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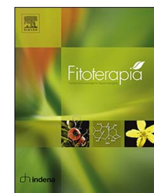

Anti-inflammatory activities of compounds from twigs of *Morus alba*

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ABSTRACT

Five new compounds, 10-oxomornigrol F (**1**), (7″*R*)-(−)-6-(7″-hydroxy-3″,8″-dimethyl-2″,8″-octadien-1″-yl) apigenin (**2**), ramumorin A (**3**), ramumorin B (**4**), and (4*S*,7*S*,8*R*)-trihydroxyoctadeca-5*Z*-enoic acid (**5**), together with 31 known compounds (**6–36**), were isolated from the twigs of *Morus alba* (Moraceae). The chemical structures of these compounds were established using spectroscopic analyses, 1D and 2D NMR, high-resolution electrospray ionization mass spectrometry (HRESIMS), and Mosher's methods. The anti-inflammatory activities of the compounds were evaluated by investigating their ability to inhibit lipopolysaccharide (LPS)-induced nitric oxide (NO) production in macrophage RAW 264.7 cells. Compounds **1**, **2**, **13**, **17**, **19**, **25–28**, and **32** showed inhibitory effects with IC_{50} values ranging from 2.2 to 5.3 μg/mL. Compounds **1**, **2**, **17**, **25**, and **32** reduced LPS-induced inducible nitric oxide synthase (iNOS) expression in a concentration-dependent manner. In addition, pretreating the cells with compound **1**, **17**, and **32** significantly suppressed LPS-induced expression of cyclooxygenase-2 (COX-2) protein.

1. Introduction

Morus alba L. (Moraceae) has long been used in traditional medicine and is widely cultivated in Korea. This species is a fast-growing tree, which can reach up to 20 m in height. The whole herb of *M. alba* is used to treat diabetes, inflammation, and obesity. Especially, the antidiabetic activity was investigated from the fruit of *Morus alba* [1]. The antimicrobial, cytotoxic, and antiviral activities of prenylated flavonoids from *M. alba* have also been reported. Three flavonols from leaf extracts of *M. alba* is known anti-inflammatory. Furthermore, the arylbenzofurans and prenylflavonoids from root barks of *M.alba* also showed significant nitric oxide production inhibitory [2]. However, the chemical composition and biological anti-inflammatory activities of these plant extracts were not extensively examined. Methanol extracts from the twigs of *M. alba* L. were found to potently inhibit NO production. Based on our ongoing studies to identify new anti-inflammatory agents from medicinal plants, we isolated and investigated five new and 31 known compounds from the twigs of *M. alba*. In this report, the purification and structural elucidation of these compounds are discussed, along with their ability to inhibit NO synthesis.

2. Experimental

2.1. General

Optical rotations were measured using a JASCO DIP 1000 digital polarimeter. UV spectra were recorded using a Thermo spectrometer. IR spectra were recorded using a JASCO FT/IR-4100 spectrometer. The 1D- and 2D–NMR spectra were obtained using Varian Unity Inova 400 MHz and 500 MHz spectrometer with tetramethylsilane (TMS) as an internal standard, and the chemical shifts were recorded in δ values (ppm). Mass spectra were recorded using a JEOL JMS-AX 300 L and SYNAPT G2 spectrometer. Silica gel (Merck, 63–200 μm particle size) and RP-18 (Merck, 75 μm particle size) were used for column chromatography. TLC was performed using Merck silica gel 60 F_{254} and RP-18 F_{254} plates.

2.2. Plant material

The dried branch of *Morus alba* L. was purchased from a folk medicine market 'Yakryoung-si' in Daegu, Korea, in January 2015. Botanical identification was performed by Professor Byung Sun Min and

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the voucher specimen CUD-1141-3 was deposited at the Herbarium of the College of Pharmacy, Catholic University of Daegu, Korea.

