Bioorganic & Medicinal Chemistry Letters 23 (2013) 2123–2127

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

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

ARTICLE INFO

Article history:
Received 3 December 2012
Revised 8 January 2013
Accepted 25 January 2013
Available online 4 February 2013

Keywords:
Piper longum
Longumoside
Amide alkaloids
Anti-HBV activity

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).

0960-894X/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2013.01.118

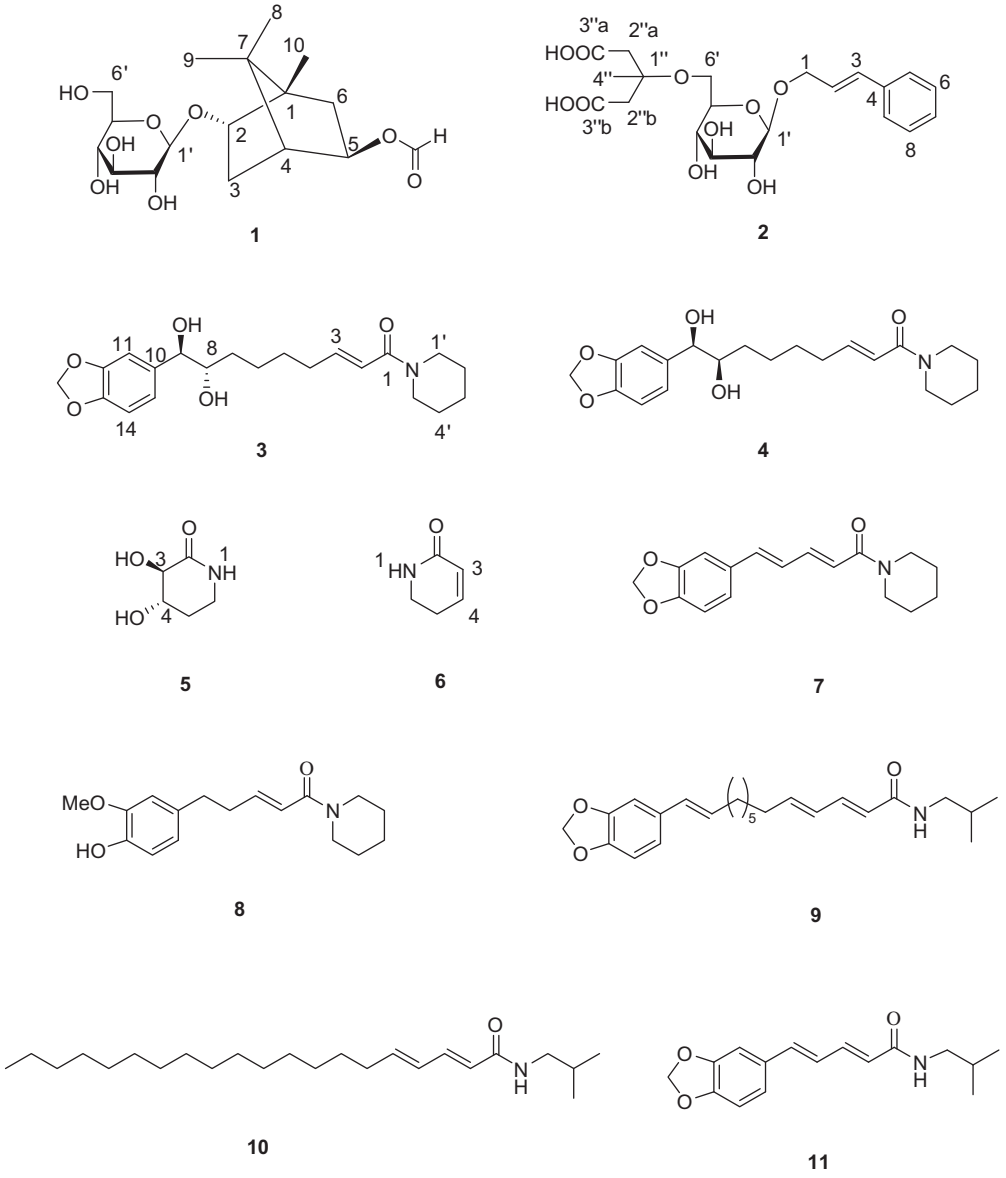


Figure 1. Structures of compounds **1**–**11**.

