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TWO NOVEL OF TERPENOIDS SKELETONS FROM *ASTER TURBINATUS* S. MOORE

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Abstract

Aster turbinatus is widely used in traditional Chinese medicine, mainly for sore throat, mammary carbuncle, carbuncle, abdominal distention, diarrhea and so on. At present, the research on its chemical constituents and pharmacological activity is deficient. In order to find its bioactive principles, the alcoholic extract of the dried root was investigated and two novel terpenoids were isolated，which were designated as asterbins **A** (1) and asterbins **B** (2). Their structures were elucidated by 1D- and 2D-NMR and HR-ESIMS.

Introduction

The genus *Aster* is the largest one of the family Asteraceae and it has estimated about 250 species in the world. The genus *Aster* is widely distributed in Asia, Europe, and North America. There are about 100 species in China and over one third of them are used in traditional herbal medicines in China (Wang 1985，, Yu *et al*. 2015). It is mainly produced in Hebei, Anhui, Henan, Gansu, North China, and Northeast China. As per the Compendium of Materia Medica, the flowers and roots of *Aster* can be used as medicine. The genus *Aster* has a good effect upon moistening lung, eliminating phlegm, and suppressing cough. Besides expectorant and antitussive activities, the genus *Aster* possesses antitumor, antibacterial, diuretic, antiviral, antioxidant, anti-inflammatory and antiulcer activities (Yang 2006, Peng *et al.* 2015, Kang *et al*. 2016).

Chemical constituents from *Aster* species are diverse，, including monoterpenoids and their glycosides, sesquiterpenes and their glycosides, diterpenoids and their glycosides, triterpenoids and their glycosides, flavonoids and their glycosides, in addition to sterols, coumarins, alkaloids, cyclic peptides, volatile oils and so on. Until now, about 44 species of *Aster* have been studied and more than 300 compounds have been isolated and identified (Yang 2006, Tian *et al*. 2013).

Two types of monoterpenes and their glycosides are reported: acyclic monoterpenes and bicyclic monoterpenes. Tsankova *et al.* (1983) isolated 6, 7-dihydtoxy-6, 7-dihydro-cis-ocimene, an acyclic monoterpene from *A. bakeranus*. Nagao *et al.* (1988) separated two monoterpene saponins from *A. tataricus*, which are Shionoside A and Shionoside B. Two monoterpenoid saponins were isolated from *A. scaber* by Jung *et al*. (2001). Cheng *et al.* (1994) and Wang *et al.* (1997) isolated Shionoside C from *A. tataricus* and gentiopicrosideo from *A. auriculatus*. A polyhydroxy monoterpene acid linked by two isoprene units was isolated from *A. farreri* (Wang *et al*. 1994). Nagao *et al.* (1988) reported the presence of different diterpenoids and their glycosides in this genus.

Terpenoids and cyclic peptides are the main characteristic components of this genus, and they are also the main active ingredients of *Aster* to relieve phlegm and cough. It has been proved that caffeoylquinic acids, aster saponins, and aster peptides, rather than shionone, may be the main constituents responsible for the expectorant and antitussive activities of *A. tataricus* and act in a synergistic way (Peng *et al.* 2015). Triterpenoid such as epi-friedelol, friedel-3-ene (Lu *et al.*

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1998), friedelin, D-friedel-14-ene-3-ol and auriculatone I has been isolated from *A. tataricus, A. albescens, A. poliothamnus, A. ageratoides* var. Ovatu (He *et al.* 1996, Tian *et al.* 2013). A lanolin type compound astertarone A was isolated from *A. lingulatus* (Yu *et al.* 1998), epishionol and 3β-hydroxyl-20, 24-diene-damanane were isolated from *A. poliothamnus* (Zhang *et al.* 1997). In addition to the above triterpenoids, most of triterpenoids isolated from *Aster* are oleanane type, such as β-amyrin, ageratoside $C_{1,}$ 2β, 3β, 16α-trihydroxyi-24α-al- olean-12-en-28-oic acid, shionone and their saponins (Tsankova *et al.* 1983, Akihisa *et al.* 1998, Lu *et al.* 1998, Kazumi *et al.* 1999, Yan *et al.* 2004, Lu *et al.* 2013, Tian *et al.* 2013). Shionane-type triterpenes, astershionones A-F, have been obtained from the roots and rhizomes of *A. tataricus* (Lu *et al.* 2013).

