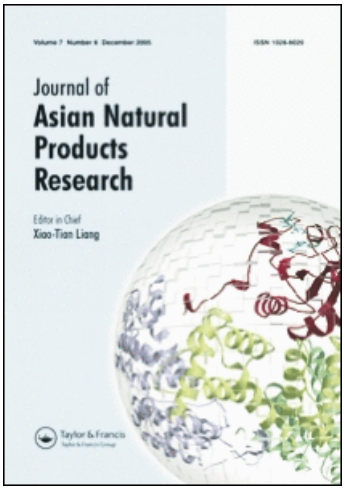

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

Triterpenoids from the stems of Myricaria paniculata

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To cite this Article Li, Shuai , Dai, Sheng-Jun , Chen, Ruo-Yun and Yu, De-Quan(2005) 'Triterpenoids from the stems of Myricaria paniculata', Journal of Asian Natural Products Research, 7: 3, 253 — 257
To link to this Article: DOI: 10.1080/10286020410001721168
URL: http://dx.doi.org/10.1080/10286020410001721168

Journal of Asian Natural Products Research, Vol. 7, No. 3, June 2005, 253–257

Triterpenoids from the stems of *Myricaria paniculata*

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(Received 1 March 2004; in final form 6 April 2004)

Two new pentacyclic triterpenoids myricarin A and B (**1** and **2**) have been isolated from the stems of *Myricaria paniculata*, together with seven known compounds, myriconal, 28-hydroxy-14-taraxeren-3-one, *epi*-friedelanol, β-sitosterol, 4-methyl stigmast-7-en-3-ol, 12-hentriacontanol and 1-triacontanol. Their structures have been established by chemical and spectroscopic methods. Cytotoxic activities of **1** and **2** have been evaluated against several different cell lines.

Keywords: *Myricaria paniculata*; Tamaricaceae; Triterpenoids; 14-Taraxeren; Myricarins A and B

1. Introduction

Myricaria paniculata (Tamaricaceae) is a traditional Tibetan herb that has been used to clear heat and toxic material, dispel mild wind and relieve exterior syndrome, promote eruption and relieve coughs. It is also reported to cure rheumatism and arthritis [1]. It is distributed in Asia and Europe. Previous phytochemical investigations of the genus *Myricaria* found flavonoids in the stems of *Myricaria alopecuroides* [2,3] and *M. longifolia* [4], gallic acids [5,6], and four different series of long-chain alkanediols from the leaves of *M. germanica* [7]. In this paper we report the isolation and structure elucidation of two new triterpenoids, myricarin A and B, from the stems of *M. paniculata*; this is for the first report of triterpenoids isolated from this genus.

2. Results and discussion

Myricarin A (**1**), colorless powder, gave a positive Liebermann–Burchard reaction. The molecular formula of **1** was established as $C_{39}H_{54}O_6$ by HREIMS. The IR spectrum of **1** suggests the presence of hydroxyl (3340 cm^{-1}), α,β-unsaturated carbonyl (1693 cm^{-1}) and phenyl (1604, 1516 cm^{-1}) groups, as well as a trisubstituted double

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Journal of Asian Natural Products Research
ISSN 1028-6020 print/ISSN 1477-2213 online © 2005 Taylor & Francis Ltd
http://www.tandf.co.uk/journals
DOI: 10.1080/10286020410001721168

bond (812 cm^{-1}). The ^1H NMR spectrum of **1** in CD_3COCD_3 exhibits signals due to three aromatic protons of an AMX system at δ 7.14 (1H, d, $J = 2.0$ Hz), 7.01 (1H, dd, $J = 8.0$, 2.0 Hz) and 6.84 (1H, d, $J = 8.0$ Hz), and two *trans* conjugated olefinic protons at δ 7.52 (1H, d, $J = 16.0$ Hz) and 6.33 (1H, d, $J = 16.0$ Hz) that were identical with the signals of caffeic acid. The presence of caffeic acid moiety in **1** was also supported by the UV maxima at 328, 245 and 207 nm. Additional signals in the ^1H NMR spectrum were typical for a triterpenoid, revealing seven tertiary methyl signals, an olefinic proton at the trisubstituted double bond (δ 5.57 1H, dd, $J = 8.0$, 3.0 Hz), and an oxygenated methine at C-3 (δ 4.69,1H, t, $J = 2.0$ Hz). The configuration of the hydroxyl group at C-3 was deduced to be in the α-position by a small $^3J_{H,H}$ coupling constant (2.0 Hz) [8]. The ^{13}C NMR spectrum and DEPT experiment of **1** show that **1** was a triterpene caffeic acid ester having a carbonyl, an ester carbonyl, four sp^2 quaternary carbons, six sp^2 methines, an oxygenated methine, six quaternary carbons, three methines, ten methylenes, and seven

Table 1. ^{13}C NMR chemical shifts (ppm) of **1**[a], **1a**[b] and **2**[a].