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

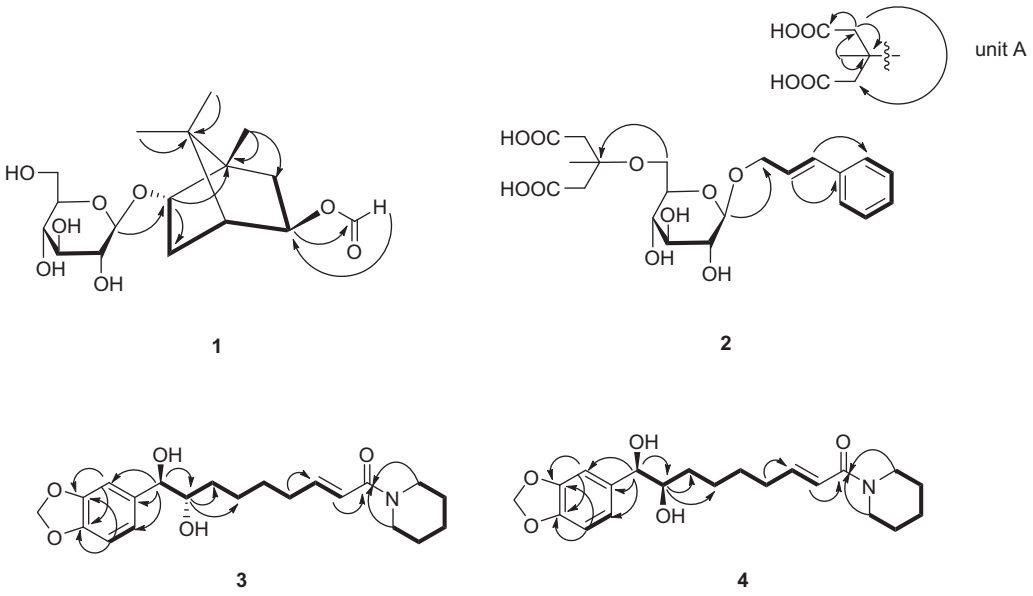


Figure 2. Selected HMBC (→) and ^{1}H–^{1}H COSY (—) correlations of compounds **1**–**4**.

Table 1
The ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) data of compounds **1** and **2** in CD_3OD

No.	1		2	
	δ_H	δ_C	δ_H	δ_C
1	—	51.5 (s)	4.47 (m, overlapped) 4.29 (dd, 12.5, 6.5)	71.0 (t)
2	3.88 (m, overlapped)	85.4 (d)	6.35 (dd, 16.0, 6.5)	126.7 (d)
3	2.34 (m, 14.0, 9.8, 5.2) 1.24 (dd, 14.0, 2.8)	35.7 (t)	6.67 (d, 16.0)	133.8 (d)
4	1.84 (d, 5.2)	51.5 (d)	—	138.2 (s)
5	4.76 (dd, 8.0, 3.2)	78.6 (d)	7.41 (d, 7.5)	127.5 (d)
6	2.64 (dd, 14.0, 8.0) 1.47 (brd, 14.4)	37.5 (t)	7.29 (t, 7.5)	129.6 (d)
7	—	48.4 (s)	7.21 (t, 7.5)	128.7 (d)
8	1.02 (s)	21. 0 (q)	7.29 (t, 7.5)	129.6 (d)
9	0.89 (s)	19.8 (q)	7.41 (d, 7.5)	127.5 (d)
10	0.99 (s)	13.3 (q)	—	—
1′	4.26 (d, 8.0)	105.9 (d)	4.37 (d, 8.0)	103.5 (d)
2′	3.18 (dd, 8.0, 8.0)	75.4 (d)	3.24 (t, 8.5)	75.0 (d)
3′	3.30 (m)	78.1 (d)	3.37(t, 9.0)	77.8 (d)
4′	3.27 (m)	71.6 (d)	3.33 (d, 9.0)	71.6 (d)
5′	3.24 (dd, 5.6, 2.0)	77.8 (d)	3.47 (m)	75.2 (d)
6′	3.88 (dd, 12.0, 2.4) 3.66 (dd, 11.6, 5.2)	62.7 (t)	4.47 (m)	64.6 (t)
–HC=O	8.03 (s)	162.7 (d)	—	—
1″	—	—	—	—
2″a	—	—	2.71 (s)	46.5 (t)
2″b	—	—	2.71 (s)	46.5 (t)
3″a	—	—	—	172.5 (s)
3″b	—	—	—	172.5 (s)
4″	—	—	1.38 (s)	27.8 (q)