Peptides are another characteristic chemical compound of this genus. Only one dipeptide was isolated from *A. tataricus* by Lu *et al.* (1998), and pentapeptides and cyclopentapeptides were isolated from *A. tataricus* (Tian *et al.* 2013). No other species have been reported to yield peptides. In addition to the above-mentioned compounds, flavonoids, anthraquinones, coumarins, lignans, sterols, alkynate glycosides have also reported from this genus (Zhou *et al.* 2014). A coumarins identified as scopolamine was isolated from *Aster*. Anthraquinones, namely chrysophanol, emodin, emodin monomethyl ether and aloe emodin have been found from *Aster*. Presence of flavonoids including quercetin, luteolin, rutin, kaempferol, hesperidin and apigenin were isolated (Tian *et al.* 2013, Kang *et al.* 2016). Many kinds of organic acids have been isolated and identified from *Aster*. They are benzoic acid, p-hydroxybenzoic acid, caffeic acid, ferulic acid, hexadecane ferulate, hexadecanoic acid and palmitic acid, and so on (Yang 2006, Lu *et al.* 2013). Two phenolic compounds, methyl 3-O-feruloyl-quinate and (+)-isolarchin 9β-D-pyranoside, have been obtained from *Aster*. Three sterols, namely stigmasterol, β-stitosterol and daucosterin, have been reported from *Aster* (Lu *et al.* 2013).

A. turbinatus also known as Yizhixiang, Baitiaogen and single aster is available in southern Jiangsu, southern Anhui, Eastern Jiangxi and Zhejiang, Eastern Fujian to the West. Its root is widely used in traditional Chinese medicine, mainly for sore throat, mammary carbuncle, carbuncle, abdominal distention, diarrhea and so on (Peng *et al.* 2015). At present, the research on its chemical composition and pharmacological activity are deficient. In order to find its bioactive principles, the chemical constituents of *A. turbinatus* were investigated and two novel terpenoids were isolated from the alcoholic extract of the whole plant. In this paper structural elucidation of the new compounds **1** and **2** is reported (Fig. 1A, 2A).

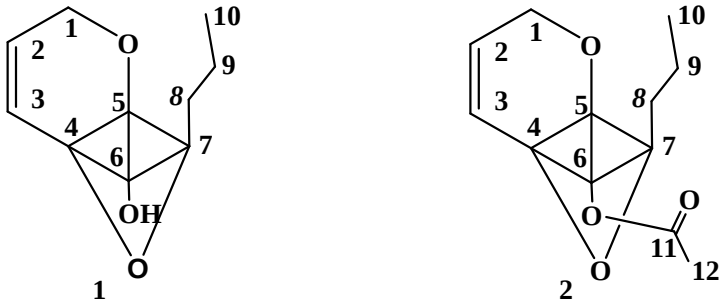

Fig. 1A, 2A

Materials and Methods

The fresh air-dried root of *Aster turbinatus* was bought from Huizhou Anhui province, in China, in October 2005 and was identified by Professor Ji Ma, School of Chinese Medicine.,

Southern Medical University, Guangzhou, P. R. China. A voucher specimen was deposited at key Laboratory of Natural Medicine for Gansu Province.

