

New hydroxy fatty acid from the root bark of *Morus alba* L.

Jae-Woo Jung · Ji-Hae Park · Kyeong-Hwa Seo ·
Eun-Ji Oh · Dae-Young Lee · Dong-Wook Lim ·
Daeseok Han · Myoung-Chong Song ·
Nam-In Baek

Received: 17 February 2015 / Accepted: 10 April 2015 / Published online: 5 May 2015
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Abstract A new hydroxyl fatty acid, named sangbaimoric acid (**1**), was isolated from the root bark of *Morus alba* L. The chemical structure of compound **1** was established as 2-methoxyoctadeca-3*Z*,5*Z*-dienoic acid on the basis of spectroscopic analyses including nuclear magnetic resonance, high-resolution electronic ionization mass spectroscopy, and infrared spectroscopy experiments.

Keywords 2-Methoxyoctadeca-3*Z*,5*Z*-dienoic acid ·
Morus alba L. · Nuclear magnetic resonance ·
Sangbaimoric acid

Introduction

The mulberry tree, *Morus alba* L. (Moraceae), a deciduous broad-leaved tree, is native to Thailand and also widely distributed in Europe, America, and Asia (Sohn et al. 2009). The root barks of mulberry trees are named “Sang-

Bai-Pi” and are used for a variety of medicinal purposes, primarily in South Asia. Previously, many phytochemical compounds such as flavonoids, Diels–Alder type adducts, coumarins, stilbenes, and triterpenoids (Hano et al. 1988; Piao et al. 2009; Jung et al. 2014) have been isolated from the root bark of *M. alba*. These compounds have been reported to show anti-oxidant, anti-inflammatory, anti-cancer, and anti-microbial activities (Dat et al. 2010; Yang et al. 2011; Yang and Lee 2012; Naik et al. 2015). In order to identify new biological constituents of the root bark of *M. alba*, we conducted a phytochemical study.

Materials and Methods

The root barks of *M. alba* were extracted with 80 % MeOH and the concentrated extract was successively partitioned by polarity gradient using ethyl acetate (EtOAc), *normal*-butylalcohol (*n*-BuOH), and H₂O. From the EtOAc fraction, repeated open column chromatography (c.c.) through the silica gel (SiO₂), octadecyl SiO₂ (ODS), and sephadex LH-20 afforded a new fatty acid, which was subsequently identified by spectroscopic data analyses including nuclear magnetic resonance (NMR), high-resolution electronic ionization mass spectroscopy (HR/EI/MS), and infrared spectroscopy (IR) experiments.

In our study, dried and powdered root barks of *M. alba* (10 kg) were extracted with 80 % MeOH (68 L × 3) at room temperature for 24 h. Next, the concentrated MeOH extract (1.7 kg) was suspended in 2 L water and successively extracted with EtOAc (2 L × 2) and *n*-BuOH (1.8 L × 3). The organic and aqueous layers were concentrated to produce residues of the EtOAc fraction (MRE, 580 g), the *n*-BuOH fraction (MRB, 114 g), and the H₂O fraction (MRW, 1006 g), respectively. The EtOAc fraction (122 g) was

J.-W. Jung · J.-H. Park · K.-H. Seo · E.-J. Oh · N.-I. Baek (✉)
Department of Oriental Medicine Biotechnology, Graduate
School of Biotechnology, Kyung Hee University,
Yongin 446-701, Republic of Korea
e-mail: nibaek@khu.ac.kr

D.-Y. Lee
Department of Herbal Crop Research, National Institute of
Horticultural and Herbal Science, RDA, Eumseong 369-873,
Republic of Korea

D.-W. Lim · D. Han
Division of Metabolism and Functionality Research, Korea Food
Research Institute, Sungnam 463-746, Republic of Korea