more 3-methyl-pentanedioic acid fragment (unit A, Fig. 2) in compound **2**. The existence of unit A in compound **2**, characterized by the proton signals at δ_H 2.71 (4H, s, H-2″a and H-2″b), 1.38 (3H, s, H-4″) in the ^{1}H NMR spectrum and the carbon resonances at δ_C 70.8 (s, C-1″), 46.5 (t, C-2″a, 2″b), 172.5 (s, C-3″a, 3″b), 27.8 (q, C-4″) in the ^{13}C NMR (DEPT), was supported by the HMBC correlations shown in Figure 2. The additional unit A was assigned to be located at C-6′ of the β-D-glucopyranose by the correlations between H-6′a, 6′b (δ_H 4.47, 4.21) and C-1″ (δ_C 70.8) in the HMBC (Fig. 2). Accordingly, compound **2** was determined to be *trans*-cinnamyl-(6-(3-*O*-3-methyl-pentanedioic acid))-β-D-glucopyranoside and named as longumoside B (**2**) (see Table 2).

Compound **3**[20] was obtained as colorless oil. Its molecular formula $C_{21}H_{29}NO_5$ was determined on the basis of the positive HRESIMS at *m*/*z* 398.1953 $[M+Na]^+$. The ^{1}H NMR spectrum displayed one *meta*-coupled aromatic proton signals at δ 6.90 (1H, d, J = 1.5 Hz, H-11), one *ortho*-*meta*-coupled aromatic proton at δ 6.81 (1H, dd, J = 7.9, 1.5 Hz, H-15), and one *ortho*-coupled aromatic proton at δ 6.75 (1H, d, J = 7.9 Hz, H-14), besides two *trans*-olefinic protons at 6.72 (1H, m, H-3), 6.38 (1H, d, J = 15.1 Hz, H-2), and one methylenedioxy singlet at δ_H 5.90 (s, 2H). The presence of one piperidine ring was deduced by the signals at δ_H 3.54 (2H, m, H-1′), 1.57 (2H, m, H-2′), 1.67 (2H, m, H-3′), 1.57 (2H, m, H-4′), 3.55 (2H, m, H-5′),[21] as well as by analysis of the ^{1}H–^{1}H COSY

Table 2
The ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) data of compounds **3** and **4** in CD_3OD

No.	**3**		**4**	
	δ_H	δ_C	δ_H	δ_C
1	—	167.7	—	167.7
2	6.38 (d, 15.1)	121.6	6.35 (d, 15.1)	121.7
3	6.72 (m)	147.7	6.72 (m)	147.6
4	2.20 (m)	33.4	2.16 (m)	33.5
5	1.46 (m)	29.5	1.45 (m)	29.5
6	1.57 (m), 1.34 (m)	26.5	1.56 (m), 1.27 (m)	26.3
7	1.57 (m), 1.34 (m)	33.1	1.27 (m)	33.3
8	3.58 (m)	76.1	3.55 (m)	76.6
9	4.40 (d, 5.6)	78.2	4.30 (d, 6.8)	78.8
10	—	137.6	—	137.5
11	6.90 (d, 1.5)	108.6	6.85 (d, 1.4)	108.3
12	—	148.2	—	148.4
13	—	148.9	—	149.0
14	6.75 (d, 7.9)	108.6	6.78 (d, 8.0)	108.8
15	6.81 (dd, 7.9, 1.5)	121.6	6.79 (dd, 8.0, 1.4)	121.6
OCH_2O	5.90 (s)	102.2	5.91 (s)	102.3
1′	3.54 (m)	48.1	3.55 (m)	48.1
2′	1.57 (m)	27.8	1.56 (m)	27.8
3′	1.67 (m)	25.5	1.67 (m)	25.5
4′	1.57 (m)	26.8	1.56 (m)	26.8
5′	3.55 (m)	44.4	3.55 (m)	44.4

