Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry 11 (2003) 1781–1788

BIOORGANIC & MEDICINAL CHEMISTRY

Synthesis and Neuroprotective Activity of Bergenin Derivatives with Antioxidant Activity

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Received 29 October 2002; accepted 13 December 2002

Abstract—Norbergenin, which is the *O*-demethyl derivative of bergenin, the main component of *Mallotus japonicus*, has been found to show moderate antioxidant activity (IC_{50} 13 μM in DPPH radical scavenging; 32 μM in superoxide anion scavenging). Modification of sugar part on norbergenin by coupling with a variety of fatty acids was employed for increasing its antioxidant activity. Selective esterification of hydroxyl groups on the sugar part enhanced greatly antioxidant activity. The most potent one is norbergenin 11-caproate, which not only exhibits stronger antioxidant activity than that of catechin but also prevents neuronal death at 10 μM on the primary culture of rat cortical neurons in DMEM supplemented with N2.

Introduction

Various reactive oxygen species (ROS), such as the hydroxyl radical and superoxide anion radical, has been known to induce lipid peroxidation as well as to damage membranes, proteins and DNA. Enzymatic and non-enzymatic antioxidative defense systems can remove ROS under normal conditions. Oxidative stress, however, occurring when antioxidant systems are inadequate and/or active oxygen species are overproduced, can damage the tissues and DNA, thus resulting in a progression of a number of human diseases such as atheriosclerosis,[1] diabetes, inflammation,[2] Alzheimer's disease[3] and senescence. Hence, antioxidants are expected to be promising drugs for treatment of these diseases by removing oxidative stress.

In our antioxidative screening program of natural products using free radical scavenging activity of the α,α-diphenyl-β-picrylhydrazyl radical (DPPH)[4] and superoxide anion (O_2^-) scavenging activity,[5] we have already reported some antioxidant natural products.[6–8] Further, we have found that norbergenin (**2**)[9] which is the *O*-demethyl derivative of bergenin (**1**)[10] show moderate antioxidant properties. Bergenin (**1**) is the main component of the bark of *Mallotus japonicus*, which has been used as a traditional drug for treatment of hyperacid and a stomach ulcer in Japan. In addition, it has been reported that bergenin has anti-HIV activity,[11] and antiarrhythmic,[12] hepatoprotective[13] and antiinflammatory effects.[14] Since norbergenin (**2**) can be regarded as a *C*-glucoside of a potent antioxidant gallic acid, we have envisaged that **2** is a natural source suitable for developing effective antioxidants. Thus, compound **2** readily available from **1** is modified by coupling with a variety of fatty acids on different position of its sugar part. In this paper, we describe the synthesis of bergenin derivatives and the relationship between their structures and antioxidant activity, as well as their neuroprotective activities on a primary culture of fetal rat cortical neurons in DMEM supplemented with N2 (Fig. 1).

Results and Discussion

Synthesis of norbergenin derivatives

Bergenin (**1**), readily obtained in 22.5% yield from MeOH extract of the bark of *M. japonicus*, was converted to norbergenin (**2**) by acetylation with acetic anhydride and deprotection of the *O*-methyl group with

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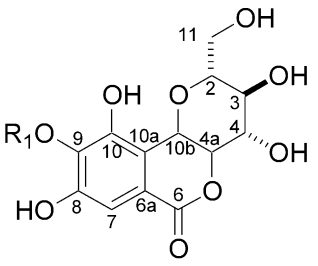
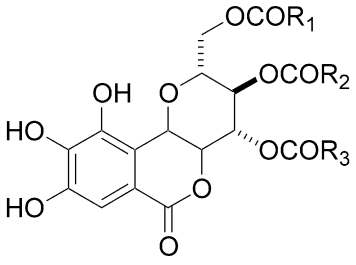
doi:10.1016/S0968-0896(02)00666-1



