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Polyacetylenes and anti-hepatitis B virus active constituents from *Artemisia capillaris*

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ABSTRACT

Three new polyacetylenes, 8-(*Z*)-decene-4, 6-diyne-1, 3, 10-triol (**1**), 1, 3*S*, 8*S*-trihydroxydec-9-en-4, 6-yne (**2**), 3*S*, 8*S*-dihydroxydec-9-en-4, 6-yne 1-*O*-*β*-D-glucopyranoside (**3**), and one new glucosyl caffeoate, 1-*O*-ethyl-6-*O*-caffeoyl-*β*-D-glucopyranose (**4**), together with 34 known compounds were isolated from *Artemisia capillaris*. The structures of the new compounds were determined by extensive spectroscopic analyses including 1D and 2D NMR, HRESIMS, $[\alpha]_D$ and CD experiments. Among them, 19 compounds showed activity inhibiting HBsAg secretion; 20 compounds showed activity inhibiting HBeAg secretion; and 25 compounds possessed inhibitory activity against HBV DNA replication according to our anti-HBV assay on HepG 2.2.15 cell line *in vitro*. The most active compound **12** could inhibit not only the secretions of HBsAg and HBeAg, but also HBV DNA replication with IC_{50} values of 15.02 μM (SI = 111.3), 9.00 μM (SI = 185.9) and 12.01 μM (SI = 139.2).

1. Introduction

Hepatitis B virus (HBV) infection is a serious global health problem; however, the current treatment strategies including vaccines, *α*-interferon and nucleoside analogues are still unsatisfied. Therefore, new anti-HBV drugs with unique mechanism are further needed. Traditional Chinese medicines (TCMs) with hepatoprotective and antiviral activity are fascinating sources for anti-HBV drug discovery, from which many anti-HBV leading compounds (silymarin, schisanthein A, oxymatrine, *etc.*) have been obtained [1]. *Artemisia capillaris* (Yin-Chen) belonging to the *Artemisia* genus of the family Compositae is a famous TCM documented in every edition of "Chinese Pharmacopoeia". The earliest recording of Yin-Chen was found in the first Chinese dispensary "Shennong Bencao Jing". This plant is mainly distributed in Shananxi, Shanxi, Anhui provinces of China, and has been widely used for hepatoprotective, choleretic, anti-inflammatory and diuretic purposes in the folk. Currently, many formulae containing Yin-Chen have been used for treating acute and chronic hepatitis in clinical [2,3]. Previous investigation suggested that coumarins, flavonoids, organic acids, polyacetylenes, chromones, *etc.* were the main constituents in *A. capillaris*, of which *p*-hydroxyacephenone, scoparone, cirsimaritin, arcapillin, quercetin, chlorogenic acid, capillarisin and capillene were revealed to be hepatoprotective and choleretic components [4,5]. Meanwhile, a series of constituents with antiplatelet aggregation and anti-HIV activity were also isolated from this herb [2]. However, its anti-HBV active constituents are still unclear.

According to our bioassay on HepG 2.2.15 cell line *in vitro*, the 90% ethanol extract of *A. capillaris* exhibited activity against the secretions of hepatitis B surface antigen (HBsAg), hepatitis B e antigen (HBeAg), and HBV DNA replication with the IC_{50} value of 460.33 μg/mL (SI = 1.4), 295.31 μg/mL (SI = 2.5) and 49.13 μg/mL (SI = 13.4), respectively. In order to clarify its anti-HBV compounds, our current investigation on the active

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parts led to the isolation of 38 compounds, including three new polyacetylenes, 8-(*Z*)-decene-4, 6-diyne-1, 3, 10-triol (**1**), 1, 3*S*, 8*S*-trihydroxydec-9-en-4, 6-yne (**2**), and 3*S*, 8*S*-dihydroxydec-9-en-4, 6-yne 1-*O*-β-D-glucopyranoside (**3**), and one new glucosyl caffeoate, 1-*O*-ethyl-6-*O*-caffeoyl-β -D-glucopyranose (**4**), together with 34 known compounds (**4–38**). Among them, 19 compounds showed activity inhibiting HBsAg secretion, 20 compounds showed activity inhibiting HBeAg secretion and 25 compounds possessed inhibitory activity against HBV DNA replication according to our anti-HBV assay on HepG 2.2.15 cell line *in vitro*. The most active compound **12** could inhibit not only the secretions of HBsAg and HBeAg but also HBV DNA replication with IC_{50} values of 15.02 μM (SI = 111.3), 9.00 μM (SI = 185.9) and 12.01 μM (SI = 139.2). In this paper, we described the isolation, structural elucidation and anti-HBV activities of the isolates.

