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NOTE

Cyclic diarylheptanoids as inhibitors of NO production from *Acer nikoense*

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Abstract We prepared a series of acerogenins A and B derivatives as inhibitors of nitric oxide (NO) production in vitro. Our results suggested that an ester group at a hydroxyl at C-2 improved inhibitory effects without cytotoxicity. A benzoyl ester derivative of acerogenin C showed the most potent inhibitory activity of NO production from lipopolysaccharide-activated macrophages.

Keywords Acerogenin · *Acer nikoense* · Aceraceae · Nitric oxide

Introduction

Nitric oxide (NO) is an important intracellular and intercellular signaling molecule as a mediator in cardiovascular, nervous, and immunological systems [1]. NO is involved in various biological reactions including vasorelaxation [2], inhibition of platelet aggregation [3], neurotransmission [4], inflammation [5], and immunoregulation [6]. In mammalian cells, NO is synthesized from L-arginine (L-Arg) by NO synthase (NOS), which is classified into three homologues; inducible-NOS (iNOS), endothelial-NOS (eNOS), and neuronal-NOS (nNOS) [7]. iNOS produces large amounts of NO in macrophages stimulated with lipopolysaccharide (LPS) and/or proinflammatory cytokines such as tumor necrosis factor (TNF) and interferon-γ (IFN-γ) [8]. Therefore, inhibition of excess NO production by iNOS might have potential therapeutic value for oxidative stress-induced inflammatory diseases and septic shock [9].

The barks of *Acer nikoense* (Aceraceae) are used as a Japanese folk medicine for hepatic disorders and eye diseases, and contain a series of acerogenins and acerosides [10] with a range of biological activities such as anti-cancer [11, 12], anti-inflammatory [13], and anti-bacterial effects [14]. In a previous report, our efforts at identifying inhibitors of SGLT1 and SGLT2 resulted in the isolation of two cyclic diarylheptanoids, acerogenins A (**1**) and B (**2**) from the barks of *Acer nikoense* [15]. Recently, Yoshikawa et al. [16] reported that **1** and **2** showed inhibition of NO production from LPS-activated macrophages without cytotoxic effects. Therefore, **1** and **2** may be valuable leads as inhibitors of NO production and have potential applications in inflammatory diseases. In this paper, we prepared a series of their derivatives to investigate the effects of two hydroxyl moieties of **1** and **2** against inhibition of NO production in vitro (Fig. 1).

Results and discussion

Acerogenins A (**1**) [17, 18] and B (**2**) [19], and acerosides I (**3**) [20] and VII (**4**) [21] were isolated from barks of *Acer nikoense* and purified using a procedure described previously [15]. Oxidation of **1** and **2** by Swern's procedure gave the acerogenins C (**5**) [22, 23] and L (**6**) [24], respectively. Dehydroxy derivative **7** was prepared by hydrogenation of the double bond from a dehydrated derivative. The esters (**8**–**16**) were synthesized using standard acylation procedures. Acerogenins A (**1**), C (**5**), and **7** were treated with acetic anhydride or benzoyl chloride in pyridine to give **8**–**11** and **16**. Esters **12**–**15** were formed from **5** and the corresponding chloride in pyridine.

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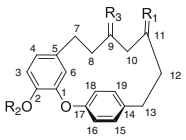

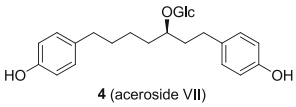

4 (aceroside VII)

