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Phytochemistry 65 (2004) 3135–3141
PHYTOCHEMISTRY
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Guangsangons F–J, anti-oxidant and anti-inflammatory Diels–Alder type adducts, from *Morus macroura* Miq.

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Received 26 November 2003; received in revised form 1 June 2004

Abstract

Five Diels–Alder type adducts, named guangsangons F, G, H, I, and J, along with two known compounds, mulberrofuran J and kuwanon J, were isolated from *Morus macroura* Miq. Their structures were determined by means of spectroscopic analyses and chemical methods. These compounds were regarded biogenetically as Diels–Alder type adducts of dehydroprenylphenols and chalcone derivatives, and ^{1}H NMR variable temperature experiments suggested that they all existed as an equilibrium mixture of conformational isomers in solution. Among the isolated new compounds, guangsangons H, I, and J displayed potent antioxidant activities and moderate anti-inflammatory activities.

Keywords: *Morus macroura* Miq.; Moraceae; Diels–Alder adduct; Guangsangon F–J

1. Introduction

Morus macroura Miq. belongs to the economically and medically important genus *Morus*, whose leaves have been an indispensable food source for silk-worms, and its root barks have been used to treat diabetes, arthritis and rheumatism in Chinese herbal medicine (Sun et al., 2001). *Morus* has 12 known species, nine of them in China. Previously, many compounds which were regarded biogenetically as Diels–Alder adducts were isolated from *M. alba*, *M. lhou*, *M. bombycis*, and related species (Nomura et al., 1982, 1983; Hano et al., 1984, 1990; Hirakura et al., 1985a,b; Shen and Lin, 2001). These biological properties, viewed together with the fact that *M. macroura* Miq. is a plant that is hitherto not so well studied, prompted a phytochemical investigation of this plant. Seven compounds (**1**–**7**) were isolated from the EtOH extract, of which guangsangon H (**3**), guangsangon I (**6**), and guangsangon J (**7**) exhibited potent antioxidant activities, with the inhibitory rates of malondialdehyde being 93.1% (**3**), 93.9% (**6**) and 91.1% (**7**) at 10 μM; and moderate anti-inflammatory activities, with the inhibitory rates of release of lysosome enzyme from polymorphonuclear leukocytes of rats being 49.4% (**3**), 43.8% (**6**) and 41.3% (**7**) at 10 μM, respectively (see Chart 1).

2. Results and discussion

The EtOH extract of *M. macroura* Miq. was partitioned successively with $CHCl_3$ and EtOAc. The EtOAc fraction was sequentially subjected to column chromatography over silica gel, silica gel RP-18 and Sephadex LH-20 to give seven compounds, including five new compounds named guangsangons F–J. The present paper deals with the isolation and structure elucidation of these compounds.

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doi:10.1016/j.phytochem.2004.07.010

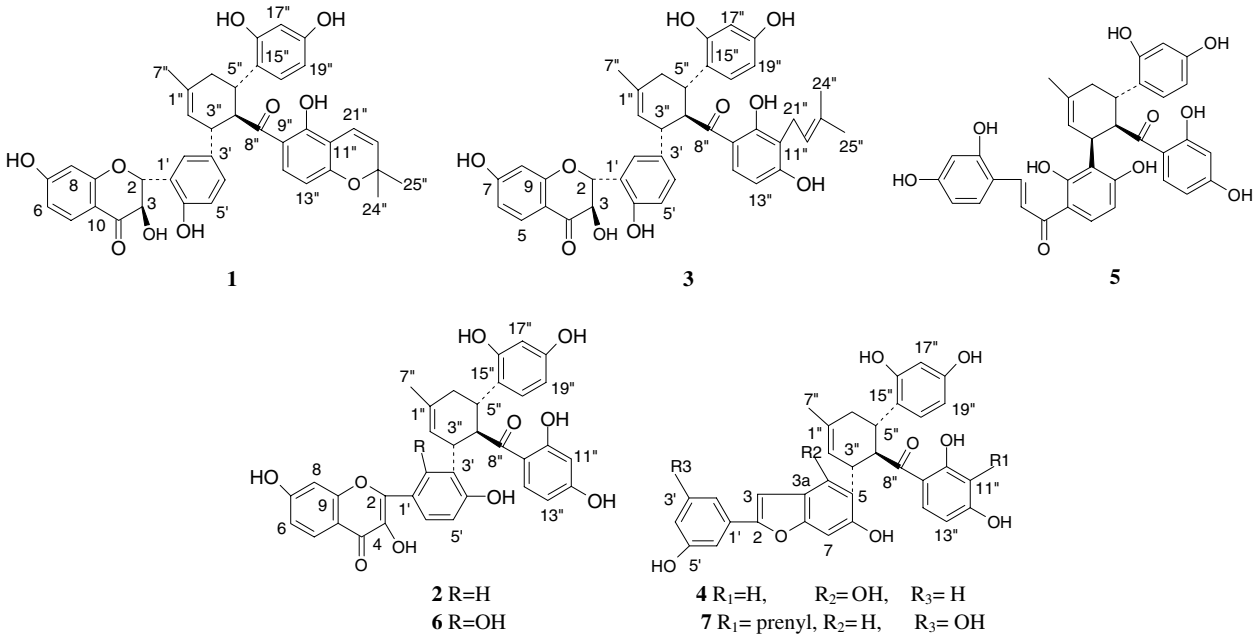

Chart 1. Diels–Alder type adducts from *Morus macroura* (**1**–**7**).

