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Four new ginkgolic acids from *Ginkgo biloba*

Jun Deguchi, Yuki Hasegawa, Ayana Takagi, Shihoko Kutsukake, Mizue Kono, Yusuke Hirasawa, Chin Piow Wong, Toshio Kaneda, Hiroshi Morita *

Faculty of Pharmaceutical Sciences, Hoshi University, Ebara 2-4-41, Shinagawa-ku, Tokyo 142-8501, Japan

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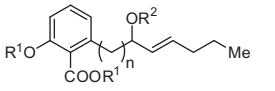
ABSTRACT

Four new compounds, 2-hydroxy-6-(12′-hydroxyheptadec-13′(*E*)-en-1-yl)benzoic acid (**1**), 2-hydroxy-6-(13′-hydroxyheptadec-11′(*E*)-en-1-yl)benzoic acid (**2**), 2-hydroxy-6-(10′-hydroxypentadec-11′(*E*)-en-1-yl)benzoic acid (**3**), and 2-hydroxy-6-(11′-hydroxypentadec-9′(*E*)-en-1-yl)benzoic acid (**4**) were isolated from the leaves of *Ginkgo biloba* and the structures of new ginkgolic acids were deduced on the basis of spectroscopic methods and chemical means. Compounds **1** and **2**, and **3** and **4** examined as an inseparable mixture of hydroxyl and double bond positional isomers, were ultimately defined by total synthesis. Compounds **1**–**4** showed moderate lipid droplets accumulation inhibitory activity on mouse pre-adipocyte cell line, MC3T3-G2/PA6.

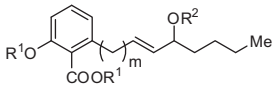
Ginkgo biloba L., although grown mainly in China and Japan, is currently well-known in various European countries. Various chemical components have been isolated from the plant, including flavonoids,[1] terpenoids (ginkgolides, bilobalides),[2] and organic acids (ginkgolic acid, cardanol).[3] While ginkgolic acids possess strong allergenic properties,[4] they show interesting biological activities, such as inhibition of SUMOylation,[5] insecticidal activity,[6] antibacterial properties against Gram-positive bacteria,[7] and inhibitory activity of glycerol-3-phosphate dehydrogenase.[8] Our efforts on identifying new natural products from the leaves of *Ginkgo biloba* resulted in the isolation of four new ginkgolic acids (**1**–**4**). Herein we would like to report the structure elucidation of **1**–**4** on the basis of spectroscopic data, chemical means, and total synthesis, and its anti-lipid droplets accumulation (LDA) activity.

The leaves of *G. biloba* (10 kg) were extracted with MeOH, and the extract was partitioned between $CHCl_3$ and H_2O. $CHCl_3$-soluble materials were subjected to a silica gel column, an ODS column, and an ODS HPLC to give mixtures of compounds **1** and **2** (0.0048%), and **3** and **4** (0.0093%) with common ginkgolic acids (17:1, **9**), (15:1, **10**), and (13:0, **11**).[3]

Compounds **1** and **2**[9] were isolated as pale yellow oils that contained isometric component. Both showed the molecular formula, $C_{24}H_{38}O_4$, which was determined by HRESIMS [*m*/*z* 389.2692, $(M-H)^-$, +0.4 mmu].



1 : n = 11 $R^1 = R^2 = H$
3 : n = 9 $R^1 = R^2 = H$
5a : n = 11 R^1 = Me, R^2 = (*S*)-MTPA
5b : n = 11 R^1 = Me, R^2 = (*R*)-MTPA
7a : n = 9 R^1 = Me, R^2 = (*S*)-MTPA
7b : n = 9 R^1 = Me, R^2 = (*R*)-MTPA



2 : m = 10 $R^1 = R^2 = H$
4 : m = 8 $R^1 = R^2 = H$
6a : m = 10 R^1 = Me, R^2 = (*S*)-MTPA
6b : m = 10 R^1 = Me, R^2 = (*R*)-MTPA
8a : m = 8 R^1 = Me, R^2 = (*S*)-MTPA
8b : m = 8 R^1 = Me, R^2 = (*R*)-MTPA

