Three New Antiviral Triterpenes from *Aster tataricus*

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Three new shionane-type triterpenes, shion-22-methoxy-20(21)-en-3-one (**1**), shion-22(30)-en-3,21-dione (**2**), shion-22-methoxy-20(21)-en-3β-ol (**3**), were isolated from the rhizomes of *Aster tataricus*. Their structures have been determined on the basis of MS, IR, 1D and 2D NMR spectral evidences. Compounds **1** and **2** show inhibitory activities on HBsAg with IC_{50} values of 0.89 and 4.49 μg mL^{-1}, respectively, **1** shows inhibitory activity on HBeAg with an IC_{50} value of 0.83 μg mL^{-1}, and **2** shows inhibitory activity on HA with an IC_{50} value of 11.18 μg mL^{-1}.

Key words: Aster tataricus, Triterpene, Antiviral, Shionane-type Triterpenes

Introduction

Aster tataricus L. f., a herb plant widely distributed in the northeast and northwest of China, has been used as a common traditional Chinese medicine for the treatment of cough and eliminating phlegm [1, 2]. According to the literature, terpenoids, saponins, coumarins, flavones, steroids, and cyclopeptides have been found in this plant, and some of these compounds show antitumor and antioxidant activities [3 – 5]. As part of our systematic investigations on chemical and bioactive constituents of TCM plants, we carried out extensive chemical studies on the rhizomes of *A. tataricus* and obtained three new shionane-type triterpenes (**1** – **3**) (Fig. 1). Compounds **1** and **2** were tested on HBV and HA bioassays. The results have indicated that **1** and **2** show inhibitory activities on Hepatitis B Surface Antigen (HBsAg) with IC_{50} values of 0.89 and 4.49 μg mL^{-1}, respectively, **1** shows inhibitory activity on Hepatitis B e Antigen (HBeAg) with an IC_{50} value of 0.83 μg mL^{-1}, and **2** shows inhibitory activity on HA with an IC_{50} value of 11.18 μg mL^{-1}. In this paper, the isolation and structure elucidation of these compounds are reported.

Results and Discussion

Compound **1** has the molecular formula $C_{31}H_{52}O_2$ according to HREIMS ([M+Na]$^+$ at m/z = 479.3867, calcd. 479.3865), which was confirmed by ^{13}C and DEPT NMR spectra. The IR spectrum of **1** showed absorption bands for C=C (1640 cm^{-1}) and C=O (1714 cm^{-1}). Its UV spectrum revealed the presence of double bonds (203 nm). The ^{1}H and ^{13}C spectra (see Tables 1 and 2) showed the presence of nine methyls (one oxygenated), ten methylenes, five methines (two olefinic carbons), and seven quaternary carbons (one oxygenated and one carbonyl), which suggested that compound **1** owns a typical shionane skeleton [6].

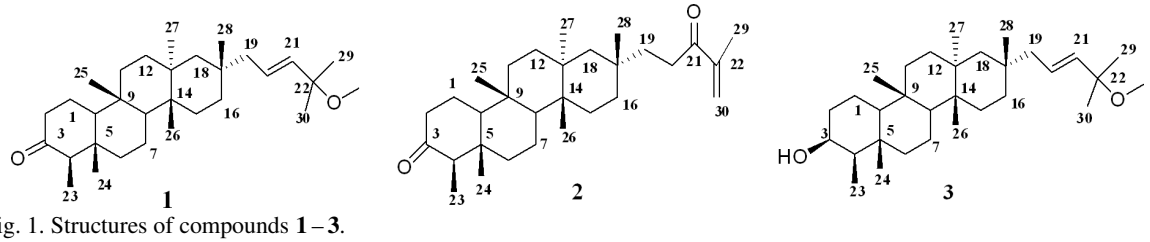
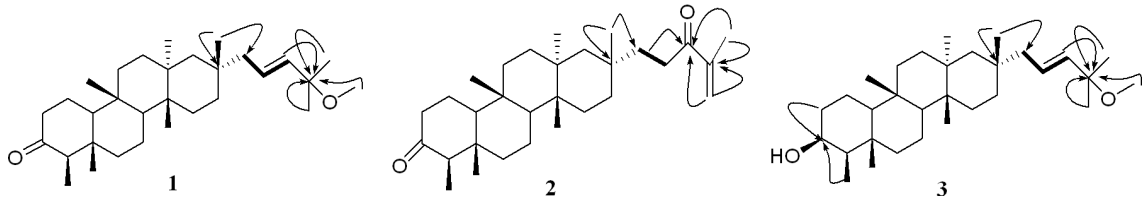


Fig. 1. Structures of compounds **1** – **3**.