2. Experimental

2.1. General experimental procedures

Mass spectra were determined by a LCMS-IT-TOF (Shimadzu, Kyoto, Japan) or a Waters AutoSpec Premier P776 (Waters, USA) mass spectrometer. Optical rotations were measured through a Jasco model 1020 digital polarimeter (Horiba, Tokyo, Japan). UV and IR (KBr) spectra were recorded on a Shimadzu UV2401PC spectrophotometer (Shimadzu, Kyoto, Japan) and a Bio-Rad FTS-135 spectrometer (Hercules, California, USA), respectively. Electronic circular dichroism (ECD) spectra were performed on an Applied Photophysics Chirascan instrument (Agilent, America). 1D and 2D NMR were recorded on Bruker AM-400, Bruker DRX-500 or AVANCE III-600 spectrometers (Bruker, Bremerhaven, Germany). Silica gel (200–300 mesh) for column chromatography and TLC plates (GF_{254}) were purchased from Qingdao Makall Chemical Company (Makall, Qingdao, China) and Qingdao Haiyang Chemical Company (Haiyang, Qingdao, China), respectively. Sephadex LH-20 (20–50 μm) for chromatography was obtained from Pharmacia Fine Chemical Co., Ltd. (Pharmacia, Uppsala, Sweden). Waters Alliance 2695 apparatus with an Agilent ZORBAX SB-C_{18} (5 μm, 9.4 × 250 mm) column (Agilent, USA) was used for HPLC separation. Fractions were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in ethanol or colored by sulfuric acid-vanillin reagent.

2.2. Plant material

The whole plants of *A. capillaris* Thunb. were collected from wide sources in Chengcheng County, Shaanxi Province, China, in April 2012 and identified by Dr. Prof. Li-Gong Lei (Kunming Institute of Botany, CAS). A voucher specimen (No. 20120428) was deposited in the Laboratory of Anti-virus and Natural Medicinal Chemistry, Kunming Institute of Botany, CAS.

2.3. Extraction and isolation

The aerial part of *A. capillaris* (10 kg) was extracted with 90% ethanol under reflux for two times, 1 h each time. The extract was concentrated *in vacuo* and partitioned between ethyl acetate (EtOAc) and water. The EtOAc part (400 g) was subjected on silica gel column chromatography (Si CC, 3 kg, 17.5 × 35 cm) eluted with MeOH–$CHCl_3$ (0:100, 10:90, 20:80, 30:70, 40:60, 50:50 *v*/*v*) to give 9 fractions (A–G). Fraction A was chromatographed on a silica gel column, eluted with EtOAc–petroleum ether (PE) (5:95) to yield compounds **8** (470 mg) and **10** (2.6 g). Purification of fraction B on Si CC with acetone–PE (5:95) afforded compounds **7** (8 mg) and **37** (44 mg). Fraction C was subjected to Si CC and further purified with Sephadex LH-20 column (MeOH–$CHCl_3$, 50:50) to give compounds **17** (3 mg), **25** (6 mg) and **30** (5 mg). Fraction D was separated by repeated Si CC [EtOAc–PE, 20:80, 30:70, 40:60] and Sephadex LH-20 columns (MeOH) to yield compounds **9** (18 mg), **11** (12 mg), **14** (20 mg), **15** (36 mg), **16** (21 mg), **19** (18 mg), **23** (10 mg), **32** (28 mg) and **33** (360 mg). Compounds **1** (10 mg), **2** (9 mg), **22** (7 mg) and **34** (10 mg) were isolated from fraction E by Si CC (acetone–PE, 30:70). Fraction F (30 g) was performed on a MCI CHP-20P gel CC (490 g, 5.04 × 50 cm) and eluted with MeOH–H_2O (30:70, 50:50, 70:30) to yield 3 sub-fractions (F-1–F-3). Fraction F-1 was loaded on a Si CC (MeOH–$CHCl_3$, 20:80) to yield compounds **26** (14 mg), **27** (19 mg) and **28** (43 mg). Fraction F-2 was chromatographed on a reversed phase C-18 column (320 g, 5.04 × 50 cm) and eluted with MeOH–H_2O to give compounds **6** (5 mg), **12** (43 mg), **18** (38 mg), **29** (13 mg) and **31** (19 mg). Fraction F-3 was purified by Sephadex LH-20 chromatography (MeOH) affording compounds **4** (45 mg), **20** (145 mg), **21** (6 mg) and **24** (43 mg). Fraction G was loaded on a silica gel column eluted with MeOH–$CHCl_3$ (10:90), then further purified with Sephadex LH-20 column (MeOH) to give compounds **13** (10 mg) and **35** (9 mg). Fraction H was purified by Sephadex LH-20 column (MeOH) to give two sub-fractions. Compound **36** (3 mg) was isolated from fraction H-1 by Sephadex LH-20 column (MeOH). Fraction H-2 was further separated by HPLC (Agilent ZORBAX SB-C_{18}, 5 μm, 9.4 × 250 mm) with MeOH–H_2O (33:67) as the eluent to afford compounds **5** (3 mg, t_R = 10 min) and **38** (39 mg, t_R = 12 min). Fraction I was performed on a silica gel column with the elution of MeOH–H_2O (30:70) to yield compound **3** (12 mg).