IR absorptions implied the presence of hydroxyl (3742 cm^{-1}) and carboxylic acid (1683 cm^{-1}) functionalities. ^1H and ^{13}C NMR spectra are presented in Table 1. Although multiple HPLC attempts were made, this mixture could not be separated. The molecular formula of **1** indicated the addition of 16 mass units to the formula for ginkgolic acid (17:1) (**9**), thus indicating an additional hydroxyl group. The gross structures of **1** and **2** were deduced from extensive analysis of HMBC spectrum in CD_3OD (Fig. 1). In the structure elucidation of **1**, the analysis of the $^1H-^1H$ COSY spectrum revealed three partial structures, **a** (C-3 to C-5), **b** (C-1′ and C-2′), and **c** (C-11′ to C-17′), and the existence of allyl alcohol (C-12′ to C-14′) in the alkyl chain. Connection between partial structures **a** and **b**, which form 5-substituted salicylic acid, could be assigned by HMBC correlations of H-3 (δ_H 6.72) to C-1 (δ_C 117.5) and H-4 (δ_H 7.20) to C-2 (δ_C 162.2) and C-6 (δ_C 146.9), and H-5 (δ_H 6.69) to C-1 and C-1′ (δ_C 36.6). The same partial structure, 5-substituted salicylic acid was revealed for **2** by the same manner. The HMBC correlations of H-12′ (δ_H 3.95) to C-10′ (δ_C 26.6) and C-14′ (δ_C 132.2) and H-13′ (δ_H 5.41) to C-11′ (δ_C 38.5) and C-15′ (δ_C 35.4) and H-14′ (δ_H 5.60)

* Corresponding author. Tel./fax: +81 354985778.
E-mail address: moritah@hoshi.ac.jp (H. Morita).

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Table 1
^{1}H NMR Data [δ_H (J, Hz)] and ^{13}C NMR Data [δ_C] of compounds **1**–**4** in CD_3OD at 300 K

Position	**1**		**2**		**3**		**4**	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
1		117.5		117.5		118.4		118.4
2		162.2		162.2		162.2		162.2
3	6.72 (1H, d, 7.8)	115.5	6.72 (1H, d, 7.8)	115.5	6.67 (1H, d, 7.8)	115.3	6.67 (1H, d, 7.8)	115.3
4	7.20 (1H, dd, 7.8, 7.8)	133.5	7.20 (1H, dd, 7.8, 7.8)	133.5	7.14 (1H, dd, 7.8, 7.8)	132.2	7.14 (1H, dd, 7.8, 7.8)	132.2
5	6.69 (1H, d, 7.8)	122.7	6.69 (1H, d, 7.8)	122.7	6.64 (1H, d, 7.8)	122.5	6.64 (1H, d, 7.8)	122.5
6		146.9		146.9		147.1		147.1
1′	2.93 (2H, br)	36.6	2.93 (2H, br)	36.6	3.00 (2H, t, 7.7)	36.5	3.00 (2H, t, 7.7)	36.5
2′	1.57 (2H, m)	33.3	1.57 (2H, m)	33.3	1.57 (2H, m)	33.3	1.57 (2H, m)	33.3
3′	1.29 (2H, m)	30.4–31.0	1.29 (2H, m)	30.4–31.0	1.29 (2H, m)	30.4–31.0	1.29 (2H, m)	30.4–31.0
4′	1.29 (2H, m)	30.4–31.0	1.29 (2H, m)	30.4–31.0	1.29 (2H, m)	30.4–31.0	1.29 (2H, m)	30.4–31.0
5′	1.29 (2H, m)	30.4–31.0	1.29 (2H, m)	30.4–31.0	1.29 (2H, m)	30.4–31.0	1.29 (2H, m)	30.4–31.0
6′	1.29 (2H, m)	30.4–31.0	1.29 (2H, m)	30.4–31.0	1.29 (2H, m)	30.4–31.0	1.29 (2H, m)	30.4–31.0
7′	1.29 (2H, m)	30.4–31.0	1.29 (2H, m)	30.4–31.0	1.29 (2H, m)	30.4–31.0	1.30 (2H, m)	30.2
8′	1.29 (2H, m)	30.4–31.0	1.29 (2H, m)	30.4–31.0	1.29 (2H, m)	26.6	1.57 (2H, m)	33.3
9′a	1.29 (2H, m)	30.4–31.0	1.30 (2H, m)	30.4–31.0	1.41 (1H, m)	38.5[a]	5.60 (1H, dt, 15.4, 6.7)	132.6
9′b					1.51 (1H, m)			
10′	1.30 (2H, m)	26.6	1.57 (2H, m)	33.3	3.95 (1H, dt, 6.8, 6.4)	73.8	5.41 (1H, m)	134.5[a]
11′a	1.41 (1H, m)	38.5[a]	5.60 (1H, dt, 15.3, 6.8)	132.2	5.41 (1H, m)	134.7[a]	3.95 (1H, dt, 6.8, 6.4)	73.8
11′b	1.50 (1H, m)							
12′a	3.95 (1H, dt, 5.9, 5.4)	73.8	5.41 (1H, m)	134.5[a]	5.60 (1H, dt, 15.4, 6.7)	132.5	1.41 (1H, m)	38.2[a]
12′b							1.51 (1H, m)	
13′	5.41 (1H, m)	134.7[a]	3.95 (1H, dt, 5.9, 5.4)	73.8	2.02 (2H, m)	35.4	1.30 (2H, m)	28.9
14′a	5.60 (1H, dt, 15.3, 6.8)	132.2	1.41 (1H, m)	38.2[a]	1.33 (2H, m)	23.5[a]	1.33 (2H, m)	23.7[a]
14′b			1.50 (1H, m)					
15′	2.02 (2H, m)	35.4	1.31 (2H, m)	28.9	0.91 (3H, t, 7.4)	14.0[a]	0.90 (3H, t, 7.4)	14.4[a]
16′	1.33 (2H, m)	23.5[a]	1.33 (2H, m)	23.7[a]				
17′	0.91 (3H, t, 7.4)	14.0[a]	0.90 (3H, t, 7.4)	14.4[a]				
COOH		175.4		175.4		175.9		175.9

