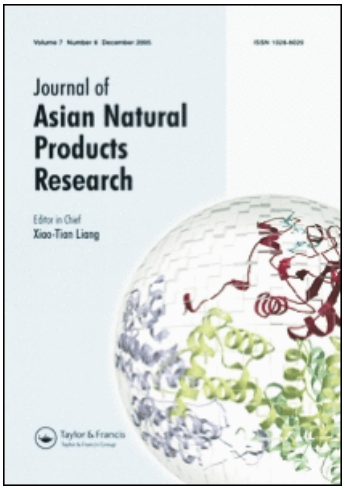


**Journal of Asian Natural Products Research**
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

# Triterpenoids from the husks of *Xanthoceras sorbifolia* Bunge

Z. -L. Li[a]; B. -Z. Yang[b]; X. Li[a]; S. -J. Wang[c]; N. Li[a]; Y. Wang[a]
[a] Research Department of Natural Medicine, Shenyang Pharmaceutical University, Shenyang, China [b] Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang, China [c] Pharmtrails Corporation, Thousand Oaks, CA, USA

**To cite this Article** Li, Z. -L. , Yang, B. -Z. , Li, X. , Wang, S. -J. , Li, N. and Wang, Y.(2006) 'Triterpenoids from the husks of *Xanthoceras sorbifolia* Bunge', Journal of Asian Natural Products Research, 8: 4, 361 — 366
**To link to this Article: DOI:** 10.1080/10286020500034642
**URL:** http://dx.doi.org/10.1080/10286020500034642

*Journal of Asian Natural Products Research*, Vol. 8, No. 4, June 2006, 361–366



*Note*

# Triterpenoids from the husks of *Xanthoceras sorbifolia* Bunge

Z.-L. LI†, B.-Z. YANG‡, X. LI†*, S.-J. WANG¶, N. LI† and Y. WANG†

†Research Department of Natural Medicine, Shenyang Pharmaceutical University, Shenyang 110016, China
‡Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, China
¶Pharmtrails Corporation, P.O. Box 1692 Thousand Oaks, CA, USA

*(Received 16 August 2004; revised 29 September 2004; in final form 11 October 2004)*

A new triterpenoid saponin, 3-*O*-[(3-*O*-α-L-arabinofuranosyl-2-*O*-β-D-galactopyranosyl)-β-D-glucuronopyranosyl]-21,22-di-*O*-angeloyl-$R_1$-barrigenol (**1**), together with four known triterpenoids, have been isolated from the husks of *Xanthoceras sorbifolia* Bunge. Their structures were elucidated based on chemical and spectral analysis. Among them, **1** was found to have activity of inhibiting the proliferation of six human tumour cell lines ($IC_{50}$ 10–40 μg/ml).

*Keywords*: *Xanthoceras sorbifolia* Bunge; Husks; Triterpenoids; Structure elucidation; Inhibition of tumour cell lines



## 1. Introduction

*Xanthoceras sorbifolia* Bunge. (Sapindaceae) is a shrub distributed in Inner Mongolia, China. Its wood and fruits are used as a folk medicine for the treatment of rheumatism, gout and enuresis of children. Some saponins and flavonoids from this plant have been reported in the literature [1–3]. The present paper describes the isolation and identification of a new triterpenoid saponin and other compounds from the husks of the titled plant (figure 1), as well as their inhibiting activities against human tumour cell lines.

## 2. Results and discussion

Compound **1** was a white needle, mp 267–268°C. The peak at *m/z* 1163 $[M + Na]^+$ in the ESI-MS, along with $^1H$ NMR and $^{13}C$ NMR, suggested a molecular formula of $C_{57}H_{88}O_{23}$. The ESI-MS also gave fragment ions at *m/z* 1008, 846 and 652 by losing a pentose,

*Corresponding author. E-mail: lixian@mail.sy.ln.cn

*Journal of Asian Natural Products Research*
ISSN 1028-6020 print/ISSN 1477-2213 online © 2006 Taylor & Francis
http://www.tandf.co.uk/journals
DOI: 10.1080/10286020500034642

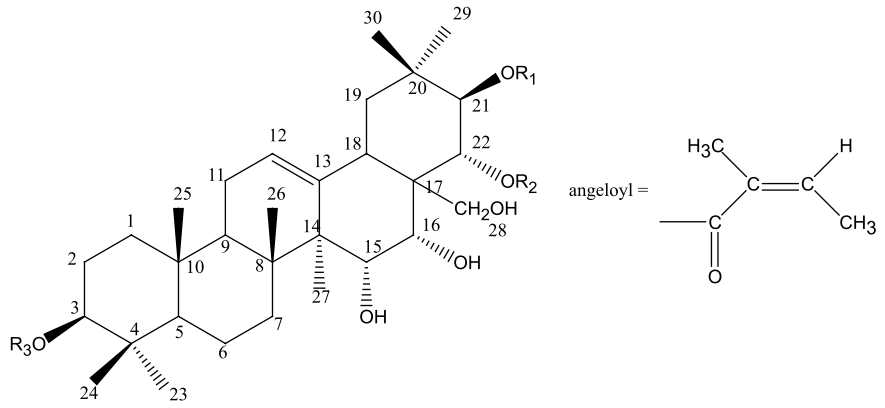


Figure 1. Structures of compounds **1**–**5**.