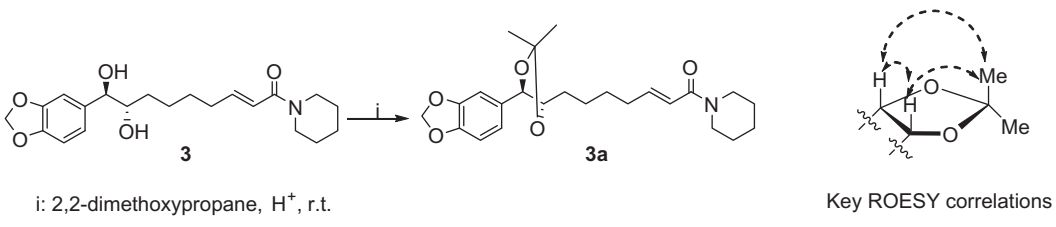
correlations (Fig. 2). The proton signals appearing at δ 4.40 and 3.58 in the ^{1}H NMR spectrum, together with the corresponding ^{13}C NMR resonances at δ_C 76.1 and 78.2, and the cross peak between δ_H 4.40 and 3.58 in the ^{1}H–^{1}H COSY spectrum (Fig. 2), demonstrated the existence of a vicinal diol. The relative configuration of the 8,9-diol was deduced as *erythro* by formation of its 8,9-acetonide and subsequent ROESY experiments (Scheme 1) on this derivative (**3a**).[22] As shown in Scheme 1. the ROESY correlations for δ_H 5.08 (1H, d, J = 6.7 Hz, H-9)/1.44 (3H, s) and δ_H 4.28 (1H, m, H-8)/1.44 (3H, s), δ_H 5.08 (1H, d, J = 6.7 Hz, H-9)/4.28 (1H, m, H-8), in combination with the evidence that there was no correlation for H-8 and H-9 to δ_H 1.56 (3H, s), suggested the *erythro* configuration of 8,9-diol functionality (Scheme 1). Detailed analyses of the ^{1}H–^{1}H COSY and HMBC (Fig. 2) correlations allowed the determination of **3** as *erythro*-1-[1-oxo-9(3,4-methylenedioxyphenyl)-8,9-dihydroxy-2*E*-nonenyl]-piperidine.

Compound **4**[23] was isolated as colorless oil and had the same molecular formula $C_{21}H_{29}NO_5$ as compound **3** established by HRESIMS (m/z 398.1951, $[M+Na]^+$, calcd 398.1943). Its NMR (^{1}H and ^{13}C NMR, DEPT, HMBC, HSQC) showed high similarity to those of compound **3**, suggesting that both compounds possessed a similar structure. However, the R_f value of the two compounds on TLC (Al_2O_3, neutral, developed by petroleum ether/*iso*-propanol/Et_2NH 90:10:1) was quite different, demonstrating that compounds **3** and **4** might be a pair of stereoisomers. By formatting its 8,9-acetonide (**4a**)[24] experiment (Scheme 2), the correlations for δ_H 4.42 (1H, d, J = 8.5 Hz, H-9)/1.53 (3H, s) and δ_H 3.69 (1H, m, H-8)/1.47 (3H, s) (Scheme 2) in the ROESY spectrum were observed, declaring the 8,9-diol should be in *threo* configuration. Thus, the structure of **4** was determined as *threo*-1-[1-oxo-9(3,4-methylenedioxyphenyl)-8,9-dihydroxy-2*E*-nonenyl]-piperidine.