2.3. Extraction and isolation

The branch of *Morus alba* L. (9.8 kg) was extracted three times (3 h × 20 L) with methanol. After solvent was removed under reduced pressure, the residue was suspended in H_2O and then partitioned with *n*-hexane, EtOAc, and *n*-BuOH, successively. By the guided-fractiona-tion-activity, the EtOAc-soluble fraction (123.8 g) was chromatographic (80 × 12 cm, 63–00 μM particle size, Merck) on a silica gel column using a stepwise gradient of $CHCl_3$:MeOH (50:1 to 0:1, each 10 L) to yield twelve fractions (Fr.1–Fr.12) according to their TLC profiles. Fraction 10 (22.0 g) was subsequently subjected to silica gel column chromatography (60 × 6.5 cm) eluting with hexane-EtOAc (4:1) to yield 4 sub-fraction (Fr. 10–1 to 10–4). Fraction 10.3 (2.5 g) was chromatographed on a silica gel column (60 × 3.5 cm) using a gradient solvent system of hexane-acetone (6:1 to 0:1) to give compounds **1** (20 mg), **2** (2.5 mg), **3** (15 mg), **4** (2.5 mg), **5** (5.8 mg), **6** (12 mg), **7** (93.4 mg), **17** (3 mg), **33** (20 mg), **34** (5 mg), **35** (6.7 mg), **36** (10 mg). Fraction 6 (12.0 g) was subjected to silica gel column chromatography (60 × 6.5 cm) using with hexane-EtOAc (8:1) to yield 6 sub-fraction (Fr. 6–1 to 6–6). Fraction 6.4 war further purified over YMC RP-18 column with MeOH-H_2O (1:1 to 4:1) as an eluent to yield **8** (32 mg), **9** (20.1 mg), **10** (14 mg), **11** (16 mg), **12** (38.2 mg), **13** (3.4 mg), **14** (2.5 mg), **15** (3.4 mg), **16** (5.3 mg), **18** (7.5 mg), **20** (7.7 mg), **22** (13.5 mg), **30** (46 mg). Fraction 8 (15 g) was repeated on a silica gel column (60 × 3.5 cm) using a gradient solvent system of hexane-acetone (5:1 to 0:1) resulted in the isolation of compounds **19** (15 mg), **21** (24.3 mg), **23** (20 mg), **24** (25 mg), **25** (8 mg), **26** (2.6 mg), **27** (50 mg), **28** (6.7 mg), **29** (4.6 mg), **31** (7 mg), **32** (20 mg).

10-Oxomornigrol F **(1)**: yellowish amorphous powder; $[\alpha]_D^{24}$ − 29.7 (*c* 0.015, acetone); UV λ_{max} (acetone) 335 nm; IR (KBr) ν_{max} 3329 (OH), 1743 (CO), 1448, 1111 cm^{-1}; ^1H and ^{13}C NMR (CD_3OCD_3) data, see Table 1; HRESIMS *m/z* 459.1418 $[M + Na]^+$ (calcd for $C_{25}H_{24}O_7Na$, 459.1420).

(7″*R*)-(−)-6-(7″-Hydroxy-3″,8″-dimethyl-2″,8″-octadien-1″-yl)api-genin **(2)**: yellowish amorphous powder; $[\alpha]_D^{24}$ − 131.2 (*c* 0.005, MeOH); UV λ_{max} (MeOH) 232, 276, 331 nm; IR (KBr) ν_{max} 3327 (OH), 1737 (CO), 1452, 1024 cm^{-1}; ^1H and ^{13}C NMR (CD_3OCD_3) data, see Table 1; HREIMS *m/z* 422.1727 $[M]^+$ (calcd for $C_{25}H_{26}O_6$, 422.1729).

Ramumorin A **(3)**: yellowish amorphous powder; $[\alpha]_D^{24}$ + 36.9 (*c* 0.04, acetone); UV λ_{max} (acetone) 329.5, 336, 371.5, 390 nm; IR (KBr) ν_{max} 3411 (OH), 1711 (CO). 1360, 1220 cm^{-1}; ^1H and ^{13}C NMR (CD_3OCD_3) data, see Table 2; HRESIMS *m/z* 679.2528 $[M + H]^+$ (calcd for $C_{40}H_{39}O_{10}$, 679.2543).

Ramumorin B **(4)**: yellowish amorphous powder; $[\alpha]_D^{24}$ + 134.0 (*c* 0.01, MeOH); UV λ_{max} (MeOH) 233, 291.5 nm; IR (KBr) ν_{max} 3336 (OH), 1750 (CO), 1450, 1024 cm^{-1}; ^1H and ^{13}C NMR (CD_3OD) data, see Table 2; HRESIMS *m/z* 649.2465 $[M]^+$ (calcd for $C_{39}H_{37}O_9$, 649.2438).

(4*S*,7*S*,8*R*)-Trihydroxyoctadeca-5*Z*-enoic acid **(5)**: colorless oil; $[\alpha]_D^{24}$ − 64.0 (*c* 0.025, MeOH); UV λ_{max} (acetone) 236.5 nm; IR (KBr) ν_{max} 3328 (OH), 1720 (CO), 1024 cm^{-1}; ^1H and ^{13}C NMR (CD_3OD) data, see Table 1; HRESIMS *m/z* 353.2304 $[M + Na]^+$ (calcd for $C_{18}H_{34}O_5Na$, 353.2304).

