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Anti-HBV active constituents from *Piper longum*

Zhi-Yong Jiang *, Wen-Feng Liu, Xue-Mei Zhang, Jie Luo, Yun-Bao Ma, Ji-Jun Chen *

State Key Laboratory of Phytochemistry and Plant Resource in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, Yunnan, China

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ABSTRACT

In the screening search for Hepatitis B virus inhibitory agents from medicinal plants, the ethanol extract of *Piper longum* Linn. was found to possess superior anti-HBV activity in vitro. Bioassay-guided fractionation coupled with repeated purification resulted in the isolation of four new compounds, involving two new glycosides longumosides A (**1**) and B (**2**) and two new amide alkaloids *erythro*-1-[1-oxo-9(3,4-methylenedioxyphenyl)-8,9-dihydroxy-2*E*-nonenyl]-piperidine (**3**), *threo*-1-[1-oxo-9(3,4-methylenedioxyphenyl)-8,9-dihydroxy-2*E*-nonenyl]-piperidine (**4**), as well as two compounds 3β,4α-dihydroxy-2-piperidinone (**5**), 5,6-dihydro-2(1*H*)-pyridinone (**6**) from natural source for the first time. The structures of the four new compounds were determined by extensive analyses of the MS, IR, 1D and 2D NMR data. Besides, the compounds **2**–**6**, together with the known compounds **7**–**11** obtained previously, were assayed for their anti-HBV activity by using Hep G 2.2.15 cell line in vitro. Results suggested the compound piperine (**7**) possessed remarkable inhibitory HBV activity, against the secretion of hepatitis B virus surface antigen (HBsAg) and hepatitis B virus e antigen (HBeAg) with the Selectivity Index (SI) values of 15.7 and 16.8, respectively.

Piper longum Linn. is a slender aromatic climber widely distributed in the tropical and subtropical regions of the world. Its fruits had long been used for the treatment of anodyne and stomach disease[1] in China. Amide alkaloids, propenylphenols, lignans, terpenes and steroids had been obtained from this plant.[2–9] Our preceding bioassay suggested the ethanol extract of *P. longum* possessed superior anti-hepatitis B virus (HBV) activity. With the aim of finding an active metabolite from this plant, the *P. longum* was phytochemically investigated and 24 known compounds had been isolated from this plant.[5,6] During our subsequent investigation on *P. longum*, four new compounds named longumosides A (**1**) and B (**2**), *erythro*-1-[1-oxo-9(3,4-methylenedioxyphenyl)-8,9-dihydroxy-2*E*-nonenyl]-piperidine (**3**), and *threo*-1-[1-oxo-9(3,4-methylenedioxyphenyl)-8,9-dihydroxy-2*E*-nonenyl]-piperidine (**4**) were isolated, besides two new natural products 3β,4α-dihydroxy-2-piperidinone (**5**), 5,6-dihydro-2(1*H*)-pyridinone (**6**) (Fig. 1). Compounds **2**–**6**, together with the isolates previously obtained with a large amount from *P. longum* involving piperine (**7**),[3,7] 1-[1-oxo-5(3-methoxyl-4-hydroxyphenyl)-2*E*-pentenyl]-piperidine (**8**),[6,10] guineesine (**9**),[3,5] (2*E*,4*E*)-*N*-isobutyleicosa-2,4-dienamide (**10**),[3] piperlonguminine (**11**),[3] were assayed for their anti-HBV activity in vitro by using HBV transfected Hep G2.2.15 cell line. Results suggested compounds **3**, **4**, **7**, and **9** possessed significant inhibitory activity against the secretion of hepatitis B virus surface antigen (HBsAg) and hepatitis B virus e antigen (HBeAg). This paper described the structural elucidation of the four new compounds and anti-HBV activities of the isolates.

The fruits of *Piper longum* Linn. were purchased in Kunming and identified by Dr. Li-Gong Lei from Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (No. 061008) was deposited in the State Key Laboratory of Phytochemistry and Plant Resource in West China, Kunming Institute of Botany, Chinese Academy of Sciences. The 90% ethanol extract of the fruits of *P. longum* (20 kg) was suspended in water and successively partitioned with petroleum ether, chloroform and *n*-BuOH. The *n*-BuOH extract (300 g) was performed with multiple chromatographic steps over silica gel, Al_2O_3, Rp-18 and sephadex LH-20 to provide compounds **1**–**6**.[11] Compounds **5** and **6**, which had been previously synthesized,[12,13] were obtained as natural products for the first time and identified as 3β,4α-dihydroxy-2-piperidinone (**5**), 5,6-dihydro-2(1*H*)-pyridinone (**6**) by extensive analyses of the NMR and MS data.