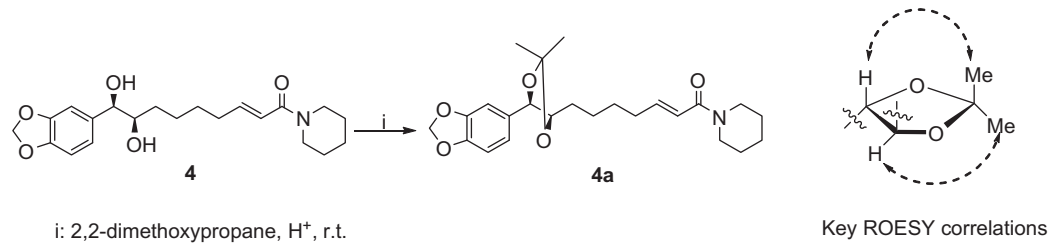
The NMR data (Table 3) of the new natural products 3β,4α-dihydroxy-2-piperidinone (**5**), 5,6-dihydro-2(1*H*)-pyridinone (**6**) were also reported for the first time by extensive analyses of their NMR spectra.

Compounds **2**–**11** were tested for their anti-HBV activity in vitro by using Hep G 2.2.15 cell line as our previous report.[25] The anti-HBV activity, cytotoxicity and Selectivity Index (SI) were summarized in Table 4. Results suggested that compounds **3**, **4**, **7**, **9** possessed remarkable inhibitory HBV activity suppressing the secretion of HBsAg and HBeAg in Hep G 2.2.15 cell line, with the IC_{50} values of 0.13, 0.11, 0.15 and 0.05 mM for HBsAg, and the IC_{50} values of 0.16, 0.11 0.14 and 0.05 mM for HBeAg, respectively. In addition, compound **7** exhibited notable SI values for HBsAg and HBeAg (Table 4) at the non-toxic concentration, suggesting the compound **7** should be paid more attention to as a potent anti-HBV agent. Compound **10** could moderately suppress the HBsAg and HBeAg secretion in the 2.2.15 cell line, and compounds **2**, **5**, **6**, **8**, **11** showed no anti-HBV activity.

The above bioactivity suggested a primary structure–activity relationship. That is, a long carbon chain (more than eight) in the amide alkaloids was helpful for the anti-HBV ability. As summarized, the compounds **3**, **4**, **9**, **10** with more than eight carbon chains exhibited significant inhibitory efficacy to the secretion of HBsAg and HBeAg. These results were in agreement with the preceding report.[26] Compound **7** possessed remarkable restraint capacity for the expression of HBsAg and HBeAg, while compounds **8** and **11** showed no anti-HBV potency, demonstrated that, in amide alkaloids with four carbon chains, the conjugated double bonds and piperidine ring might be necessary for the inhibitory HBV efficacy.



Scheme 1.



Scheme 2.

Table 3
The ^{1}H (500 MHz) and ^{13}C NMR(125 MHz) data of compounds **5**–**6**

No.	**5**[a]		**6**[b]	
	δ_H	δ_C	δ_H	δ_C
1	5.60 (br s)	—	6.56 (br s)	—
2	—	174.5	—	166.6
3	3.95 (d, 8.4)	70.6	5.89 (d, 10.0)	124.3
4	3.91 (m)	74.1	6.64 (dt, 9.7, 4.2)	141.5
5	2.14 (m), 1.90 (m)	29.0	2.34 (m)	23.4
6	3.85–3.90 (m)	38.8	3.42 (m)	39.0

[a] Measured in CD_3OD.
[b] Measured in $CDCl_3$.