Guangsangon F (**1**) was obtained as a brown amorphous powder, and gave a dark green color with the methanolic ferric chloride test, and was positive to the magnesium hydrochloric acid test. The molecular formula was determined to be $C_{40}H_{36}O_{10}$ by HRFAB-MS, which showed a quasi-molecular ion peak at *m*/*z* 675.2244 $[M-H]^-$. The UV spectrum exhibited maxima at 239 and 279 nm. The IR spectrum displayed absorption bands at 3275, 1650, 1625, 1606, 1506 and 1458 cm^{-1} assignable to hydroxyl, conjugated carbonyl and benzene ring moieties. The ^1H NMR spectrum of **1** displayed the following proton signals: a singlet at δ 13.48 (1H, *s*, HO-10″) for a hydrogen bonded hydroxyl group; one set of ABX aromatic protons at δ 6.22 (1H, *d*, $J = 2.5$ Hz, H-17″), 6.08 (1H, *dd*, $J = 2.5$, 9.0 Hz, H-19″) and 6.88 (1H, *d*, $J = 9.0$ Hz, H-20″) which were assignable to a 2,4-dihydroxyphenyl moiety; two pairs of *ortho*-coupled aromatic protons at δ 5.94 (1H, *d*, $J = 9.5$ Hz, H-13″), 7.05 (1H, *d*, $J = 9.5$ Hz, H-14″), 6.46 (1H, *d*, $J = 10.0$ Hz, H-21″) and 5.54 (1H, *d*, $J = 10.0$ Hz, H-22″), as well as two methyl protons at δ 1.32 (3H, *s*, H-25″) and 1.33 (3H, *s*, H-24″) which were assignable to the methyls of a 2,2-dimethylchromene moiety; two sets of ABX aromatic protons at δ 6.44 (1H, *d*, $J = 2.5$ Hz, H-8), 6.63 (1H, *dd*, $J = 2.5$, 9.0 Hz, H-6), 7.74 (1H, *d*, $J = 9.0$ Hz, H-5), and 6.59 (1H, *d*, $J = 8.0$ Hz, H-3′), 7.06 (1H, *dd*, $J = 2.0$, 8.0 Hz, H-4′) and 7.56 (1H, *d*, $J = 2.0$ Hz, H-6′), as well as a pair of vicinal-coupled aliphatic protons at δ 4.52 (1H, *d*, $J = 10.0$ Hz, H-3) and 5.01 (1H, *d*, $J = 10.0$ Hz, H-2), which were attributable to a dihydroflavonol moiety; and proton resonances corresponding to a trisubstituted methylcyclohexene ring at δ 5.40 (1H, *s*, H-2″), 4.31 (1H, *br s*, H-3″), 4.37 (1H, *br s*, H-4″), 3.67 (1H, *br s*, H-5″), 2.19 (1H, *m*, H-6″), 2.59 (1H, *m*, H-6″) and 1.77 (3H, *s*, H-7″). The long-range correlations between H-20″ and C-5″, as well as H-6′ (or H-4′) and C-3″ (Fig. 1), suggested that the 2,4-dihydroxyphenyl and dihydroflavonol moieties were connected to the methylcyclohexene ring at C-5″ and C-3″. Therefore, the 2,2-dimethylchromene moiety was connected to the methylcyclohexene ring at C-4″.

In the ^1H NMR spectrum (CD_3COCD_3) of **1**, the proton signals of the methylcyclohexene ring were broadened. This observation suggested that **1** existed as an equilibrium mixture of conformational isomers in solution, and hence variable temperature studies were then carried out. Thus, recorded in DMSO-d_6 at 100 °C, the proton signals of the methylcyclohexene ring was resolvable as shown in Fig. 2. The coupling constants between H-4″ and H-5″, as well as H-4″ and H-3″ were observed to be 10.0 Hz, which suggested H-3″, H-4″ and H-5″ to be axial and the corresponding bulky groups at C-3″, C-4″ and C-5″ were all equatorial; additionally, the relative configurations of three bulky groups were all-*trans* as shown in Fig. 2. The stereochemistry of **1** with the absolute configuration of *R* (C-2), *R* (C-3), *R* (C-3″), *R* (C-4″) and *S* (C-5″) was determined by analysis of CD spectrum, which showed a positive Cotton effect at 334 nm, along with two negative Cotton effects at 298 and 304 nm (Gaffield, 1970; Hano et al., 1988a,b).

Guangsangon G (**2**) was isolated and purified as a yellow amorphous powder, and gave a reddish brown color with the methanolic ferric chloride test, and