1 : R_1=Me
2 : R_1=H

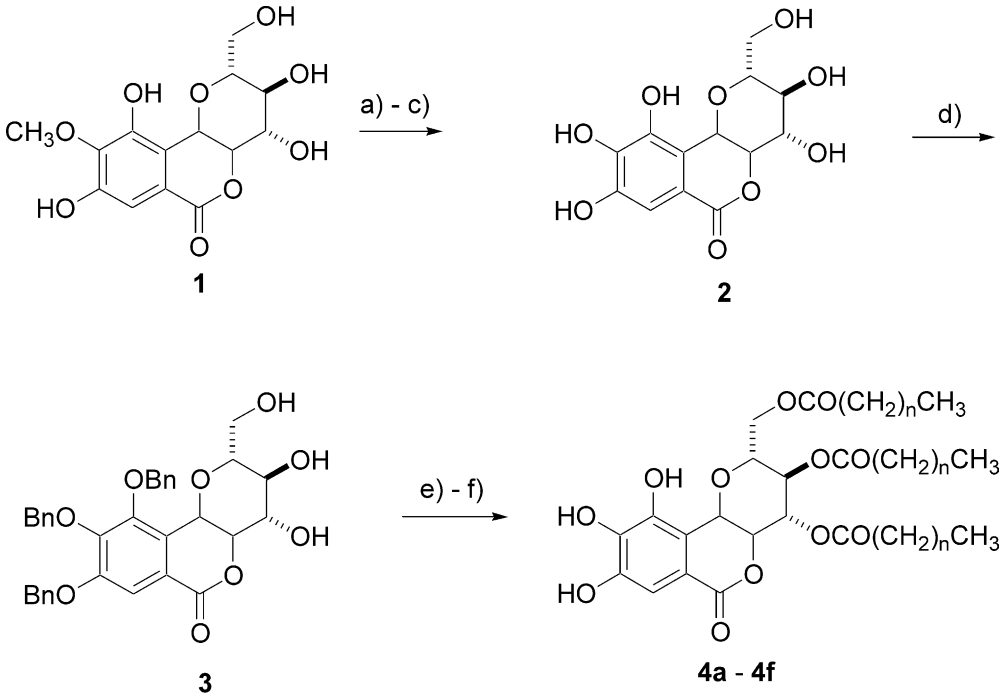


4a : $R_1=R_2=R_3=CH_3$
4b : $R_1=R_2=R_3=(CH_2)_2CH_3$
4c : $R_1=R_2=R_3=(CH_2)_4CH_3$
4d : $R_1=R_2=R_3=(CH_2)_6CH_3$
4e : $R_1=R_2=R_3=(CH_2)_{14}CH_3$
4f : $R_1=R_2=R_3=(CH_2)_{16}CH_3$
5 : $R_1=(CH_2)_4CH_3$, R_2=H, R_3=H
6 : R_1=H, $R_2=(CH_2)_4CH_3$, R_3=H
7 : R_1=H, R_2=H, $R_3=(CH_2)_4CH_3$

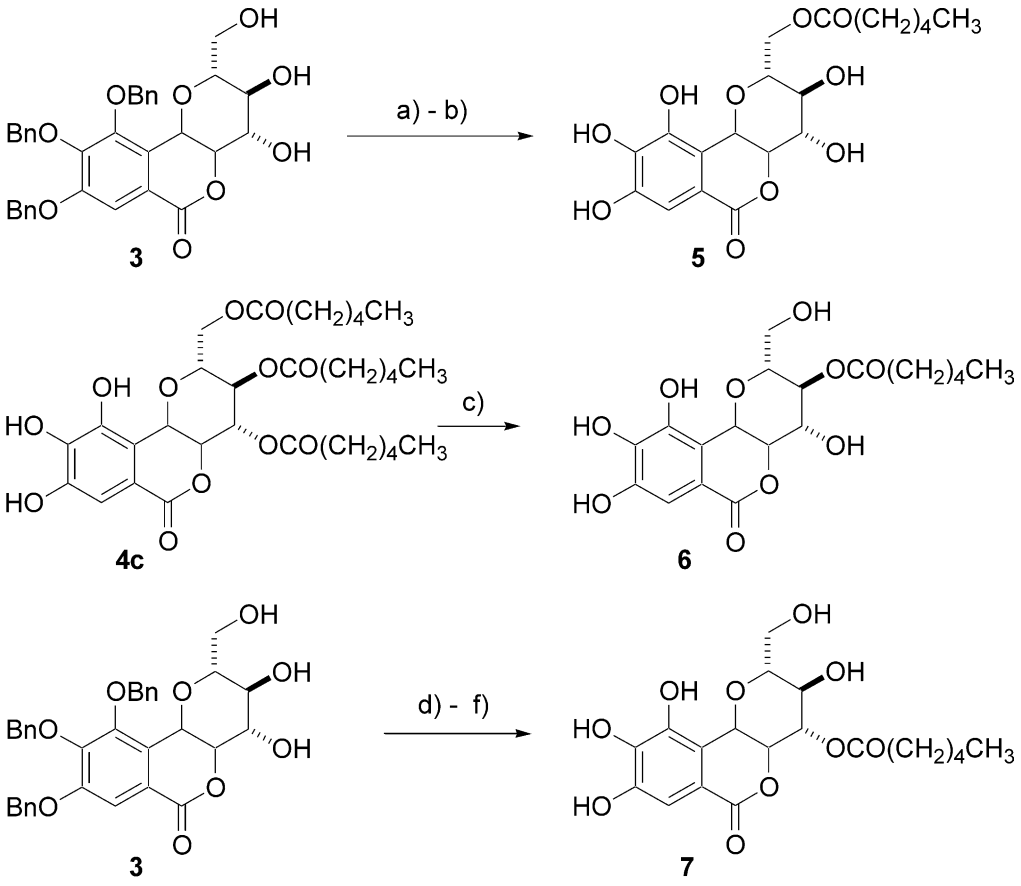
Figure 1. Structure of bergenin derivatives.

BCl_3 followed by hydrolysis with K_2CO_3 in 71% over three steps. The phenolic hydroxyl groups in **2** were selectively benzylated with benzyl bromide under the basic conditions to give **3**. In order to increase lipophilic properties of **2**, hydroxyl groups on the sugar part of **3** were esterified with various chain lengths of fatty acids. The acids with the chain lengths of $n=0$ and $n=2$ were coupled with acetic anhydride and butyric anhydride in pyridine to give triacetate and tributyrate, respectively. The fatty acids with the chain lengths of $n=4$, 6, 14 and 16 were coupled with 1,3-dicyclohexylcarbodiimide (DCC) and fatty acid with corresponding chain length to give triacylate. Finally, hydrogenation of triacylate led to a series of triacylated norbergenins (**4a–4f**) (Scheme 1). Among triacylated derivatives **4a–4f**, norbergenin 11-caproate (**4c**) was found to significantly enhance antioxidant activity.