Table 4
Anti-HBV activities of compounds **2**–**11**

Compounds	CC_{50}[a] (mM)	HBsAg		HBeAg	
		IC_{50}[a] (mM)	SI[b]	IC_{50}(mM)	SI
2	>3.75	>3.75	—	>3.75	—
3	0.32	0.13	2.5	0.16	2.0
4	0.21	0.11	1.9	0.11	1.9
5	>1.22	>1.22	—	>1.22	—
6	9.90	>14.0	—	>14.0	—
7	2.35	0.15	15.7	0.14	16.8
8	>2.70	2.70	—	>2.70	—
9	0.15	<0.05	>3.0	<0.05	>3.0
10	1.58	0.58	2.7	1.07	1.5
11	0.32	>4.51	—	>4.51	—
3TC[c]	31.18	28.82	1.1	30.35	1.0

All values are the mean of two independent experiments.
[a] IC_{50}: 50% inhibitory concentration; CC_{50}: 50% cytotoxic concentration.
[b] $SI = CC_{50}/IC_{50}$.
[c] 3TC: Lamivudine, an antiviral agent used as the positive control.

In conclusion, the *P. longum* was found to possess anti-HBV activity for the first time and several anti-HBV active constituents had been isolated from this plant. It is the first report of the presence of glycosides in *P. longum*. Interestingly, compound **7**, the main amide alkaloid in the *Piper* genus, exhibited challenging anti-HBV activity, suggesting it is necessary to further investigate on the traditional Chinese medicine. These results provide a scientific support for the new therapeutic use of this plant and warrant advance studies to develop new agents for the prevention and treatment of HBV infection.

Acknowledgments

This work was financially supported by the National Natural Science Foundation of China (NSFC No. 21162041) and the Independent Innovation Project for the post-graduate (11HXYJS05) from the Key Laboratory of Chemistry in Ethnic Medicinal Resources, State Ethnic Affairs Commission & Ministry of Education, Yunnan University of Nationalities. The authors are grateful to the staff of the analytical group of the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, for the measurements of all spectra.

