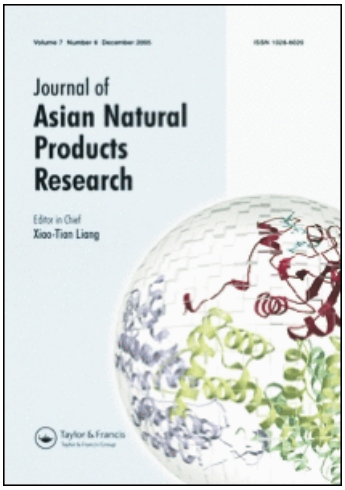

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

A new Diels-Alder type adduct and two new flavones from the stem bark of *Morus yunanensis* Koidz

Xi-Qiang Cui[a]; Lei Wang[a]; Ren-Yi Yan[a]; Yong-Xia Tan[a]; Ruo-Yun Chen[a]; De-Quan Yu[a]
[a] Key Laboratory of Bioactive Substances and Resources Utilization of Chinese Herbal Medicine, Ministry of Education and Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China

To cite this Article Cui, Xi-Qiang , Wang, Lei , Yan, Ren-Yi , Tan, Yong-Xia , Chen, Ruo-Yun and Yu, De-Quan(2008) 'A new Diels-Alder type adduct and two new flavones from the stem bark of *Morus yunanensis* Koidz', Journal of Asian Natural Products Research, 10: 4, 315 — 318
To link to this Article: DOI: 10.1080/10286020701833537
URL: http://dx.doi.org/10.1080/10286020701833537

Journal of Asian Natural Products Research
Vol. 10, No. 4, April 2008, 315–318

A new Diels–Alder type adduct and two new flavones from the stem bark of *Morus yunanensis* Koidz

Xi-Qiang Cui, Lei Wang, Ren-Yi Yan, Yong-Xia Tan, Ruo-Yun Chen* and De-Quan Yu

Key Laboratory of Bioactive Substances and Resources Utilization of Chinese Herbal Medicine, Ministry of Education and Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China

(*Received 5 September 2007; final version received 19 November 2007*)

Fractionation of the ethanolic extract of the stem bark of *Morus yunanensis* resulted in the isolation of a new Diels–Alder type adduct and two new flavones, named yunanensin A (**1**), yunanensol A (**2**) and yunanensol B (**3**), respectively, together with a known flavone (**4**). Their structures were determined on the basis of spectroscopic analysis and chemical methods. Among them, compound **1** showed moderate antioxidant and significant cytotoxic activities, and compound **2** showed potent anti-inflammatory activity.

Keywords: *Morus yunanensis* Koidz; Diels–Alder type adduct; flavone; cytotoxic

1. Introduction

The stem bark of the mulberry tree (*Morus alba* L. and other plants of the genus *Morus*) has been used in traditional Chinese medicine as antiphlogistics, diuretics and expectorants.[1] Previously, many novel phenolic compounds and Diels–Alder type adducts of dehydroprenylphenols and chalcone derivatives were isolated from the genus *Morus*.[2,3] *Morus yunanensis* Koidz is distributed in the southern part of China, especially in Yunnan Province. In the course of seeking novel bioactive compounds, the stem bark of *M. yunanensis* has been studied. In this paper, we describe the isolation and structure elucidation of the three new compounds, as well as the evaluation of their antioxidative, anti-inflammatory and cytotoxic activities.

2. Results and discussion

The EtOAc fraction of the ethanolic extract from the stem bark of *Morus yunanensis* was chromatographed successively on silica gel column, Sephadex LH-20, silica gel RP-18 and RP–HPLC to give compounds **1–4**.