Table 1. ^{1}H NMR data of compounds **1** – **3** (400 MHz, in $CDCl_3$; multiplicities and *J* values in Hz in parentheses).

H	**1**	**2**	**3**
1	1.98 (m), 1.70 (m)	1.95 (m), 1.71 (overlap)	1.48 (m), 1.58 (overlap)
2	2.39 (overlap), 2.30 (m)	2.37 (m), 2.30 (m)	1.58 (overlap), 1.89 (m)
3			3.73 (m)
4	2.25 (q, 6.5)	2.24 (q, 6.7)	1.23 (overlap)
6	1.25 (overlap), 1.73 (m)	1.28 (overlap), 1.71 (overlap)	0.93 (overlap), 1.72 (m)
7	1.50 (overlap), 1.32 (overlap)	1.47 (overlap), 1.31 (overlap)	1.37 (overlap)
8	1.32 (overlap)	1.38 (overlap)	1.23 (overlap)
10	1.60 (m)	1.55 (m)	0.93 (overlap)
11	1.40 (overlap), 1.50 (overlap)	1.38 (overlap),1.47 (overlap)	1.37 (overlap)
12	0.87 (overlap), 1.57 (m)	0.91 (m), 1.52 (m)	0.87 (overlap), 1.48 (overlap)
15	1.25 (overlap), 1.32 (overlap)	1.31 (overlap)	1.30 (m)
16	1.40 (overlap), 1.65 (m)	1.60 (m), 1.90 (m)	1.58 (overlap), 1.37 (overlap)
18	1.09 (m), 1.25 (overlap)	1.06 (m), 1.28 (overlap)	1.04 (m), 1.23 (overlap)
19	2.39 (overlap), 2.02 (m)	2.64 (m)	2.37 (dd, 13.9, 7.6), 2.00 (dd, 17.5, 8.5)
20	5.57 (m)	1.64 (m), 1.38 (overlap)	5.57 (m)
21	5.38 (d, 15.6)		5.38 (d, 15.8)
23	0.86 (d, 7.8)	0.85 (d, 6.9)	0.93 (overlap)
24	0.70 (s)	0.69 (s)	0.95 (s)
25	0.92 (s)	0.90 (s)	0.91 (s)
26	0.89 (s)	0.87 (s)	0.87 (s)
27	1.14 (s)	1.10 (s)	1.09 (s)
28	0.89 (s)	0.87 (s)	0.87 (s)
29	1.27 (s)	1.86 (s)	1.26 (s)
30	1.27 (s)	5.75 (s), 5.95(s)	1.26 (s)
OCH_3	3.14 (s)		3.15 (s)



1 **2** **3**

Fig. 2. Key ^{1}H-^{1}H COSY (▬) and HMBC (H→C) correlations of **1** – **3**.

The NMR data of **1** were similar to that of shionone [7] on rings A–D. However, different carbon and proton chemical shifts for C-19, C-20, C-21, C-22, C-29, and C-30 indicated that the structure of **1** differed from shionone in the side chain. In the H-H COSY spectrum, the cross peaks between δ_H = 2.39 (1H, overlap, H-19a), 2.02 (1H, m, H-19b) and δ_H = 5.57 (1H, m, H-20), δ_H = 5.57 (1H, m, H-20) and δ_H = 5.38 (1H, d, *J* = 15.6 Hz, H-21) indicated the presence of a -CH_2-CH=CH- fragment. The correlations between δ_H = 5.38 (1H, d, *J* = 15.6 Hz, H-21), 1.27 (6H, s, H-29, 30), 3.14 (3H, s, H-OMe) and δ_C = 74.9 (C-22) revealed that the side chain of **1** was a -CH_2-CH=CH-$C(CH_3)_2(OCH_3)$ fragment (Fig. 2). Thus, the structure of **1** was established as shion-22-methoxy-20(21)-en-3-one.