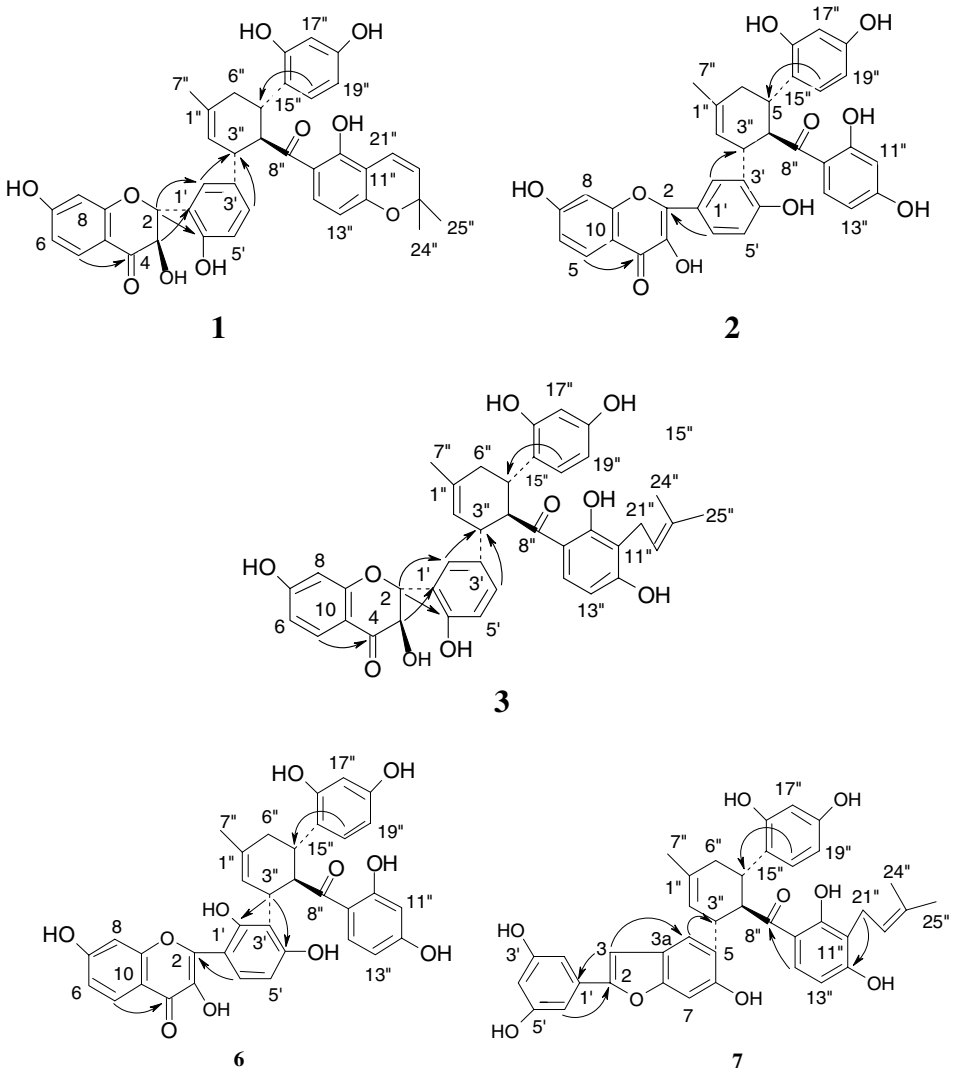

Fig. 1. Key HMBC (curved arrows) correlations for **1**, **2**, **3**, **6**, and **7**.

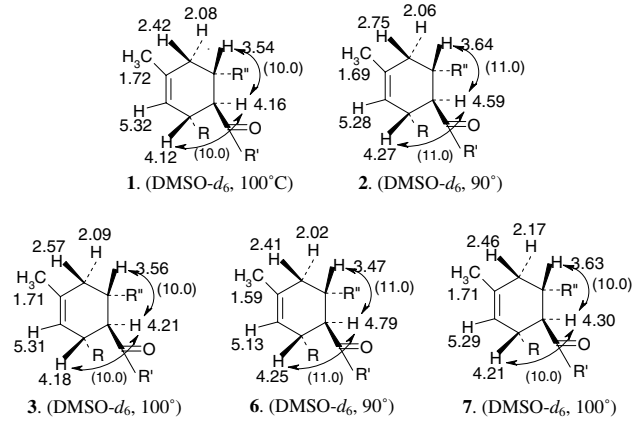

Fig. 2. ^{1}H NMR chemical shifts and key coupling constants of the methylcyclohexene ring protons for **1**, **2**, **3**, **6** and **7**.

was positive to the magnesium hydrochloric acid test. The HRFAB-MS showed a quasi-molecular ion peak at *m*/*z* 607.1623 $[M - H]^-$, consistent with a molecular formula of $C_{35}H_{28}O_{10}$. The UV spectrum displayed maxima at 213, 259, 285 and 320 nm, and showed a red shift upon addition of aluminum chloride, suggesting the presence of a hydrogen bonded carbonyl group (Sherif et al., 1980). The IR spectrum showed absorption bands attributable to hydroxyl (3340 cm^{-1}), conjugated carbonyl (1650, 1622 cm^{-1}) and benzene ring (1606, 1506 cm^{-1}) moieties. The ^{1}H NMR spectrum of **2** exhibited proton signals as follows: a singlet at δ 13.32 (1H, *s*, OH-10″) which was considered due to a hydrogen bonded hydroxyl group; one set of ABX aromatic protons which were ascribable to a 2,4-dihydroxyphenyl moiety at δ 6.28 (1H, *d*, $J = 2.0$ Hz, H-17″), 6.11 (1H, *dd*, $J = 2.0$, 9.0 Hz, H-19″) and 6.87 (1H, *d*, $J = 9.0$ Hz, H-20″); another set of ABX aromatic