Compound **1**: pale yellow oil; [α]21 D: −20.6 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε): 284 (3.69), 268 (3.80), 254 (3.65), 241 (3.37) nm; IR (KBr) ν_{max}: 3381, 2954, 2928, 2888, 2232, 1743, 1630, 1416, 1385, 1334, 1050, 1024 cm^{-1}; ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) data, see Tables 1–2; HREIMS *m*/*z* 180.0784 ($C_{10}H_{12}O_3$, calcd for 180.0786).

Compound **2**: pale yellow oil; [α]23 D: −94.9 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε): 283 (2.80), 267 (2.89), 253 (2.88), 241 (2.90) nm; IR (KBr) ν_{max}: 3395, 2959, 2930, 2890, 2152, 1641, 1410, 1312, 1050, 1021 cm^{-1}; ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) data, see Tables 1–2; HREIMS *m*/*z* 180.0781 ($C_{10}H_{12}O_3$, calcd for 180.0786).

Compound **3**: pale yellow oil; [α]18 D: −51.4 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε): 284 (3.05), 268 (3.12), 254 (3.12), 242 (3.13) nm; IR (KBr) ν_{max}: 3419, 2925, 2894, 1641, 1419, 1384, 1078, 1032 cm^{-1}; ^1H NMR (500 MHz) and ^{13}C NMR (100 MHz) data, see Tables 1–2; HRESIMS *m*/*z* 365.1266 ($[M + Na]^+$, $C_{16}H_{22}O_8Na^+$, calcd for 365.1207).

Compound **4**: brown gum; [α]27 D:-25.8 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ε): 329 (4.26), 216 (4.27) nm; IR (KBr) ν_{max}: 3423, 2977, 2920, 1692, 1632, 1605, 1521, 1446, 1383, 1284, 1181, 1053 cm^{-1}; ^1H NMR (400 MHz) and ^{13}C NMR

Table 1
^{1}H NMR data of compounds **1–4** (**1–3** in CD_3OD, **4** in acetone-d_6, δ in ppm, J in Hz).

Position	1	2	3	4
1	3.72 (m)	3.70 (m)	3.72 (m) 3.99 (m)	—
2	1.89 (m)	1.89 (m)	1.97 (m)	6.31 (d, 15.9)
3	4.59 (t, 6.8)	4.55 (t, 6.7)	4.63 (t, 6.5)	7.56 (d, 15.9)
4	—	—	—	—
5	—	—	—	7.17 (d, 1.8)
6	—	—	—	—
7	—	—	—	—
8	5.64 (d, 11.0)	4.88 (overlap)	4.87 (overlap)	6.86 (d, 8.1)
9	6.23 (dt, 11.0, 6.4)	5.91 (ddd, 17.0, 10.2, 5.5)	5.91 (ddd, 16.6, 9.8, 5.4)	7.05 (dd, 8.1, 1.8)
10	4.30 (d, 6.4)	5.20 (d, 10.2) 5.40 (dd, 17.0, 1.2)	5.20 (d, 9.8) 5.39 (d, 16.6)	—
1'	—	—	4.27 (d, 7.8)	4.30 (d, 7.5)
2'	—	—	3.17 (m)	3.54 (m)
3'	—	—	3.30 (m)	3.39 (m)
4'	—	—	3.27 (m)	3.41 (m)
5'	—	—	3.36 (m)	3.19 (m)
6'	—	—	3.68 (dd, 11.9, 4.5)	4.30 (m)
	—	—	3.87 (dd, 11.7, 1.0)	4.47 (dd, 11.8, 1.9)
1''	—	—	—	3.84 (m)
				3.55 (m)
2''	—	—	—	1.14 (t, 7.1)