	1	*1a*	*2*
1	33.2	32.8	33.2
2	22.7	25.1	22.7
3	77.6	76.2	77.6
4	36.8	37.3	36.8
5	50.8	48.8	50.8
6	18.6	18.8	18.6
7	41.2	40.8	41.2
8	39.2	39.2	39.2
9	49.3	49.2	49.3
10	38.2	38.1	38.2
11	17.4	17.2	17.4
12	33.6	33.4	33.6
13	37.4	37.1	37.4
14	160.8	160.8	160.8
15	116.5	116.6	116.4
16	31.9	31.3	31.9
17	50.8	51.4	50.8
18	41.8	41.4	41.8
19	35.3	35.4	35.3
20	29.0	29.3	29.0
21	33.8	33.7	33.8
22	30.9	30.7	30.9
23	27.7	28.2	27.7
24	21.5	22.2	21.5
25	15.0	15.4	14.9
26	25.9	26.3	25.9
27	22.0	22.4	21.9
28	178.7	183.8	178.5
29	31.7	32.3	31.7
30	28.9	28.6	28.9
1′	127.1		126.4
2′	114.6		130.3
3′	145.6		116.0
4′	147.9		159.8
5′	115.7		116.0
6′	121.8		130.3
7′	144.6		144.4
8′	115.8		116.5
9′	166.3		166.4

[a] Measured in CD_3COCD_3 at 125 MHz for ^{13}C spectra.
[b] Measured in $CDCl_3$ at 125 MHz for ^{13}C spectra.

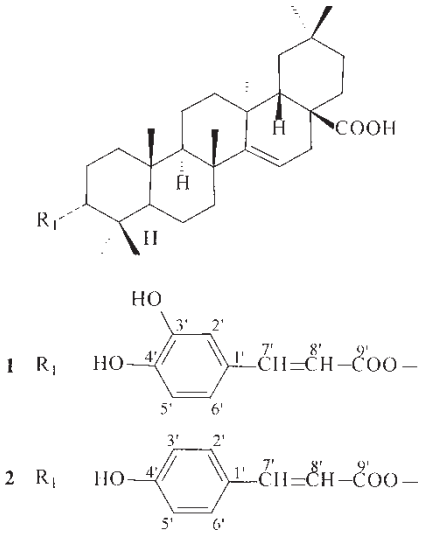

Figure 1. The structures of **1** and **2**.

tertiary methyls. The olefinic signals at δ 160.8 and 115.7 for C-14 and C-15 in the ^{13}C NMR spectrum indicate a $\Delta^{14,15}$-taraxeren skeleton in compound **1** [9]. The EI-MS spectrum of **1** shows fragment ion peaks at *m/z* 300, 285, 203, 189, indicating that **1** is a $\Delta^{14,15}$-taraxeren derivative [10]. Alkaline hydrolysis of **1** afforded a known compound that was identified as isoaleuritolic acid (**1a**) by ^{13}C NMR data [11]. Assignment of the ^{13}C NMR signals of **1** was performed by comparison with those of **1a** reported in the literature (table 1), isomyricadiol and acetyl aleuritolic acid [9]. Thus the structure of compound **1** was determined to be isoaleuritolic acid 3-(3′,4′-dihydroxy)caffeate (figure 1).

Myricarin B (**2**), a colorless powder, also gave a positive Liebermann–Burchard reaction. It has a molecular formula of $C_{39}H_{54}O_5$ by HR-FABMS. Comparison of its ^1H and ^{13}C NMR data with those of **1** revealed that **2** is very similar to **1** (table 1), having the same triterpenoid moiety, the only difference being the substitution group of C-3. The ^1H NMR spectrum of **2** shows aromatic proton signals at δ 7.52 (2H, d, $J = 8.7$ Hz) and 6.87 (2H, d, $J = 8.7$ Hz), characteristic of an AA′BB′ system, which are compatible with the presence of a *p*-hydroxybenzoyl moiety, and the olefinic proton signals at δ 7.59 (1H, d, $J = 16.0$ Hz) and 6.39 (1H, d, $J = 16.0$ Hz) are identical to those of *trans*-cinnamoyl. Thus the structure of **2** was elucidated as isoaleuritolic acid 3-*p*-hydroxycinnamate.

Bioassay experiments using the MTT method [12] revealed that compounds **1** and **2** have no cytotoxicity against Bel-7402, HCT-8, BGC823, A549 and MCF-7 human tumor cell lines (table 2).

Table 2. Cytotoxicities (IC_{50} μg ml^{-1}) of **1** and **2** against some human cell lines by the MTT method.