Compound **1** was obtained as brown amorphous powder, and gave a dark green colour with methanolic ferric chloride. The HRFAB–MS indicated a quasi-molecular ion at *m/z* 625.1810 $[M + H]^+$, corresponding to the molecular formula of $C_{39}H_{28}O_8$. Its IR spectrum revealed the presence of hydroxyl (3385 cm^{-1}) and benzene ring moieties (1606, 1585 cm^{-1}). The UV absorption maxima at 220, 346 and 365 nm demonstrated the presence of a highly conjugated system in the structure. The ^1H NMR and ^{13}C NMR spectral data exhibited structural feature of a ketalised Diels–Alder type adduct. The ^1H NMR spectrum showed signals of 2-arylbenzofuran moiety at δ 6.80 (1H, dd, $J = 2.4$, 8.4 Hz), 6.96 (1H, d, $J = 2.4$ Hz), 7.05 (1H, s), 7.06 (1H, d, $J = 1.8$ Hz), 7.08 (1H, d, $J = 1.8$ Hz) and 7.40 (1H, d, $J = 8.4$ Hz). The presence of the following moieties was also supported by comparing the ^1H NMR spectral data with those of mulberrofuran K[4] and albonal B,[5] namely, a 5-oxygenated 2,2-dimethylchromene moiety at δ 6.10 (1H, d, $J = 8.7$ Hz), 6.72 (1H, d, $J = 8.7$ Hz), 6.46 (1H, d, $J = 9.9$ Hz), 5.50 (1H, d, $J = 9.9$ Hz), 1.28, 1.30 (s, each 3H); a 2,4-dioxygenated phenyl moiety at δ 6.48 (1H, d, $J = 2.4$ Hz), 6.52 (1H, dd, $J = 2.4$, 8.1 Hz), 7.67 (1H, d, $J = 8.1$ Hz); and an arylated methylcyclohexene ring at δ 8.39 (1H, s), 7.56 (1H, s), and 2.48 (3H, s).

From the above results, two possible structures were considered (**1**, **1′**). In order to discriminate the two possible structures, selected NOE experiments were carried out. From the key NOE observations (H-25″/H-17″ and H-24″/H-17″), structure **1** was proposed to be the structure of compound **1** (Figure 1). All of the ^1H NMR and ^{13}C NMR assignments were confirmed by HMBC and HSQC experiments.

Compound **2** was obtained as yellow powder. The molecular formula of $C_{25}H_{28}O_7$ was determined by HRFAB–MS at *m/z* 441.1907 $[M + H]^+$. Its IR spectrum showed absorption bands at 3346, 1651, 1624 cm^{-1}, assignable to hydroxyl, conjugated carbonyl and benzene ring. The UV spectrum displayed absorption

*Corresponding author. Email: ruoyunchen@hotmail.com

ISSN 1028-6020 print/ISSN 1477-2213 online

DOI: 10.1080/10286020701833537
http://www.informaworld.com

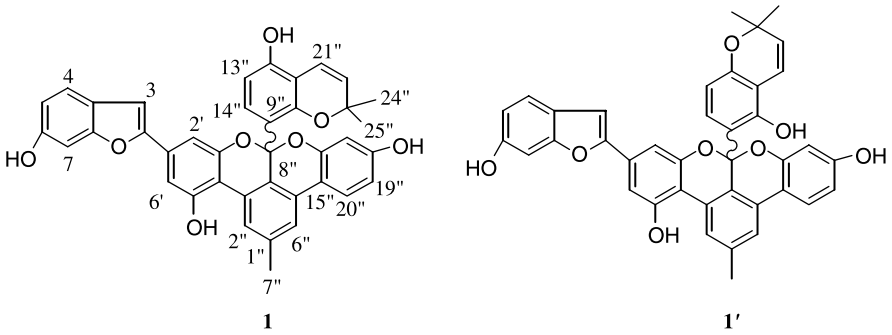

Figure 1. The possible structures of compound **1**.