Since compound **4c** with caproyl ester ($n=4$) showed the most potent antioxidant activity, regioselective monoesterfication of **2** with hexanoic acid was employed to know which hydroxyl group on the sugar part is essential for increasement of antioxidant activity as outlined in Scheme 2. Esterification of the hydroxyl group at C-11 was accomplished by coupling **3** with DCC and hexanoic acid (1.5 equiv) followed by hydrogenation with Pd/C to gave **5**. On the other hand, compound **6** having hexanoic acid ester on the C-3 position was obtained from acid hydrolysis of 4**c**. Compound **7** esterified on the C-4 position was obtained as follows: both the hydroxyl groups at C-11 and C-3 in **3** were protected as an acetal group with *p*-anisaldehyde-dimethylacetal and pyridinium *p*-toluenesulfonate (PPTS), and then the free hydroxyl group at C-4 was



Scheme 1. Reagents and conditions: (a) $(CH_3CO)_2O$, Py, 96%; (b) BCl_3, CH_2Cl_2; (c) 10% K_2CO_3, two steps 74%; (d) K_2CO_3, BnBr, DMF/acetone, 66%; (e) (i) $(CH_3CO)_2O$, Py; or (ii) $CH_3(CH_2)_nCOOH$, DCC, DMAP, Py; (f) H_2/Pd/C, EtOH/CH_2Cl_2.



Scheme 2. Reagents and conditions: (a) 1.5 equiv $CH_3(CH_2)_4COOH$, DCC, DMAP, py; (b) H_2/Pd/C, EtOH/CH_2Cl_2; (c) HCl, MeOH, 28%; (d) *p*-anisaldehyde dimethylacetal, PPTS, CH_2Cl_2, 89%; (e) $CH_3(CH_2)_4COOH$, EDCl, DMAP, py, 82%; (f) H_2/Pd/C, EtOH/CH_2Cl_2, 72%.

coupled with 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDCl) and hexanoic acid, finally, all of the benzyl groups and the *p*-methoxy denzilidene were removed by hydrogenation, to giving rise to **7**.

Free radical scavenging activity

The DPPH radical scavenging activity of bergenin derivatives (**4**–**7**) is summarized in Table 1. First of all, it should be emphasized that the radical scavenging degree for *O*-demethylated compound **2** is enhanced seventy times more than that of bergenin (**1**). Compound **4c** and **4d**, which bear ester groups with C6 and C8 alkylating chains, respectively, showed the highest activity among a series of tri-ester derivatives (**4a**–**4f**) and their potencies were comparable to that of catechin as a positive standard compound. These results clearly indicate that hexanoic acid is appropriate for a modification of the sugar part in **2**. Next, our attention has been directed to which hydroxyl group on the sugar part is significance for being esterified with hexanoic acid. Compound **5**, having caproyl group on the C-11 position was found to show most potent activity, whereas **6** and **7** having the same ester on the C-3 and C-4 position respectively decreased the DPPH radical scavenging activities slightly than **4c**.

The superoxide anion radical scavenging activity also were examined (Table 1). As the number of the methylene group is increasing, the superoxide anion radical scavenging activity (**4a**–**4c**) is tending to be enhanced in parallel with the DPPH radical scavenging activity. Compounds (**4d**–**4f**) with the alkyl chains higher than $n = 6$ failed to be tested due to poor solubility in water. On the other hand, caproates (**5**–**7**) showed high comparable activity regardless of the esterified positions of hydroxyl groups. Judging from these results, the modification of the primary hydroxyl group on the C-11

Table 1. Antioxidant activity of bergenin derivatives (**1**–**7**)

	IC_{50} (μM)[a]	
	DPPH radical[b]	O_2^-[c]
1	921	NT[d]
2	13	32
4a	32	72
4b	12	62
4c	9	44
4d	9	NT[d]
4e	25	NT[d]
4f	25	NT[d]
5	8	3
6	15	3
7	16	2
Catechin	15	2

[a]Inhibitory activity was expressed as the mean of 50% inhibitory concentration of triplicate determination, obtained by interpolation of concentration-inhibition curves.
[b]Chemically stable radical scavenging activity [α,α-diphenyl-β-picrylhydrazyl (DPPH) radical].
[c]Superoxide anion scavenging activity.
[d]Not tested due to poor solubility.

position in norbergenin (**2**) with appropriate groups allows to provide a potential way for developing a promising antioxidant agent.

Neuroprotective activity

The brain consumes about 20% of oxygen in body, and the generation of ROS in brain is assumed to be so much. Therefore, the ROS plays some risky roles in a progression of many neurodegenerative pathologies of the central nervous system such as Parkinson's and Alzheimer's diseases. It should be noted that the search and synthesis for efficient antioxidants can provide a defensive system against a variety of diseases caused by a ROS. In a previous paper,[15] we reported that mastigophorenes A and B, which was isolated from *Mastigophora diclados*,[16] showed not only potent antioxidant activity but also neuroprotective effect on neuronal death in the primary culture of fetal rat cortical neurons. Additionally, some antioxidants such as α-tocopherol,[17] 1,4-benzoxazines,[18–20] flavanoid glycosides[21] and a triterpene ester[22] were reported to also show neuroprotective activity. Thus, the antioxidants appear to have close relation with neuroprotective effect.