a hexose, and a hexuronic acid from $[M]^+$ successively. The $^1H$ NMR spectrum showed seven methyl singlets at $\delta$ 1.84, 1.31, 1.26, 1.16, 1.09, 0.98, 0.81, an olefinic proton at $\delta$ 5.48 (1H, brs), a signal at $\delta$ 3.21 (1H, dd, $J = 12.0$, 4.0 Hz, H-3), two signals at $\delta$ 6.72, 6.33 (each 1H, d, $J = 10.3$ Hz) which were assigned to protons at C-21 and C-22 bearing an ester group, respectively. Furthermore, the $^1H$ NMR data suggested the presence of two angeloyl functionalities: two olefinic protons at $\delta$ 5.97, 5.77 (each 1H, q, $J = 7.2$ Hz), four vinylic methyls at $\delta$ 2.01, 1.72 (each 3H, m), $\delta$ 2.09, 1.95 (each 3H, d, $J = 7.2$ Hz). In the $^{13}C$ NMR spectrum (table 1), 30 typical carbon signals of $R_1$-barrigenol were observed, together with those signals of angeloyl moieties. Based on those observations and the comparison of its NMR data with those of **5**, the aglycone moiety of **1** was identified as 21,22-di-*O*-angeloyl- $R_1$-barrigenol.

The sugar part of the molecule consisted of three residues characterised by signals for three anomeric carbons in $^{13}C$ NMR at $\delta$ 105.2, 104.9, 111.2, and attached to protons at $\delta$ 4.89 (d, $J = 7.3$ Hz), 5.32 (d, $J = 7.5$ Hz), 6.03 (brs) respectively in HMQC spectrum. Acid hydrolysis, TOCSY and HMQC experiments allowed the full identification of the sugar residues composed of an α-L-arabinofuranose, a β-D-galactopyranose and a β-D-glucuropyranoic acid, whose anomeric configurations were determined by the coupling constants and the comparison of $^{13}C$ NMR data with those in the literature [4]. In the HMBC spectrum (figure 2), the long-range correlations between H-1 ($\delta$ 5.32) of galactose with C-2 ($\delta$ 78.9) of glucuronic acid, H-1 ($\delta$ 6.03) of arabinose with C-3 ($\delta$ 86.4) of glucuronic acid, H-1 ($\delta$ 4.89) of glucuronic acid with C-3 ($\delta$ 89.9) of the genin, suggested the sequencing and the attachment position of the sugar moiety. Consequently, the structure of **1** was concluded to be 3-*O*-[(3-*O*-α-L-arabinofuranosyl-2-*O*-β-D-galactopyranosyl)-β-D-glucuronopyranosyl]-21,22-di-*O*-angeloyl-$R_1$-barrigenol.{INSERT FIGURE 2 HERE}

With MTT and SRB methods, compounds **1**–**5** were tested for their anti-tumour activity *in vitro* against six human tumour cell lines (HL-60, PC-3MIE8, BGC-823, MDA-MB-435, Bel-7402 and HeLa). **1** was found to be the most active against all the cell lines, with $IC_{50}$ values varying between 10 and 40 μg/ml (table 2); the other compounds were inactive, which suggested that glycosidation at C-3 of the sapogenol was necessary to the activity.

Table 1. $^{13}C$ NMR data of compound **1** (75 MHz, pyridine-$d_5$; $\delta$ in ppm).

| *No.* | ***1*** | *No.* | ***1*** |
|---|---|---|---|
| 1 | 39.7 | C-21-angeloyl | |
| 2 | 26.7 | 1 | 167.8 |
| 3 | 89.9 | 2 | 129.0 |
| 4 | 39.0 | 3 | 137.4 |
| 5 | 55.7 | 4 | 15.9 |
| 6 | 18.9 | 5 | 21.0 |
| 7 | 36.4 | C-22-angeloyl | |
| 8 | 41.1 | 1 | 168.2 |
| 9 | 47.2 | 2 | 129.2 |
| 10 | 37.0 | 3 | 136.6 |
| 11 | 24.0 | 4 | 15.7 |
| 12 | 125.5 | 5 | 20.7 |
| 13 | 143.7 | Sugar moiety | |
| 14 | 47.8 | GlcUA | |
| 15 | 67.6 | 1 | 105.2 |
| 16 | 73.4 | 2 | 78.9 |
| 17 | 48.4 | 3 | 86.4 |
| 18 | 41.5 | 4 | 71.7 |
| 19 | 47.0 | 5 | 77.3 |
| 20 | 36.8 | 6 | 172.0 |
| 21 | 78.1 | Gal | |
| 22 | 73.6 | 1 | 104.9 |
| 23 | 28.0 | 2 | 73.5 |
| 24 | 16.8 | 3 | 75.2 |
| 25 | 15.8 | 4 | 69.8 |
| 26 | 17.6 | 5 | 76.7 |
| 27 | 21.3 | 6 | 61.9 |
| 28 | 63.2 | Ara | |
| 29 | 29.6 | 1 | 111.2 |
| 30 | 20.3 | 2 | 83.6 |
| | | 3 | 77.7 |
| | | 4 | 85.5 |
| | | 5 | 62.4 |



