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Antiviral flavonoids from the root bark of *Morus alba* L.

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Abstract

A prenylated flavonoid, moralbanone, along with seven known compounds kuwanon S, mulberroside C, cyclomorusin, eudraflavone B hydroperoxide, oxydihydromorusin, leachianone G and α-acetyl-amyrin were isolated from the root bark of *Morus alba* L. Leachianone G showed potent antiviral activity ($IC_{50}=1.6$ μg/ml), whereas mulberroside C showed weak activity ($IC_{50}=75.4$ μg/ml) against herpes simplex type 1 virus (HSV-1). Their structures were elucidated by spectroscopic methods.

Keywords: *Morus alba*; Moraceae; Moralbanone; Leachianone; Mulberroside; Flavonoid; Antiviral; Herpes

1. Introduction

The cortex of the root bark of *Morus alba* L. (Moraceae) is a herb recorded in the Pharmacopoeia of the People's Republic of China (2000 edition) for removing heat from the lung, relieving asthma and inducing diuresis (Pharmacopoeia Commission of People's Republic of China, 2000). This and other species of *Morus* have been shown to exhibit anti-HIV, anti-oxidative, anti-hypotensive and cytotoxic activities (Nomura et al., 1978, 1980; Luo et al., 1995; Hosseinzadeh and Sadeghi, 1999; Kim et al., 1999; Doi et al., 2001; Shi et al., 2001). Plants of this genus are known to be rich in flavonoids (Nomura, 1999, 2001), a group of chemicals shown to have potent antiviral activities against herpes simplex virus, rhinovirus, rotavirus, human immunodeficiency virus, and various respiratory viruses (Alves et al., 1999; Lin et al., 1999; Bae et al., 2000; Bunyapraphatsara et al., 2000; Desideri et al., 2000; Abdel-Kader, 2001; Ma et al., 2002). In the course of our ongoing search for antiviral agents from natural sources (Jiang et al., 2001a,b; Ma et al., 2001a,b; Li et al., 2002), the root bark of *M. alba* was investigated. In this paper, we report the isolation and structural elucidation of one new compound (**1**) together with seven known compounds (**2**–**8**) (Fig. 1) and their antiviral activities against herpes simplex type 1 virus (HSV-1).

2. Results and discussion

Compound **1** was obtained as a yellow powder and its molecular formula was determined to be $C_{30}H_{34}O_6$ by HRESIMS. The ^{1}H NMR spectrum of **1** showed signals of ABX-type aromatic protons (B-ring) at δ 7.73 (1H, *d*, $J=9.0$ Hz), 6.50 (1H, *d*, $J=2.0$ Hz), 6.41 (1H, *dd*, $J=9.0$, 2.0 Hz), two isolated aromatic protons (δ 6.97, 6.27), protons of an (*E*, *E*)-3,7,11-trimethyl-2,6,10-dodecatrienyl (farnesyl) group [δ 5.17 (1H, *t*, $J=6.8$ Hz), 4.97 (2H, *m*), 3.42 (2H, *d*, $J=6.0$ Hz), 1.99 (2H, *m*), 1.93 (2H, *m*), 1.87 (2H, *m*), 1.79 (2H, *m*), 1.75 (3H, *s*), 1.59 (3H, *s*), 1.49 (3H, *s*), 1.45 (3H, *s*)] and protons of four hydroxyl groups (δ 12.98, 10.74, 10.69, 10.17). The presence of the farnesyl group was also confirmed by ^{13}C spectroscopic analogues and NOE correlations. The C-2′ and C-4′ positions in ring B were substituted by hydroxyl groups, which can be determined by HMBC correlations (Fig. 2). The signal at the 4′-OH group has correlations with C-3′ (δ 103.2) and C-5′ (δ 107.8), whereas the 2′-OH (δ 10.69) functionality has correlations with C-1′ (δ 108.9). Accordingly, the farnesyl group must be located at either the C-6 or C-8 positions of ring A. However, according to the chemical shifts of C-6 (98.0) and C-8 (105.8), the