1 : R_1 = OH (β), H, R_2 = H, R_3 = H_2 (acerogenin A)
2 : R_1 = H_2, R_2 = H, R_3 = OH, H (acerogenin B)
3 : R_1 = OH (β), H, R_2 = Glc, R_3 = H_2 (aceroside I)
5 : R_1 = O, R_2 = H, R_3 = H_2 (acerogenin C)
6 : R_1 = H_2, R_2 = H, R_3 = O (acerogenin L)
7 : R_1 = H_2, R_2 = H, R_3 = H_2
8 : R_1 = OAc (β), H, R_2 = Ac, R_3 = H_2
9 : R_1 = OBz (β), H, R_2 = Bz, R_3 = H_2
10 : R_1 = O, R_2 = Ac, R_3 = H_2
11 : R_1 = O, R_2 = Bz, R_3 = H_2
12 : R_1 = O, R_2 = $CH_3(CH_2)_5CO$, R_3 = H_2
13 : R_1 = O, R_2 = $(CH_3)_2CHCO$, R_3 = H_2
14 : R_1 = O, R_2 = Bz (p-Cl), R_3 = H_2
15 : R_1 = O, R_2 = Bz (p-OMe), R_3 = H_2
16 : R_1 = H_2, R_2 = Bz, R_3 = H_2
17 : R_1 = H_2, R_2 = Bn, R_3 = H_2
18 : R_1 = OH (β), H, R_2 = Me, R_3 = H_2
19 : R_1 = OH (β), H, R_2 = Bn, R_3 = H_2
20 : R_1 = O, R_2 = Me, R_3 = H_2
21 : R_1 = O, R_2 = Bn, R_3 = H_2
22 : R_1 = H_2, R_2 = Me, R_3 = O
23 : R_1 = N-OH, R_2 = H, R_3 = H_2

Fig. 1 Structures of acerogenins A (**1**) and B (**2**) and acerosides I (**3**) and VII (**4**) and their derivatives **5**–**23**

Incorporation of ethers at C-2 was accomplished by treatment of **1** and **5**–**7** with the corresponding alkyl halides in the presence of K_2CO_3 in acetone to provide compounds **17**–**22**. Reaction of **5** with hydroxylamine hydrochloride provided the oxime **23** [26].

The NO inhibitory activities of acerogenins A (**1**) and B (**2**), acerosides I (**3**) and VII (**4**) and their derivatives **5**–**23** were evaluated as shown in Table 1. Although acerogenins A (**1**) and B (**2**) inhibited NO production at IC_{50} 74 and 88 μM, respectively, in the previous report [16], only acerogenin A (**1**) showed a weak inhibitory activity of NO production from LPS-activated macrophages in our assay system (**1**: IC_{50} 162.3 μM; **2**: IC_{50} >200 μM). No inhibitory activity of acerosides I (**3**) and VII (**4**) indicated that a hydrophilic group such as glucose diminished the activity. Acerogenin C (**5**) showed moderate activity (IC_{50} 61.4 μM) along with **1** and **2**. On the other hand, acerogenin L (**6**), possessing a carbonyl group at C-9, showed no inhibitory activity (>100 μM) even though the structure was very similar to that of **5**. Removing a hydroxyl group at C-11 of **1** built the cytotoxic derivative **7** (Table 1).

Some of the nine esters **8**–**16** showed improvement of the inhibitory activity. Derivatives **8**, **12**, and **13** with bis-esters and an alkyl ester such as acetyl, isobutyl, and heptanoyl group diminished the inhibitory activity. Interestingly, a benzoyl derivative **11** of acerogenin C (**5**) increased the inhibitory activity. Introduction of the methoxy and chloro substituents as representative for electro-withdrawing and electro-donor moiety at the *para* position resulted in a completely loss of the inhibitory activity. Derivatives **16** and **17** showed no improvement to the inhibitory activity though they weakened the cytotoxic effects.

Similar patterns were observed in the ether derivatives **18**–**22**. Methyl ethers **18**, **20**, and **22** showed little effects on the inhibitory activity. A benzyl group of **19** and **21** led to an increase in the inhibitory activity similar to the benzoyl group. In comparison with **5** and **23**, the oxime group had no influence on the activity.

In conclusion, we synthesized and evaluated a series of acerogenin derivatives. Of them, the benzoyl ester **11** derived from acerogenin C (**5**) exhibited potent inhibitory activity of NO production from LPS-activated macrophages without cytotoxic effect. In addition, we found that the presence of the substituents on the side chain at C-9 and C-11 and the hydroxyl at C-2, and the position of each substituent, have influence on the potency of inhibition of NO production as well as cytotoxicity.