The three norbergenin derivatives **2**, **4c** and **5**, the stronger antioxidants than the others, were evaluated for neuroprotective effect. The primary cultures performed in DMEM medium[23] supplemented with N2 was used for this purpose and all viability of rat cortical neurons was measured by the WST-8 reduction assay. We have already established an assay system[24] to primarily evaluate neuroprotective effect of compounds by using the primary culture of rat cortical neurons plated at cell density 2×10^5 cells cm^{-2} in serum-free medium supplemental with N2, the culture conditions of which naturally can cause neuronal death within 5 days. As shown in Figure 2, compounds **4c** and **5** enhanced cell viability at 1 and 10 μM, respectively. But, lower concentration did not show positive effect on neuronal survival. Morphological evaluations as shown in Figure 3, the control culture (a) shows a small number of neurons, but the cultures (b) and (c), in the presence of **4c** and **5**, increased the number of survival neurons. On the other hand, **2** showed no effect on neuronal survival at concentration of 0.01–10 μM. However, neuroprotective activity of **4c** and **5** is not dose-dependent and seems to be limited to a narrow range of concentration. We have not figured out why their effective dose-range is so

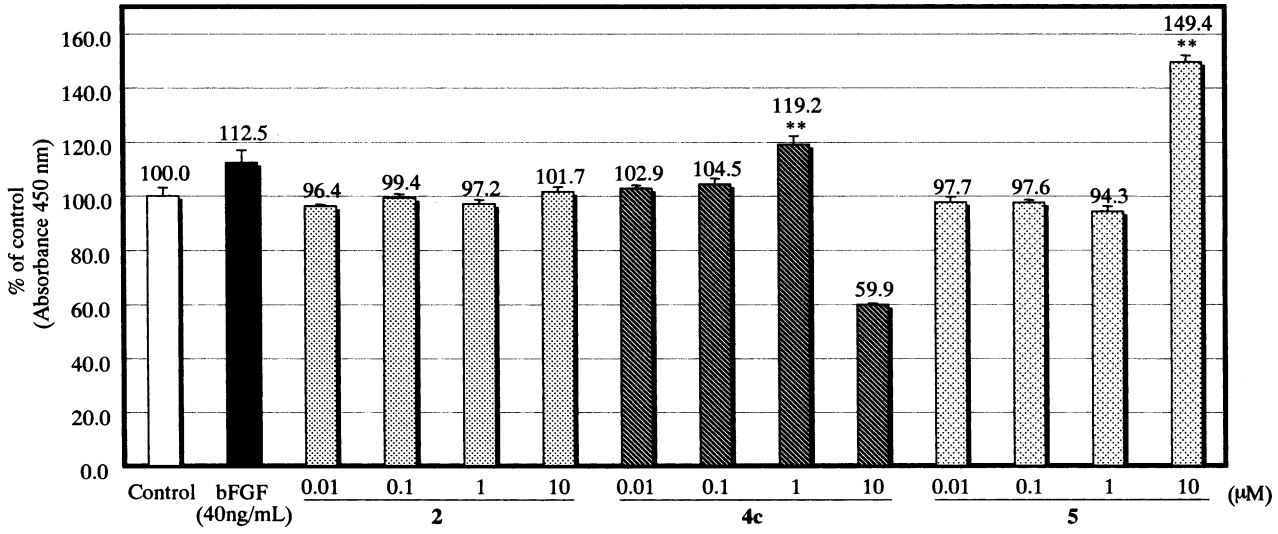


Figure 2. Cell viability of rat cortical neurons enhanced by compounds **2**, **4c** and **5** in primary clutures. Effects of survival were assessed by the WST-8 reduction assay. The data are expressed as means SE ($n=6$); $^{**}p<0.01$ versus control.

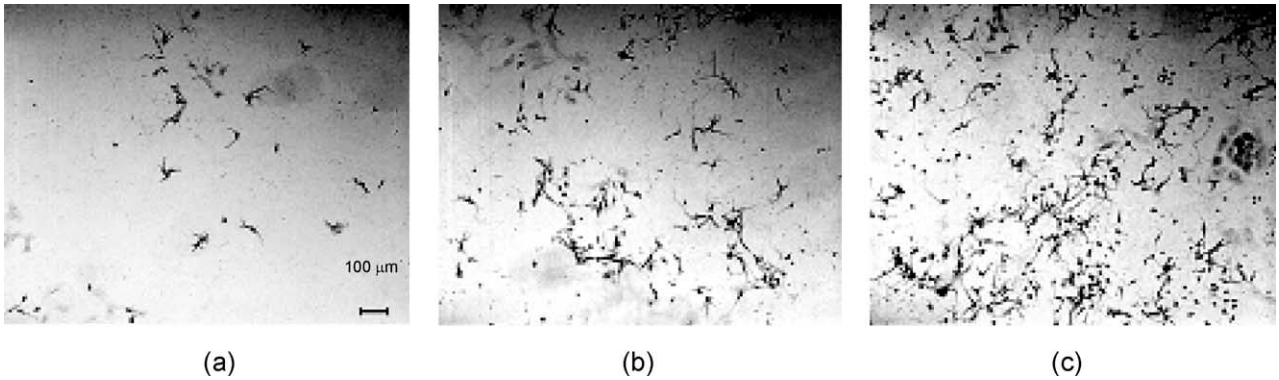


Figure 3. Enhancement of survival of rat cortical neurons by bergenin derivatives in primary cultures. After the neuronal cells (2×10^5 cells/cm^2) cultured for 3 days in the presence of 0.5% EtOH and **5c** and **6** were fixed by 4% paraformaldehyde-PBS, the immunohistochemical staining for the microtuble associated protein-2 (MAP-2) were performed. Pictures taken with 100 magnifications. (a) 0.5% EtOH, (b) 10 μM **5c**, and (c) 1 μM **6**.

