

A New Megastigmane Diglycoside from *Litsea glutinosa* (Lour.) C. B. Rob.

Yun-Song Wang,^a Zhen Liao,^b Yan Li,^c Rong Huang,^a Hong-Bing Zhang^a and
Jing-Hua Yang^{*,a}

^aKey Laboratory of Medicinal Chemistry for Natural Resource, Ministry of Education,
School of Chemical Science and Technology, Yunnan University, Kunming 650091, P. R. China

^bYunnan Academy of Tobacco Science, Kunming 650031, P. R. China

^cState Key Laboratory of Phytochemistry and Plant Resources in West China,
Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, P. R. China

Estudo fotoquímico das folhas e galhos de *Litsea glutinosa* (Lour.) C. B. Rob. forneceu um novo diglicosídeo megastimano (6*S*, 7*E*, 9*R*)-6, 9-dihidroxi-4, 7-megastigmadieno-3-ona-9-*O*-[α -L-arabinofuranosil-(1 $\rightarrow$ 6)]- β -D-glucopiranosídeo (**1**), juntamente com os glicosídeos (6*S*, 7*E*, 9*R*)-roseosido (**2**), (7'*R*, 8'*R*)-3, 5'-dimetoxi-9, 9'-dihidroxi-4, 7'-epóxi lignina 4'- β -D-glucopiranosídeo (**3**), álcool (7'*R*, 8'*S*)-dihidrodéhidrodiconifenil 9'-*O*- β -D-xilopiranosídeo (**4**) e pinoresinol 3-*O*- β -D-glucopiranosídeo (**5**). Suas estruturas foram estabelecidas com base em métodos espectroscópicos e químicos. Compostos **2-5** são reportados pela primeira vez nestas espécies. Composto **1** foi avaliado para atividades citotóxicas de linhagens de células tumorais humanas (células leucemia mielóide HL-60, carcinoma hepatocelular SMMC-7721, câncer de pulmão A-549, câncer da mama MCF-7 e câncer do colo SW480), para as quais provou-se ser inativo (IC₅₀ > 40 μ M).

Phytochemical study on the leaves and twigs of afforded the new megastigmane diglycoside (6*S*, 7*E*, 9*R*)-6, 9-dihydroxy-4, 7-megastigmadien-3-one-9-*O*-[α -L-arabinofuranosyl-(1 $\rightarrow$ 6)]- β -D-glucopyranoside (**1**), along with glycosides (6*S*, 7*E*, 9*R*)-roseoside (**2**), (7'*R*, 8'*R*)-3, 5'-dimethoxy-9, 9'-dihydroxy-4, 7'-epoxylignan 4'- β -D-glucopyranoside (**3**), (7'*R*, 8'*S*)-dihydrodehydrodiconifenyl alcohol 9'-*O*- β -D-xylopyranoside (**4**) and pinoresinol 3-*O*- β -D-glucopyranoside (**5**). Their structures were established on the basis of extensive spectroscopic and chemical methods. Compounds **2-5** were reported for the first time in this species. Compound **1** was evaluated for cytotoxic activities against human tumor cell lines (myeloid leukemia HL-60, hepatocellular carcinoma SMMC-7721, lung cancer A-549, breast cancer MCF-7 and colon cancer SW480 cells), for which it was proved to be inactive (IC₅₀ > 40 μ M).

Keywords: Lauraceae, *Litsea glutinosa* (Lour.) C. B. Rob., megastigmane glycosides, lignan glycosides

Introduction

Litsea (Lauraceae) is a genus of about 200 species mainly growing in tropical and subtropical Asia, some distributed in Australia and from North America to subtropical South America. In China, this genus is represented by 72 species and mostly distributed in the south and southwestern parts of the country, where many of them are known for their edible fruits and medicinal properties.¹ Previous phytochemical studies have indicated

that *Litsea* species contain structurally diverse and biologically active aporphine alkaloids,²⁻⁴ butanolides^{5,6} and sesquiterpenes.⁷⁻¹⁰

Litsea glutinosa (Lour.) C. B. Rob. is an evergreen tree known in China as "Chan Gao Shu". The leaves and twigs of *L. glutinosa* (Lour.) have been used as a demulcent and mild astringent for diarrhea and dysentery, whereas the roots are used in local folk medicine to poultice sprains and bruises.¹ Earlier phytochemical studies on this plant have been reported,¹¹⁻¹⁶ and these include the isolation of an arabinoxylan,¹² an abscisic acid derivative and lignans,¹³ aporphine alkaloids¹⁴ and a flavone glycoside.¹⁵