Table 1 Effects of acerogenins, acerosides, and their derivatives on NO production inhibitory activity

Compounds	Inhibition (IC_{50}, μM)	Cell viability (%)[a]
1	162.3	106
2	>200	106
3	>100	100
4	>100	100
5	61.4	87
6	>100	122
7	23.9	6
8	>100	86
9	68.2	106
10	47.4	88
11	13.0	86
12	92.7	47
13	69.0	27
14	>100	27
15	>100	110
16	66.5	73
17	74.6	90
18	>100	107
19	39.0	106
20	82.6	95
21	35.9	90
22	65.7	96
23	68.7	100

[a] All compounds were evaluated at 50 μM

Further studies on **11**, such as the mechanisms of inhibition of NO production, are necessary to develop a unique anti-inflammatory drug.

Experimental section

General experimental procedures

Optical rotations were measured on a JASCO P-1030 polarimeter. UV spectra were obtained on an Ultrospec 2100 pro spectrophotometer, and IR spectra on a JASCO FTIR-230 spectrometer. ^{1}H NMR spectrum was recorded on a Bruker AV 400 spectrometer at 300 K, and chemical shifts were reported using residual $CHCl_3$ (δ_H 7.26) as internal standard. Mass spectra were obtained with a Micromass LCT spectrometer.

Synthesis of acerogenin C (**5**)

DMSO (141 μL, 2.0 mmol) was added to a stirred solution of oxalyl chloride (113 μL, 1.3 mmol) in CH_2Cl_2 (7 mL) at −78 °C under argon atmosphere. After the mixture had been stirred for 5 min, a solution of **1** (200 mg, 0.67 mmol) in CH_2Cl_2 was added, and stirring was continued for an additional 15 min. Et_3N (472 μL, 3.4 mmol) was added and the mixture was stirred for 1 h at 0 °C. H_2O was then added and extracted with ethyl acetate. The combined organic phase was dried with Na_2SO_4. Purification with column chromatography (hexane/ethyl acetate = 9:1) gave **5** (129.2 mg, 65 %) as a white powder. Spectroscopic data corresponded to those in Ref. [23].

Synthesis of acerogenin L (**6**)

The compound was prepared in a manner similar to that of **5** from **2** (26.8 mg) with 65 % yield (13 mg) as a white powder. Spectroscopic data corresponded to those in Ref. [24].

Synthesis of 11-dehydroxy acerogenin A (**7**)

p-TsOH (5.7 mg, 0.03 mmol) was added to a stirred toluene solution of **1** (100 mg, 0.34 mmol) and the reaction mixture was stirred at 100 °C for 1 h. Saturated aqueous $NaHCO_3$ was added to the mixture and extracted with ethyl acetate. The combined organic phases were washed with brine, dried over Na_2SO_4, and concentrated in vacuo. Purification with column chromatography (hexane/ethyl acetate = 9:1) gave dehydrated compound (84 mg, 88 %) as a white powder. The dehydrated compound obtained (32 mg, 0.1 mmol) and 10 % Pd/C (3 mg) were combined in MeOH (8 mL) and the reaction vessel was evacuated and back-filled with H_2 (1 atm). The reaction mixture was stirred under H_2 for 1 h, then filtered over a plug of silica gel topped with Celite (MeOH eluent) to give **7** (17 mg, 60 %). UV (MeOH) λ_{max} (ε) 279.5 (1500) and 203 (27000); IR (KBr) 3442, 1510, and 1214 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.64 (2H, m), 0.78 (2H, m), 1.03 (2H, m), 1.23 (2H, m), 1.50 (2H, m), 2.36 (2H, m), 2.60 (2H, m), 5.67 (1H, d, J = 2.3 Hz), 6.52 (1H, dd, J = 2.3, 8.0 Hz), 6.76 (1H, d, J = 8.0 Hz), 6.95 (2H, d, J = 8.6 Hz), 7.17 (2H, d, J = 8.6 Hz); ESIMS *m/z* 305 $(M + Na)^+$. HRESIMS *m/z* 305.15228 [calcd. for $C_{19}H_{22}NaO_2$ $(M + Na)^+$, 305.15175].