References and notes

1. Pharmacopoeia Committee of China In *Chinese Pharmacopoeia*; China Medical Science Press: Beijing, 2010; Vol. 1, p. 219.
2. Parmar, V. S.; Jain, S. C.; Brsht, K. S.; Jain, R.; Taneja, P.; Jha, A.; Tyagi, O. D.; Prasad, A. K.; Wengel, J.; Olsen, C. E.; Boll, P. M. *Phytochemistry* **1997**, *46*, 597.
3. Wu, S. H.; Sun, C. R.; Pei, S. F.; Lu, Y. B.; Pan, Y. J. *J. Chromatogr., A* **2004**, *1040*, 193.
4. Tabuneng, W.; Bando, H.; Amiya, T. *Chem. Pharm. Bull.* **1983**, *31*, 3562.
5. Liu, W. F.; Jiang, Z. Y.; Chen, J. J.; Zhang, X. M.; Ma, Y. B. *China J. Chin. Mater. Med.* **2009**, *34*, 1101.
6. Liu, W. F.; Jiang, Z. Y.; Chen, J. J.; Zhang, X. M.; Ma, Y. B. *China J. Chin. Mater. Med.* **2009**, *34*, 2891.
7. Araujo-Junior, J. X. D.; Da-Cunha, E. V. L.; Chaves, M. C. D. O.; Gray, A. I. *Phytochemistry* **1997**, *44*, 559.
8. Das, B.; Kashinatham, A.; Srinivas, K. V. *Planta Med.* **1996**, *62*, 582.
9. Lee, S. W.; Kim, Y. K.; Kim, K.; Lee, H. S.; Choi, J. H.; Lee, W. S.; Jun, C. D.; Park, J. H.; Lee, J. M.; Rho, M. C. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4544.
10. Inatani, R.; Nakatani, N.; Fuwa, H. *Agric. Biol. Chem.* **1981**, *45*, 667.
11. The air-dried fruits of *P. longum* (20 kg) were powdered and extracted with 90% ethanol under reflux for three times, 2 h each time. After concentrated in vacuum, the extract was suspended in water and successively partitioned with petroleum ether, chloroform and *n*-BuOH to give petroleum ether (A). chloroform (B), *n*-BuOH (C) and aqueous (D) fractions. The *n*-BuOH extract (Fr. C, 300 g) was performed on a silica gel CC with a gradient elution of $CHCl_3$/MeOH (100:0–0:100) to afford eight fractions (Frs. C.1-8). The Fr. C.1 (10 g) was performed on a silica gel CC (petroleum ether/Me_2CO 90:10, 80:20, 70:30, 60:40, 50:50) to give five fractions (Fr. C.1.1–Fr. C.1.5). The Fr. C.1.3 (2 g) was successively separated by silica gel CC and finally purified by Al_2O_3 (neutral) CC with a eluent of petroleum ether/iso-propanol/Et_2NH (90:10:1) to yield compounds **3** (113 mg) and **4** (209 mg). The Fr. C.2 (5 g, eluted by $CHCl_3$/MeOH 95:5–90:10) was subjected to a silica gel CC and eluted with petroleum ether/Me_2CO/diethylamine (80:20:2) to yield compound **6** (800 mg). Fr. C.3 (15.5 g, eluted by $CHCl_3$/MeOH 80:20) was chromatographed on a silica gel CC (petroleum ether/Me_2CO/diethylamine 70:30:3) to provide three fractions (Fr. C.3.1–Fr. C.3.3). The Fr. C.3.3 (1.0 g) was separated by MCI CC (MeOH/H_2O 80:20–100:0) and further purified by sephadex LH-20 to give compound **5** (300 mg). Fr. C.4 (32.0 g) was chromatographed on a silica gel CC (EtOAc/MeOH 86:14) to yield four fractions (Fr. C.4.1–Fr. C.4.4). Fr. C.4.2 was subjected to an MCI CC (MeOH/ H_2O 70:30–100:0) and further purified by sephadex LH-20 to obtain compound **1** (8 mg). Fr. C.4.3 was subsequently purified by a silica gel CC with an eluent of $CHCl_3$/MeOH/HCOOH (500:35:2) to supply compound **2** (250 mg).
12. Winter, D. K.; Drouin, A.; Lessard, J.; Claude, S. *J. Org. Chem.* **2010**, *75*, 2610.
13. Williams, S. J.; Hoos, R.; Withers, S. G. *J. Am. Chem. Soc.* **2000**, *122*, 2223.
14. Longumoside A (**1**): colorless amorphous powder; $[\alpha]_D^{22.4}$ −13.5 (*c* 0.46, MeOH); IR (KBr) ν_{max} 3426, 2956, 2925, 2874, 2855, 1722, 1678, 1459, 1432, 1376, 1204, 1180, 1134, 1074 cm^{-1}; (500 MHz, CD_3OD) and ^{13}C NMR^{13}C NMR (125 MHz, CD_3OD), see Table 1; ESIMS (negative): *m*/*z* 359 $[M-H]^-$, 395 $[M+Cl]^-$; HRESIMS (negative) *m*/*z* 395.1481 $[M+Cl]^-$ (calcd for $C_{17}H_{28}O_8Cl$: 395.1472).