The air-dried root of *A. turbinatus* (2 kg) was extracted with C_2H_5OH (95%) at room temperature (30 L × 5, each extraction lasted 5 days). The combined extract was evaporated to dryness under reduced pressure. The residue (63 g) was then suspended in H_2O (1.5 L), and extracted with ethylacetate (1.0 L × 3) by the separating funnel. ethylacetate soluble extract (18 g) was subjected to column chromatography over silica gel (200~300 mesh, 200 g) using petroleum ether (60 - 90ºC) with increasing volume of acetone (v : v = 40 : 1, 20 : 1, 15 : 1, 10 : 1, 5 : 1, 2 : 1, 1 : 1, each about 0.5 l) as eluent. The fractions A1, A2 were collected according to TLC analysis. Fraction A1 and A2 (2.0 g) was further fractionated on a silica gel column (200 ~ 300 mesh, 100 g) eluting with petroleum ether-ethylacetate (v : v = 18 : 1) to give A1 (57 mg) and A2 (48 mg). Fraction A1 gave compound **1** (30 mg) on a silica gel eluting with petroleum ether-ethyl acetate (v : v = 15 : 1). Fraction A2 was purified by colum chromatography over a silica gel (200 ~ 300 mesh, 40 g) eluting with petroleum ether-acetone (v : v = 18 : 1) to yield compound **2** (28 mg). In addition, four known compounds were obtained by column chromatography.

Optical rotations were recorded in CH_3OH using a Perkin Elmer model 341 polarimeter. UV spectra were measured on a Spect 50-UV/Vis instrument (Analytic Jena AG). IR spectra were recorded on an FTS165-IR instrument (BioRad, USA). 1D NMR spectra and 2D NMR were acquired on a Varian INOVA-400 FT-NMR spectrometer (USA) in $CDCl_3$ TMS as internal standard. HR-ESIMS were obtained on a Bruker Daltonics APEX II spectrometer. Silica gel (200 ~ 300 mesh) used for column chromatography (CC) and silica GF_{254} for TLC was supplied by the Qingdao Marine Chemical Factory, Qingdao, P. R. China. Spots on TLC plate were detected on TLC by visualization under UV light or by spraying with 98% H_2SO_4-EtOH (v : v = 5 : 95) followed by heating at 110ºC for 3 min.

Results and Discussion

The alcoholic extract of the root part of *Aster turbinatus* was repeatedly separated by silica gel column chromatography to yield two new terpenoids **1** and **2**, the structures of which were by extensive spectroscopic analysis. So far the knowledge goes compounds **1** and **2** were previously unreported. In addition，friedelin (filedelin), shionone, epifriedelinol, kaempferol were isolated and obtained from alcoholic extract of the root part. The structures of these compounds were identified by 1D-, 2D-NMR and HR-ESIMS.

Compound **1** is a colorless needle, mp 38 - 40℃, $[\alpha]_D^{20}$+ 3º (c 1.0, $CHCl_3$). The molecular formula was assigned as $C_{10}H_{12}O_3$, which indicated five degrees of unsaturation, on the basis of HR-ESIMS ($[M+Na]^+$ = 203.0680; Calcd. 203.0679), and results from ^1H, ^{13}C NMR and DEPT analysis. The IR spectrum (film) indicated the presence of hydroxyl (3295 cm^{-1}), methyl (2964 cm^{-1}, 2880 cm^{-1}), methylene (2933 cm^{-1}, 2873 cm^{-1}), an ether bond (1093 cm^{-1}) and trans olefinic bond (952 cm^{-1}). The ^1H NMR spectrum displayed signals of one methyl at δ 0.95 (3H, t, J = 7.0 Hz), three methylenes at δ 1.53 (2H, q, J = 7.0Hz) and 2.26 (2H, t, J = 7.0 Hz) and δ 4.18 (2H, dd, J = 2.0, 5.2 Hz), two double-bond protons at δ 5.75 (1H, brd, J = 16Hz) and δ 6.32 (1H, dt, J = 5.2, 16 Hz) that they were trans-isomeric. In the ^{13}C NMR spectrum, there were one methyl (δ 13.4), three methylene (δ 62.7, 21.6, 21.4), two olefinc bond carbons (δ 144.5, 109.2) and four quaternary carbons (δ 84.5, 74.9, 72.8, 65.1) which should be connected (jointed) with oxygen atoms (Table 1, , Fig. 1B).