Synthesis of acerogenin A diacetate (**8**)

Ac_2O (500 μL) was added to a pyridine solution of **1** (4.4 mg, 14.8 μmol) and the solution was stirred at room temperature for 30 min. The reaction mixture was worked up in the usual way and the product was purified by column chromatography (hexane/ethyl acetate = 9:1) to give **8** (5 mg, 88 %) as a white powder. Spectroscopic data corresponded to those in Ref. [24].

Synthesis of acerogenin A dibenzoate (**9**)

Benzoyl chloride (6.0 μL, 52 μmol) was added to a stirred $CHCl_3$ solution of **1** (4.7 mg, 15.7 μmol) and DMAP (catalytic amount) at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred for 30 min. Ice-cold water (5 mL) and ethyl acetate were added to the reaction mixture. The organic layer was concentrated in vacuo and then purified by column chromatography (hexane/ethyl acetate = 9:1) to give **9** (2.0 mg, 25 %) as a white powder. $[\alpha]_D$ +33; UV (MeOH) λ_{max} (ε) 270 (3100), 225 (25000), and 200.5 (41000); IR (KBr) 1738, 1649, 1598, 1509, and 1421 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.00 (2H, m), 1.42 (2H, m), 1.70 (3H, m), 2.08 (1H, m), 2.56 (2H, m), 2.81 (1H, m), 2.98 (1H, m), 4.64 (1H, m), 5.90 (1H, d, J = 2.0 Hz), 6.73 (1H, dd, J = 2.0, 8.1 Hz), 7.05 (1H, d, J = 8.1 Hz), 7.06 (1H, dd, J = 2.4, 8.3 Hz), 7.21 (1H, dd, J = 2.4, 8.3 Hz), 7.28 (1H, dd, J = 2.4, 8.3 Hz), 7.33 (1H, dd, J = 2.4, 8.3 Hz), 7.45 (2H, dd, J = 7.0, 7.0 Hz), 7.53 (2H, dd, J = 7.0, 7.0 Hz), 7.57 (1H, overlapped), 7.64 (1H, dddd, J = 1.3, 1.3, 7.0, 7.0 Hz), 8.05 (2H, brd, J = 7.0 Hz), 8.30 (2H, brd, J = 7.0 Hz); ESIMS *m/z* 529 $(M + Na)^+$. HRESIMS *m/z* 529.19909 [calcd. for $C_{33}H_{30}NaO_5$ $(M + Na)^+$, 529.20154].

Synthesis of acerogenin C acetate (**10**)

The compound was prepared in a manner similar to that of **8** from **5** (2.0 mg) with 96 % yield (2.2 mg) as a white powder. UV (MeOH) λ_{max} (ε) 365.5 (10000) and 200.5 (10000); IR (KBr) 1766, 1707, 1510, and 1201 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.06 (2H, m), 1.38 (2H, m),

1.89 (2H, m), 2.37 (3H, s), 2.50 (2H, m), 2.60 (2H, m), 2.98 (2H, m), 5.75 (1H, d, $J = 2.0$ Hz), 6.70 (1H, dd, $J = 2.0$, 8.5 Hz), 6.92 (1H, d, $J = 8.5$ Hz), 7.02 (2H, d, $J = 8.5$ Hz), 7.16 (2H, d, $J = 8.5$ Hz); ESIMS *m/z* 361 $(M + Na)^+$. HRESIMS *m/z* 361.14060 [calcd. for $C_{21}H_{22}NaO_4$ $(M + Na)^+$, 361.14158].