15. Kitajima, J.; Okamura, C.; Ishikama, T.; Tanaka, Y. *Chem. Pharm. Bull.* **1998**, *46*, 1595.
16. Inoshiri, S.; Saiki, M.; Kohda, H.; Otsuka, H.; Yamasaki, K. *Phytochemistry* **1988**, *21*, 2869.
17. Longumoside B (**2**): colorless amorphous powder; $[\alpha]_D^{25.8}$ −26.32 (*c* 0.27, CH_3OH); UV (CH_3OH) λ_{max} ($\log\varepsilon$) = 251 (4.17) nm; IR (KBr) ν_{max} 3425, 3084, 3060, 3027, 2974, 2922, 1726, 1600, 1579, 1495, 1450, 1203, 1077, 970, 747, 694 cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD), see Table 1; ESIMS (negative): *m*/*z* = 439 $[M-H]^-$; HRESIMS (negative): *m*/*z* = 439.1604 $[M-H]^-$ (calcd for $C_{21}H_{27}O_{10}$: 439.1618).
18. Tolonen, A.; Pakonen, M.; Hohtola, A.; Jalonen, J. *Chem. Pharm. Bull.* **2003**, *51*, 467.
19. Zapesochnaya, G. G.; Kurkin, V. A. *Chem. Nat. Compd.* **1982**, *18*, 685.
20. Erythro-1-[1-oxo-9(3,4-methylenedioxyphenyl)-8,9-dihydroxy-2*E*-nonenyl]-piperidine (**3**): colorless oil; $[\alpha]_D^{22.4}$ −32.41 (*c* 0.11, CH_3OH); UV (CH_3OH) λ_{max} ($\log\varepsilon$) = 283 (3.59) nm; IR (KBr) ν_{max} 3454, 2936, 2858, 1655, 1597, 1503, 1488, 1444, 1248, 1038 cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD), see Table 2; ESIMS (positive): *m*/*z* = 398 $[M+Na]^+$; HRESIMS (positive): *m*/*z* = 398.1953 $[M+Na]^+$ (calcd for $C_{21}H_{29}NO_5Na$: 398.1943).
21. Wei, K.; Li, W.; Koike, K.; Pei, Y. P.; Chen, Y. J.; Nikaido, T. *J. Nat. Prod.* **2004**, *67*, 1005.
22. *Preparation of the acetonide (**3a**) from compound **3**.* A solution of **3** (1.2 mg, 3.2 μmol) in 2,2-dimethoxypropane (0.5 mL) was treated with Dowex 50 W-X8 (H^+ form, 20 mg). The mixture was stirred at room temperature for 3 h. The resin was removed by filtration. Removing of the solvent from the filtrate in vacuum to yield **3a** (1.2 mg). **3a**: colorless oil; ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.71–6.81 (4H, m, H-3, 11, 14, 15), 6.18 (1H, dd, *J* = 15.2 Hz, H-2), 5.96 (1H, s, O–CH_2–O), 5.08 (1H, d, *J* = 6.7 Hz, H-9), 4.28 (1H, m, H-8), 3.58, 3.45 (each 1H, m, H-5′, 1′), 1.56 (3H, s, $(CH_3)_2$–C–), 1.44 (3H, s, $(CH_3)_2$–C–).
23. Threo-1-[1-oxo-9(3,4-methylenedioxyphenyl)-8,9-dihydroxy-2*E*-nonenyl]-piperidine (**4**): colorless oil; $[\alpha]_D^{16.0}$ −23.58 (*c* 0.12, CH_3OH); UV (CH_3OH) λ_{max} ($\log\varepsilon$) = 284 (3.58) nm; IR (KBr) ν_{max} 3462, 2937, 2858, 1656, 1612, 1503, 1488, 1444, 1247, 1037 cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD), see Table 2; ESIMS (positive): *m*/*z* = 398 $[M+Na]^+$; HRESIMS (positive): *m*/*z* = 398.1951 $[M+Na]^+$ (calcd for $C_{21}H_{29}NO_5Na$, 398.1943).
24. *Preparation of the acetonide (**4a**) from compound **4**.* A solution of **4** (1.5 mg, 4.0 μmol) in 2,2-dimethoxypropane (0.5 mL) was treated with Dowex 50W-X8 (H^+ form, 20 mg). The mixture was stirred at room temperature for 3 h. The resin was removed by filtration. Removing of the solvent from the filtrate in vacuum to yield **4a** (1.5 mg). **4a**: colorless oil; ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.75–6.81 (4H, m, H-3, 11, 14, 15), 6.20 (1H, dd, *J* = 15.1 Hz, H-2), 5.96 (1H, s, O–CH_2–O), 4.42 (1H, d, *J* = 8.5 Hz, H-9), 3.69 (1H, m, H-8), 3.58, 3.45 (each 1H, m, H-5′, 1′), 1.53 (3H, s, $(CH_3)_2$–C–), 1.47 (3H, s, $(CH_3)_2$–C–).
25. Jiang, Z. Y.; Zhang, X. M.; Zhang, F. X.; Liu, N.; Zhao, F.; Zhou, J.; Chen, J. *J. Planta Med.* **2006**, *72*, 951.
26. Mehta, A.; Carrouée, S.; Conyers, B.; Jordan, R.; Butters, T.; Dwek, R. A.; Block, T. M. *Hepatology* **2001**, *33*, 1488.