In the ^1H-^1H COSY spectrum of **1**, there were significant cross-peaks (Table 1), suggesting the presence of structural sets (Fig. 1B). These substituents and structural sets could be put

together by key relative peaks in HMBC (Table 1). In the HMBC experiment, the C-4 at δ 72.8 was correlated with H-3 (δ 5.75) and H-2 (δ 6.32) proved the C-4 at δ 72.8) was attached to C-3 at δ 109.2. In HMBC, the C-5 at δ 74.9 correlated with H-8 (δ 2.26) and H-3 (δ 5.75) proved the C-5 at δ 74.9 was connected with C-7 at δ 84.5 and C-4 at δ 72.8. The C-5 at δ 74.9 was jointed with C-1 at δ 62.7 by one oxygen atom, as a result of the correlation between C-5 (δ 74.9) and H-1 (δ 4.18) in HMBC. The C-6 at δ 65.1 correlated with H-3 (δ 5.75) and H-8 (δ 2.26) in HMBC proved the C-6 at δ 65.1 was connected with C-4 at δ 72.8 and C-7 at δ 84.5. The C-7 at δ 84.5 should be connected with C-8 at δ 21.6, because of the correlation between C-7 (δ 84.5) and H-8 (δ 2.26), as well as H-9 (δ 1.53). Thus, the structure of compound **1** was elucidated (Fig. 1A), named asterbins **A**, which were consistent with the dates in Table 1 (HR-ESIMS, 1D- and 2D-NMR, IR, and unsaturation).

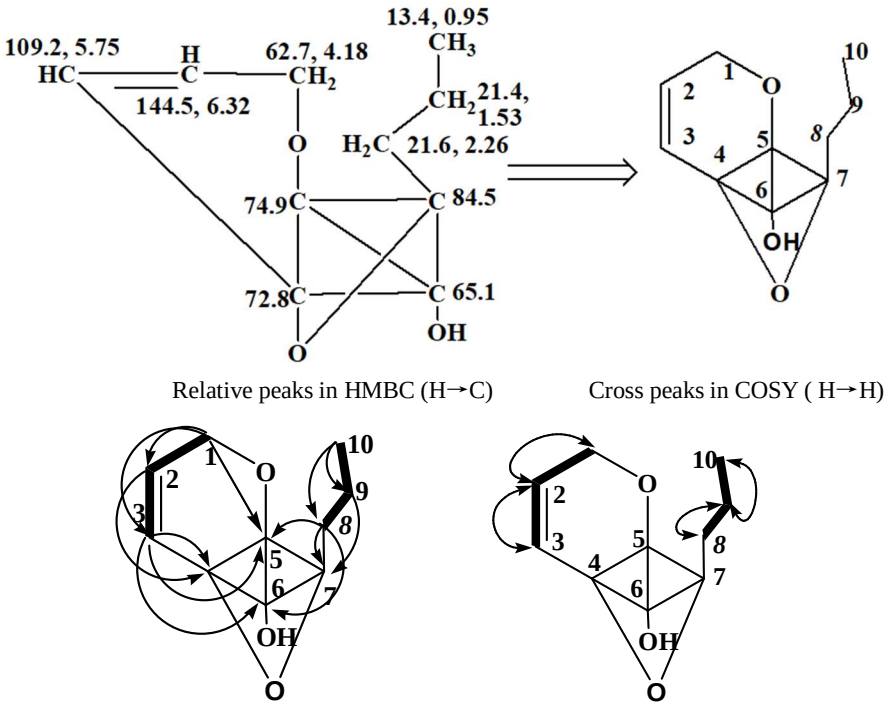

Fig. 1B.