[a] The ^{13}C NMR data on **1**–**4** were defined on the synthetic samples.

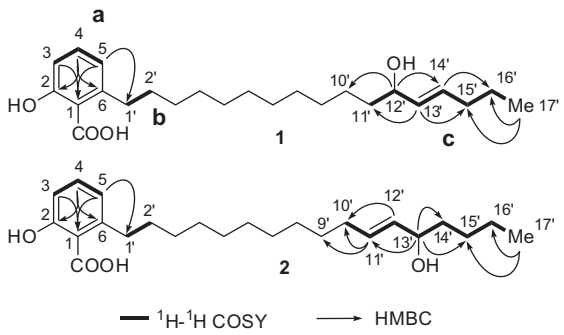


Figure 1. ^{1}H–^{1}H COSY and key HMBC correlations used to establish the structures of **1** and **2**.

to C-16′ (δ_C 23.5), and H_3-17′ (δ_H 0.91) to C-15′ and C-16′ for **1** and HMBC correlations of H-11′ (δ_H 5.60) to C-9′ (δ_C 30.4–31.0) and C-10′ (δ_C 33.3) and H-12′ (δ_H 5.41) to C-10′ and H-13′ (δ_H 3.95) to C-11′ (δ_C 132.2), C-14′ (δ_C 38.2), and C-15′ (δ_C 28.9), and H-17′ (δ_H 0.90) to C-15′ and C-16′ (δ_C 23.7) for **2** indicated the position of each hydroxyl group and double bond, which indicated that the two-component mixture was realized to be composed of hydroxyl group and double bond positional isomers. The characteristic signals, which appeared as a doublet of triplet at δ_H 5.60 (dt, $J_{13',14'}$ = 15.3 Hz, $J_{14',15'}$ = 6.8 Hz) for **1** and δ_H 5.60 (dt, $J_{11',12'}$ = 15.3 Hz, $J_{10',11'}$ = 6.8 Hz) for **2** were assigned *E* configuration for their double bonds, respectively. Therefore, the structures of compounds **1** and **2** were assigned as 2-hydroxy-6-(12′-hydroxyheptadec-13′(*E*)-en-1-yl)benzoic acid and 2-hydroxy-6-(13′-hydroxyheptadec-11′(*E*)-en-1-yl)benzoic acid, respectively.

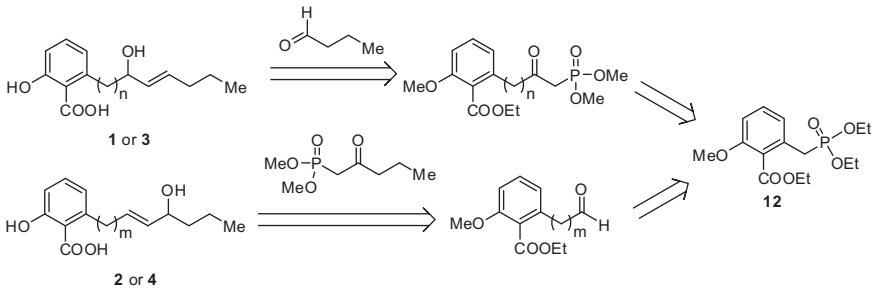
Based on the no optical rotation of mixture of **1** and **2**, the absolute configurations at C-12′ for **1** and C-14′ for **2** could be deduced as racemic forms. The absolute configurations at C-12′ and C-14′ were confirmed by the modified Mosher method.[10] Treatment of mixture of **1** and **2** with iodomethane in acetone followed by reaction with (*R*)- and (*S*)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (MTPACl) afforded (*S*)- and (*R*)-MTPA esters. Both the (*S*)-MTPA and (*R*)-MTPA esters (**5a**, **5b**, **6a**, and **6b**) showed an identical NMR spectrum, indicating that the compounds **1** and **2** were racemate.