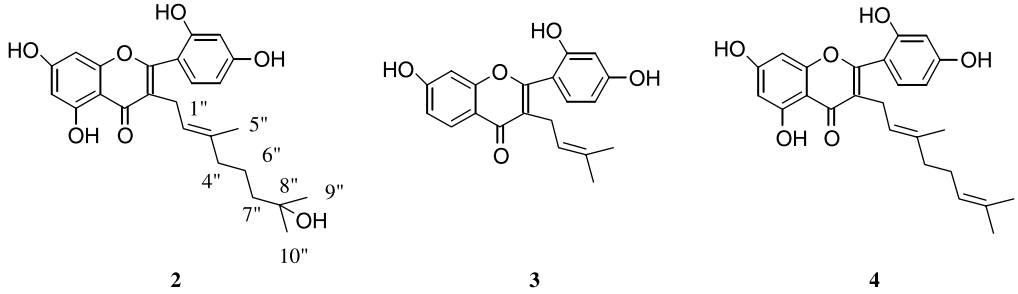

Figure 2. The structures of compounds **2**–**4**.

maxima at 204, 257, 325 nm. The ^{1}H NMR spectrum of **2** was very similar to that of compound **4**[6] except for the aliphatic proton signals, showing a set of *meta*-coupled proton signals at δ 6.22 (1H, d, $J = 1.6$ Hz), 6.30 (1H, d, $J = 1.6$ Hz) and proton signals of an ABX system at δ 7.19 (1H, d, $J = 8.4$ Hz), 6.50 (1H, dd, $J = 2.4$, 8.4 Hz), and 6.53 (1H, d, $J = 2.4$ Hz). Comparison of the ^{1}H NMR, ^{13}C NMR and MS spectral data with those of **4**, it was deduced that one of the double bonds in **4** was hydrated. The ^{13}C NMR signal at δ 70.2 indicated that compound **2** bore an aliphatic hydroxyl group. The location of the hydroxyl group was determined to be at C-8″ by HMBC correlations between C-8″ and H-6″, H-7″, H-9″, H-10″. The E-configuration of the $\Delta^{2'',3''}$ double bond was deduced from the NOE correlation between H-5″ and H-1″. Accordingly, the structure of compound **2** was elucidated as 3-[(2″E)-8″-hydroxyl-3″,8″-dimethyloct-2″-enyl]-5,7,2′,4′-tetrahydroxyflavone (Figure 2).

Compound **3** was obtained as yellow powder. The HRFAB–MS showed a quasi-molecular ion at *m/z* 339.1227 $[M + H]^+$, consistent with a molecular formula of $C_{20}H_{18}O_5$. The UV spectrum exhibited absorption maxima at 205, 304 nm. The ^{1}H NMR spectrum of **3** showed proton signals of a γ,γ-dimethylallyl group at δ 1.40, 1.53 (s, each 3H), 3.10 (2H, d, $J = 6.9$ Hz), 5.21 (1H, m); and proton signals of two ABX systems at δ 7.97 (1H, d, $J = 8.7$ Hz), 6.93 (1H, dd, $J = 2.1$, 8.7 Hz), 6.80 (1H, d, $J = 2.1$ Hz), 7.16 (1H, d, $J = 8.4$ Hz), 6.49 (1H, dd, $J = 2.4$, 8.4 Hz), and 6.56 (1H, d, $J = 2.4$ Hz). The ^{1}H NMR and ^{13}C NMR (Table 2) assignments together with HMBC correlations (H-1″/C-3, 2, 4) revealed the prenyl group at C-3. Thus, compound **3** was assigned as 3-prenyl-7,2′,4′-trihydroxyflavone (Figure 2). The 7-hydroxyflavone is rare in genus *Morus*.

Compounds **1**–**4** were evaluated for their antioxidative, anti-inflammatory and cytotoxic activities, and the inhibitory ratios and IC_{50} values were shown in

Table 1. ^{13}C NMR (acetone-d_6, 125 MHz) spectral data for compound **1**.