Compound **2** is a colorless gum, $[\alpha]_D^{20}$ + 1° (c 1.0, $CHCl_3$). The molecular formula assigned as $C_{12}H_{14}O_4$, indicated six degrees of unsaturation, on the basis of HR-ESIMS ($[M+Na]^+$ = 245.0783; Calcd. 245.0784), and results from ^1H, ^{13}C NMR and DEPT analysis. The IR spectrum (film) indicated the presences of methyl (2966 cm^{-1}), methylene (2936cm^{-1}, 2877 cm^{-1}), carbonyl (1745 cm^{-1}), an ether bond (1048 cm^{-1}) and trans olefinic bond (952 cm^{-1}). The ^1H NMR spectrum displayed signals of one methyl at δ 0.95 (3H, t, J = 7.0 Hz); three methylene at δ1.53 (2H, q, J = 7.0Hz) and δ2.26 (2H, t, J = 7.0Hz) and δ 4.56 (2H, dd, J = 1.6, 6.0 Hz); two double-bond protons at δ 5.73 (1H, brd, J = 16Hz) and δ 6.21 (1H, dt, J = 6.0, 16 Hz) that they were trans-isomeric. In the ^{13}C NMR spectrum, there were two methyl (δ 20.7, 13.4), three methylene (δ 63.9, 21.6, 21.4), two olefinic bond carbons (δ 138.9, 112.2), an ester carbonyl (δ 170.4) and four quaternary

carbons (δ 84.9, 75.7, 72.2, 64.9), which should be connected (jointed) with oxygen atoms (Table 1).

Table 1. Data of ^{1}H- (400MHz), ^{13}C-NMR (100MHz), ^{1}H-^{1}H COSY and HMBC for compound 1 (δ_{ppm}, J_{Hz}).

No.	δ_H	δ_C	COSY	HMBC
1	4.18, dd (2.0, 5.2)	62.7 t	H-2	H-2, H_a-3
2	6.32, dt (5.2, 16)	144.5 d	H-1, H-3	H-1, H_a-3
3	5.75, brd (16)	109.2 d	H-2	H-1
4	-	72.8 s		H-2, H-3
5	-	74.9 s		H-1, H-3, H-8
6	-	65.1 s		H-3, H-8
7	-	84.5 s		H-8, H-9
8	2.26, t (7.0)	21.6 t	H-9	H-9, H-10
9	1.53, q (7.0)	21.4 t	H-8, H-10	H_a-8, H_b-10
10	0.95, t (7.0)	13.4 q	H-9	H-8, H-9

*Assignments were aided by spin splitting patterns, DEPT, HMQC, HMBC experiments, and chemical shift values (δ). The δ values are in ppm and are referenced to either the residual $CHCl_3$ (7.26 ppm) or $CDCl_3$ (77.2 ppm) signals.