Synthesis of acerogenin C benzoate (**11**)

The compound was prepared in a manner similar to that of **9** from **5** (2.0 mg) with 96 % yield (2.7 mg) as a white powder. UV (MeOH) λ_{max} (ε) 273.5 (2900), 228.5 (25000), and 201 (40000); IR (KBr) 1741, 1707, 1510, and 1201 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.08 (2H, m), 1.41 (2H, m), 1.92 (2H, m), 2.53 (2H, m), 2.60 (2H, m), 2.96 (2H, m), 5.82 (1H, d, $J = 2.1$ Hz), 6.75 (1H, dd, $J = 2.1$, 8.1 Hz), 7.05 (1H, d, $J = 8.1$ Hz), 7.06 (2H, d, $J = 8.5$ Hz), 7.15 (2H, d, $J = 8.5$ Hz), 7.52 (2H, dd, $J = 7.1$, 7.1 Hz), 7.63 (1H, dd, $J = 7.1$, 7.1 Hz), 8.28 (2H, d, $J = 7.1$ Hz); ESIMS *m/z* 423 $(M + Na)^+$. HRESIMS *m/z* 423.15791 [calcd. for $C_{26}H_{24}NaO_4$ $(M + Na)^+$, 423.15723].

Synthesis of acerogenin C heptanoate (**12**)

The compound was prepared in a manner similar to that of **9** from **5** (4.7 mg) with 6 % yield (0.4 mg) as a white powder. UV (MeOH) λ_{max} (ε) 274 (400) and 200.5 (17000); IR (KBr) 1762, 1707, 1508, and 1208 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.87 (3H, t, $J = 7.0$ Hz), 1.06 (2H, m), 1.31 (4H, m), 1.41 (4H, m), 1.80 (2H, m), 1.88 (2H, m), 2.49 (2H, m), 2.63 (4H, m), 2.98 (2H, m), 5.75 (1H, d, $J = 2.0$ Hz), 6.69 (1H, dd, $J = 2.0$, 8.5 Hz), 6.92 (1H, d, $J = 8.5$ Hz), 7.03 (2H, d, $J = 8.1$ Hz), 7.15 (2H, d, $J = 8.1$ Hz); ESIMS *m/z* 431 $(M + Na)^+$. HRESIMS *m/z* 431.22202 [calcd. for $C_{26}H_{32}NaO_4$ $(M + Na)^+$, 431.21983].

Synthesis of acerogenin C isobutylate (**13**)

The compound was prepared in a manner similar to that of **9** from **5** (5.0 mg) with 58 % yield (3.6 mg) as a white powder. UV (MeOH) λ_{max} (ε) 276.5 (1400) and 202.5 (35000); IR (KBr) 1759, 1708, 1593, 1504, and 1253 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.05 (2H, m), 1.35–1.39 (6H + 2H, overlapped), 1.88 (2H, m), 2.50 (2H, m), 2.59 (2H, m), 2.90 (1H, m), 2.97 (2H, m), 5.76 (1H, d, $J = 1.8$ Hz), 6.69 (1H, d, $J = 8.0$ Hz), 6.91 (1H, dd, $J = 1.8$, 8.0 Hz), 7.02 (2H, d, $J = 8.3$ Hz), 7.15 (2H, d, $J = 8.3$ Hz); ESIMS *m/z* 389 $(M + Na)^+$. HRESIMS *m/z* 389.17312 [calcd. for $C_{23}H_{26}NaO_4$ $(M + Na)^+$, 389.17288].

Synthesis of acerogenin C *p*-chlorobenzoate (**14**)

The compound was prepared in a manner similar to that of **9** from **5** (3.8 mg) with 81 % yield (4.2 mg) as a white powder. UV (MeOH) λ_{max} (ε) 273.5 (2800), 236 (25000), and 200.5 (62000); IR (KBr) 1742, 1594, 1504, and 1262 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.09 (2H, m), 1.42 (2H, m), 1.90 (2H, m), 2.53 (2H, m), 2.60 (2H, m), 2.97 (2H, m), 5.80 (1H, d, $J = 2.0$ Hz), 6.75 (1H, dd, $J = 2.0$, 8.0 Hz), 7.04 (2H + 1H, overlapped), 7.15 (2H, d, $J = 8.6$ Hz), 7.49 (2H, d, $J = 8.6$ Hz), 8.21 (2H, d, $J = 8.6$ Hz); ESIMS *m/z* 457 $(M + Na)^+$. HRESIMS *m/z* 435.13780 [calcd. for $C_{26}H_{24}ClO_4$ $(M + H)^+$, 435.13631].