Compound **2** was assigned the molecular formula $C_{30}H_{48}O_2$ by its HRESIMS data at *m*/*z* = 463.3561 $[M+Na]^+$ (calcd 463.3552). The NMR spectra of **2** were quite similar to that of **1** on the rings A–D and differences again existed on the side chain. In the ^{13}C NMR spectrum of **2** signals of one methoxy, one methyl and two olefinic carbons have disappeared, and new signals occurred for one carbonyl, one methylene, one methine and one quaternary carbon. A cross peak between δ_H = 2.64 (2H, m, H-19) and δ_H = 1.64 (1H, m, H-20a), 1.38 (1H, m, H-20b) in the H-H COSY spectrum, correlations between δ_H = 2.64 (2H, m, H-19), 1.86 (3H, s, H-29), 5.75 (1H, s, H-30a), 5.95 (1H, s, H-30b) and δ_C = 202.9 (C-21), δ_H = 1.86 (3H, s, H-29), 5.75 (1H, s, H-30a), 5.95 (1H, s, H-30b) and δ_C = 144.6 (C-22) in the HMBC spectrum revealed that the side chain of **2** was a -CH_2-CH_2-C(O)-$C(CH_3)(CH_2)$ fragment (Fig. 2). Thus, the structure of **2** was established as shion-22(30)-en-3,21-dione.

Table 2. ^{13}C NMR data of compounds **1** – **3** (100 MHz, in $CDCl_3$; multiplicities in parentheses).

C	**1**	**2**	**3**	C	**1**	**2**	**3**
1	22.3 (t)	22.3 (t)	15.8 (t)	16	33.8 (t)	33.1 (t)	33.8 (t)
2	41.5 (t)	41.5 (t)	35.2 (t)	17	32.1 (s)	31.4 (s)	32.1 (s)
3	213.2 (s)	213.2 (s)	72.7 (d)	18	45.4 (t)	45.0 (t)	45.5 (t)
4	58.1 (d)	58.1 (d)	49.1 (d)	19	46.2 (t)	37.6 (t)	46.1 (t)
5	42.1 (s)	42.1 (s)	37.8 (s)	20	127.5 (d)	34.2 (t)	127.6 (d)
6	41.0 (t)	41.0 (t)	41.5 (t)	21	137.9 (d)	202.9 (s)	137.8 (d)
7	17.9 (t)	17.9 (t)	17.2 (t)	22	74.9 (s)	144.6 (s)	75.2 (s)
8	49.8 (d)	49.8 (d)	49.9 (d)	23	6.8 (q)	6.8 (q)	11.6 (q)
9	38.4 (s)	38.4 (s)	38.1 (s)	24	14.6 (q)	14.6 (q)	16.4 (q)
10	59.5 (d)	59.5 (d)	61.4 (d)	25	19.6 (q)	19.6 (q)	19.9 (q)
11	35.2 (t)	35.2 (t)	35.1 (t)	26	15.1 (q)	15.2 (q)	15.0 (q)
12	32.2 (t)	32.2 (t)	32.3 (t)	27	21.1 (q)	20.8 (q)	21.1 (q)
13	36.9 (s)	36.8 (s)	36.9 (s)	28	33.0 (q)	32.8 (q)	33.0 (q)
14	38.4 (s)	38.5 (s)	38.5 (s)	29	26.0 (q)	17.8 (q)	26.0 (q)
15	29.2 (t)	29.1 (t)	29.1 (t)	30	26.0 (q)	124.3 (t)	26.0 (q)
				OMe	50.4 (q)		50.3 (q)

Compound **3** has the molecular formula $C_{31}H_{54}O_2$ established by HREIMS at m/z = 458.4143 $[M]^+$ (calcd. 458.4124). The IR spectrum of **3** showed absorption bands for OH (3471 cm^{-1}) and C=C (1630 cm^{-1}) groups. The ^1H and ^{13}C spectra (see Tables 1 and 2) indicated that compounds **3** and **1** are almost the same except for differences on ring A, but the data of **3** were also quite similar to that of epishionol on ring A [8, 9]. Thus, the structure of **3** was established as shion-22-methoxy-20(21)-en-3β-ol, which was confirmed by the presence of cross peaks between δ_H = 1.89 (1H, m, H-2), 0.93 (3H, overlap, H-23) and δ_C = 72.7 (C-3) in the HMBC spectrum (Fig. 2).

Compounds **1** and **2** were tested on HBV and HA bioassays. Results have indicated that **1** and **2** show inhibitory activities on HBsAg (IC_{50} = 0.89 and 4.49 $\mu g\,mL^{-1}$), **1** shows inhibitory activity on HBeAg (IC_{50} = 0.83 $\mu g\,mL^{-1}$), and **2** shows inhibitory activity on HA (IC_{50} = 11.18 $\mu g\,mL^{-1}$).