protons which were assignable to the 2,4-dihydroxybenzoyl unit at δ 5.93 (1H, *d*, $J = 2.0$ Hz, H-11″), 6.06 (1H, *dd*, $J = 2.0$, 9.0 Hz, H-13″) and 7.81 (1H, *d*, $J = 9.0$ Hz, H-14″); two other sets of ABX aromatic protons that were attributable to a flavonol moiety at δ 8.01 (1H, *d*, $J = 8.5$ Hz, H-5), 6.97 (1H, *d*, $J = 8.5$, 2.0 Hz, H-6), 7.17 (1H, *d*, $J = 2.0$ Hz, H-8), and 8.22 (1H, *d*, $J = 2.0$ Hz, H-2′), 7.85 (1H, *dd*, $J = 2.0$, 8.5 Hz, H-6′) and 6.78 (1H, *d*, $J = 2.0$ Hz, H-5′); and protons attributable to a trisubstituted methylcyclohexene ring at δ 1.78 (1H, *s*, H-7″), 2.08 (1H, *m*, H-6″), 2.81 (1H, *m*, H-6″), 3.67 (1H, *br s*, H-5″), 4.64 (1H, *br s*, H-4″), 4.40 (1H, *br s*, H-3″) and 5.40 (1H, *s*, H-2″). In the HMBC experiment, H-20″ and H-2′ showed correlations with C-5″ and C-3″, respectively, which proved that the 2,4-dihydroxyphenyl and flavonol moieties were attached to the methylcyclohexene ring at C-5″and C-3″. Thus, the 2,4-dihydroxybenzoyl moiety was connected to the methylcyclohexene ring at C-4″. When measured at room temperature (CD_3COCD_3), the protons at C-3″, C-4″ and C-5″ in the methylcyclohexene ring also showed broadened signals to such an extent that the spectrum could not be satisfactorily analyzed. When recorded at 90 °C in DMSO-d_6, the proton resonances were resolvable as follows (Fig. 2): δ 4.27 (1H, *br d*, $J = 10.0$ Hz, H-3″), 4.59 (1H, *br t*, $J = 10.0$ Hz, H-4″) and 3.64 (1H, *br s*, H-5″). Both coupling constants of H-4″/H-3″ and H-4″/H-5″ were 11.0 Hz, which suggested that **2** had the same disposition concerning three bulky groups on the methylcyclohexene ring as **1**, and therefore had the same relative configuration as **1** (all-*trans*). In a previous paper (Hano et al., 1988a,b), it was reported that the absolute configurations of the chiral centers on the methylcyclohexene ring in the all-*trans* adducts were established as *R* (C-3″), *R* (C-4″) and *S* (C-5″), while in the *cis*-*trans* adducts as *S* (C-3″), *R* (C-4″) and *S* (C-5″). Furthermore, the all-*trans* adducts exhibited negative optical rotation values while the *cis*-*trans* adducts showed positive ones. Since **2** with an all-*trans* configuration displayed a negative optical rotation, the absolute configurations of three chiral carbons were assigned as *R* (C-3″), *R* -(C-4″) and *S* (C-5″).

Guangsangon H (**3**) was obtained as a yellow amorphous powder. The molecular formula was determined to be $C_{40}H_{38}O_{10}$ by HRFAB-MS, which displayed a quasi-molecular ion peak at *m*/*z* 677.2407 $[M - H]^-$. A comparison of the NMR (^1H, ^{13}C) spectral data of **1** with those of **3** indicated the latter differed from **1** only in the moiety at C-4″ of the methylcyclohexene ring. Instead of a 2,2-dimethylchromene moiety of **1**, a 2,4-dihydroxy-3-prenylbenzoyl group was present in **3**. According to the results of ^1H NMR variable temperature experiment and CD measurement, the configuration of **3** was found to be in agreement with **1**.

Guangsangon I (**6**) was isolated as a brown amorphous powder. In the HRFAB-MS, **6** gave a quasi-molecular ion peak at *m*/*z* 625.1688 $[M + H]^+$, corresponding to a molecular formula of $C_{35}H_{28}O_{11}$. By analysis of the NMR (^1H, ^{13}C) spectroscopic data, compound **6** was found to be identical with **2** except for the difference of B ring of the flavonol moiety. In contrast to the 7,4′-dihydroflavonol unit of **2**, a 7,2′, 4′-trihydroflavonol unit was connected to the methylcyclohexene ring of **6**. Based on the results of ^1H NMR variable temperature experiment and optical rotation measurement, **6** was suggested to possess the same configuration as **2**.