Synthesis of acerogenin C *p*-methoxybenzoate (**15**)

The compound was prepared in a manner similar to that of **9** from **5** (3.0 mg) with 79 % yield (3.2 mg) as a white powder. UV (MeOH) λ_{max} (ε) 260 (17000) and 203.5 (43000); IR (KBr) 1734, 1601, 1507, and 1260 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.08 (2H, m), 1.42 (2H, m), 1.89 (2H, m), 2.46 (2H, m), 2.59 (2H, m), 3.00 (2H, m), 3.90 (3H, s), 5.80 (1H, d, $J = 2.0$ Hz), 6.74 (1H, dd, $J = 2.0$, 8.3 Hz), 6.99 (2H, d, $J = 9.0$ Hz), 7.04 (1H, d, $J = 8.3$ Hz), 7.05 (2H, d, $J = 8.5$ Hz), 7.15 (2H, d, $J = 8.5$ Hz), 8.25 (2H, d, $J = 9.0$ Hz); ESIMS *m/z* 453 $(M + Na)^+$. HRESIMS *m/z* 431.18780 [calcd. for $C_{27}H_{27}O_5$ $(M + H)^+$, 431.18585].

Synthesis of 11-dehydroxy acerogenin A benzoate (**16**)

The compound was prepared in a manner similar to that of **9** from **7** (2.0 mg) with 99 % yield (2.7 mg) as a white powder. UV (MeOH) λ_{max} (ε) 273.5 (3600) and 228.5 (23000); IR (KBr) 2928, 1742, 1593, 1505, and 1260 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.72 (2H, m), 0.87 (2H, m), 1.12 (2H, m), 1.36 (2H, m), 1.57 (2H, m), 2.52 (2H, m), 2.66 (2H, m), 5.93 (1H, d, $J = 2.1$ Hz), 6.73 (1H, dd, $J = 2.1$, 8.3 Hz), 7.04 (1H, d, $J = 8.3$ Hz), 7.07 (2H, dd, $J = 2.0$, 8.6 Hz), 7.23 (2H, dd, $J = 2.0$, 8.6 Hz), 7.53 (2H, dd, $J = 7.3$, 7.3 Hz), 7.64 (1H, dd, $J = 2.0$, 7.3 Hz), 8.29 (2H, d, $J = 7.3$ Hz); ESIMS *m/z* 409 $(M + Na)^+$. HRESIMS *m/z* 409.17819 [calcd. for $C_{26}H_{26}NaO_3$ $(M + Na)^+$, 409.17796].

Synthesis of 11-dehydroxy acerogenin A benzyl ether (**17**)

K_2CO_3 (3 mg) and BnBr (3 μL, 27 μmol) were added to a stirred acetone solution of **7** (2.0 mg, 7.1 μmol) and the reaction mixture was refluxed for 24 h, concentrated under reduced pressure and purified by column chromatography (hexane/ethyl acetate = 9:1) to give **17** (1.2 mg, 45 %) as