M.-C. Song
Intelligent Synthetic Biology Center, KAIST, Daejeon 305-701,
Republic of Korea

fractionated by SiO₂ c.c. (12.5 × 17 cm) eluting with *n*-hexane–EtOAc (4:1 → 2:1 → 1:1, 27 L of each) and CHCl₃–MeOH (10:1, 27 L) to yield 41 fractions (MRE-1 to MRE-41). Fraction MRE-23 [848 mg, elution volume/total volume (Ve/Vt) 0.422–0.528] was applied to ODS c.c. (4.5 × 6 cm) and eluted with MeOH–H₂O (3:1 → 8:1, 3 L of each), yielding 12 fractions (MRE-23-1 to MRE-23-12). Subfraction MRE-23-9 (120 mg, Ve/Vt 0.507–0.651) was subjected to sephadex LH-20 c.c. (1.5 × 60 cm) and eluted with MeOH–H₂O (4:1, 0.7 L) to yield five fractions (MRE-23-9-1 to MRE-23-9-5) including compound **1**, MRE-23-9-1 [12 mg (yield 0.0006 %), Ve/Vt 0.00–0.12, TLC (ODS) R_f 0.51, MeOH–H₂O = 12:1].

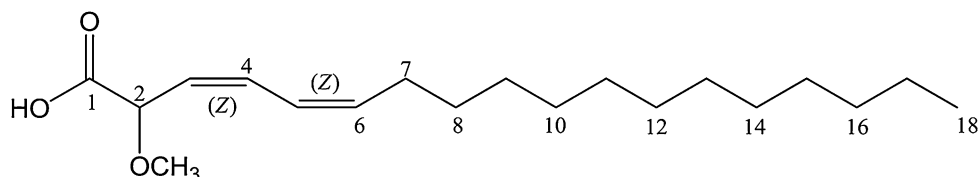
Compound **1** (sangbaimoric acid): Yellow oil; [α]_D²⁵ +19.1° (*c* 0.82, MeOH); IR (KBr, ν): 3450, 1732, 1681 cm⁻¹; HR/EI/MS *m/z* 310.2513 [M]⁺ (calcd. for C₁₉H₃₄O₃ 310.2509); ¹H-NMR (400 MHz, CD₃OD, δ _H): 6.48 (1H, dd, *J* = 10.8, 10.8 Hz, H-4), 5.99 (1H, dd, *J* = 10.8, 10.8 Hz, H-5), 5.45 (1H, dt, *J* = 10.8, 8.0 Hz, H-6), 5.41 (1H, dd, *J* = 10.8, 8.0 Hz, H-3), 3.61 (1H, d, *J* = 8.0 Hz, H-2), 3.22 (3H, s, –OCH₃), 2.19 (2H, m, H-7), 1.39 (2H, m, H-8), 1.31–1.28 (14H, m, H-9 ~ H-15), 0.89 (3H, t, *J* = 6.4 Hz, H-18); ¹³C-NMR (100 MHz, CD₃OD, δ _C): 172.88 (C-1), 134.39 (C-3), 133.64 (C-6), 129.44 (C-4), 128.97 (C-5), 83.75 (C-2), 56.32 (–OCH₃), 36.62 (C-8), 32.49 (C-16 or 17), 30.53 ~ 30.36 (C-10, 11, 12, 13, 14, 15), 28.57 (C-7), 26.39 (C-9), 23.56 (C-16 or 17), 14.39 (C-18).

Results and Discussion

The fatty acid is a carboxylic acid with a long aliphatic chain that was either saturated or unsaturated. C16 and C18 unsaturated fatty acids are more common in the plant kingdom. In addition, hydroxyl fatty acids with C16 and C18 chains are observed often, and have been reported to possess several biological activities such as anti-bacterial (Kurata et al. 2010), anti-inflammatory (Masayuki et al. 1998), and anti-melanogenesis effects (Fujita et al. 2010). In this study, we isolated and identified a new hydroxyl fatty acid from the root barks of *M. alba*. Interestingly, this compound is a hydroxyl fatty acid with a C-2 hydroxylated moiety, which has been rarely reported in the literature.