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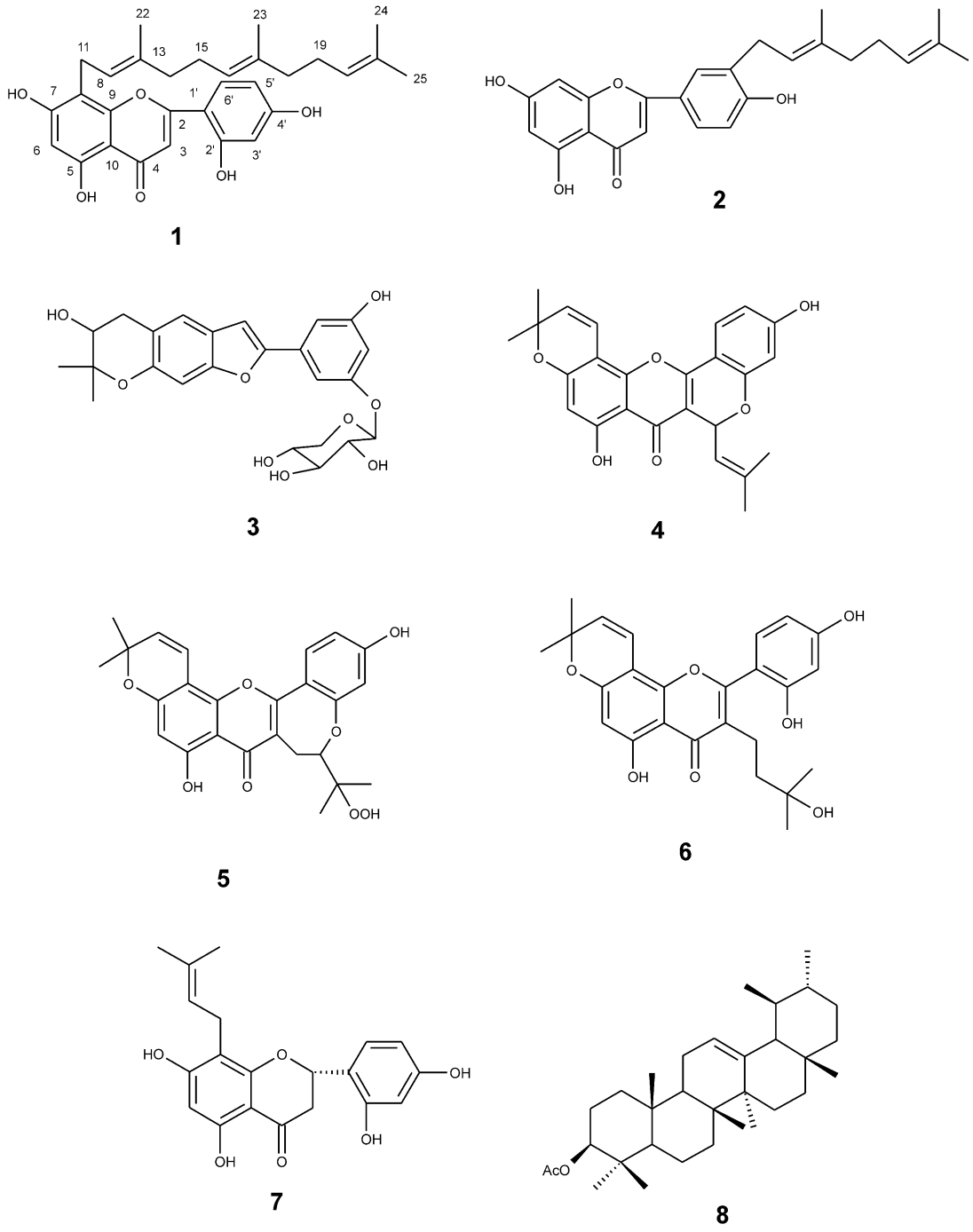

Fig. 1. Structural formulae of compounds **1**–**8**.

farnesyl group should be at C-8. Otherwise, the chemical shifts C-6 and C-8 would be around δ 112.0 and δ 93.9, respecitvely, as in albanins D and E (Fukai and Nomura, 1991). This substitution was also confirmed by the HMBC correlations between 11-H and C-11 (105.8), as well as C-9 (154.6) and C-7 (161.2). On the basis of these observations, the structure of compound **1** is 8-(3,7,11-trimethyl-2,6,10-dodecatrienyl)-2-(2,4-dihydroxyphenyl)-5,7-dihydroxy-4*H*-1-benzopyran-4-one. It is here named as moralbanone.

The spectral data for the known compounds **2** (Fukai et al., 1985), **3** (Hirakura et al., 1986), **4** (Nomura et al., 1976), **5** (Fujimoto et al., 1984), **6** (Nomura et al., 1978), **7** (Iinuma et al., 1993) and **8** (Pisova and Soucek, 1973) were in agreement with those reported in the literature.

Leachianone G (**7**) showed potent antiviral activity against HSV-1 on vero cells ($IC_{50}=1.6$ μg/ml, $CC_{50}=15.5$ μg/ml). Mulberroside C (**3**) also demonstrated weak antiviral activity against HSV-1 ($IC_{50}=75.4$ μg/ml, $CC_{50}=250$ μg/ml). The other compounds were

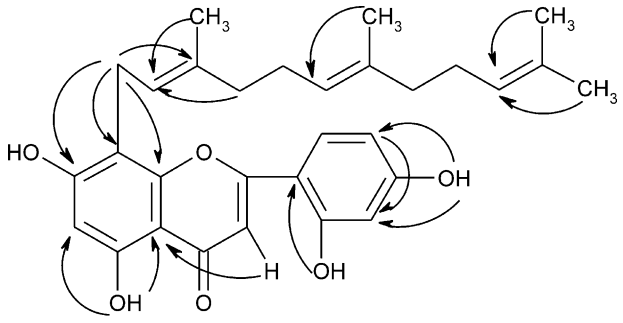

Fig. 2. HMBC correlations for compound **1**.

inactive with IC_{50} values over 100 μg/ml. Acyclovir, which was used as a positive control, also showed potent anti-HSV-1 activity (IC_{50} = 1.3 μg/ml, CC_{50} = 167 μg/ml).