## 3. Experimental

### *3.1 General experimental procedures*

Melting points were measured on a Yanaco MPS3-hot-stage without correction. The UV spectrum was recorded on a Shimadzu UV-260 UV–Vis instrument. ESI-MS was performed on a Finnigan LCQ spectrometer. Optical rotation was measured on a Perkin-Elmer 241 polarimeter. One- and two-dimensional NMR spectra were recorded on a Bruker-ARX-300 or an INOVA-BMU-500 spectrometer, using TMS as an internal standard.

### *3.2 Plant material*

The husks of *Xanthoceras sorbifolia* Bunge. were collected at Shenhe district, Shenyang, China. The plant was identified by Associate Professor Yang Bai-Zhen. A voucher specimen (No. 0154620) is deposited in the Herbarium Department of the Institute of Applied Ecology, Chinese Academy of Sciences.

### *3.3 Extraction and isolation*

Dried husks of *Xanthoceras sorbifolia* Bunge. (4.0 kg) were extracted (three times) with 70% aqueous ethanol. After evaporation of ethanol *in vacuo*, the extract was suspended in water

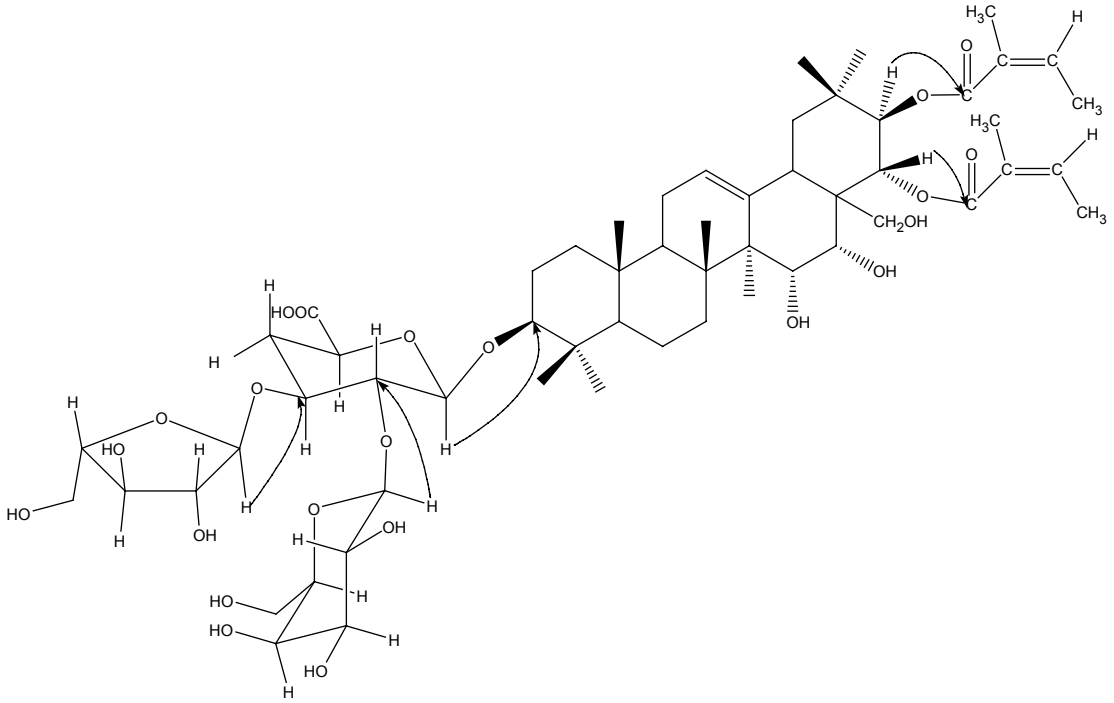


Figure 2. The main HMBC correlations for compound **1**.