(100Hz) data, see Tables 1–2; HRESIMS m/z 393.1147 ($[M + Na]^+$, $C_{17}H_{22}O_9Na^+$, calcd for 393.1156).

2.4. Acid hydrolysis of compound **3**

Compound **3** (4 mg) was refluxed with 2 M HCl (5 mL) at 80 °C for 5 h. After neutralization with $NaHCO_3$ and extraction with $CHCl_3$, the aqueous layer was concentrated and detected by TLC over silica gel (H_2O–MeOH–$CHCl_3$, 4:40:60). The presence of glucose was confirmed by comparison with authentic samples (*Rf* 0.4). The aqueous part was further purified by Si CC and identified to be D-glucose based on its $[\alpha]_D$ value ([α]13 D + 46.6, *c* 0.089, MeOH).

Table 2
^{13}C NMR data[a,b] of compounds **1–4** (**1–3** in CD_3OD, **4** in acetone-d_6, δ in ppm).

Position	1	2	3	4
1	59.0, t	57.6, t	66.7, t	167.5, s
2	41.3, t	39.8, t	38.8, t	115.2, d
3	60.3, d	58.8, d	60.1, d	145.9, d
4	85.9, s	80.5, s	82.0, s	127.5, s
5	69.1, s	67.6, s	68.9, s	115.4, d
6	74.9, s	68.7, s	70.1, s	146.2, s
7	78.6, s	78.1, s	79.5, s	148.8, s
8	109.2, d	62.5, d	63.9, d	116.3, d
9	147.7, d	136.5, d	138.0, d	122.5, d
10	61.1, t	115.5, t	116.8, t	—
1'	—	—	104.5, d	103.9, d
2'	—	—	75.0, d	74.8, d
3′	—	—	77.9, d	71.3, d
4′	—	—	71.5, d	77.8, d
5′	—	—	78.0, d	74.8, d
6′	—	—	62.6, t	64.3, t
1″	—	—	—	65.3, t
2″	—	—	—	15.5, q

2.5. Anti-HBV assay on HepG 2.2.15 cell line in vitro

The anti-HBV assay was performed according to our previous report, with tenofovir (Jiangxi Chenyang Pharmaceutial Co., Ltd, China, purity >97.6%) as the positive control [6].

3. Results and discussion

Compound **1** had a molecular formula of $C_{10}H_{12}O_3$ by HREIMS ($[M]^+$ m/z 180.0784, calcd for 180.0786). The absorption bands at 3381, 2232 and 1630 cm^{-1} in the IR spectrum indicated the presence of hydroxyl, alkynyl and alkenyl groups. The UV absorptions at 284, 268, 254 and 241 nm were typical for polyacetylenes [7]. The ^{13}C NMR (DEPT) spectrum of **1** displayed 10 carbons, including three methylenes, three methines and four quaternary carbons, of which two alkynyls [δ_C 85.9 (s, C-4), 69.1 (s, C-5), 74.9 (s, C-6) and 78.6 (s, C-7)] and one alkenyl [δ_C 109.2 (d, C-8) and 147.7 (d, C-9)] were obviously recognized. The above analyses suggested that compound **1** should be a C_{10} polyacetylene. The NMR data of compound **1** were similar to these of the known compound 8-(*E*)-decene-4, 6-diyne-1, 3, 10-triol (**1a**) [7], except for the different coupling constants between H-8 and H-9 ($J_{H\text{-}8/H\text{-}9}$ = 11.0 Hz in **1**, $J_{H\text{-}8/H\text{-}9}$ = 15.9 Hz in **1a**). From the above analysis, the double bond was deduced as *Z* configuration in **1** instead of *E* configuration in **1a**. Since there was only one chiral center (C-3) in both of the two compounds, the absolute configuration of compound **1** was determined to be *R* identical to **1a** from the similar $[\alpha]_D$ value. Thus, compound **1** was determined as 8-(*Z*)-decene-4, 6-diyne-1, 3, 10-triol as shown in Fig. 1.