Guangsangon J (**7**) was isolated and purified as a brown amorphous powder, and gave a dark green color with the methanolic ferric chloride test. The HRFAB-MS showed a quasi-molecular ion peak at *m*/*z* 647.2289 $[M - H]^-$, corresponding to a molecular formula of $C_{39}H_{36}O_9$. The UV spectrum exhibited maxima at 212, 301 and 329 nm assignable to the presence of an 2-arylbenzofuran moiety (Nomura and Fukai, 1981); moreover, its absorption bands did not show a bathochromic shift in the presence of aluminum chloride, suggesting that a prenyl group was located *ortho* to a hydrogen bonded hydroxyl group (Sherif et al., 1980). In the IR spectrum, the absorption bands due to hydroxyl (3349 cm^{-1}), conjugated carbonyl (1650 cm^{-1}) and benzene ring (1616, 1506 and 1456 cm^{-1}) moieties were observed. The ^1H NMR spectrum of **7** showed proton signals as follows: one singlet at δ 13.47 (1H, *s*, HO-10″) that was assignable to a hydrogen bonded hydroxyl group; one set of ABX type aromatic protons at δ 6.22 (1H, *d*, $J = 1.5$ Hz, H-17″), 6.11 (1H, *d*, $J = 1.5$, 8.5 Hz, H-19″) and 6.93 (1H, *d*, $J = 8.5$ Hz, H-20″) that were consistent with a 2,4-dihydroxyphenyl group; a pair of *ortho*-coupled aromatic protons at δ 5.98 (1H, *d*, $J = 8.5$ Hz, H-13″) and 7.35 (1H, *d*, $J = 8.5$ Hz, H-14″) due to the presence of a 2,4-dihydroxy-3-prenylbenzoyl moiety; one set of AX_2 aromatic protons at δ 6.35 (1H, *t*, $J = 2.0$ Hz, H-4′) and 6.80 (2H, *d*, $J = 2.0$ Hz, H-2′ and H-6′), as well as three singlets at δ 6.96 (1H, *s*, H-3), 7.41 (1H, *s*, H-4) and 6.74 (1H, *s*, H-7) that were assignable to a 2-arylbenzofuran moiety; nine protons attributable to a prenyl group at δ 3.11 (2H, *d*, $J = 7.0$ Hz, H-21″), 5.05 (1H, *t*, $J = 7.0$ Hz, H-22″), 1.64 (3H, *s*, H-24″) and 1.53 (3H, *s*, H-25″); and protons for a trisubstituted methylcyclohexene ring at δ 5.41 (1H, *s*, H-2″), 4.32 (1H, *br s*, H-3″), 4.49 (1H, *br s*, H-4″), 3.69 (1H, *br s*, H-5″), 2.21 (1H, *m*, H-6″), 2.60 (1H, *m*, H-6″) and 1.74 (3H, *s*, H-7″). The location of the 2,4-dihydroxyphenyl, 2,4-dihydroxy-3-prenylbenzoyl and 2-arylbenzofuran moieties on the methylcyclohexene ring was determined by HMBC correlations, and key HMBC correlations are shown in Fig. 1. Owing to the slow conversion of conformational isomers, the protons at C-3″, C-4″ and C-5″ also showed broad signals

Table 1
^{13}C NMR spectral data of **1**, **2**, **3**, **6** and **7** in acetone-d_6 (125 MHz, 25 °C)

Compound	1	2	3	6	7
2	84.9	145.6	84.8	147.9	155.3
3	73.9	137.9	73.4	137.4	102.3
3α					123.5
4	193.1	173.2	193.0	173.9	122.8
5	133.3	127.5	131.4	127.5	128.5
6	113.2	115.1	111.7	115.7	155.4
7	164.6	163.5	164.5	163.6	98.2
7α					154.1
8	103.9	103.6	103.8	102.9	
9	165.8	158.0	162.2	158.6	
10	115.6	115.6	112.8	108.7	
1′	129.5	124.1	129.9	112.7	131.2
2′	155.9	130.2	156.3	159.1	103.9
3′	116.1	131.4	115.5	118.8	159.8
4′	128.4	157.5	127.8	156.9	102.7
5′	131.2	116.3	130.8	108.7	159.8
6′	129.4	128.1	129.8	128.8	103.9
1″	134.8	134.5	134.4	134.3	134.5
2″	125.4	125.5	125.6	125.4	125.8
3″	42.2	42.5	41.7	39.2	43.2
4″	51.2	50.6	50.8	46.2	51.1
5″	42.2	42.5	41.7	39.2	43.2
6″	37.7	37.3	37.8	38.6	37.8
7″	23.4	23.4	23.3	23.4	23.5
8″	209.7	209.4	209.0	209.4	209.6
9″	116.4	115.8	115.3	115.7	115.8
10″	160.3	164.9	166.2	165.3	163.8
11″	108.7	102.8	114.3	102.5	115.4
12″	159.7	159.8	163.3	164.9	162.0
13″	108.2	107.5	107.4	107.1	107.7
14″	133.4	133.9	130.8	133.1	133.5
15″	121.7	121.7	121.7	122.1	121.7
16″	157.4	157.9	157.1	156.9	157.4
17″	103.7	103.3	103.7	103.5	103.6
18″	156.5	157.2	155.6	156.5	156.6
19″	107.6	107.8	107.3	108.1	107.2
20″	129.8	129.8	129.5	132.6	129.5
21″	115.4		22.1		22.2
22″	128.3		123.5		123.5
23″	78.0		130.9		131.1
24″	28.3		17.6		17.8
25″	28.4		25.6		25.8

(CD_3COCD_3). Recorded in DMSO-d_6 at 100 °C, the corresponding proton signals were as follows (Fig. 2): δ 4.21 (1H, *br d*, $J = 10.0$ Hz, H-3″), 4.30 (1H, *br t*, $J = 10.0$ Hz, H-4″) and 3.46 (1H, *br s*, H-5″). The proton at C-4″ was observed to be coupled with H-3″ (J, 10.0 Hz) and also with H-5″ (J, 10.0 Hz), suggesting that three protons were all axial and **7** had the same relative configuration (all-*trans*) as compounds mentioned above. Since compound **7** exhibited a negative optical rotation, the absolute configurations of three unsymmetrical carbons were assigned as *R* (C-3″), *R* (C-4″) and *S* (C-5″) (Hano et al., 1988a,b), respectively.

Two known compounds, mulberrofuran J (**4**) and kuwanon J (**5**) were identified by comparison of their physical and spectral data ($[\alpha]_D$, UV, IR, ^1H NMR, ^{13}C NMR, MS) with reported values or those of commercial samples.