2.4. Preparation of (S)- and (R)-MTPA ester derivatives

(*R*)-MTPA (α-methoxy-α-(trifluoromethyl)phenyl-acetyl) ester of aglycone **(a)** was prepared using Mosher's esterification method performed in NMR tubes. Aglycone (1.0 mg) and 4-(dimethylamino)-pyridine (0.2 mg) were transferred into an NMR tube, and this mixture was dried under vacuum. Deuterated pyridine (0.5 mL) and (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenyl-acetyl chloride (6.0 μL) were added into the NMR tube immediately under a N_2 gas stream, then the NMR tube was sealed and shaken to mix the sample and MTPA chloride evenly. The reaction NMR tube was permitted to stand at room temperature and monitored every 2 h by ^1H NMR. The derivatives of aglycone were afforded completely after 6 h, and then the reaction was stopped by shaking the unsealed NMR tube under air. In the manner described for (*R*)-MTPA ester of aglycone, (*S*)-MTPA ester of aglycone **(b)** (1.0 mg) was reacted in a second NMR tube with (*R*)-MTPA chloride (6.0 μL), and 4-(dimethylamino)-pyridine (0.2 mg) at room tempera-ture for 6 h using deuterated pyridine (0.5 mL) as solvent, to afford the (*S*)-MTPA derivative of aglycone.

(*S*)-MTPA ester of **2** (**2a**): ^1H NMR data (400 MHz, pyridine-d_5) δ_H 1.281 (2H, m, H-6″), 1.339 (3H, d, *J* = 0.8 Hz, H-10″), 1.679 (3H, s, H-

Table 1
^1H NMR and ^{13}C NMR data of **1**, **2** and **5**.

Position	**1**[a] δ_H (*J* in Hz)	δ_C	**2**[a] δ_H (*J* in Hz)	δ_C	**5**[b] δ_H (*J* in Hz)	δ_C	Position	**1**[a] δ_H (*J* in Hz)	δ_C	**2**[a] δ_H (*J* in Hz)	δ_C
1						177.1	1′		112.7		123.0
2		163.7		164.8	2.25, t (7.0)	35.0	2′		162.1	7.91, d (8.4)	129.1
3		117.3	6.62, d (1.6)	104.1	1.58, t (7.0)	26.1	3′	6.54, d (2.0)	104.2	7.02, d (8.4)	116.8
4		183.1		183.1	4.03, dd (6.5, 12.4)	73.0	4′		157.8		161.8
4a		105.0		105.2			5′	6.46, dd (2.0, 8.4)	108.4	7.02, d (8.4)	116.8
5		161.0		160.2	5.60–5.72, ovl[c] (12.4)	136.6	6′	7.17, d (8.4)	132.5	7.91, d (8.4)	129.1
6	6.34, s	99.3		112.3	5.60–5.72, ovl[c] (12.4)	131.1	1″			3.37, d (7.2)	21.9
7		162.6		162.5	3.39, m	75.8	2″			5.32, t (7.2)	123.4
8		107.3	6.60, s	94.1	3.89	76.5	3″				135.5
8a		156.8		156.5			4″			1.80, s	16.3
9	3.39, d (6.8)	22.4			1.23–1.47, m	33.6	5″			2.00, m	36.5
10	5.22, t (6.8)	123.5			1.23–1.48, m	38.3	6″			1.58, m	34.6
11		131.9			1.23–1.49, m	26.6	7″			3.97, t (6.4)	75.3
12	1.59, s	18.1			1.23–1.50, m	26.4	8″				149.3
13	1.58, d (1.2)	26.1			1.23–1.51, m	30.5	9″a			4.58, d (0.8)	110.3
14	3.83, s	35.5			1.23–1.52, m	30.4	9″b			4.71, d (0.8)	
15		198.9			1.23–1.53, m	30.2	10″			1.66, d (0.8)	17.8
16		145.4			1.23–1.54, m	33.1	5-OH	12.8, s		13.29, s	
17b	5.79, s	125.1			1.23–1.55, m	23.7					
17a	6.08, s										
18	1.80, s	18.0			0.89, t (7.5)	14.4					

[a] ^1H-NMR (400 MHz) and ^{13}C NMR (100 MHz) measured in CD_3OCD_3.
[b] ^1H-NMR (500 MHz) and ^{13}C NMR (125 MHz) measured in CD_3OD.
[c] Overlapped with other signal.

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