Compound **2**, had the same molecular formula of $C_{10}H_{12}O_3$ as compound **1** by HREIMS ($[M]^+$ m/z 180.0781, calcd for 180.0786). The NMR spectra of compound **2** were almost identical to those of the known compound 1, 3*R*, 8*R*-trihydroxydec-9-en-4, 6-yne (**2a**) from the congener plant *Artemisia monosperma* [8], indicating the same relative configuration. However, the $[\alpha]_D$ value of compound **2** (−94.9) was

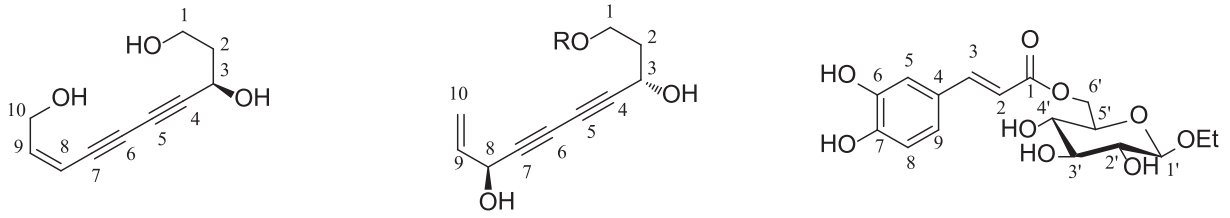

Fig. 1. Structures of compounds **1–4**.

opposite to **2a** (+125), suggesting that compound **2** should be the enantiomer of **2a**. Based on the above analyses, compound **2** was assigned as 1, 3*S*, 8*S*-trihydroxydec-9-en-4, 6-yne.

Compound **3** had a molecular formula of $C_{16}H_{22}O_8$ by HRESIMS ($[M + Na]^+$ *m/z* 365.1266, calcd for 365.1207). The ^{13}C NMR data of compound **3** was similar to compound **2** except for an additional glucosyl group and the obvious down-field shift of C-1 from δ_C 57.6 in **2** to 66.7 in **3**. Therefore, compound **3** was proposed as a glycoside of **2** with the glycosidation at C-1, which was confirmed by the correlations of δ_H 4.26 (H-1′, d, J = 7.8 Hz) to δ_C 66.7 (C-1, t) in the HMBC spectrum (Fig. 2). The β-glucose was deduced from the coupling constant (J = 7.8 Hz) of the anomeric proton. Acid hydrolysis of **3** provided D-glucose, which was confirmed by comparison with an authentic sample on TLC and $[\alpha]_D$ experiment ([α]13 D: +46.6, *c* 0.089, MeOH). The identical ECD spectra shown in Fig. 3 suggested that compounds **2** and **3** had the same absolute configuration. Therefore, compound **3** was assigned as 3*S*, 8*S*-dihydroxydec-9-en-4, 6-yne 1-*O*-β-D-glucopyranoside.