narrow. These results suggest that increase of lipophilic property of norbergenin (**2**) can enhance antioxidant activity, which can be further related to protecting neurons from damage by ROS.[25]

Conclusion

We have synthesized a series of bergenin derivatives aimed at increase of antioxidant activity and lipophilic properties. Norbergenin 11–caprorate (**5**) showed not only the highest antioxidant activity but also could prevent neuronal death on the primary culture of rat cortical neurons. These results indicated that the modification of the primary hydroxyl group at the C-11 position in norbergenin with fatty acid made a contribution to increase antioxidant activity. As we have found relationship between antioxidant activity and neuroprotective activity in norbergenin derivatives, synthetic studies of norbergenin derivatives, the C-11 hydroxyl group of which is modified with various fatty acids and/or other functional groups, will continue to exploit more efficient and potent neuroprotective agent. Moreover, our studies are under way to clarify neuroprotective profile of **4c** and **5** against neuronal death caused by reactive oxygen species.

Experimental

General

IR spectra were recorded on Jasco 5300 FT-IR. ^{1}H and ^{13}C NMR spectra were taken on a JEOL ECP-400 spectrometer. Chemical shifts are expressed in δ unit (part per million downfield from tetramethylsilane. Mass spectra (MS) were recorded on a JEOL AX-500. Column chromatography was carried out on Kiselgel 60 (70–230, 230–400 mesh), Wakogel C-300, Sephadex LH-20 and DIAION HP-20. Analytical thin-layer chromatographies were performed with Merck precoated TLC plates, and spots were visualized with ultraviolet light and $CeSO_4/H_2SO_4$.

Plant material. The bark of *M. japonicus* were purchased from Nakai Koshindo (Kobe, Japan).

Bergenin (1). The dried powder bark of *M. japonicus* (10 kg) were extracted with MeOH at room temperature to give 410 g of extract. The extract (24 g) was chromatographed on silica gel (C-300) eluted successively $CHCl_3$, $CHCl_3$/MeOH (9:1), $CHCl_3$/MeOH (4:1) and $CHCl_3$/MeOH (1:1) to yield five fractions. Fraction third were subjected to column chromatography on Sephadex LH-20 and eluted with MeOH to give bergenin (5.35 g).

Norbergenin (2). To a solution of bergenin (10 g, 30.8 mmol) in pyridine (20 mL), acetic anhydride (20 mL) was added, and the mixture was stirred for 24 h. The reaction mixture was poured into water and extracted with EtOAc. The combined organic layers were washed with brine, dried with $MgSO_4$, and concentrated in vacuo to give pentaacetate bergenin (15.9 g, 96%). The obtained residue (4.4 g, 8.4 mmol) was dissolved in CH_2Cl_2 (100 mL) and then BCl_3 (1.0 M in CH_2Cl_2, 30 mL, 30 mmol) was added at 0 °C and stirred for 1 h. After stirring at room temperature for 24 h, the reaction mixture was poured into an ice-water. The organic solvent was removed in vacuo, and then the water layer added to 10% K_2CO_3 solution, and stirred for 1 h. The reaction mixture was acidified with 2 M HCl, and then water layer absorbed with HP-20 (500 mL). The fraction eluted with MeOH was concentrated in vacuo, and then residue purified by recrystallization to afford **2** (1.89 g, 74%) as a white powder. Mp 250 °C (dec.); HR-EIMS *m/z* 314.0635 (calcd 314.0638 for $C_{13}H_{14}O_9$); IR 3385, 1707, 1622, 1477, 1325 cm^{-1}; ^{1}H NMR (Py-d_5) δ 4.20 (3H, m), 4.46 (1H, dd, J = 8.4, 9.5 Hz), 4.60 (1H, dd, J = 9.5, 10.3 Hz), 4.64 (1H, dd, J = 5.1, 10.3 Hz), 5.25 (1H, d, J = 10.3 Hz), 7.86 (1H, s).

Tribenzyl norbergenin (3). To a solution of **2** (515 mg, 1.64 mmol) in DMF (6 mL) and acetone (6 mL) was added K_2CO_3 (1.13 g, 8.2 mmol) and benzyl bromide (0.98 mL, 8.2 mmol) at 0 °C. After stirring at room temperature for 24 h, the reaction mixture was diluted with water and extracted with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was chromatographed on silica gel to afford **3** (635 mg, 66%) as a white powder. Mp 160 °C; HR-FABMS *m/z* 585.2114 (calcd 585.2100 for $C_{32}H_{34}O_9Na$); IR 3335, 1734, 1593, 1444, 1332 cm^{-1}; ^{1}H NMR (Py-d_5) δ 4.07 (1H, m), 4.43 (2H, m), 4.52 (2H, m), 4.62 (1H, dd, J = 9.2, 9.7 Hz), 5.08 (1H, d, J = 9.7 Hz), 5.20 (2H, s), 5.24 (1H, d, J = 10.6 Hz), 5.29 (1H, d, J = 10.6 Hz), 5.39 (1H, d, J = 10.6 Hz), 5.44 (1H, d, J = 10.6 Hz), 7.30–7.43 (9H, m), 7.51–7.76 (7H, m).