Experimental Section

General

Optical rotations were taken on a Horiba SEAP-300 polarimeter. ^1H, ^{13}C NMR and 2D NMR spectra were recorded on a Bruker AM-400 NMR spectrometer with TMS as internal standard, δ in ppm, J in Hz. MS data were obtained on a VG Autospec-3000 spectrometer. IR spectra were obtained on a Bio-Red FTS-135 spectrometer. UV spectra were taken on a 2401PC spectrometer.

Plant materials

Rhizomes of *A. tataricus* were bought in the market of Chinese traditional medicine in Kunming, Yunnan, P. R. China, in September 2008. The material was identified by Prof. Xi Wen Li, Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (no. 200704) was deposited in the herbarium of Kunming Institute of Botany.

Extraction and isolation

The dried and powdered rhizomes (50 kg) of *A. tataricus* were extracted three times with methanol at r. t. The extracts were concentrated under reduced pressure and afforded 5.5 kg of methanol extract. The methanol extract was respectively partitioned by EtOAc, *n*-BuOH and H_2O. The EtOAc layer (1.55 kg) was subjected to column chromatography (10 kg SiO_2) and eluted by $CHCl_3$-MeOH with increasing polarity. The fraction from $CHCl_3$ (840 g) was subjected to CC (SiO_2; petroleum ether-acetone mixtures of increasing polarity), giving fractions Fr. 1 – 6. Fr. 4 (40 g) from petroleum ether-acetone (20 : 1) was further purified by repeated chromatography over silica gel (petroleum ether-acetone, from 30 : 1 to 10 : 1 and petroleum ether-ethyl acetate, from 30 : 1 to 8 : 1) and gel sephadex LH-20 ($CHCl_3$-CH_3OH 2 : 1) to yield compounds **1** (10 mg), **2** (63 mg) and **3** (35 mg).

*Shion-22-methoxy-20(21)-en-3-one (**1**)*

Colorless needle-shaped crystals. – $[\alpha]_D^{27}$ = −45.5 (c = 0.27, $CHCl_3$). – UV (MeOH): $\lambda(\lg\varepsilon)$ = 203.0 (3.44), 267.4 (2.08). – IR (KBr): ν = 2972, 2930, 2867, 1714, 1463, 1452, 1387, 1377, 1172, 1078 cm^{-1}. – ^1H and ^{13}C NMR spectral data: see Tables 1 and 2. – HRMS ((+)-ESI): m/z = 479.3867 (calcd. 479.3865 for $C_{31}H_{52}O_2$, $[M+Na]^+$). – MS ((+)-FAB): m/z = 457 $[M+H]^+$.

*Shion-22(30)-en-3,21-dione (**2**)*

Colorless powder. – $[\alpha]_D^{27}$ = −46.8 (c = 0.42, $CHCl_3$). – UV (MeOH): $\lambda(\lg\varepsilon)$ = 195.4 (3.48), 219.0 (3.75), 296.8

(2.82), 335.8 (2.49), 375.6 (2.40). – IR (KBr): ν = 2949, 2930, 2863, 1712, 1680, 1466, 1452, 1390, 1378, 1078 cm^{-1}. – ^1H and ^{13}C NMR spectral data: see Tables 1 and 2. – HRMS ((+)-ESI): m/z = 463.3561 $[M+Na]^+$ (calcd. 463.3552 for $C_{30}H_{48}O_2$, $[M+Na]^+$). – MS ((+)-ESI): m/z = 463 $[M+Na]^+$.

*Shion-22-methoxy-20(21)-en-3β-ol (**3**)*

Colorless acicular solid. – $[\alpha]_D^{24}$ = −27.2 (c = 0.09, $CHCl_3$). – UV (MeOH): λ (lg ε) = 192.6 (3.10), 202.4 (3.42), 218 (2.93). – IR (KBr): ν = 3471, 2930, 2870, 1630, 1461, 1380, 1174, 1074 cm^{-1}. – ^1H and ^{13}C NMR spectral data: see Tables 1 and 2. – HRMS ((+)-EI): m/z = 458.4143 $[M]^+$ (calcd. 458.4124 for $C_{31}H_{54}O_2$, $[M]^+$). – MS ((+)-EI): m/z = 458 $[M]^+$.

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