*e-mail: yangjh@ynu.edu.cn

The present study resulted in the characterization of a new megastigmane diglycoside (**1**), along with known compounds (6*S*, 7*E*, 9*R*)-roseoside (**2**), (7'*R*, 8'*R*)-3, 5'-dimethoxy-9, 9'-dihydroxy-4, 7'-epoxylignan 4'-β-D-glucopyranoside (**3**), (7'*R*, 8'*S*)-dihydro-dehydrodiconifenyl alcohol 9'-*O*-β-D-xylopyranoside (**4**), and pinoresinol 3-*O*-β-D-glucopyranoside (**5**), from the EtOH extract of *L. glutinosa* leaves and twigs. Compound **1** was evaluated for cytotoxic activities against five tumor cell lines, for which it was proved to be inactive.

Experimental

General

Commercial silica-gel plates (Qing Dao Marine Chemical Group Co.) were used for thin layer chromatography (TLC) analyses. Normal silica was used for normal (Qing Dao Marine Chemical Group Co.) and reversed-phase CC (Merk). UV spectra were measured on a Shimadzu UV-2401PC spectrophotometer ($\lambda_{\max}$ in nm). Optical rotation was obtained on a Horiba SEAP-300 spectropolarimeter. Infrared (IR) spectra were measured on a Bio-Rad FTS-135 infrared spectrophotometer ($\nu_{\max}$ in cm^{-1}). ^1H (500 MHz) and ^{13}C NMR (125 MHz) spectra, as well as 2D-NMR spectra, were recorded on a Bruker DRX-500 spectrometer, chemical shifts δ in ppm related to TMS (coupling constant J in Hz). Electrospray ionization mass spectrometry (ESI-MS) spectra were acquired on a VG-Autospec 3000 mass spectrometers.

Plant material

Leaves and twigs of *L. glutinosa* (Lour.) C. B. Rob. were collected in Xishuangbanna in Yunnan Province (People's Republic of China) in May 2003, and identified by Professor Yu Chen of Kunming Institute of Botany. A voucher specimen is deposited in the Key Laboratory of Medicinal Chemistry for Natural Resource (Ministry of Education, School of Chemical Science and Technology, Yunnan University).

Extraction and isolation

Powdered leaves and twigs of *L. glutinosa* (Lour.) (12.0 kg) were repeatedly extracted with EtOH at room temperature. Then, the extract was concentrated under reduced pressure to give a brown syrup, which was partitioned in H_2O with solvents of increasing polarity to yield a petroleum ether-fraction (80 g), an EtOAc-fraction (54 g) and a *n*-BuOH-fraction (108 g). The *n*-BuOH-soluble fraction was subjected to silica-gel column chromatography and

eluted with CHCl_3 -MeOH (9:1-1:1) to afford eight fractions (I-VIII). Fraction V (15 g) was further separated by reversed-phase silica-gel column chromatography and eluted with H_2O -MeOH (7:3, 6:4, 5:5), and then Sephadex LH-20 column chromatography (MeOH elution) to give **2** (3 mg), **3** (5 mg) and **5** (10 mg). Fraction VI (21 g) was subjected to silica-gel column chromatography and eluted with CHCl_3 -MeOH (9:1-1:1), and then to RP C-18 (H_2O -MeOH 7:3, 6:4) to yield compound **4** (6 mg). Fraction VII (14 g) was rechromatographed on a silica-gel column and eluted with CHCl_3 containing increasing amounts of MeOH, and then to Sephadex LH-20 column chromatography (MeOH elution) to give compound **1** (4 mg).

(6*S*, 7*E*, 9*R*)-6, 9-dihydroxy-4, 7-megastigmadien-3-one-9-*O*-[α -*L*-arabinopyranosyl-(1 $\rightarrow$ 6)]-β-D-glucopyranoside (**1**)

White amorphous powder; $[\alpha]_{\text{D}}^{21} -16^\circ$ (*c* 0.55, MeOH); IR (KBr) $\nu_{\max}/\text{cm}^{-1}$ 3460, 1642, 1548, 1413; UV (MeOH) $\lambda_{\max}/\text{nm}$ 250; ^1H and ^{13}C NMR: see Table 1; HRESIMS (m/z) calcd for $\text{C}_{24}\text{H}_{38}\text{O}_{12}$: 517.5525, found: 517.5513 $[\text{M}-\text{H}]^-$.

Acid hydrolysis of compound 1

A solution of **1** (1 mg) in 1 mol L^{-1} HCl (2 mL) was heated at 80 °C for 2 h. The reaction mixture was diluted with H_2O and then extracted with Et_2O . D-glucose and L-arabinose were identified from the aqueous phase by TLC comparison with authentic substances.

