ fibrils was apparent in the absence of acteoside (**1a**) (Fig. 3A), while the fibril formation was significantly reduced after 24 h by treating with 50 μM acteoside (**1a**) (Fig. 3B). These results suggest that acteoside (**1a**) had an anti-aggregative effect on Aβ42.

CD spectra were measured to investigate changes in the secondary structure of Aβ42 in the presence and absence of acteoside (**1a**). The incubation conditions were the same as those used for preparing the samples for the Th-T assay. The sample solution was loaded into quartz cells with a 10-mm path length, and CD spectra were recorded at 200–270 nm with a J-820 spectropolarimeter (Jasco).

In the incubation of Aβ42 alone, the negative peak at 220 nm was increased after incubating for 12 h, meaning that random organization had transformed the protein into a β-sheet structure (Fig. 3C). However, the addition of acteoside (**1a**) attenuated the changes in the negative CD signals at 220 nm, indicating that acteoside (**1a**) had inhibited the β-sheet formation in Aβ42 (Fig. 3D).

We investigated in this study by the Th-T assay the structure-activity relationship of acteoside (**1a**) and its derivatives for the inhibition of Aβ42 aggregation. In addition, the modulation of the inhibitory activity towards the fibril formation and β-sheet transformation of Aβ42 by acteoside (**1a**) was observed from the TEM and CD spectra (Fig. 3).

Based on the structure-activity relationship studies of acteoside (**1a**) and its derivatives (Figs. 1 and 2), two catechol moieties that were present at a position a little away through a glucose proved to be indispensable for the inhibition of Aβ42 aggregation. This result does not contradict with our previous report about the protective effects of CQA on Aβ42 aggregation.[7] In that structure-activity relationship study, we suggested the caffeoyl group in CQA to be essential for the inhibitory activity. We thus speculated that auto-oxidation of the catechol moiety in the caffeoyl group would be related to the mechanism for the inhibition of Aβ42 aggregation.[7] Such covalent modification may have destabilized the β-sheet structure in amyloidogenic polypeptides. Since acteoside (**1a**) has two catechol moieties, this mechanism may also be applied to that for acteoside (**1a**) inhibiting Aβ42 aggregation. A further structural analysis to clarify the interaction between acteoside (**1a**) and Aβ42 may be helpful to elucidate the mechanism.

To the best of our knowledge, this is the first report to show that acteoside (**1a**) possessed an anti-amyloidogenic effect. Acteoside (**1a**) could therefore be a promising therapeutic option for inhibiting the Aβ42-mediated pathology in AD.