Table 2
Biological activity of compounds, **3**, **6** and **7** at concentrations of 10 μM

Compound	3	6	7	Vit E	ginkgolide B
Antioxidant (%)	93.1	93.9	91.1	33.4	
Anti-inflammatory (%)	49.4	43.8	41.3		58.9

Compounds, **3**, **6** and **7** were evaluated for their antioxidant and anti-inflammatory activities using established methods (Lu and Liu, 1991; Nie and Wang, 2003), and the inhibitory rates of malondialdehyde and release of lysosome enzyme from polymorphonuclear leukocytes of rats were showed in Table 2. Based on the bioassay results, it is concluded that these compounds possess antioxidant activity better than that of the positive control (Vit E), and also had anti-inflammatory activities close to that of the positive control (ginkgolide B).

3. Experimental

3.1. General

UV spectra were obtained on a Shimadzu UV-160 spectrophotometer, whereas NMR spectra were recorded on a Varian Unity INOVA 500 at 500 MHz (^1H) and 125 MHz (^{13}C), with TMS as internal standard. IR spectra were recorded on a Perkin–Elmer 683 infrared spectrometer with KBr disks, whereas optical rotations were measured on a Perkin–Elmer 241 polarimeter. FABMS and HRFAB-MS were recorded on an Autospec-Ultima ETOF MS spectrometer. Silica gel (200–300 mesh) for CC and silica gel GF254 for prep. TLC were obtained from Qingdao Marine Chemical Factory (Qingdao, People's Republic of China). Precoated plates of silica gel GF254 and silica gel RP-18 F254s (Merck) were used for TLC, and detected under UV light. HPLC separation was performed on CONSTA METRIC 3200 and a UV detector at 254 nm.

3.2. Plant material

Morus macroura Miq. was collected in Xishuangbanna, in Yunnan Province, Peoples Republic of China, in November 2002, and identified by Professor Kai-Jiao Jiang, Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences & Peking Union Medical College. A voucher specimen (EX97038) has been deposited at the Herbarium of Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College.

3.3. Extraction and isolation

The air-dried stem bark (9.8 kg) of *Morus macroura* Miq. was finely cut and extracted with EtOH (3 × 10 l, 3 h) under reflux condition. Evaporation of the solvent under reduced pressure provided an ethanolic extract (684.2 g), which was dissolved and suspended in H_2O (3.0 l), and partitioned with $CHCl_3$ (3 × 4 l), and EtOAc (3 × 4 l). The resulting EtOAc fraction (95.4 g) was subjected to CC on silica gel (10 × 120 cm), eluted with $CHCl_3$ and MeOH in increasing polarity, and these fractions were combined according to TLC monitoring to give eight fractions. Fraction IV (8.6 g) was subjected to reversed-phase silica gel chromatograph [eluted with MeOH–H_2O (30:70–50:50–70:30, v/v)] giving kuwanon J (**5**, 13 mg) and a mixture (26 mg). The latter was further separated by C_{18} HPLC (MeOH–H_2O, 40/60) to give guangsangon F (**1**, 9 mg) and guangsangon H (**3**, 10 mg). Fraction V (13.9 g) was subjected to reversed-phase silica gel CC [eluted by MeOH–H_2O, 50:50, v/v] and purified by prep. TLC [$CHCl_3$–MeOH, 7:3, v/v] to give mulberrofuran J (**4**, 13 mg) and guangsangon G (**2**, 8 mg). Fraction VI (13.7 g) was separated by CC on reversed-phase silica gel [eluted by MeOH–H_2O, 30:70–50:50:70:30, v/v], and purified by prep. TLC [$CHCl_3$–MeOH, 7/3, v/v] and CC over Sephadex LH-20 to give guangsangon I (**6**, 83 mg), and guangsangon J (**7**, 16 mg).

3.4. Guangsangon F (*1*)

Brown amorphous powder; $[\alpha]_D^{21} = -112.1°$ (c = 0.13, MeOH); UV (MeOH) λ_{max}: 239, 279 nm; IR (KBr) ν_{max}: 3275, 1650, 1625, 1606, 1506, 1458 cm^{-1}; ^1H NMR (CD_3COCD_3): δ 13.48 (1H, *s*, HO-10″), 7.74 (1H, *d*, J = 9.0 Hz, H-5), 7.56 (1H, *d*, J = 2.0 Hz, H-6′), 7.06 (1H, *dd*, J = 2.0, 8.0 Hz, H-4′), 7.05 (1H, *d*, J = 9.5 Hz, H-14″), 6.88 (1H, *d*, J = 9.0 Hz, H-20″), 6.63 (1H, *d*, J = 9.0, 2.5 Hz, H-6), 6.59 (1H, *d*, J = 8.0 Hz, H-3′), 6.46 (1H, *d*, J = 10.0 Hz, H-21″), 6.44 (1H, *d*, J = 2.5 Hz, H-8), 6.22 (1H, *d*, J = 2.5 Hz, H-17″), 6.08 (1H, *dd*, J = 2.5, 9.0 Hz, H-19″), 5.94 (1H, *d*, J = 9.5 Hz, H-13″), 5.54 (1H, *d*, J = 10.0 Hz, H-22″), 5.40 (1H, *s*, H-2″), 5.01 (1H, *d*, J = 10.0 Hz, H-2), 4.52 (1H, *d*, J = 10.0 Hz, H-3), 4.31 (1H, *br s*, H-3″), 4.37 (1H, *br s*, H-4″), 3.67 (1H, *br s*, H-5″), 2.59 (1H, *m*, H-6″), 2.19 (1H, *m*, H-6″), 1.77 (3H, *s*, H-7″), 1.33 (3H, *s*, H-24″), 1.32 (3H, *s*, H-25″); ^{13}C NMR data see Table 1; CD ($c = 7.40 \times 10^{-4}$ M, MeOH): $\Delta\varepsilon_{298}$ −14.0, $\Delta\varepsilon_{304}$ −13.8, $\Delta\varepsilon_{332}$ +6.1; HRFAB-MS *m/z*: 675.2244 (calcd for $C_{40}H_{35}O_{10}$, $[M - H]^-$, 675.2236).