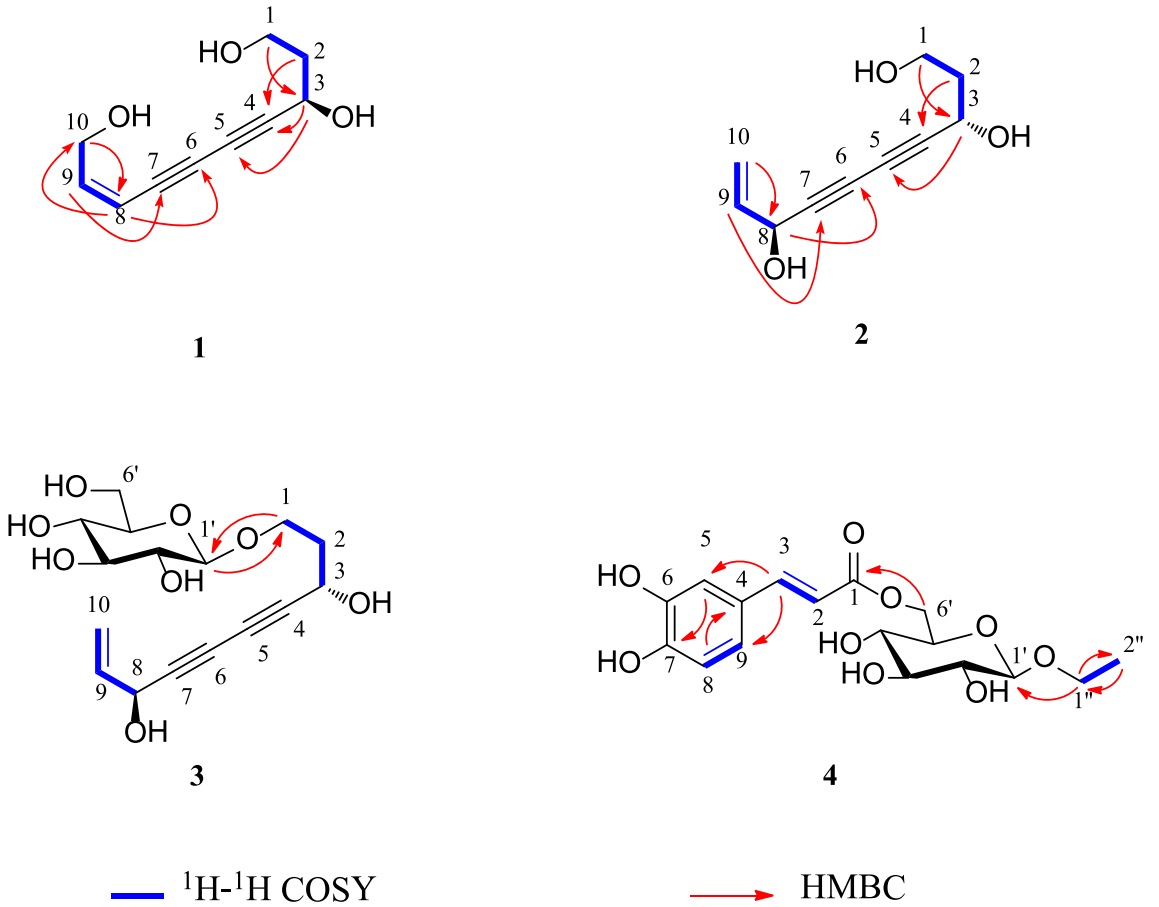

Fig. 2. Selected HMBC and COSY correlations of compounds **1–4**.

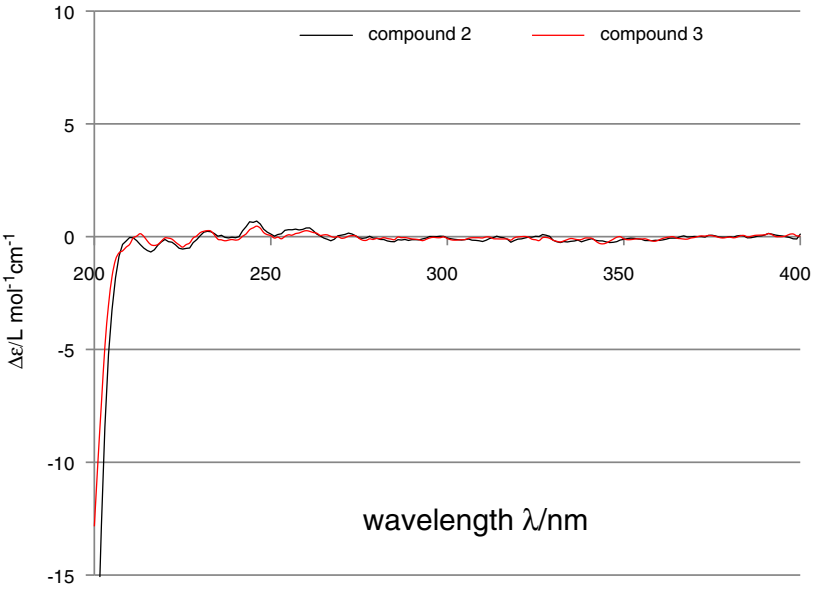

Fig. 3. Experimental ECD spectra of compounds **2** and **3**.