Table 2. $IC_{50}$ values of compound **1** against six human tumour cells.

| | *HL-60* | *PC-3MIE8* | *BGC-823* | *MDA-MB-435* | *Bel-7402* | *HeLa* |
|---|---|---|---|---|---|---|
| $IC_{50}$ (μg/ml) | 41.92 | 40.98 | 40.24 | 18.58 | 39.32 | 34.23 |

and partitioned with EtOAc and n-BuOH for three times successively. The EtOAc extract (24 g) was subjected to column chromatography over silica gel (200–300 mesh), using $CHCl_3$/MeOH as eluent to yield **5** (12 mg, 100:1.0), **4** (15 mg, 100:1.5), **3** (30 mg, 100:4.5), **2** (10 mg, 100:7.5), respectively. The n-BuOH extract (125 g) was chromatographed on silica gel (200–300 mesh) to give **1** (990 mg, 100:45–100:50).

Compound **1**: white needle (MeOH), mp 267–268°C. UV $\lambda_{max}$ (MeOH): 211.6 nm. $[\alpha]_D^{20}$ + 19.1 [*c* 0.8, MeOH/$H_2O$ (1:1)]. $^1$H NMR (300 MHz, pyridine-$d_5$), aglycone moiety: $\delta$ 3.21 (1H, dd, $J = 12.0$, 4.0 Hz, H-3), 5.48 (1H, brs, H-12), 6.72, 6.33 (each 1H, $J = 10.3$ Hz, H-21, 22), 3.73, 3.50 (each 1H, $J = 10.2$ Hz, H-28); angeloyl moiety: $\delta$ 5.97, 5.77 (each 1H, q, $J = 7.2$ Hz), 2.01, 1.72 (each 3H, m), 2.09, 1.95 (each 3H, d, $J = 7.2$ Hz); sugar moiety: $\delta$ 4.89 (1H, d, $J = 7.3$ Hz, glcUA-H-1), 5.32 (1H, d, $J = 7.5$ Hz, gal-H-1), 6.03 (1H, brs, ara-H-1). $^{13}$C NMR (75 MHz, pyridine-$d_5$): see table 1.

The $^1$H and $^{13}$C NMR data of **2**–**5** were in great coincidence with those of the known compounds in the literature [2,5,6].

### *3.4 Acid and alkaline hydrolysis of compound 1*

**1** (20 mg) was hydrolysed by refluxing with a mixture of HCl/$H_2O$/EtOH (2:1:2) (10 ml) In a water bath for 6 h. The hydrolysate was partitioned between EtOAc and $H_2O$, the aqueous layer was neutralised to pH 7 with NaOH, then thoroughly dried. The comparison with authentic samples on silica gel (BAW, upper phase) showed the sugars were composed of D-galactose, D-glucuronic acid and L-arabinose. The EtOAc extract was chromatographed on silica gel with $CHCl_3$/MeOH (100:1.0) to give the aglycone (7 mg, mp 272–274°C), which showed the identical $R_f$ value with 21,22-di-*O*-angeloyl-$R_1$-barrigenol (compound **5**) on a silica gel TLC [$CHCl_3$/MeOH (20:1)]. The solution of aglycone (5 mg) in 5% KOH/MeOH (5 ml) was refluxed for 2 h and neutralised with $H_2SO_4$. After removing the solvent, the residue was extracted with EtOAc to give a white needle (2 mg, mp 293–295°C), which was identical with $R_1$-barrigenol (compound **2**) on a silica gel TLC [$CHCl_3$/MeOH (6:1)].

### *3.5 Anti-tumour activity assay*

The MTT and SRB methods were used with three concentration levels (1, 10, 100 μg/ml). $IC_{50}$ values were calculated using the LOGIT method.

## Acknowledgements

We are grateful to the Analytical Centre, Shenyang Pharmaceutical University, for recording UV, ESI-MS and NMR spectra. Sincere thanks are also due to the State Key Laboratory of Natural and Biomimetic Drugs, Health Science Centre, Peking University, for activity testing.

## References

[1] Y.J. Chen, T. Takeda, Y. Ogihara, Y. Iitaka. *Chem. Pharm. Bull.*, **32**, 3378 (1984).
[2] Y.J. Chen, T. Takeda, Y. Ogihara. *Chem. Pharm. Bull.*, **33**, 127 (1985).
[3] C.M. Ma, N. Nakamura, M. Hattori, H. Kakuda, J.C. Qiao, H.L. Yu. *J. Nat. Prod.*, **63**, 238 (2000).
[4] L. Voutquenne, C. Lavaud, G. Massiot, C. Delaude. *Phytochemistry*, **49**, 2081 (1998).
[5] M.D. Greca, A. Fiorentino, P. Monaco, L. Previtera. *Phytochemistry*, **35**, 201 (1994).
[6] E. Aurada, J. Jurenitsch, W. Kubelka. *Plant Med.*, **50**, 391 (1984).