Compound **1**[14] was obtained as colorless amorphous powder and had the molecular formula $C_{17}H_{28}O_8$, established by the negative HRESIMS at *m*/*z* 395.1481 $[M+Cl]^-$ (395.1472, calcd for $C_{17}H_{28}O_8Cl$). In the IR spectrum, the absorptions ascribable to hydroxyl (3426 cm^{-1}) and ester-carbonyl (1722 cm^{-1}) groups were observed. The ^1H NMR spectrum showed three methyl signals at δ_H 1.02 (3H, s, H-8), 0.99 (3H, s, H-10), 0.89 (3H, s, H-9), as well as an

* Corresponding authors at. Present addresses: Key Laboratory of Chemistry in Ethnic Medicinal Resources, State Ethnic Affairs Commission & Ministry of Education, School of Chemistry and Biotechnology, Yunnan University of Nationalities, Jingming South Road, Chenggong New District, Kunming 650500, Yunnan, China. Tel.: +86 871 5910017; fax: +86 871 5913013 (Z.-Y.J.), State Key Laboratory of Phytochemistry and Plant Resource in West China, Kunming Institute of Botany, Chinese Academy of Sciences. Lanhei Road 132#, 650204, Kunming, Yunnan, China.

E-mail addresses: jiangzy2010@163.com, jiangzy@mail.kib.ac.cn (Z.-Y. Jiang), chenjj@mail.kib.ac.cn (J.-J. Chen).

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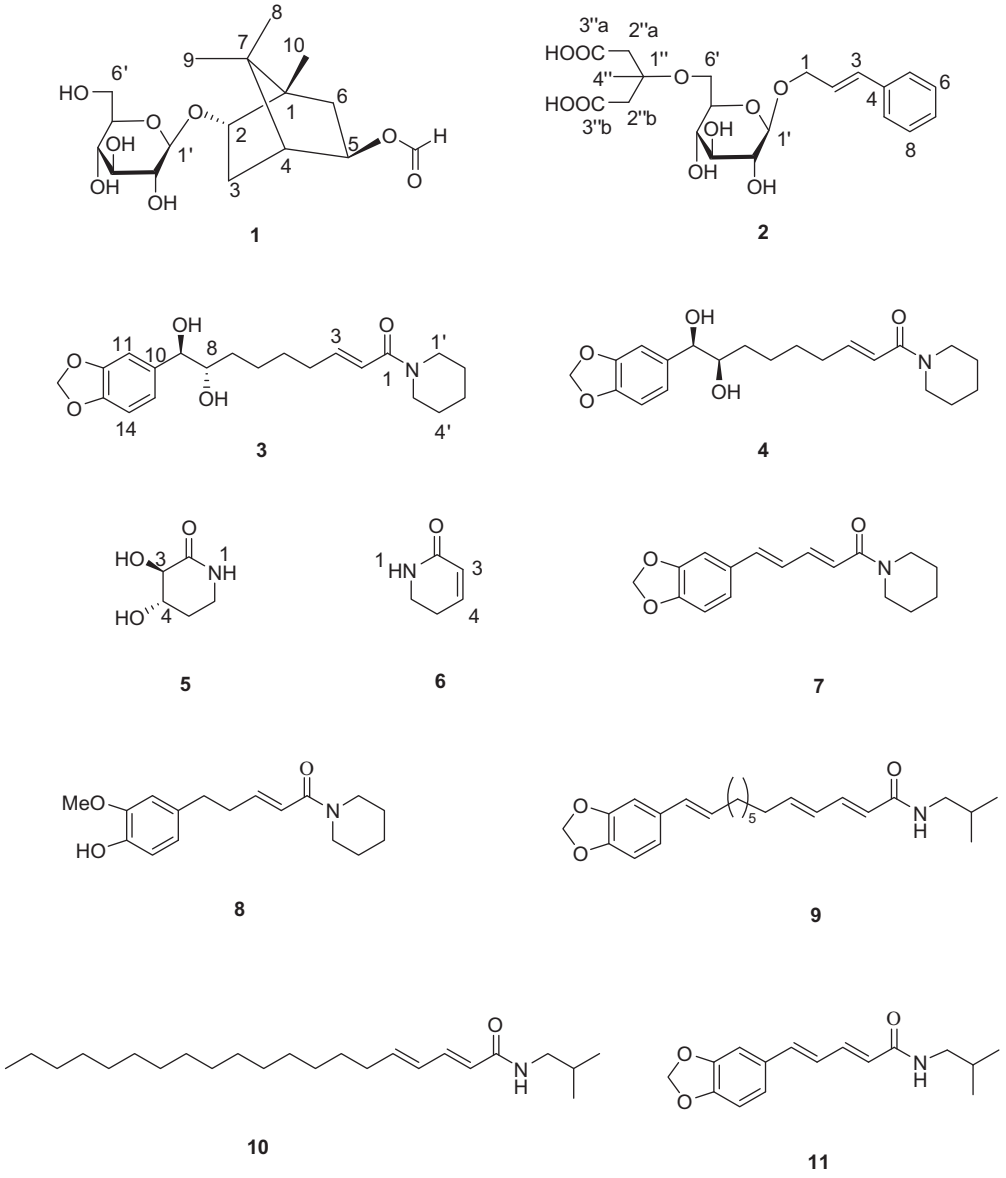