a white powder. UV (MeOH) λ_{max} (ε) 276.5 (1500) and 203 (29000); IR (KBr) 2925, 2857, 1584, 1509, and 1264 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.67 (2H, m), 0.82 (2H, m), 1.06 (2H, m), 1.25 (2H, m), 1.52 (2H, m), 2.36 (2H, m), 2.62 (2H, m), 5.19 (2H, s), 5.73 (1H, d, $J = 2.1$ Hz), 6.50 (1H, dd, $J = 2.1$, 8.8 Hz), 6.76 (1H, d, $J = 8.8$ Hz), 6.99 (2H, d, $J = 8.4$ Hz), 7.19 (2H, d, $J = 8.4$ Hz), 7.27 (1H, dd, $J = 7.1$, 7.1 Hz), 7.33 (2H, dd, $J = 7.1$, 7.1 Hz), 7.47 (2H, d, $J = 7.1$ Hz); ESIMS *m/z* 395 $(M + Na)^+$. HRESIMS *m/z* 395.20065 [calcd. for $C_{26}H_{28}NaO_2$ $(M + Na)^+$, 395.19870].

Synthesis of acerogenin A methyl ether (**18**)

K_2CO_3 (10 mg) and MeI (4.0 μL, 67 μmol) were added to a stirred acetone solution of **1** (4.0 mg, 13.4 μmol) and the reaction mixture was refluxed for 24 h, concentrated under reduced pressure and purified by column chromatography (hexane/ethyl acetate = 9:1) to give **18** (3.7 mg, 88 %) as a white powder. $[\alpha]_D$ −25; UV (MeOH) λ_{max} (ε) 271.5 (1100) and 200.5 (16000); IR (KBr) 2880, 1509, 1419, and 1215 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.95 (2H, m), 1.08 (2H, m), 1.25 (1H, m), 1.48 (1H, m), 1.66 (1H, m), 1.88 (1H, m), 2.45 (2H, m), 2.69 (1H, m), 2.99 (1H, m), 3.30 (1H, m), 3.95 (3H, s), 5.62 (1H, d, $J = 2.1$ Hz), 6.64 (1H, dd, $J = 2.1$, 8.2 Hz), 6.81 (1H, d, $J = 8.2$ Hz), 6.97 (1H, dd, $J = 2.2$, 8.3 Hz), 7.17 (1H, dd, $J = 2.2$, 8.3 Hz), 7.20 (1H, dd, $J = 2.2$, 8.3 Hz), 7.28 (1H, dd, $J = 2.2$, 8.3 Hz); ESIMS *m/z* 335 $(M + Na)^+$. HRESIMS *m/z* 335.16027 [calcd. for $C_{20}H_{24}NaO_3$ $(M + Na)^+$, 335.16231].

Synthesis of acerogenin A benzyl ether (**19**)

The compound was prepared in a manner similar to that of **17** from **1** (4.0 mg) with 88 % yield (4.6 mg) as a white powder. $[\alpha]_D$ +22; UV (MeOH) λ_{max} (ε) 278 (2200) and 203.5 (48000); IR (KBr) 1587, 1509, and 1211 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.90 (2H, m), 1.08 (2H, m), 1.25 (1H, m), 1.55 (2H, m), 1.88 (1H, m), 2.44 (2H, m), 2.68 (1H, m), 2.98 (1H, m), 3.29 (1H, m), 5.25 (2H, s), 5.66 (1H, d, $J = 2.1$ Hz), 6.58 (1H, dd, $J = 2.1$, 8.2 Hz), 6.82 (1H, d, $J = 8.2$ Hz), 6.95 (1H, dd, $J = 2.5$, 8.3 Hz), 7.17 (1H, dd, $J = 2.5$, 8.3 Hz), 7.20 (1H, dd, $J = 2.5$, 8.3 Hz), 7.28 (1H, dd, $J = 2.5$, 8.3 Hz), 7.31 (1H, dd, $J = 8.0$, 8.0 Hz), 7.38 (2H, dd, $J = 8.0$, 8.0 Hz), 7.51 (2H, d, $J = 8.0$ Hz); ESIMS *m/z* 411 $(M + Na)^+$. HRESIMS *m/z* 411.19661 [calcd. for $C_{26}H_{28}NaO_3$ $(M + Na)^+$, 411.19361].