No.	δ	No.	δ	No.	δ
2	156.9	6′	106.6	13″	106.9
3	103.0	1″	139.5	14″	130.4
3a	129.5	2″	126.0	15″	115.6
4	122.1	3″	131.9	16″	159.7
5	133.4	4″	123.9	17″	105.3
6	154.6	5″	129.9	18″	153.8
7	98.4	6″	120.9	19″	110.7
7a	156.9	7″	22.1	20″	124.9
1′	122.5	8″	118.8	21″	117.5
2′	106.4	9″	110.3	22″	129.2
3′	156.6	10″	154.2	23″	76.2
4′	111.9	11″	104.0	24″	27.9
5′	156.6	12″	153.1	25″	27.6

Table 2. ^{13}C NMR (acetone-d_6, 100 MHz) spectral data for compounds **2**, **3** and **4**.

No.	2	3	4	No.	2	3	4
2	162.4	161.0	162.4	5′	108.0	107.8	108.0
3	121.7	122.9	121.8	6′	132.3	132.3	132.3
4	182.9	177.6	183.0	1″	24.3	25.8	24.4
5	163.4	127.8	163.4	2″	122.5	123.2	122.6
6	99.2	117.1	99.2	3″	136.0	131.5	135.8
7	164.8	163.3	164.7	4″	40.8	25.3	40.4
8	94.2	102.9	94.2	5″	15.8	17.6	16.0
9	159.2	159.2	159.3	6″	23.1		27.3
10	105.1	113.5	105.3	7″	44.0		125.1
1′	113.0	115.2	113.0	8″	70.2		131.6
2′	157.2	161.1	157.2	9″	29.3		25.8
3′	103.8	103.7	103.8	10″	29.3		17.7
4′	161.3	157.0	161.4				

Table 3. Antioxidative and anti-inflammatory activities of compounds **1**–**4** at concentration of 10^{-5} mol/L.

Compound	1	2	3	4	Vit E
Antioxidant (%)	100	−3.0	13.0	9.1	38
Anti-inflammatory (%)	5.3	93.9	18.3	45.2	

Table 4. Cytotoxic activity of compound **1**.

Cell lines	Compound **1** [IC_{50} (μg/ml)]
A549	0.922
Bel7402	5.387
BGC823	0.863
HCT-8	5.378
A2780	2.384

Tables 3 and 4. Based on the bioassay results, it is concluded that compound **1** could inhibit liver microsomal lipid peroxidation induced by Fe^{2+}-Cys system, and exhibit significant cytotoxic activities against A549, Bel7402, BGC-823, HCT-8 and A2780 cell lines. Compound **2** has potent inhibitory activity on the release of β-glucuronidase from rat PMNs induced by PAF.

3. Experimental

3.1 General experimental procedures

The optical rotations were measured on a Perkin–Elmer 241 digital polarimeter. IR spectra were carried out on an IMPACT 400 spectrometer. UV spectra were determined with an Hitachi UV-240 spectrophometer. ^1H NMR (500 MHz), ^{13}C NMR (125 MHz), NOE difference, HSQC, HMBC and ^1H NMR (400 MHz), ^{13}C NMR (100 MHz) spectra were run on an INOVA-500 spectrometer and a Mercury-400 spectrometer with TMS as internal standard. HR-FAB–MS were performed on a VG-Auto spect-300 mass spectrometer, and ESI–MS on an Agilent 1100 LC/MSD Trap-SL mass spectrometer. Silica gel (Qingdao Marine Chemical Factory, 160–200 mesh), Sephadex LH-20 (Pharmacia) and RP-18 (Merck, 40–60 μm) were used for column chromatography, and silica gel GF-254 (QingDao Marine Chemical Factory) was used for TLC. HPLC separations were performed on a preparation YMC-Pack ODS-A column (10 μm, 250 × 20 mm I.D.) equipped with Shimadzu SPD-6A UV spectrophotometric detector and Shimadzu LC-6AD series pumping system.