Compound **1** was isolated as a yellow oil, was UV absorption active, and appeared as a brown spot color on TLC plates upon spraying with 10 % sulfuric acid followed by heating. The molecular weight was determined to be 310 from the molecular ion peak *m/z* 310 [M]⁺ in the EI/MS spectrum, and a molecular formula of C₁₉H₃₄O₃ was elucidated on the basis of the high-resolution molecular ion peak *m/z* 310.2509 [M]⁺ (calcd for 310.2513, C₁₉H₃₄O₃) in the HR/EI/MS. The IR absorbance bands of hydroxyl (3450 cm⁻¹), carbonyl (1732 cm⁻¹), and double bond (1681 cm⁻¹) groups were observed. In the ¹H-NMR spectrum, four olefinic methine signals at δ _H 6.48 (1H, dd, *J* = 10.8, 10.8 Hz, H-4), 5.99 (1H, dd, *J* = 10.8, 10.8 Hz, H-5), 5.45 (1H, dt, *J* = 10.8, 8.0 Hz, H-6), and 5.41 (1H, dd, *J* = 8.0, 10.8 Hz, H-3) were observed. In the oxygenated proton region, one oxygenated methine signal at δ _H 3.61 (1H, d, *J* = 8.0 Hz, H-2) and one oxygenated methyl signal at δ _H 3.22 (3H, s, –OCH₃) were detected. In the high magnetic fields, nine methylene signals including overlapping signals at δ _H 2.19 (2H, m, H-7), 1.39 (2H, m, H-8), and 1.31–1.28 (14H, m, H-9 ~ 15) were detected, along with a terminal methyl signal at 0.89 (3H, t, *J* = 6.4 Hz, H-18). According to the above-described proton signals, compound **1** was assumed to be an unsaturated fatty acid that included one hydroxyl group. The ¹³C-NMR spectrum showed 19 carbon signals including one methoxy carbon signal. Specifically, the observed carbon signals were as follows: one carbonyl at δ _C 172.88 (C-1); four olefine methines at δ _C 134.39 (C-3), 133.64 (C-6), 129.44 (C-4), and 128.97 (C-5); one oxygenated methine at δ _C 83.75 (C-2); and one methoxy at δ _C 56.32 (–OCH₃). In addition, at high magnetic fields, 11 methylene carbon signals at δ _C 23.56–36.62 (C-7 ~ 17) and terminal methyl carbon signal at δ _C 14.39 (C-18) were observed, indicating the presence of a C18 fatty acid with two double bonds, a hydroxyl group, and a methoxy group. The locations of the functional groups in compound **1** were determined by 2D NMR (gCOSY, gHSQC, gHMBC) experiments. In the gCOSY experiments, the connectivity of C-2 to C-8 and C-16 to C-18 was successfully determined on the basis of the correlations among neighboring proton signals. In the gHMBC experiment, the oxygenated methine proton signal at δ _H 3.61 (H-2) showed the cross

Fig. 1 Chemical structure of compound **1** isolated from the root bark of *Morus alba* L.



sangbaimoric acid (**1**)

peaks with the carbonyl carbon signal at δ_C 172.88 (C-1) and the methoxy signal at δ_C 56.32 (–OCH₃), indicating the position of the carbonyl, methoxy, and hydroxy groups. Likewise, the stereostructures of both double bonds were, respectively, identified as 3Z and 5Z from the coupling constants (*J*) between the olefine methine proton signals (H-3/H-4: *J* = 10.8 Hz, H-5/H-6: *J* = 10.8 Hz). The stereochemistry of the chiral carbon, C-2, was not specified in this study. Taken together, the structure of compound **1** was determined to be 2-methoxyoctadeca-3Z,5Z-dienoic acid, a new hydroxy fatty acid, which we named sangbaimoric acid (Fig. 1).

Acknowledgments This study was financially supported from the Korea Food Research Institute (Grant Number 20140398U0054101S 00100) and the Kyung Hee University (sabbatical year project: 20150142), Republic of Korea.

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