Phytochemistry Letters 10 (2014) 230–234

Contents lists available at ScienceDirect

Phytochemistry Letters

journal homepage: www.elsevier.com/locate/phytol

Two new anti-HBV lignans from *Herpetospermum caudigerum*

Jin-Qian Yu [a], Wei Hang [b], Wen-Juan Duan [a], Xiao Wang [a,b,*], Dai-Jie Wang [a], Xue-Mei Qin [c,**]

[a] Shandong Analysis and Test Center, Shandong Academy of Sciences, Jinan, 250014, PR China
[b] College of Pharmacy, Shandong University of Traditional Chinese Medicine, Jinan, 250014, PR China
[c] Department of Hematology, Qilu Hospital, Shandong University, Jinan, 250014, PR China

ARTICLE INFO

Article history:
Received 13 May 2014
Received in revised form 28 September 2014
Accepted 1 October 2014
Available online 16 October 2014

Keywords:
Herpetospermum caudigerum Wall
Cucurbitaceae
Lignan
Anti-HBV
HepG 2.2.15 cell

ABSTRACT

Two new lignans, named (+)-(7′*S*, 7″*S*, 8′*R*, 8″*R*)-4, 4′, 4″-trihydroxy-3, 5′, 3″-trimethoxy-7-oxo-8-ene [8-3′, 7′-O-9″, 8′-8″, 9′-O-7″] lignoid (**1**) and (1*S*)-4-Hydroxy-3-[2-(4-hydroxy-3-methoxy-phenyl)-1-hydroxymethyl-2-oxo-ethyl]-5-methoxy-benzaldehyde (**2**), along with five known (**3**–**7**) ones, have been isolated from the 95% ethanol extract of the seeds of *Herpetospermum caudigerum* Wall. The structures of the new compounds, including the absolute configurations, were elucidated by spectroscopic and CD analysis. Compounds **1**, **2**, and **7** displayed inhibitory activities on HBsAg secretion with IC_{50} values of 20.5, 0.34, and 4.89 μM, while **1**, **2**, and **7** displayed inhibitory activities on HBeAg secretion with IC_{50} values of 3.54, 4.83×10^{-4}, and 8.02 μM, and cytotoxicity on HepG 2.2.15 cells with CC_{50} values of 12.7, 2.96×10^{5}, and 11.4 μM, respectively.

1. Introduction

Herpetospermum caudigerum Wall (*H. pedunculosum* (SER.) BAILL.), an annual medical herb from the family of Cucurbitaceae, is widely distributed in southwest China, Nepal, and northeast India. The dried ripe seeds of *H. caudigerum* have been popularly known and used for the treatment of liver diseases, cholic diseases, and dyspepsia as a Tibetan medicinal herb in traditional Chinese medicine (TCM) (Brend, 1995; Cheng, 1985). The main native organic compounds isolated from *H. caudigerum* are lignans (Kaouadji and Favre, 1987). Our previous biological screening of the ethyl acetate soluble fraction and the ethanol extract revealed significant inhibitory activities against hepatitis b virus. This prompted us to carry out bioassay-guided isolation studies on the ethyl acetate soluble fraction of the title plant. In this paper, we describe the isolation and structure elucidation of two new compounds: (+)-(7′*S*, 7″*S*, 8′*R*, 8″*R*)-4, 4′, 4″-trihydroxy-3, 5′, 3″-trimethoxy-7-oxo-8-ene [8-3′, 7′-O-9″, 8′-8″, 9′-O-7″] lignoid (**1**) and (1*S*)-4-hydroxy-3-[2-(4-hydroxy-3-methoxy-phenyl)-1-hydroxymethyl-2-oxo-ethyl]-5-methoxy-benzaldehyde (**2**), and five known compounds (**3**–**7**), as well as the evaluation of their anti-HBV activities. The compounds **1**, **2**, and **7** showed significant inhibitory activities against hepatitis b virus *in vitro* (Fig. 1).