Compounds **3** and **4**[11] were isolated as pale yellow oils that contained isometric component. The molecular formula for both constituents was established by HRESIMS as $C_{22}H_{34}O_4$ ($[M-H]^-$ m/z 361.2379). This molecular formula of **3** and **4** indicated the addition of 16 mass units to the formula for ginkgolic acid (15:1) (**10**) and lack of two methylenes from **1** and **2**. The 1D and 2D NMR spectroscopic data of **3** and **4** were indistinguishable from those of **1** and **2**. According to the above data, the structures of **3** and **4** were assigned as 2-hydroxy-6-(10′-hydroxypentadec-11′(*E*)-en-1-yl)benzoic acid and 2-hydroxy-6-(11′-hydroxypentadec-9′(*E*)-en-1-yl)benzoic acid.

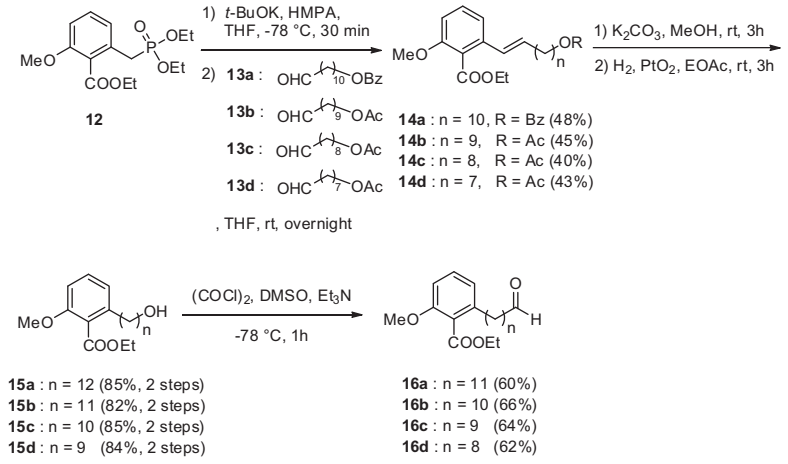
The absolute configurations at C-10 of **3** and C-11 of **4** were confirmed by the modified Mosher method, which generated esters (**7a**, **7b**, **8a**, and **8b**) showed an identical NMR spectrum, indicating that the compounds **3** and **4** were racemate.

While some new ginkgolic acids or related natural products, anacardic acids, which possessing new length alkyl chains, have been isolated from plant resources,[12] ginkgolic acids possessing hydroxyl group and *E* double bond on its alkyl chain were still on few report. Each new compound might be biosynthetically produced by an oxidative transformation of known ginkgolic acids (17:1) and (15:1).

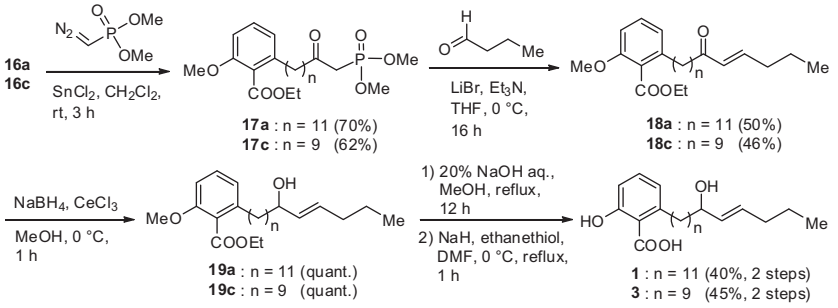
In order to confirm the position of each substituent and spectral data, and evaluate their each biological activity, we synthesized compounds **1**–**4**. Our retrosynthetic analysis of **1**–**4** is outlined in Scheme 1. Generation of *E* geometry double bond and allyl alcohol could be constructed by Horner–Wadsworth–Emmons (HWE)[13] reaction and the reduction under Luche conditions.[14] Preparing a