Compound **4** had a molecular formula of $C_{17}H_{22}O_9$ by positive HRESIMS (*m/z* 393.1147 $[M + Na]^+$, calcd for 393.1156). The presence of hydroxyl (3423 cm^{-1}), carbonyl (1692 cm^{-1}), and aromatic ring (1605, 1521, 1446 cm^{-1}) was deduced from the IR spectrum. The NMR spectra of compound **4** were similar to those of 1-*O*-methyl-6-*O*-caffeoyl-β-*D*-glucopyranose (**4a**) [9], and the only difference was that the methoxyl group in **4a** was changed to be ethoxyl group in **4**. The above deduction was supported by the HMBC correlations from H-1″ to C-1′ and from H-6′ to C-1. The coupling constant of 7.5 Hz for H-1′ indicated the β configuration of the glucosyl group. Its absolute configuration was proposed as D-glucosyl due to the similar $[\alpha]_D$ values of compounds **4** (−25.8) with **4a** (−18.1).

The other 34 compounds were determined as [8-(*E*)-decene-4, 6-diyne-1, 10-diol (**5**) [10], deca-9-en-4, 6-diyne-1, 8-diol 1-*O*-β -D-glucopyranoside (**6**) [11], dendroarboreol B (**7**) [12], (3*S*)-16, 17-didehydrofalcarinol (**8**) [12], (3*S*, 8*S*)-16, 17-dehydrofalcarindiol (**9**) [12]]; 4 terpenoids [phytol (**10**) [13], 7-eudesm-4 (15)-ene-1β, 6α-diol (**11**) [14], pumilaside A [15] (**12**), (6*R*, 9*R*)-3-oxo-α -ionol-9-*O*-β -D-glucopyranoside (**13**) [16]]; 5 coumarins [umbelliferone (**14**) [17], scopoletin (**15**) [18], isoscopoletin (**16**) [18], scoparone (**17**) [19], esculetin (**18**) [18]]; 3 flavonoids [rhamnocitrin (**19**) [20], isorhamnetin 3-*O*-β -D-glucopyranoside (**20**) [21], naringenin 7-*O*-β -D-glucopyranoside (**21**) [22]], 2 lignans [(+)-epipinoresinol (**22**) [23], (+)-medioresinol (**23**) [24]]; 11 benzenoids [salicylic acid (**24**) [25], 4-hydroxy-3, 5-dimethoxybenzaldehyde (**25**) [26], 3, 4-dihydroxybenzaldehyde (**26**) [27], 4-hydroxybenzoic acid (**27**) [28], ethyl 4-hydroxybenzoic acid (**28**) [29], protocatechuic acid (**29**) [30], 1-(4-hydroxy-3-methoxyphenyl) propan-1-one (**30**) [31], caffeic acid (**31**) [30], ethyl *p*-hydroxy-trans-cinnamate (**32**) [32], ethyl caffeoate (**33**) [32], sonchifolinin B (**34**) [33]]; 4 other compounds [6-amino-9-[1-(3, 4-dihydroxyphenyl) ethyl]-9*H*-purine (**35**) [34], 4-methoxynicotinic acid (**36**) [35], stigmast-4-en-3-one (**37**) [36] and tuberonic acid (**38**) [37]] by comparing the data with literatures.

According to our anti-HBV assay on HepG 2.2.15 cells *in vitro*, 26 compounds showed activity inhibiting the secretions of HBsAg, HBeAg and HBV replication (Table 3). Three caffeic acid derivatives (**4**, **31**, **33**) possessed potent anti-HBV activity against HBV DNA replication with IC_{50} values of 6.84, 16.83 and 25.77 μM, indicating that caffeoyl moiety might be the active group [38]. Of the 5 coumarins (**14**–**18**), compound **18** with hydroxyl groups in both C-6 and C-7 exhibited the most promising activity against HBV DNA replication with an IC_{50} value of 33.76 μM. Other aromatic compounds such as furofurans lignans (**22** and **23**), flavonoids (**19**, **20** and **21**), and benzenoids (**24**–**30**, **32** and **34**) showed moderate anti-HBV activity, of which compounds **22**, **23**, **24**, **26** and **29** could inhibit HBV DNA replication with IC_{50} values in the range of 9.38 to 32.60 μM. Importantly, two eudesmane sesquiterpenoids (**11** and **12**) exhibited promising activity against HBV DNA replication with IC_{50} values of 19.70 and 12.01 μM, with high SI values of 105.5 and 139.2. In addition to the inhibition on HBV DNA, compound **12** could also suppress the secretions of HBsAg and HBeAg with the IC_{50} values of 15.02 μM (SI = 111.3) and 9.00 μM (SI = 185.9). Compound **35** as a heterozygote of purine and phenol possessed potent activity against HBV DNA replication with an IC_{50} value of 13.06 μM (SI = 148.6), as well as activity against the secretions of HBsAg and HBeAg. This is the first investigation on the anti-HBV constituents of *A. capillaris*, which will provide valuable information for understanding its active principles.