2. Results and discussion

Compound **1** was obtained as a white amorphous powder with $[\alpha]_D^{20}$ +60.8 (*c* = 0.625, CH_3OH, 20 °C) and determined to possess the molecular formula $C_{30}H_{30}O_9$ by its pseudo-molecular ion peak at *m*/*z* 535.1967 $[M+H]^+$ in the positive HRESI-MS experiment. The IR spectrum showed the absorption bands for hydroxyl (3393 cm^{-1}), carbonyl (1651 cm^{-1}), and olefinic (1592, 1512 cm^{-1}) groups. The ^1H NMR spectrum of compound **1** exhibited the characteristic signals attributable to eight aromatic protons, including two ABX spin systems at δ_H 6.77 (d, *J* = 8.4 Hz, H-5″), 6.81 (dd, *J* = 1.8, 8.4 Hz, H-6″), 6.95 (d, *J* = 1.8 Hz, H-2″) and δ_H 6.77 (d, *J* = 7.8 Hz, H-5), 7.44 (dd, *J* = 1.8, 7.8 Hz, H-6), 7.45 (d, *J* = 1.8 Hz, H-2) and two proton AB doublets at δ_H 6.94 (d, *J* = 2.4 Hz, H-2′) and 6.95 (d, *J* = 2.4 Hz, H-6′). According to the heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple bonding connectivity (HMBC) spectra, the NMR chemical shifts of the carbons signals could be assigned unambiguously. The ^1H NMR spectrum and ^1H–^1H COSY spectrum also allowed identification of the H_2-9 protons as two alkenyl singlets at δ_H 5.96 (1H, s, H_a-9) and 5.66 (1H, s, H_b-9), which

* Corresponding author at: Shandong Analysis and Test Center, Shandong Academy of Sciences, 19 Keyuan Street, Jinan, Shandong 250014, China. Tel.: +86 0531 82605319; fax: +86 531 8296 4889.
** Corresponding author.
E-mail addresses: yujinqian87528@126.com (J.-Q. Yu), hangwei_1003@sina.com (W. Hang), duanwj4048@126.com (W.-J. Duan), wxjn1998@126.com (X. Wang), wangdaijie@126.com (D.-J. Wang), Qinxm21@aliyun.com (X.-M. Qin).