Figure 1. Structures of compounds **1**–**11**.

anomeric proton signal due to a β-linkage sugar unit at δ 4.26 (1H, d, J = 8.0 Hz, H-1′). The ^{13}C NMR spectrum of compound **1** displayed 17 carbon resonances involving three methyls, four methylenes, two methines and two quaternary carbons, besides the carbons signals assignable to a β-D-glucopyranose moiety. Detailed analyses of the NMR data revealed that compound **1** should be a pinane-type monoterpenoid, with a similar skeleton to angelicoidenol 2-*O*-β-D-glucopyranoside.[15,16] The only difference between compound **1** and angelicoidenol 2-*O*-β-D-glucopyranoside was that compound **1** had one more formyl unit. Considering that the carbon signal C-5 was down-shifted to δ 78.6 from 74.9 in angelicoidenol 2-*O*-β-D-glucopyranoside, the additional formyl was restricted at C-5. This was verified by the HMBC (Fig. 2) correlation between H-5 (δ_H 4.76) and the carbonyl (δ_C 162.7). The absolute configuration of compound **1** was confirmed by comparing the NMR data with those of (+)-angelicoidenol 2-*O*-β-D-glucopyranoside and (−)-angelicoidenol 2-*O*-β-D-glucopyranoside. As previous reported,[15,16] the chemical shifts of C-2 in (+)-angelicoidenol 2-*O*-β-D-glucopyranoside (2*S*,5*R*) and (−)-angelicoidenol 2-*O*-β-D-glucopyranoside (2*R*,5*S*) were 85.2 and 82.9, respectively, well suggesting that compound **1**, whose chemical shifts of C-2 was 85.4, should possess the same 2*S*,5*R* configuration as (+)-angelicoidenol 2-*O*-β-D-glucopyranoside. The approximate optical rotation value of −13.5° to the report[16] further supported the above deduction. Consequently, compound **1** was elucidated as 5-formyl-(+)-angelicoidenol 2-*O*-β-D-glucopyranoside and named to be longumoside A (**1**).

Compound **2**[17] was obtained as colorless amorphous powder. Its molecular formula was determined as $C_{21}H_{28}O_{10}$ based on the negative HRESIMS at m/z 439.1604 $[M-H]^-$ (439.1618, calcd for $C_{21}H_{27}O_{10}$). The IR spectrum showed absorption bands for hydroxyl (3425 cm^{-1}), carbonyl (1726 cm^{-1}) and aromatic ring (1600, 1495, and 1450 cm^{-1}) functionalities. The ^{1}H NMR spectrum exhibited a *trans*-double bond at δ_H 6.35 (1H, dt, J = 16.0, 6.5 Hz, H-2), 6.67 (1H, d, J = 16.0 Hz, H-3), an anomeric proton signal at δ_H 4.37 (1H, d, J = 8.0 Hz, H-1′) corresponding to a β-linkage sugar moiety, and five aromatic protons assignable to a single-substituted phenyl ring (Table 1). In the ^{13}C NMR spectrum of **2**, the signals due to a methyl, four methylenes, twelve methines and four quaternary carbons were presented. Comparison of the ^{1}H and ^{13}C NMR data of compound **2** with those of *trans*-cinnamyl-β-D-glucopyranoside[18,19] demonstrated that compound **2** was structurally similar to *trans*-cinnamyl-β-D-glucopyranoside except that there was one