General method of preparation of triacyl norbergenin

Method A. To a solution of **3** (57 mg, 0.098 mmol) in pyridine (2 mL) was added acetic anhydride (1 mL), and the mixture was stirred for 12 h. The reaction mixture was diluted with water, extracted EtOAc and washed with $Cu(NO_3)_2$ solution and brine, dried over $MgSO_4$ and concentrated in vacuo to give triacetate. A solution of triacetate in EtOH (2 mL) and CH_2Cl_2 (1 mL) containing palladium carbon (Pd/C, Pd: 10%, 15 mg) was stirred under an atmospheric pressure of hydrogen for 12 h. The reaction mixture was filtrated, concentrated, and the residue was purified by column chromatography (*n*-hexane/EtOAc = 2:1) to afford **4a** (43 mg, 100%). In the same manner, **4b** was prepared.

Method B. To a solution of **3** (101 mg, 0.17 mmol) in pyridine (3 mL) was added 1,3-dicyclohexylcarbodiimide (DCC) (206 mg, 1.0 mmol) and 4-dimethyl- aminopyridine (DMAP) (122 mg, 1 mmol). To this stirring mixture was added hexanoic acid (0.13 mL, 1.0 mmol). The reaction mixture was added, stirring at room temperature for 12 h. The reaction mixture was diluted with water, washed with water, $Cu(NO_3)_2$ solution and brine, and dried over $MgSO_4$ and concentrated in vacuo. The residue was chromatographed on silica gel to afford tricaproate. The crude product obtained

after evaporation of the solvent was subjected to the same hydrogenation as Method A, and then the residue was purified by column chromatography (*n*-hexane/EtOAc = 6:1) to afford **4c** (144 mg, 100%). In the same manner, **4d–4f** were prepared.

Norbergenin 3,4,11-triacetate (4a). HR-FABMS *m/z* 463.0878 (calcd 463.0877 for $C_{19}H_{21}O_{12}Na$); IR 3409, 1730, 1622, 1528, 1478, 1318 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.09 (3H, s, Ac), 2.10 (3H, s, Ac), 2.12 (3H, s, Ac), 4.03 (1H, dd, *J* = 5.3, 9.7 Hz, H-2), 4.16 (1H, dd, *J* = 5.3, 12.6 Hz, H-11), 4.30 (1H, dd, *J* = 9.2, 10.2 Hz, H-4a), 4.44 (1H, d, *J* = 12.6 Hz, H-11), 4.98 (1H, d, *J* = 10.2 Hz, H-10b), 5.08 (1H, dd, *J* = 9.7, 9.7 Hz, H-3), 5.47 (1H, dd, *J* = 9.2, 9.7 Hz, H-4), 7.28 (1H, s, H-7).

Norbergenin 3,4,11-tributyrate (4b). HR-FABMS *m/z* 547.1810 (calcd 547.1791 for $C_{25}H_{32}O_{12}Na$); IR 3420, 1738, 1622, 1524, 1478 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.94 (6H, t, *J* = 7.3 Hz), 0.97 (3H, t, *J* = 7.3 Hz), 1.61 (4H, q, *J* = 7.3 Hz), 1.69 (2H, q, *J* = 7.3 Hz), 2.28–2.37 (6H, m), 4.03 (1H, dd, *J* = 6.2, 9.5 Hz, H-2), 4.13 (1H, dd, *J* = 6.2, 12.4 Hz, H-11), 4.29 (1H, dd, *J* = 10.3, 10.6 Hz, H-4a), 4.47 (1H, d, *J* = 12.4 Hz, H-11), 5.00 (1H, d, *J* = 10.6 Hz, H-10b), 5.12 (1H, dd, *J* = 9.5, 9.5 Hz, H-3), 5.52 (1H, dd, *J* = 9.5, 10.3 Hz, H-4), 7.28 (1H, s, H-7).

Norbergenin 3,4,11-tricaproate (4c). Mp 190 °C; HR-FABMS *m/z* 631.2708 (calcd 631.2731 for $C_{31}H_{44}O_{12}Na$); IR 3406, 1743, 1620, 1469, 1311 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.86 (3H, t, *J* = 7.0 Hz), 0.88 (3H, t, *J* = 7.7 Hz), 0.90 (3H, t, *J* = 6.6 Hz), 1.21–1.30 (12H, m), 1.58–1.66 (6H, m), 2.29–2.43 (6H, m), 4.03 (1H, ddd, *J* = 2.0, 6.2, 9.9 Hz, H-2), 4.11 (1H, dd, *J* = 6.2, 12.1 Hz, H-11), 4.30 (1H, dd, *J* = 10.3, 10.6 Hz, H-4a), 4.47 (1H, dd, *J* = 2.0, 12.1 Hz, H-11), 5.01 (1H, d, *J* = 10.6 Hz, H-10b), 5.11 (1H, dd, *J* = 9.5, 9.9 Hz, H-3), 5.52 (1H, dd, *J* = 9.5, 10.3 Hz, H-4), 7.29 (1H, s, H-7).