http://dx.doi.org/10.1016/j.phytol.2014.10.001
1874-3900/

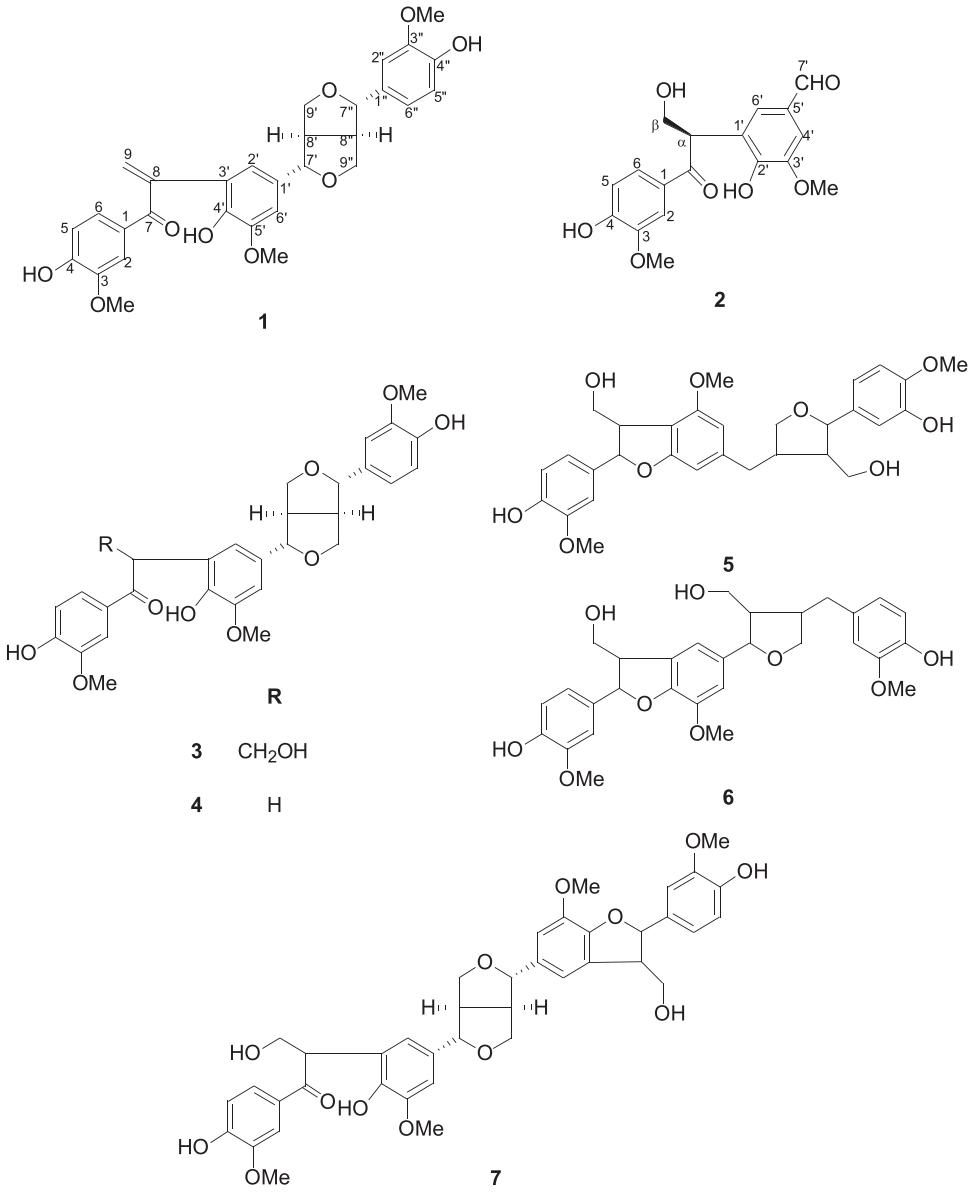

Fig. 1. The structures of compounds **1**–**7**.

directly correlated with δ_C 121.9 (C-9) by HMQC experiment. In addition, the spectrum showed resonances due to a bistetrahydrofuran group at δ_H 4.73 (d, J = 4.2 Hz, H-7′), 4.71 (d, J = 4.8 Hz, H-7″), 3.15 (overlapped, H-8′, 8″), 4.24 (dd, J = 7.2, 16.2 Hz, H_a-9′, 9″), 3.84 (overlapped, H_b-9′, 9″), together with three methoxyls at δ_H 3.85, 3.83, and 3.80, respectively. Analysis of the ^{13}C NMR and DEPT spectra (Table 1) showed resonances for 30 carbons: 1 carbonyl carbon and 11 olefinic quaternary carbons (including 6 oxygenated ones), 12 methines (including 8 olefinic), 3 methylenes (2 oxygenated and 1 end-alkene), and 3 methoxyls, among which 27 were assigned to the bistetrahydrofuran-type sesquilignan skeleton. The ^{1}H and ^{13}C NMR resonances of compound **1** was superimposable over those of herpetrione (**3**) (Kaouadji and Favre, 1983), and the only difference was that the hydroxymethyl group at C-9 in herpetrione (**3**) was substituted by an end-alkenes signal in compound **1** at C-9. This difference was supported by correlations between δ_H 5.96 (1H, s, H_a-9), 5.66 (1H, s, H_b-9) and both 197.0 (C-7) and 132.2 (C-3′) (Fig. 2). Furthermore, the coupling constants of $J_{7',8'}$, and $J_{7'',8''}$ (4.2 Hz) and the shifts of H-7′/H-7″, H-8′/H-8″, and C-8′/C-8″ indicated that the aryl groups were pseudoequatorial and cis-oriented with H-8′/H-8″ in **1** (Kim et al., 2010; Xiong et al., 2011). The CD data (positive at 249 and 288 nm) and specific rotation {$[\alpha]_D^{20}$ +60.8 (c = 0.625, CH_3OH, 20 °C)} of **1** were opposite to those of (−)-(7*R*, 7′*R*, 8*S*, 8′*S*)-4′-hydroxy-3, 3′, 4, 5, 5′-pentamethoxy-7, 9′:7′, 9-diepoxylignane (Xiong et al., 2011). Therefore, the structure of compound **1** was concluded to be (+)-(7′*S*, 7″*S*, 8′*R*, 8″*R*)-4, 4′, 4″-trihydroxy-3, 5′, 3″-trimethoxy-7-oxo-8-ene [8-3′, 7′-O-9″, 8′-8″, 9′-O-7″] lignoid.

Compound **2** was obtained as a brown amorphous powder with $[\alpha]_D^{20}$ −40.0 (c = 0.25, CH_3OH, 20 °C) and determined to possess the molecular formula $C_{18}H_{18}O_7$ by its pseudo-molecular ion peak at m/z 347.1112 $[M+H]^+$ in the positive HRESI-MS experiment. The IR spectra showed the absorption bands for hydroxyl (3413 cm^{-1}), carbonyl (1679, 1657 cm^{-1}), and olefinic (1591 cm^{-1}) groups. The ^{1}H NMR spectrum of compound **2** revealed the diagnostic signals assignable to a 1,3,4-trisubstituted benzoketone and a 1′,2′,3′,5′-tetrasubstituted benzoketone, which were indicated from the two pairs of deshielded proton signals appeared at the aromatic region: δ_H 6.77 (d, J = 8.4 Hz, H-5), 7.63 (dd, J = 1.8, 8.4 Hz, H-6), 7.66 (d, J = 1.8 Hz, H-2), and 7.29 (d, J = 1.2 Hz, H-6′), 7.34 (d, J = 1.2 Hz,