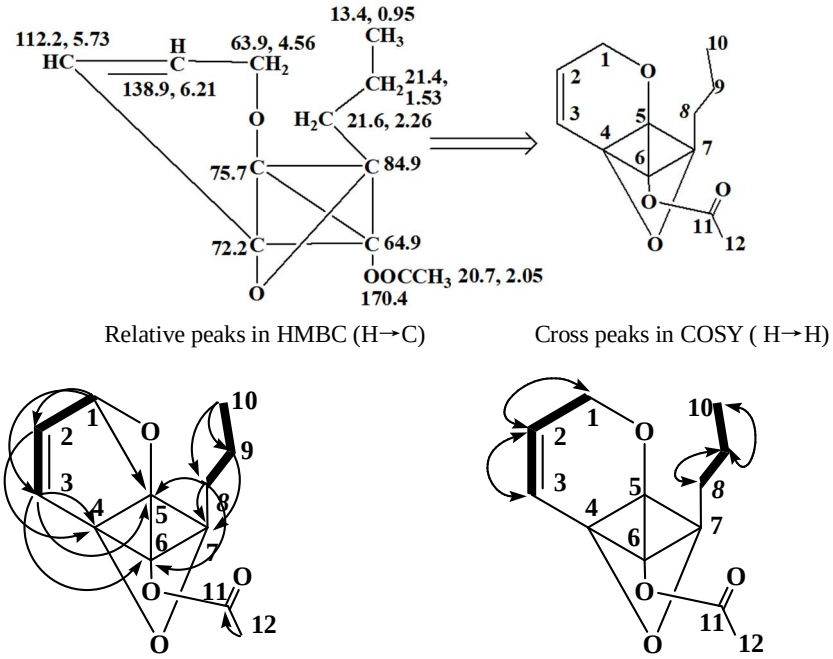

Fig. 2B.

In the ^{1}H-^{1}H COSY spectrum of 2, there were significant cross-peaks (Table 1), suggesting the presence of two structural sets (Fig. 2B). These substituents and structural sets could be put together by key relative peaks in HMBC (Table 1). In the HMBC experiment, the C-4 at δ 72.2 correlated with H-3 (δ 5.73) and H-2 (δ 6.21) proved the C-4 at δ 72.2 was attached to C-3 at

δ 112.2. In HMBC, the C-5 at δ 75.7 correlated with H-8 (δ 2.26) and H-3 (δ 5.73) proved the C-5at δ 75.7 was connected with C-7 at δ 84.9 and C-4 at δ 72.2. The C-5 at δ 75.7 was jointed with C-1 at δ 63.9 by one oxygen atom, as a result of the correlation between C-5 (δ 75.7) and H-1 (δ 4.56) in HMBC. The C-6 at δ 64.9 correlated with H-3 (δ 5.73) and H-8 (δ 2.26) in HMBC proved the C-6 at δ 64.9 was connected with C-4 at δ 72.2 and C-7 at δ 84.9. The C-7 at δ 84.9 should be connected with C-8 at δ 21.6, because of the correlation between C-7 (δ 84.9) and H-8 (δ 2.26), as well as H-9 (δ 1.53). The C-11 at δ 170.4 correlated with H-12 (δ2.05) proved the C-12 at δ 20.7 was attached to C-11at δ 170.4. Assignments of the ^{1}H and ^{13}C signals by 2D NMR revealed that **2** was an analogue of Compound **1** and only more a ester carbonyl sets which, only, should be connected with C-6. Thus, the structure of compound **2** was elucidated as shown in Fig. 2A, named asterbins B, which were consistent with the data (HR-ESIMS, 1D- and 2D-NMR, IR, and unsaturation (Table 2).

Table 2. Data of ^{1}H- (400MHz), ^{13}C-NMR (100MHz), ^{1}H-^{1}H COSY and HMBC for compound 2* (δ_{ppm}, J_{Hz}).

No.	δ_H	δ_C	COSY	HMBC
1	4.56, dd (1.6, 5.0)	63.9 t	H-2	H-2, H_a-3
2	6.21, dt (6.0, 16)	138.9 d	H-1, H-3	H-1, H_a-3
3	5.73, brd (16)	112.2 d	H-2	H-1
4	-	72.2 s		H-2, H-3
5	-	75.7 s		H-1, H-3, H-8
6	-	64.9 s		H-3, H-8
7	-	84.9 s		H-8, H-9
8	2.26, t (7.0)	21.6 t	H-9	H-9, H-10
9	1.53, q (7.0)	21.4 t	H-8, H-10	H_a-8, H_b-10
10	0.95, t (7.0)	13.4 q	H-9	H-8, H-9
11	-	170.4 s		H-12
12	2.05, s	20.7q		

*Assignments were aided by spin splitting patterns, DEPT, HMQC, HMBC experiments, and chemical shift values (δ). The δ values are in ppm and are referenced to either the residual $CHCl_3$ (7.26 ppm) or $CDCl_3$ (77.2 ppm) signals.

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