3.2 Plant material

The stem bark of *Morus yunanensis* Koidz was collected in County of Simaopuhe, Yunnan Province, China, in October 2003, and identified by Professor Shaorong Guo, Institute of Medical Plant Development, Chinese Academy of Medical Science and Peking Union Medical College. A voucher specimen (No. 20280) 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 barks (9 kg) of *Morus yunanensis* were finely cut and extracted with 95% EtOH (3 × 10 L, 3 h) under reflux. After evaporation of the solvents under reduced pressure, the residue (750 g) was submitted to chromatography over a silica gel column (160–200 mesh, 10 × 60 cm, 1.0 kg) and eluted with petroleum ether, $CHCl_3$, EtOAc, CH_3COCH_3 and MeOH, successively. The EtOAc fraction (201 g) was chromatographed over a silica gel column (160–200 mesh, 8 × 130 cm, 2.5 kg) using $CHCl_3$-MeOH as gradient eluent [(95:5–9:1–8:2–7:3–1:1, v/v)/MeOH] to provide 13 fractions.

Fraction 2 (18 g) was purified by silica gel column chromatography (160–200 mesh, 5–100 cm, 600 g), eluted with petroleum ether/CH_3COCH_3 (95:5–9:1–8:2–7:3–1:1, v/v) to give 10 fractions. Fraction 2-7 (2.3 g) was subjected to a Sephadex LH-20 column chromatography using MeOH as eluent to give 8 fractions. Fraction 2-7-2 (133 mg) was purified by RP-18 column chromatography (MeOH/H_2O 8:2) to yield compound **4** (30 mg). Fraction 2-7-8 (1.1 g) was further submitted on Sephadex LH-20 column chromatography to gain compound **1** (60 mg).

Fraction 4 (9.5 g) was subjected to silica gel column chromatography (160–200 mesh, 5 × 50 cm, 300 g), eluted with petroleum ether/CH_3COCH_3 (9:1–8:2–7:3–1:1, v/v) to give 13 fractions. Fractions 4-4 (760 mg), 4-5 (350 mg) were submitted to Sephadex LH-20 column chromatography respectively. Fraction 4-4-3 (112 mg)

was then purified on RP-HPLC with an ODS column (flow rate 8 ml/min) with MeOH/H_2O (7:3) to yield compound **2** (20 mg) ($t_R = 20.1$ min). By the same method, compound **3** (68 mg) ($t_R = 15.8$ min) was obtained on RP-HPLC from 4-5-2 (120 mg).

3.3.1 Compound *1*

Amorphous brown powder, $[\alpha]_D^{25}$ +12.0 (*c* 0.075, MeOH); IR: ν_{max}^{KBr} cm^{-1}:3385 (OH), 1606, 1585 (C═C); UV: λ_{max} (MeOH) 220, 346, 365 nm; ^{1}H NMR (acetone-d_6, 500 MHz): δ 8.39 (1H, s, H-2″), 7.67 (1H, d, $J = 8.1$ Hz, H-20″), 7.56 (1H, s, H-6″), 7.40 (1H, d, $J = 8.4$ Hz, H-4), 7.08 (1H, d, $J = 1.8$ Hz, H-2′), 7.06 (1H, d, $J = 1.8$ Hz, H-6′), 7.05 (1H, s, H-3), 6.96 (1H, d, $J = 2.4$ Hz, H-7), 6.80 (1H, dd, $J = 2.4$, 8.4 Hz, H-5), 6.72 (1H, d, $J = 8.7$ Hz, H-14″), 6.52 (1H, dd, $J = 2.4$, 8.1 Hz, H-19″), 6.48 (1H, d, $J = 2.4$ Hz, H-17″), 6.46 (1H, d, $J = 9.9$ Hz, H-21″), 6.10 (1H, d, $J = 8.7$ Hz, H-13″), 5.50 (1H, d, $J = 9.9$ Hz, H-22″), 2.48 (3H, s, H-7″), 1.28, 1.30 (s, each 3H, H-24″,25″); ^{13}C NMR spectral data (acetone-d_6): Table 1; HR-FAB–MS: *m/z* 625.1810 $[M + H]^+$ (calcd for $C_{39}H_{29}O_8$, 625.1862); ESI–MS: *m/z* 625 $[M + H]^+$, 647 $[M + Na]^+$, 663 $[M + K]^+$.