Bioassays

Human cancer cell lines myeloid leukemia HL-60, hepatocellular carcinoma SMMC-7721, lung cancer A-549, breast cancer MCF-7 and colon cancer SW480 cells were used in the cytotoxic assay, which was performed according to the MTT (3-(4,5-dimethylthiazol-2-yl)-3,5-di-phenyltetrazolium bromide) method in 96-well microplates.¹⁶ Cells were cultured in RPMI-1640 or DMEM medium (Hyclone, USA) and supplemented with 10% fetal bovine serum (Hyclone, USA) in 5% CO_2 at 37 °C.

Results and Discussion

Compound **1**, $[\alpha]_{\text{D}}^{21} -16^\circ$ (*c* 0.55, CH_3OH) was isolated as an amorphous powder. The molecular formula $\text{C}_{24}\text{H}_{38}\text{O}_{12}$ was deduced from the high resolution fast atom bombardment mass spectrometry (HR-FABMS) exhibiting a quasi-molecular ion at m/z 517.5513 $[\text{M}-\text{H}]^-$ (calcd. for 517.5525). Compound **1** showed UV absorption at

Table 1. ^1H and ^{13}C NMR spectral data for compounds **1** and **2** (in MeOD)

Position	1		2	
	δ_{H} / ppm (mult, integral, J / Hz)	δ_{C}	δ_{H} / ppm (mult, integral, J / Hz)	δ_{C}
1	—	42.5 s	—	42.4 s
2 β	2.54 (d, 1H, 17.0)	50.8 t	2.50 (d, 1H, 17.0)	50.7 t
2 α	2.17 (d, 1H, 17.0)	50.8 t	2.16 (d, 1H, 17.0)	50.7 t
3	—	201.4 s	—	201.2 s
4	5.90 (s, 1H)	127.3 d	5.85 (s, 1H)	127.2 d
5	—	167.4 s	—	167.3 s
6	—	80.1 s	—	80.0 s
7	5.85 (d, 1H, 15.7)	131.7 d	5.86 (1H, overlap)	131.6 d
8	5.84 (dd, 1H, 15.7, 6.4)	134.9 d	5.85 (1H, overlap)	135.3 d
9	4.42 (quint., 1H, 6.4)	76.8 d	4.41 (1H, m)	77.3 d
10	1.29 (d, 3H, 6.4)	21.1 q	1.29 (d, 3H, 6.6)	21.2 q
11	1.04 (s, 3H)	23.5 q	1.02 (3H, s)	23.4 q
12	1.03 (s, 3H)	24.7 q	1.01 (3H, s)	24.7 q
13	1.92 (d, 3H, 1.5)	19.8 q	1.93 (3H, d, 1.5)	19.6 q
Glu-1'	4.35 (d, 1H, 7.8)	102.6 d	4.33 (1H, d, 7.8)	102.8 d
Glu-2'	3.16 (dd, 1H, 7.9 and 9.5)	75.2 d	3.18 (m, 1H)	75.3 d
Glu-3'	3.40 (m, 1H)	76.7 d	3.33 (m, 1H)	78.1 d
Glu-4'	3.31 (m, 1H)	71.9 d	3.33 (m, 1H)	71.7 d
Glu-5'	3.29 (m, 1H)	78.0 d	3.23 (m, 1H)	78.2 d
Glu-6'	4.00 (m, 1H)	68.0 t	3.84(brd, 1H, 10.7)	62.9 t
	3.58 (dd, 1H, 5.7 and 11.2)		3.63 (brd, 1H, 10.7)	
Ara-1''	4.94 (d, 1H, 1.7)	109.9 d		
Ara-2''	4.00 (m, 1H)	83.1 d		
Ara-3''	3.82 (dd, 1H, 3.3 and 6.0)	78.9 d		
Ara-4''	3.97 (m, 1H)	85.9 d		
Ara-5''	3.74 (dd, 1H, 3.2 and 11.8)	63.1 t		
	3.64 (dd, 1H, 5.4 and 11.8)			

250 nm and an IR band at 1642 cm^{-1} , characteristic of an α,β -unsaturated carbonyl.