Synthesis of acerogenin C methyl ether (**20**)

The compound was prepared in a manner similar to that of **18** from **5** (4.7 mg) with 72 % yield (1.5 mg) as a white powder. Spectroscopic data corresponded to those in Ref. [24].

Synthesis of acerogenin C benzyl ether (**21**)

The compound was prepared in a manner similar to that of **17** from **5** (2.0 mg) with 35 % yield (0.9 mg) as a white powder. UV (MeOH) λ_{max} (ε) 199.5 (8300); IR (KBr) 1706, 1514, and 1261 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.06 (2H, m), 1.36 (4H, m), 2.43 (2H, m), 2.60 (2H, m), 2.98 (2H, m), 5.22 (2H, s), 5.67 (1H, d, $J = 2.0$ Hz), 6.58 (1H, dd, $J = 2.0$, 8.2 Hz), 6.84 (1H, d, $J = 8.2$ Hz), 7.01 (2H, d, $J = 8.4$ Hz), 7.17 (2H, d, $J = 8.4$ Hz), 7.32 (1H, dd, $J = 7.4$, 7.4 Hz), 7.39 (2H, dd, $J = 7.4$, 7.4 Hz), 7.50 (2H, d, $J = 7.4$ Hz); ESIMS *m/z* 409 $(M + Na)^+$. HRESIMS *m/z* 409.18009 [calcd. for $C_{26}H_{26}NaO_3$ $(M + Na)^+$, 409.17796].

Synthesis of acerogenin L methyl ether (**22**)

The compound was prepared in a manner similar to that of **18** from **6** (2.0 mg) with 72 % yield (1.5 mg) as a white powder. Spectroscopic data corresponded to those in Ref. [25].

Synthesis of acerogenin C oxime (**23**)

Sodium acetate (7.0 mg, 85 μmol) and hydroxylamine hydrochloride (60 mg, 85 μmol) were added sequentially to a stirred EtOH solution of **5** (10.0 mg, 34 μmol) under argon and stirred for 2 h at room temperature. The reaction mixture was poured into water and the precipitated product was purified by recrystallization from EtOH to give **23** (1.4 mg, 13 %) as a white powder. UV (MeOH) λ_{max} (ε) 276.5 (1000) and 203 (56000); IR (KBr) 3479, 3372, 1599, 1509, and 1222 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.20 (2H, m), 1.38 (2H, m), 1.81 (2H, m), 2.51 (2H, m), 2.71 (2H, m), 3.16 (2H, m), 5.71 (1H, d, $J = 2.0$ Hz), 6.61 (1H, dd, $J = 2.0$, 8.3 Hz), 6.86 (1H, d, $J = 8.3$ Hz), 7.02 (2H, d, $J = 8.6$ Hz), 7.21 (2H, d, $J = 8.6$ Hz); ESIMS *m/z* 312 $(M + H)^+$. HRESIMS *m/z* 312.15748 [calcd. for $C_{19}H_{22}NO_3$ $(M + H)^+$, 312.15997].

NO inhibition assay

J774.1 cells were cultured in RPMI-1640 medium supplemented with 10 % fetal bovine serum and penicillin/streptomycin. J774.1 cells were seeded onto 96-well microtiter plates at 1×10^5 cells in 100 μL per well, and were preincubated for 12 h at 37 °C in a humidified atmosphere containing 5 % CO_2. The cells were cultured in the medium containing LPS (5 μg/mL) with or without the test sample at different concentrations for 24 h. The NO

production was then determined by Griess assay: 100 μL of the supernatant of the cultured medium were transferred to 96-well microtiter plates, and then 100 μL of Griess reagent (1 % sulfanilamide, 0.1 % *N*-L-naphthylethylenediamine dihydrochloride in 2.5 % H_3PO_4) were added. After incubation at room temperature for 15 min, the absorbance at 540 and 620 nm was measured with a microplate reader (Benchmark Plus microplate spectrometer, Bio-Rad). L-NMMA [27] (98 %), an NOS inhibitor, was used as a positive control (IC_{50} = 73 μM).

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