3.3.2 Compound *2*

Yellow powder, IR ν_{max}^{KBr} cm^{-1}:3346 (OH), 1651 (conj. C═O), 1624 (C═C); UV: λ_{max} (MeOH) 204, 257, 325 nm; ^{1}H NMR (acetone-d_6, 400 MHz): δ 7.19 (1H, d, $J = 8.4$ Hz, H-6′), 6.50 (1H, dd, $J = 2.4$, 8.4 Hz, H-5′), 6.53 (1H, d, $J = 2.4$ Hz, H-3′), 6.30 (1H, d, $J = 1.6$ Hz, H-8), 6.22 (1H, d, $J = 1.6$ Hz, H-6), 5.11 (1H, t, $J = 6.8$ Hz, H-2″), 3.11 (2H, d, $J = 6.8$ Hz, H-1″), 1.86 (2H, t, $J = 7.6$ Hz, H-4″), 1.42 (2H, m, H-7″), 1.35 (2H, m, H-6″), 1.46, 1.15, 1.15 (each 3H, s, H-5″,9″,10″); ^{13}C NMR spectral data (acetone-d_6): Table 2; HR-FAB–MS: *m/z* 441.1907 $[M + H]^+$ (calcd for $C_{25}H_{29}O_7$, 441.1908); ESI–MS: *m/z* 441 $[M + H]^+$, 463 $[M + Na]^+$, 479 $[M + K]^+$.

3.3.3 Compound *3*

Yellow powder, IR ν_{max}^{KBr} cm^{-1}:3343 (OH), 1743 (C═O), 1618 (C═C); UV: λ_{max} (MeOH) 205, 304 nm; ^{1}H NMR (acetone-d_6, 400 MHz): δ 7.97 (1H, d, $J = 8.7$ Hz, H-5), 7.16 (1H, d, $J = 8.4$ Hz, H-6′), 6.93 (1H, dd, $J = 2.1$, 8.7 Hz, H-6), 6.80 (1H, d, $J = 2.1$ Hz, H-8), 6.56 (1H, d, $J = 2.4$ Hz, H-3′), 6.49 (1H, dd, $J = 2.4$, 8.4 Hz, H-5′), 5.21 (1H, m, H-2″), 3.10 (2H, d, $J = 6.9$ Hz, H-1″), 1.53, 1.40 (s, each 3H, H-4″,5″); ^{13}C NMR spectral data (acetone-d_6): Table 2; HR-FAB–MS: *m/z* 339.1227 $[M + H]^+$ (calcd for $C_{20}H_{19}O_5$, 339.1233); ESI–MS: *m/z* 339 $[M + H]^+$, 361 $[M + Na]^+$, 377 $[M + K]^+$.

Acknowledgement

This research programme was supported by the National Natural Science Foundation of China (No. 20572133).

References

[1] T. Nomura and T. Fukai, *Planta Med.* **42**, 79 (1981).
[2] J. Kang, R.Y. Chen, and D.Q. Yu, *Planta Med.* **72**, 52 (2006).
[3] S.J. Dai, Z.B. Ma, Y. Wu, R.Y. Chen, and D.Q. Yu, *Phytochemistry* **65**, 3135 (2004).
[4] Y. Hano, H. Kohno, M. Itou, and T. Nomura, *Chem. Pharm. Bull.* **33**, 5294 (1985).
[5] T. Fukai, Y. Hano, K. Hirakura, T. Nomura, J. Uzawa, and K. Fukushima, *Chem. Pharm. Bull.* **33**, 3195 (1985).
[6] D. Lee, K.P.L. Bhat, H.H.S. Fong, N.R. Farsworth, J.M. Pezzuto, and A.D. Kinghorn, *J. Nat. Prod.* **64**, 1286 (2001).