The ^1H NMR spectrum of **1** showed signals assignable to a double bond proton at δ 5.90 (s), two vinyl protons at δ 5.85 (d, J 15.7 Hz) and δ 5.84 (dd, J 15.7 and 6.4 Hz), an oxygenated methine at δ 4.42, an isolated methylene (δ 2.54 and 2.17, each d, J 17.0 Hz), a vinyl methyl (δ 1.92, d, J 1.5 Hz), a secondary methyl (δ 1.29, d, J 6.4 Hz) and two tertiary methyl singlets at δ 1.04 and 1.03 (Table 1). The ^{13}C NMR spectrum of **1** showed 24 carbon signals, including those characteristic of a disaccharide moiety. Analysis of 1D and 2D NMR spectra indicated that the aglycone portion of **1** was identical to that of the megastigmane glycosides (6*S*, 7*E*, 9*R*)-roseoside (**2**).¹⁷ ^1H and ^{13}C NMR data of the disaccharide moiety suggested the presence of glucopyranosyl and arabinofuranosyl units.^{17,18} This was confirmed by acid hydrolysis of **1** that afforded D-glucose and L-arabinose, which were identified by direct comparison with authentic samples. The J values of the anomeric protons at 4.35 (d, H-1', J 7.8 Hz, δ_{C} 102.6d) and δ 4.94 (d, H-1'', J 1.7 Hz, δ_{C} 109.9d) established the sugar units as β -D-glucopyranosyl and α -L-arabinofuranosyl in **1**.

The locations of the sugar residues in **1** were established by the presence of the NOESY (nuclear Overhauser enhancement spectroscopy) cross peak between H-1' (δ 4.35) and H-9 (δ 4.42), and HMBC (heteronuclear multiple bond correlation) correlation between H-1' and C-9 (δ 76.8). These results demonstrated the glucosyl moiety are connected to the 9-OH group of the aglycone through a glycosidic linkage.¹⁷ Furthermore, the C-6' signal of the inner β -D-glucopyranosyl unit was observed at δ_{C} 68.0, indicating the involvement of this carbon in a glycosidic linkage.¹⁹ The other anomeric proton (arabinose) at δ 4.94 was also correlated with C-6' of glucose at δ 68.0 (Figure 2). From the above data, compound **1** appears to be the arabinofuranosyl-(1'' $\rightarrow$ 6')-glucopyranosyl analogue of roseoside. Literature data report that most megastigmane glycosides possesses the 1'' $\rightarrow$ 6' linkage in their disaccharide unit.^{19,20}

Stereochemical assignments of **1** could be deduced from NOESY, COSY and CD (circular dichroism) experiments. The ^1H - ^1H COSY spectra showed long-range couplings between H-2 β and H₃-11, H-2 α and H-4, whereas NOE (nuclear Overhauser effect) cross-peaks

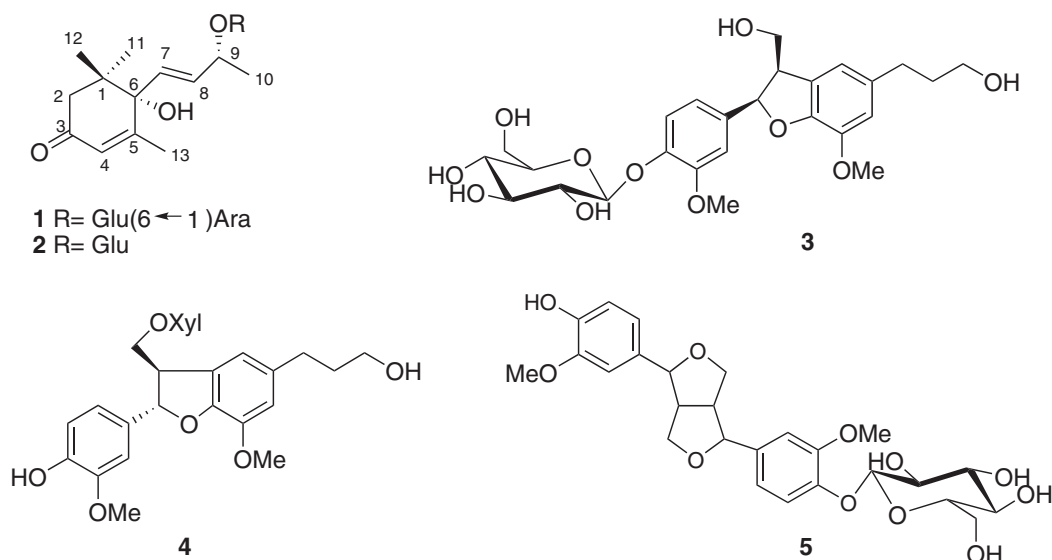


Figure 1. Chemical structures of compounds 1-5.