3.5. Guangsangon G (*2*)

Yellow amorphous powder; $[\alpha]_D^{21} = -469.1°$ (c = 0.11, MeOH); UV (MeOH) λ_{max}: 213, 259, 285, 320 nm; IR (KBr) ν_{max}: 3340, 1650, 1622, 1606, 1506 cm^{-1}; ^1H NMR (CD_3COCD_3): δ 13.32 (1H, *s*, HO-10″), 8.22 (1H, *d*, J = 2.0 Hz, H-2′), 8.01 (1H, *d*, J = 2.0 Hz, H-5), 7.85 (1H, *dd*, J = 2.0, 8.5 Hz, H-6′), 7.81 (1H, *d*, J = 9.0 Hz, H-14″), 7.17 (1H, *d*, J = 2.0 Hz, H-8), 6.97 (1H, *dd*, J = 2.0, 8.5 Hz, H-6), 6.87 (1H, *d*, J = 9.0 Hz, H-20″), 6.78 (1H, *d*, J = 8.5 Hz, H-5′), 6.28 (1H, *d*, J = 2.0 Hz, H-17″), 6.11 (1H, *dd*, J = 2.0, 9.0 Hz, H-19″), 6.06 (1H, *dd*, J = 2.0, 9.0 Hz, H-13″), 5.93 (1H, *d*, J = 2.0 Hz, H-11″), 5.40 (1H, *s*, H-2″), 4.64 (1H, *br s*, H-4″), 4.40 (1H, *br s*, H-3″), 3.67 (1H, *br s*, H-5″), 2.81 (1H, *m*, H-6″), 2.08 (1H, *m*, H-6″) and 1.78 (3H, *s*, H-7″); ^{13}C NMR data see Table 1; HRFAB-MS *m/z*: 607.1623 (calcd for $C_{35}H_{27}O_{10}$, $[M - H]^-$, 607.1610).

3.6. Guangsangon H (*3*)

Yellow amorphous powder; $[\alpha]_D^{21} = -127.9°$ (c = 0.15, MeOH); UV (MeOH) λ_{max}: 245, 259, 267, 302 nm; IR (KBr) ν_{max}: 3302, 1676, 1614, 1512, 1458 cm^{-1}; ^1H NMR (CD_3COCD_3): δ 13.37 (1H, *s*, HO-10″), 7.72 (1H, *d*, J = 8.5 Hz, H-5), 7.55 (1H, *d*, J = 2.0 Hz, H-6′), 7.40 (1H, *d*, J = 8.0 Hz, H-14″), 7.05 (1H, *dd*, J = 2.0, 8.5 Hz, H-4′), 6.89 (1H, *d*, J = 8.0 Hz, H-20″), 6.59 (1H, *d*, J = 8.5, 2.0 Hz, H-6), 6.56 (1H, *d*, J = 2.0 Hz, H-8), 6.59 (1H, *d*, J = 8.5 Hz, H-3′), 6.21 (1H, *d*, J = 8.0 Hz, H-13″), 6.20 (1H, *d*, J = 2.0 Hz, H-17″), 6.06 (1H, *dd*, J = 2.0, 8.0 Hz, H-19″), 5.37 (1H, *s*, H-2″), 5.08 (1H, *t*, J = 7.0 Hz, H-22″), 4.99 (1H, *d*, J = 12.0 Hz, H-2), 4.52 (1H, *d*, J = 12.0 Hz, H-3), 4.37 (1H, *br s*, H-3″), 4.39 (1H, *br s*, H-4″), 3.66 (1H, *br s*, H-5″), 3.11 (2H, *d*, J = 7.0Hz, H-21″), 2.18 (1H, *m*, H-6″), 2.53 (1H, *m*, H-6″), 1.75 (3H, *s*, H-7″), 1.65 (3H, *s*, H-24″), 1.54 (3H, *s*, H-25″); ^{13}C NMR data see Table 1; CD ($c = 8.11 \times 10^{-4}$ M, MeOH): $\Delta\varepsilon_{285}$ −18.1, $\Delta\varepsilon_{298}$ −22.8, $\Delta\varepsilon_{330}$ +2.0; HRFAB-MS *m/z*: 677.2407 (calcd for $C_{40}H_{37}O_{10}$, $[M - H]^-$, 677.2392).