Conflict of interest statement

There are no conflicts of interests among all authors in this manuscript.

Table 3
Anti-HBV activities of compounds **4**, **11**, **12**, **14–35** and **38**.[a]

Compounds	CC_{50} [μM]	HBsAg[b]		HBeAg[c]		DNA[d]	
		IC_{50} [μM]	SI[e]	IC_{50} [μM]	SI	IC_{50} [μM]	SI
4	>1059.89	28.08	>37.7	126.70	>8.4	6.84	>155.0
11	2079.54	155.29	13.4	107.52	19.3	19.70	105.5
12	1672.27	15.02	111.3	9.00	185.9	12.01	139.2
14	488.76	855.74	<1.0	717.22	<1.0	448.15	1.1
15	1819.11	885.36	2.1	2911.25	<1.0	682.14	2.7
16	1625.68	451.09	3.6	1206.56	1.4	761.67	2.1
17	2892.33	516.26	4.7	110.24	21.8	206.31	11.7
18	>5452.75	43.60	>125.1	811.29	>6.7	33.76	161.5
19	>4084.97	<32.67	>125.1	<32.67	>125.1	490.87	>8.3
20	>2584.29	446.74	>5.8	331.92	>7.8	16.42	>157.4
21	>2552.63	370.69	>6.9	389.72	>6.5	41.59	>61.4
22	480.08	56.11	8.6	50.98	9.4	9.38	51.2
23	1086.20	836.73	1.3	381.82	2.8	10.92	99.5
24	4331.38	3620.51	1.2	4652.54	<1.0	25.80	167.9
25	1504.70	2343.88	<1.0	3717.16	<1.0	763.22	2.0
26	1392.46	<55.72	>25.0	<55.72	>25.0	27.17	51.2
27	>7002.79	3531.43	>2.0	159.87	>43.8	274.94	>25.5
28	4617.47	<44.88	>101.6	<44.88	>101.6	59.52	77.6
29	>8339.68	308.57	>27.0	66.69	>125.1	32.60	>255.8
30	2759.50	543.48	5.1	120.66	22.9	481.66	5.7
31	>4302.83	2166.50	>2.0	67.17	>64.1	16.83	>255.6
32	3258.54	802.74	4.1	454.15	7.2	1046.46	3.1
33	5049.38	<52.79	>95.7	<52.79	>95.7	25.77	195.9
34	3067.44	1643.93	1.9	446.31	6.9	198.69	15.4
35	1902.25	1423.21	1.3	527.34	3.6	13.06	148.6
38	>4945.35	>4945.35		>4945.35		166.06	>29.8
Tenofovir[f]	>8710.80	5531.26	>1.6	>8710.80		3.34	>2604.2

[a] All values are the mean of two independent experiments.
[b] HBsAg: HBV surface antigen.
[c] HBeAg: HBV e antigen.
[d] DNA: HBV DNA replication.
[e] CC_{50} = 50% cytotoxic concentration, IC_{50} = 50% inhibitory concentration, SI (selectivity index) = CC_{50}/IC_{50}.
[f] Tenofovir, an antiviral agent used as a positive control.

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Appendix A. Supplementary data

1D and 2D NMR, HREIMS, HRESIMS, IR, UV and $[\alpha]_D$ spectra of compounds **1–4**, ECD spectra for **2** and **3**, and structures of known compounds (**5–38**) are available as Supporting Information. Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.fitote.2014.03.017.

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