Compound	*Bel-7402*	*BGC823*	*HCT-8*	*A549*	*MCF-7*
1	>10	>10	>10	>10	>10
2	>10	>10	>10	>10	>10

3. Experimental

3.1 General experimental procedures

Melting points were measured on an XT-4 micro-melting point apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. UV spectra were obtained on a Shimadzu UV 260 spectrometer. IR spectra were recorded on an IMPACT-400 FT infrared spectrometer. NMR spectra were recorded on a Varian INOVA-500 NMR spectrometer with TMS as internal standard. EIMS, FABMS, HR-EIMS and HR-FABMS spectra were measured on an Autospec–Ultima ETOF Spec mass spectrometer.

3.2 Plant material

Dried stems of *Myricaria paniculata* were collected on the wild field of Xining, Qinghai Province, China, in April 2001, and identified by Professor Duo Jie. A voucher specimen has been deposited in the Herbarium of the Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College.

3.3 Extraction and isolation

The dried stems of *M. paniculata* (9.5 kg) were extracted twice with hot 95% EtOH. The combined EtOH extracts were then concentrated *in vacuo* to give a syrup (556 g), followed by suspension in water. The suspension was extracted with light petroleum, EtOAc and n-BuOH successively. The EtOAc extract (112 g) was subjected to column chromatography over silica gel, eluting with $CHCl_3$–MeOH (0–100%), to give nine fractions, A–I. Fraction C (18.6 g) was further subjected to column chromatography over silica gel, eluting with $CHCl_3$–MeOH (100:3–5), to afford **1** (1.1 g), **2** (12 mg).

3.3.1 Myricarin A (1). Colorless powder, 278–280°C (dec.), $[\alpha]_D^{26}$ − 12 (*c* 0.01,MeOH). UV (MeOH) λ_{max} (nm) (log ε): 207 (0.65), 245 (0.41), 328 (0.70). IR (KBr) ν (cm^{-1}): 3340 (OH), 2943, 2866, 1693 (C=O), 1604, 1516, 1444, 1279, 1174, 812. ^{1}H NMR (CD_3COCD_3) δ (ppm): 7.14 (1H, d, J = 2.0 Hz, H-2′), 6.84 (1H, d, J = 8.0 Hz, H-5′), 7.01 (1H, dd, J = 8.0, 2.0 Hz, H-6′), 7.52 (1H, d, J = 16.0 Hz, H-7′), 6.33 (1H, d, J = 16.0 Hz, H-8′), 5.57 (1H, dd, J = 8.0, 3.0 Hz, H-15), 4.69 (1H, t, J = 2.7 Hz, H-3), 1.01 (3H, s, CH_3), 1.00 (6H, s, 2 × CH_3), 0.96 (3H, s, CH_3), 0.95 (3H, s, CH_3), 0.92 (3H, s, CH_3), 0.88 (3H, s, CH_3). ^{13}C NMR: see table 1. EIMS *m/z* 618[M]$^+$ (3), 572 (4), 439 (32), 423 (19), 377 (9), 285 (6), 269 (12), 248 (34), 234 (23), 219 (26), 203 (32), 189 (60), 163 (100). HR-EIMS *m/z* 618.3895 ([M]$^+$, calcd for $C_{39}H_{54}O_6$, 618.3920).

3.3.2 Myricarin B (2). Colorless powder, 246–248°C (dec.), $[\alpha]_D^{26}$ − 13 (*c* 0.01, MeOH). UV (MeOH) λ_{max} (nm) (log ε): 207 (0.35), 225 (0.32), 300 (0.56). IR (KBr) ν (cm^{-1}): 3369 (OH), 2943, 2864, 1687 (C=O), 1604, 1514, 1444, 1205, 1167, 831. ^{1}H NMR (CD_3COCD_3) δ (ppm): 7.52 (2H, d, J = 8.7 Hz, H-2′,6′), 6.87 (2H, d, J = 8.7 Hz, H-3′, 5′), 7.59 (1H, d, J = 16.0 Hz, H-7′), 6.39 (1H, d, J = 16.0 Hz, H-8′), 5.57 (1H, dd, J = 8.0, 3.0 Hz, H-15), 4.70 (1H, t, J = 2.7 Hz, H-3), 1.01 (3H, s, CH_3), 1.00 (6H, s, 2 × CH_3), 0.96 (3H, s, CH_3), 0.95 (3H, s, CH_3), 0.92 (3H, s, CH_3), 0.88 (3H, s, CH_3). ^{13}C NMR: see table 1. FAB-MS

(positive) *m/z*: 603[M + H]$^+$ (4), 497 (4), 439 (36), 275 (15), 189 (16), 165 (13), 147 (100). HR-FABMS (positive) *m/z* 603.4038 ([M + H]$^+$, calcd for $C_{39}H_{55}O_5$, 603.4050).

Acknowledgements

The authors are grateful to the Department of Instrumental Analysis, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College for measuring the UV, IR, NMR and MS spectral data. We also thank Professor Duo Jie for the plant identification and Professor X.G. Chen for biological testing.

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