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References and Notes

1) Haass C and Selkoe DJ, *Nat. Rev. Mol. Cell Biol.*, **8**, 101–112 (2007).
2) Glenner GG and Wong CW, *Biochem. Biophys. Res. Commun.*, **120**, 885–890 (1984).
3) Masters CL, Simms G, Weinman NA, Multhaup G, McDonald BL, and Beyreuther K, *Proc. Natl. Acad. Sci. USA*, **82**, 4245–4249 (1985).
4) Han J, Miyamae Y, Shigemori H, and Isoda H, *Neuroscience*, **169**, 1039–1045 (2010).
5) Miyamae Y, Kurisu M, Han J, Isoda H, and Shigemori H, *Chem. Pharm. Bull.*, **59**, 502–507 (2011).
6) Miyamae Y, Han J, Sasaki K, Terakawa M, Isoda H, and Shigemori H, *Cytotechnology*, **63**, 191–200 (2011).
7) Miyamae Y, Kurisu M, Murakami K, Han J, Isoda H, Irie K, and Shigemori H, *Bioorg. Med. Chem.*, **20**, 5844–5849 (2012).
8) Wang H, Xu Y, Yan J, Zhao X, Sun X, Zhang Y, Guo J, and Zhu C, *Brain Res.*, **1283**, 139–147 (2009).
9) Acteoside-tetramethylether (**1b**). ^{1}H-NMR (MeOD, 500 MHz) δ: 7.73 (1H, d, J = 15.9 Hz), 7.27 (1H, d, J = 2.0 Hz), 7.22 (1H, dd, J = 8.4, 1.9 Hz), 7.02 (1H, d, J = 8.4 Hz), 6.95 (1H, d, J = 2.0 Hz), 6.90 (1H, dd, J = 2.5, 8.3 Hz), 6.85 (1H, dd, J = 9.0, 2.0 Hz), 6.47 (1H, d, J = 15.9 Hz), 5.25 (1H, d, J = 1.7 Hz), 4.96 (1H, t, J = 9.5 Hz), 4.43 (1H, d, J = 7.9 Hz), 4.14 (1H, m), 3.96 (1H, dd, J = 1.8, 4,2 Hz), 3.90 (1H, d, J = 2.6 Hz), 3.90–3.83 (12H, m), 3.80 (1H, m), 3.69–3.54 (5H, m), 3.44 (1H, d, J = 8.0, 9.2 Hz), 3.34 (1H, d, J = 3.1 Hz), 2.92 (2H, m), 1.14 (1H, d, J = 6.2 Hz). ^{13}C-NMR (MeOD, 125 MHz) δ: 168.9, 153.8, 151.6, 151.2, 149.8, 148.3, 133.9, 129.6, 125.1, 123.1, 116.9, 115.0, 114.0, 113.4, 112.4, 105.0, 103.8, 82.3, 77.0, 76.9, 74.6, 73.2, 72.9, 72.8, 71.6, 71.2, 63.2, 57.4, 57.3, 57.2, 57.1, 37.5, 19.2. HR-ESI-MS (positive ion) m/z: 703.2600 $(M + Na)^+$ (calcd. for $C_{33}H_{44}O_{15}Na$: 703.2578).
10) Oraposide-tetramethylether (**2b**). ^{1}H-NMR (MeOD, 500 MHz) δ: 7.72 (1H, d, J = 13.6 Hz), 7.29 (1H, d, J = 2.0 Hz), 7.23 (1H, dd, J = 2.0, 8.5 Hz), 7.04 (1H, s), 7.03 (1H, d, J = 8.4 Hz), 6.97 (1H, s), 6.96 (1H, s), 6.51 (1H, d, J = 15.9 Hz), 5.25 (1H, d, J = 1.7 Hz), 4.72 (1H, dd, J = 2.7, 10.5 Hz), 4.61 (1H, dd, J = 2.0, 12.0 Hz), 4.57 (1H, d, J = 7.8 Hz), 4.43 (1H, dd, J = 5.6, 12.0 Hz), 4.02 (2H, m), 3.91 (1H, d, J = 3.5 Hz), 3.86 (1H, d, J = 3.9 Hz), 3.92–3.85 (12H, m), 3.81 (1H, dd, J = 2.1, 5.6 Hz), 3.73 (1H, dd, J = 3.4, 9.5 Hz), 3.69 (1H, m), 3.62 (1H, d, J = 9.4 Hz), 3.43 (1H, m), 3.40 (1H, m), 1.30 (3H, d, J = 6.2 Hz). ^{13}C-NMR (MeOD, 125 MHz) δ: 169.6, 152.5, 151.6, 151.3, 151.2, 147.6, 132.1, 129.6, 124.9, 120.7, 115.6, 113.4, 112.8, 112.3, 112.1, 102.8, 99.9, 82.8, 79.7, 79.1, 78.1, 74.8, 73.6, 73.0, 72.9, 71.0, 70.7, 65.3, 57.3, 57.2, 18.7. HR-ESI-MS (positive ion) m/z: 701.2407 $(M + Na)^+$ (calcd. for $C_{33}H_{42}O_{15}Na$: 701.2421).
11) Naiki H and Gejyo F, *Methods Enzymol.*, **309**, 305–318 (1999).