Norbergenin 3,4,11-tricaprylate (4d). Mp 198 °C; HR-FABMS *m/z* 715.3643 (calcd 715.3670 for $C_{37}H_{56}O_{12}Na$); IR 3256, 1732, 1597, 1467, 1358 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.89 (9H, t, *J* = 7.0 Hz), 1.24–1.29 (24H, m), 1.59–1.66 (6H, m), 2.26–2.42 (6H, m), 4.01 (1H, ddd, *J* = 1.8, 5.9, 9.9 Hz, H-2), 4.10 (1H, dd, *J* = 5.9, 12.8 Hz, H-11), 4.30 (1H, dd, *J* = 9.5, 10.6 Hz, H-4a), 4.50 (1H, dd, *J* = 1.8, 12.8 Hz, H-11), 4.97 (1H, d, *J* = 10.6 Hz, H-10b), 5.11 (1H, dd, *J* = 9.5, 9.9 Hz, H-3), 5.52 (1H, dd, *J* = 9.5, 9.5 Hz, H-4), 7.31 (1H, s, H-7).

Norbergenin 3,4,11-tripalmitate (4e). HR-FABMS *m/z* 1051.7470 (calcd 1051.7426 for $C_{61}H_{104}O_{12}Na$); IR 3408, 1745, 1620, 1467, 1313 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (9H, t, *J* = 7.0 Hz), 1.26 (72H, m), 1.59–1.66 (6H, m), 2.30–2.40 (6H, m), 4.01 (1H, dd, *J* = 5.9, 9.9 Hz, H-2), 4.11 (1H, dd, *J* = 5.9, 12.4 Hz, H-11), 4.29 (1H, dd, *J* = 9.9, 10.6 Hz, H-4a), 4.48 (1H, d, *J* = 12.4 Hz, H-11), 4.96 (1H, d, *J* = 10.6 Hz, H-10b), 5.09 (1H, dd, *J* = 9.5, 9.9 Hz, H-3), 5.51 (1H, dd, *J* = 9.5, 9.9 Hz, H-4), 7.31 (1H, s, H-7).

Norbergenin 3,4,11-tristearate (4f). HR-FABMS *m/z* 1135.8310 (calcd 1135.8367 for $C_{67}H_{116}O_{12}Na$); IR 3418, 1745, 1620, 1467, 1315 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (9H, t, *J* = 7.0 Hz), 1.24–1.26 (84H, m), 1.59–1.66 (6H, m), 2.30–2.40 (6H, m), 3.99 (1H, m, H-2), 4.11 (1H, dd, *J* = 6.2, 12.5 Hz, H-11), 4.29 (1H, dd, *J* = 10.3, 10.6 Hz, H-4a), 4.49 (1H, d, *J* = 12.5 Hz, H-11), 4.96 (1H, d, *J* = 10.6 Hz, H-10b), 5.09 (1H, dd, *J* = 9.5, 9.9 Hz, H-3), 5.51 (1H, dd, *J* = 9.5, 10.3 Hz, H-4), 7.31 (1H, s, H-7).

Preparation of regioselective esterification of norbergenin

Norbergenin 11–caproate (5). To a solution of **3** (500 mg, 0.86 mmol) in pyridine (20 mL) was added hexanoic acid (0.16 mL, 1.29 mmol), DCC (530 mg, 2.57 mmol) and DMAP (314 mg, 2.57 mmol). The mixture was stirred at room temperature for 12 h. The reaction mixture was diluted with water, washed with water, $Cu(NO_3)_2$ solution and brine, and dried over $MgSO_4$. Evaporation of the solvent gave a crude product, which was chromatographed on silica gel ($CHCl_3$/MeOH = 24:1) to afford monoester (105 mg, 18%) and starting material **3** (129 mg, 30%). A solution of monoester (105 mg, 0.15 mmol) in ethanol (2 mL) and CH_2Cl_2 (2 mL) containing palladium carbon (Pd/C, Pd: 10%, 15 mg) was stirred under an atmospheric pressure of hydrogen for 24 h. The reaction mixture was filtrated, concentrated, and the residue was purified by column chromatography ($CHCl_3$/MeOH = 24:1) to afford **5** (27 mg, 42%). Mp 175 °C; HR-FABMS *m/z* 413.1399 (calcd 413.1447 for $C_{19}H_{25}O_{10}$); IR 3384, 1719, 1618, 1509, 1466, 1307 cm^{-1}; ^1H NMR (Py-d_5) δ 0.72 (3H, t, *J* = 6.6 Hz), 1.13 (4H, m), 1.57 (2H, m), 2.34 (2H, m), 4.09 (1H, dd, *J* = 8.8, 9.2 Hz, H-3), 4.25 (1H, td, *J* = 8.1, 9.2 Hz, H-2), 4.43 (1H, dd, *J* = 8.8, 9.5 Hz, H-4), 4.57 (1H, dd, *J* = 9.5, 10.3 Hz, H-4a), 4.61 (1H, dd, *J* = 8.1, 11.7 Hz, H-11), 5.17 (1H, dd, *J* = 8.1, 11.7 Hz, H-11), 5.25 (1H, d, *J* = 10.3 Hz, H-10b), 7.86 (1H, s, H-7).