3.7. Guangsangon I (*6*)

Brown amorphous powder; $[\alpha]_D^{21} = -470.5°$ (c = 0.17, MeOH); UV (MeOH) λ_{max}: 212, 281, 326 nm; IR (KBr) ν_{max}: 3344, 1699, 1628, 1604, 1506, 1458 cm^{-1}; ^1H NMR (CD_3COCD_3): δ 13.12 (1H, *s*, HO-10″), 8.02 (1H, *d*, J = 2.0 Hz, H-5), 7.81 (1H, *d*, J = 9.0 Hz, H-14″), 7.18 (1H, *d*, J = 9.0 Hz, H-6′), 6.95 (1H, *dd*, J = 2.0, 9.0 Hz, H-6), 6.91 (1H, *d*, J = 2.0 Hz, H-8), 6.82 (1H, *d*, J = 9.0 Hz, H-20″), 6.37 (1H, *d*, J = 9.0 Hz, H-5′), 6.21 (1H, *d*, J = 2.0 Hz, H-17″), 6.07 (1H, *dd*, J = 2.0, 9.0 Hz, H-19″), 6.03 (1H, *dd*, J = 2.0, 9.0 Hz, H-13″), 5.94 (1H, *d*, J = 2.0 Hz, H-11″), 5.35 (1H, *s*, H-2″), 5.10 (1H, *br s*, H-4″), 4.53 (1H, *br s*, H-3″), 3.73 (1H, *br s*, H-5″), 2.48 (1H, *m*, H-6″), 2.18 (1H, *m*, H-6″), and 1.68 (3H, *s*, H-7″); ^{13}C NMR data see Table 1; HRFAB-MS *m/z*: 625.1688 (calcd for $C_{35}H_{27}O_{11}$, $[M + H]^+$, 625.1701).

3.8. Guangsangon J (7)

Brown amorphous powder; $[\alpha]_D^{21} = -419.7°$ ($c = 0.16$, MeOH); UV (MeOH) λ_{max}: 212, 301, 329 nm; IR (KBr) ν_{max}: 3349, 1650, 1616, 1506, 1456 cm^{-1}; ^1H NMR (CD_3COCD_3): δ 13.47 (1H, *s*, HO-10″), 7.35 (1H, *d*, $J = 8.5$ Hz, H-14″), 7.41 (1H, *s*, H-4), 6.96 (1H, *s*, H-3), 6.93 (1H, *d*, $J = 8.5$ Hz, H-20″), 6.80 (2H, *d*, $J = 2.0$ Hz, H-2′ and H-6′), 6.74 (1H, *s*, H-7), 6.35 (1H, *t*, $J = 2.0$ Hz, H-4′), 6.22 (1H, *d*, $J = 1.5$ Hz, H-17″), 6.11 (1H, *d*, $J = 1.5$, 8.5 Hz, H-19″), 5.98 (1H, *d*, $J = 8.5$ Hz, H-13″), 5.41 (1H, *s*, H-2″), 5.05 (1H, *t*, $J = 7.0$ Hz, H-22″), 4.32 (1H, *br s*, H-3″), 4.49 (1H, *br s*, H-4″), 3.69 (1H, *br s*, H-5″), 3.11 (2H, *d*, $J = 7.0$ Hz, H-21″), 2.60 (1H, *m*, H-6″), 2.21 (1H, *m*, H-6″), 1.74 (3H, *s*, H-7″), 1.64 (3H, *s*, H-24″), 1.53 (3H, *s*, H-25″); ^{13}C NMR data see Table 1; HRFAB-MS *m/z*: 647.2289 (calcd for $C_{39}H_{35}O_9$, $[M - H]^-$, 647.2286).

3.9. Anti-inflammatory activity testing

Effects of compounds **3**, **6**, **7**, and ginkgolide B on the release of β-glucuronidase in rat polymorphonuclear (PMN) leukocytes induced by PAF (Nie and Wang, 2003). Rat PMNs were incubated with vehicle or various concentrations of compounds at 37 °C for 15 min. Then cytochalasin B (10 μM) was added. After 5 min, PAF (1 μM) was added prior to the termination of the reaction. The supertnants of the reaction was incubated with phonolphthalin glucuranic acid (0.4 mM) at 37 °C for 18 h. The results were quantified by reading the absorbance at 550 nm under the condition of pH 10.4. Based on the above methods, the inhibitory rates of **3**, **6**, **7**, and ginkgolide B were determined to be 49.4% ($P < 0.01$), 43.8% ($P < 0.001$), 41.3% ($P < 0.01$), and 58.9%, respectively.

3.10. Antioxidant activity testing

The antioxidant activities of **3**, **6**, **7**, and Vit E were determined by the content of malondialdehyde (MDA) (Lu and Liu, 1991), a compound that was produced during microsomal lipid peroxidation induced by ferrous-cysteine. MDA was detected by using the thiobarbituric acid (TBA) method. Briefly, 1 mg/ml microsomal protein, different concentration compound or vehicle and 0.2 mM cysteine in 0.1 M PBS was incubated for 15 min at 37 °C, added 0.5 mM ferrous chloride, mixed and incubated for 15 min at 37 °C again. The equal volume of 20% TCA was added to terminate the reaction and centrifuged for 10 min at 714 g. The supernatants reacted with 0.67% TBA for 10 min at 100 °C. After cooling, the MDA was quantified by determining the absorbance at 532 nm, and the inhibition rates were calculated. In the end, the inhibitory rates of compounds, **3**, **6**, **7**, and Vit E were established as 93.1%, 93.9%, 91.1%, and 33.4%, respectively.

Acknowledgements

We are grateful to Mr. Feng Liang, Jing-Hua Ni, and Ms. Ying-Hong Wang (National Research Center for Analysis of Drugs and Metabolites) for the measurement of FABMS, HRFAB-MS, UV, IR, and NMR spectra, respectively. We also gratefully acknowledge Prof. Wen-Jie Wang and Ms. Huai-Ling Wei (Department of Pharmacology, Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College) for the bioactivity screenings.

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