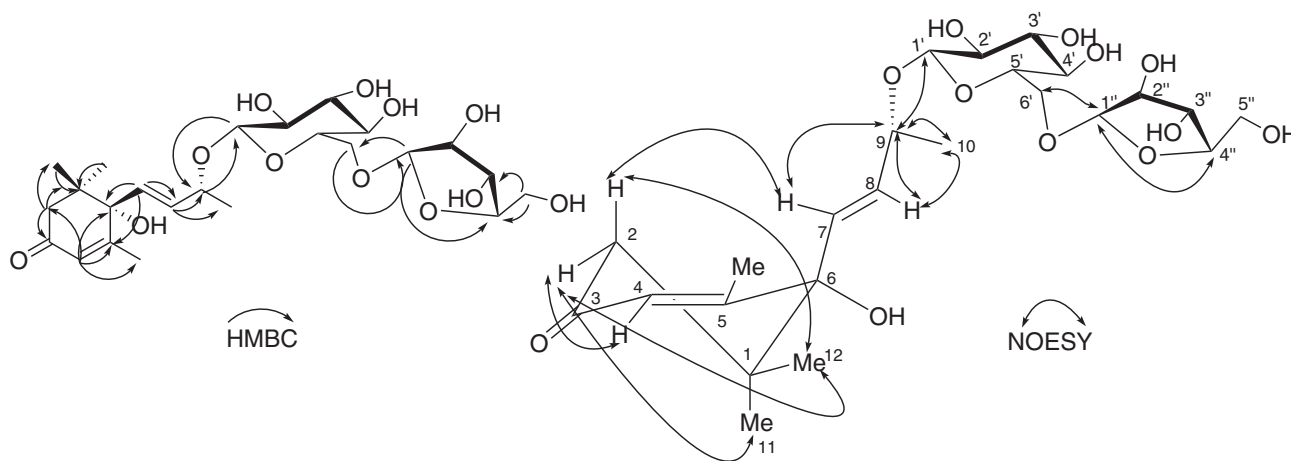


Figure 2. Key HMBC and NOESY correlations of 1.

were observed between H-7 and H-2 β , H-2 β and H₃-12, H-2 α and H₃-11, H-2 α and H₃-12 (Figure 2). These results demonstrated that the side-chain (C-7-C-10) was in a pseudo-axial orientation to the half-chair conformation of the cyclohexenone ring. The CD spectrum of **1** showed a positive Cotton effect at 240 nm, similar to that of **2**, thus indicating a 6*S*-configuration. The absolute configuration at C-9 of the aglycon was assigned as *R* on the basis of a diagnostic chemical shift of the C-9 signal (δ 76.8) in the ¹³C NMR spectrum.²¹ Consequently, the structure of **1** was determined to be (6*S*, 7*E*, 9*R*)-6,9-dihydroxy-4,7-megastigmadien-3-one-9-*O*-[α -L-arabinofuranosyl-(1'' $\rightarrow$ 6')]- β -D-glucopyranoside (Figure 1). Earlier study reported the isolation of (6*S*, 7*E*, 9*R*)-6, 9-dihydroxy-4, 7-megastigmadien-3-one-9-*O*-[α -L-arabinopyranosyl-(1 $\rightarrow$ 6)]- β -D-glucopyranoside from *Lonicera gracilipes* Var. *glandulosa*.²²

The structures of known compounds **2-5** were identified as (6*S*, 7*E*, 9*R*)-roseoside (**2**),¹⁷ (7'*R*, 8'*R*)-3, 5'-dimethoxy-9, 9'-dihydroxy-4, 7'-epoxylignan 4'- β -D-glucopyranoside (**3**),²¹ (7'*R*, 8'*S*)-dihydrodehydrodiconifenyl alcohol 9'-*O*- β -D-xylopyranoside (**4**)²³ and pinoresinol 3-*O*- β -D-glucopyranoside (**5**),²⁴ by comparison of physical data and spectroscopic literature data. These glycosides are reported for the first time from the genus *Litsea*.

Naturally occurring megastigmane derivatives are an expanding class of compounds. Several oxidation steps and glycosylation at the megastigmane scaffold afforded several derivatives with anti-proliferative, anticancer and cytotoxic effects.^{18,19,25-28} Compound **1** was tested for its cytotoxicity effects in the human tumor cell lines myeloid leukemia HL-60, hepatocellular carcinoma SMMC-7721, lung cancer A-549, breast cancer MCF-7 and colon cancer SW480 cells, but it proved to be inactive (IC₅₀ > 40 μ M).

Supplementary Information

^1H and ^{13}C NMR, ^1H - ^1H COSY, HSQC, HMBC, NOESY NMR spectra of compound **1** are available free of charge at <http://jbcs.sbq.org.br> as PDF file.

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