Norbergenin 3–caproate (6). To a solution of **4c** (46 mg, 0.075 mmol) in MeOH (3 mL) was added 1 M HCl (0.3 mL) and the mixture was refluxed for 4 h. The reaction mixture was concentrated, and the residue was purified by column chromatography ($CHCl_3$/MeOH = 24:1) to afford **6** (11 mg, 28%). Mp 165 °C; HR-FABMS *m/z* 413.1419 (calcd 413.1447 for $C_{19}H_{25}O_{10}$); IR 3387, 1719, 1618, 1476, 1317 cm^{-1}; ^1H NMR (Py-d_5) δ 0.75 (3H, t, *J* = 6.6 Hz), 1.18 (4H, m), 1.60 (2H, m), 2.38 (2H, m), 4.09 (1H, dd, *J* = 9.9, 12.1 Hz, H-11), 4.23 (1H, dd, *J* = 2.2, 12.1 Hz, H-11), 4.30 (1H, dd, *J* = 8.7, 9.9 Hz, H-2), 4.58 (1H, dd, *J* = 9.2, 9.5 Hz, H-4), 4.66 (1H, dd, *J* = 9.5, 10.3 Hz, H-4a), 5.31 (1H, d, *J* = 10.3 Hz, H-10b), 5.63 (1H, dd, *J* = 8.8, 9.2 Hz, H-3), 7.86 (1H, s, H-7).

Norbergenin 4–caproate (7). To a solution of **3** (103 mg, 0.18 mmol) in CH_2Cl_2 (3 mL) was added *p*-anisaldehyde dimethyl acetal (0.30 mL, 1.76 mmol) and pyridinium *p*-toluenesulfonate (PPTS, 89 mg, 0.35 mmol). The reaction mixture was stirred for 24 h. The reaction mixture was diluted with water, extracted with EtOAc, washed water and brine, dried $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography ($CHCl_3$/MeOH = 24:1) to afford an acetal compound (110 mg,

89%). To a solution of the acetal compound (110 mg, 0.16 mmol) in pyridine (2 mL) was added hexanoic acid (0.12 mL, 0.93 mmol), EDC (180 mg, 0.94 mmol) and DMAP (114 mg, 0.94 mmol). The mixture was stirred at room temperature for 24 h. The reaction mixture diluted with water, washed with water, $Cu(NO_3)_2$ solution and brine, and dried over $MgSO_4$. Evaporation of the solvent gave a crude product, which was chromatographed on silica gel ($CHCl_3$/EtOAc = 49:1) to afford monoester (102 mg, 82%). A solution of the monoester (17 mg, 0.02 mmol) in ethanol (1 mL) and CH_2Cl_2 (1 mL) containing palladium carbon (Pd/C, Pd: 10%, 5 mg) was stirred under an atmospheric pressure of hydrogen for 24 h. The reaction mixture was filtrated, concentrated, and the residue was purified by column chromatography (EtOAc) to afford **7** (6 mg, 72%). Mp 168 °C; HR-FABMS *m*/*z* 413.1419 (calcd 413.1447 for $C_{19}H_{25}O_{10}$); IR 3393, 1718, 1617, 1522, 1477, 1319, 1089 cm^{-1}; ^1H NMR (CD_3OD) δ 0.92 (3H, t, *J* = 6.8 Hz), 1.37 (4H, m), 1.68 (2H, m), 2.43 (2H, t, *J* = 7.7 Hz), 3.62 (1H, dd, *J* = 9.2, 9.2 Hz, H-3), 3.73 (2H, m, H-2 and 11), 4.02 (1H, d, *J* = 9.2 Hz, H-11), 4.23 (1H, dd, *J* = 9.7, 10.2 Hz, H-4a), 5.05 (1H, d, *J* = 10.2 Hz, H-10a), 5.37 (1H, dd, *J* = 9.2, 9.7 Hz, H-4), 7.07 (1H, s, H-7).

Radical scavenging activity

A solution of 180 μL of DPPH (0.1 mM) in EtOH was added to 20 μL of a solution of the bergenin derivatives in EtOH. After 20 min, the absorbance at 517 nm was measured. The scavenging activity of the tested compound was measured as the decrease in absorbance of the DPPH expressed as a percentage of the absorbance of a control DPPH solution without test compounds.

Superoxide anion scavenging assay

Superoxide anion scavenging activity was determined by xanthine/xanthine-oxidase reduced WST-1 system which was using the SOD assay kit (Dojindo Co. Ltd.)[26,27] according to the manufacturer's procedure.

Cell culture

Primary cell cultures were prepared as described.[23] All operations were carried out under sterile conditions. The neuronal cells were separated from the cerebral hemispheres of fetal 18-day SD rat and suspended in 10% FAB/MEM, then seeded at 5×10^5 cells cm^{-2} into poly-L-lysine-coated 24 well-culture plates. After 24 h, the medium was changed into the serum-free medium, Dulbeco's modified Eagle's medium (DMEM) supplemented with N2,[28] in the presence or absence of the compounds at the concentrations of 0.01, 0.1, 1 and 10 μM. After being incubated for 3 days, the cells were fixed with 4% paraformaldehyde/PBS for anti-MAP-2 immunohistochemical stain and then morphological evaluation was carried out.

Assay of neuronal survival

Neuronal survival was determined by the WST-8 reduction assay, which was performed using the cell counting kit-8 (Dojindo Co. Ltd.)[29] according to the manufacture's procedure.

Acknowledgements

The authors thank Ms. Ikuko Okamoto for measuring MS spectra. This work is supported in a part by a Grant-in Aid for Scientific Research (No. 12480175) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan, and the Open Research Center Fund from the Promotion and Mutual Aid Corporation for Private School of Japan.

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