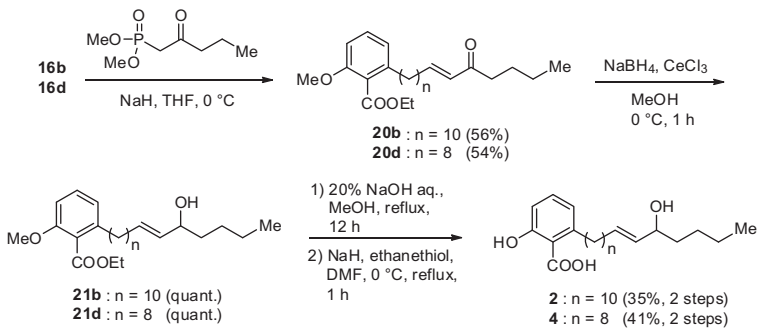
Scheme 1. Retrosynthetic analysis for compounds **1**–**4**.



Scheme 2. Synthesis of aldehydes **16a**, **16b**, **16c**, and **16d**.



Scheme 3. Synthesis of **1** and **3**.



Scheme 4. Synthesis of **2** and **4**.

common starting intermediate **12**, we applied the improved methodology described by Yamagiwa et al.[15]

The Horner–Emmons olefination was carried out in order to extend the side chain at the C-6 position; phosphonate **12** was treated with *n*-BuLi at −78 °C and reacted with different aldehydes (**13a**,[16] **13b**,[17] **13c**,[18] and **13d**[19]) to afford the products **14a–d**. Furthermore, hydrolysis of these esters and hydrogenation of the double bond using 5% platinum on carbon as catalyst gave saturated alcohols **15a**, **15b**, **15c**, and **15d**. Oxidation of the alcohol by Swern's procedure[20] afforded the aldehydes **16a**, **16b**, **16c**, and **16d** (Scheme 2).

We set the stage for a daring two-step sequence consisting of (1) Roskamp reaction[21] for the introduction of the desired β-ketophosphonate, and (2) coupling to the other fragment by a HWE reaction to afford *E* geometry double bond of **1** and **3**. Reaction of **16a** and **16c** with dimethyl(diazomethyl) phosphonate[22] and $SnCl_2$ gave **17a** and **17c**, and then reaction with butanal in the presence of LiBr and triethylamine in THF produced **18a** and **18c** in 50% and 46% yields, respectively. Enones **18a** and **18c** were regioselectively reduced to **19a** and **19c** under Luche condition in quantitative yield. The two protecting groups were removed by successive treatment with sodium hydroxide in MeOH and with sodium ethanethiolate in DMF,[23] to give the desired compounds **1** and **3** in an overall yield of 3.4% from **12** for **1** (9 steps) and 3.0% from **12** for **3** (9 steps) (Scheme 3). The spectral data of synthetic compounds **1** and **3** were identical to those of authentic samples and we have achieved the supplement of samples for biological evaluation.

For the synthesis of other compounds, **2** and **4**, enones **20b** and **20d** were synthesized from aldehydes **16b** and **16d** and a known β-keto phosphonate[24] by HWE reaction, which was then regioselectively reduced to **21b** and **21d** under Luche condition in quantitative yield. The protecting groups were removed by the same manner as **1** and **3** to give the desired compounds **2** and **4** in an overall yield of 5.4% from **12** for **2** (8 steps) and 5.8% from **12** for **4** (8 steps) (Scheme 4). The spectroscopic data of the synthetically obtained compounds **2** and **4** were identical to those of authentic samples.

During our investigation for LDA inhibitors from plant natural products, we found a promising small molecule, ceramicine B from *C. ceramicus*,[25–27] showing anti-LDA activity on mouse pre-adipocyte cell line, MC3T3-G2/PA6.[28] Each synthetic compound (**1–4**) was tested for its anti-LDA activity on mouse pre-adipocyte cell line, MC3T3-G2/PA6 and was found to show moderate LDA inhibitory activity (IC_{50} **1**: 57.6 μM, **2**: 60.7 μM, **3**: 74.0 μM, **4**: 76.7 μM) as compared to positive control berberine (15.1 μM). LDA inhibitory assay procedure was performed as previously described.[25,27]

Acknowledgments

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Supplementary data

Supplementary data (scanned copies of NMR spectra including ^{1}H NMR, ^{13}C NMR, ^{1}H–^{1}H COSY, HSQC, HMBC, and ROESY spectra) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2014.05.076.

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