3. Experimental

3.1. General experimental procedures

UV spectra were run on a Beckman DU 650 spectrophotometer in MeOH. The NMR spectra were recorded on an INDVA-500 spectrometer in DMSO-d_6. Mass spectra were obtained with a Finnigan MAT TSQ 7000 mass spectrometer. HRESIMS measurements were made on an APEX 47e FTMS spectrometer.

3.2. Plant material

The root bark of *Morus alba* was purchased from the Chinese Pharmacy of Guangzhou University of Traditional Chinese Medicine, Guangzhou, PR China. A voucher specimen (No. 2391) was deposited at the Museum, Institute of Chinese Medicine, The Chinese University of Hong Kong.

3.3. Extraction and isolation

The dried root bark (1.7 kg) was extracted with hot aqueous EtOH (3:7, 3×6 L) under conditions of reflux. With the resulting extracts combined and concentrated under reduced pressure to obtain a residue (180 g). The residue was suspended in 1 l water and filtered three times to obtain insoluble and aqueous portions. The insoluble residue (82.52 g) was next subjected to silica gel CC ($CHCl_3$:MeOH, 8:2), Sephadex LH-20 (MeOH:H_2O, 6: 4) and Rp-18 [MeOH: H_2O (7:3, 6:4)], respectively, to give moralbanone (**1**) (7 mg), kuwanon S (**2**) (8 mg), mulberroside C (**3**) (53 mg), cyclomorusin A (**4**) (35 mg), eudraflavone B hydroperoxide (**5**) (27 mg), oxydihydromorusin (**6**) (30 mg), leachianone G (**7**) (9 mg) and α-acetyl-amyrin (**8**) (32 mg).

Moralbanone (**1**): yellow powder; UV (MeOH) λ_{max} (log ε) 308 (4.32), 373 (4.41). HRESIMS: m/z 491.2421 [calc. for $C_{30}H_{34}O_6$ $(M+H)^+$, 491.2387]. ^{1}H NMR (DMSO-d_6, 500 MHz): 12.98 (1H, *s*, 5-OH), 10.74 (1H, *s*, 2′-OH), 10.69 (1H, *s*, 7-OH), 10.17 (1H, *s*, 4′-OH), 7.73 (1H, *d*, J = 9.0 Hz, 6′-H), 6.97 (1H, *s*, 3-H), 6.50 (1H, *d*,J = 2.0 Hz, 3′-H), 6.41 (1H, *dd*, J = 9.0, 2.0 Hz, 5′-H), 6.27 (1H, *s*, 6-H), 5.17 (1H, *t*, J = 6.8 Hz, 12-H), 4.97 (2H, *m*, 16, 20-H), 3.42 (2H, *d*, J = 6.0 Hz, 11-H), 1.99 (2H, *m*, 15-H), 1.93 (2H, *m*, 14-H), 1.87 (2H, *m*, 19-H), 1.79 (2H, *m*, 18-H), 1.75 (3H, *s*, 22-H), 1.59 (3H, *s*, 25-H), 1.49 (3H, *s*, 23-H), 1.45(3H, *s*, 24-H). ^{13}C NMR (DMSO-d_6, 125 MHz) δ 182.1 (C-4), 161.6 (C-2), 161.6 (C-4′), 161.2 (C-7), 158.9 (C-5), 158.7 (C-2′), 154.6 (C-9), 134.2(C-17), 134.2 (C-13), 130.4 (C-21), 129.5 (C-6′), 124.0 (C-20), 123.6 (C-16), 122.6 (C-12), 108.9 (C-1′), 107.8 (C-5′), 106.5 (C-3), 105.8 (C-8), 103.5 (C-10), 103.2 (C-3′), 98.0 (C-6), 39.2 (C-14), 39.2 (C-18), 26.0 (C-19), 25.8 (C-15), 25.4 (C-25), 21.2 (C-11), 17.4 (C-24), 16.0 (C-22), 15.7 (C-23).

3.4. Antiviral assays

The antiviral activities and cytotoxic effects of compounds **1**–**8** as well as control antiviral drug acyclovir were determined using the viral cytopathic effect assay. The procedures used for the antiviral and cytotoxicity assays have been previously described (Ma et al., 2001b). HSV-1 (15577) strain and vero cells were obtained from American Type Culture Collection. The antiviral activity of each sample was expressed in μg/ml as 50% inhibitory concentration (IC_{50}), and cytotoxicity on vero cells was expressed as 50% cytotoxic concentration (CC_{50}).

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