Astertarone B, a Hydroxy-Triterpenoid Ketone from the Roots of *Aster tataricus* L.

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The structure of astertarone B isolated from the root extract of *Aster tataricus* L. (Compositae) was established to be 4β-hydroxy-4-epishion-21-en-3-one based on spectroscopic methods.

Key words *Aster tataricus*; Compositae; root; triterpenoid ketone; astertarone B

The roots of *Aster* (*A.*) *tataricus* L. (Compositae) are used as the Chinese crude drug Asteris Radix (Japanese name: shion) and are combined in various traditional Chinese prescriptions for use as cough medicines, expectorants and diuretics[1]. Constituents of the root extracts have been investigated, and the presence of a triterpenoid ketone, shionone (shion-21-en-3-one),[2—4] seven triterpenoid glycosides, aster saponins A—G,[5—7] and three monoterpenoid glycosides, shionosides A—C,[7,8] have been reported. Our recent study on the methanol extract of *A. tataricus* roots has led to the isolation and structural elucidation of a new triterpenoid ketone, astertarone A (D: A-friedoeuph-21-en-3-one), along with epishionol (shion-21-en-3β-ol).[9] In this paper, we report the isolation and characterization of yet another new triterpenoid ketone, designated astertarone B (**1**), in addition to spinasterone [(22*E*)-5α-stigmasta-7,22-dien-3-one], from the extract.

Column chromatography of the hexane soluble portion of the methanol extract of *A. tataricus* roots on silica gel afforded a triterpenoid ketone fraction. Crystallization of the ketone fraction from acetone–methanol, followed by preparative HPLC of the filtrate portion, eventually yielded compound **1** and spinasterone. Identification of spinasterone was performed by spectral comparison with an authentic compound.

Compound **1**, with a molecular formula of $C_{30}H_{50}O_2$ determined from its high-resolution mass spectrum (HR-MS) (M^+, *m/z* 442.3836), gave IR absorptions at 3473 (hydroxyl group), 1712 (ketone), and 833 cm^{-1} (trisubstituted double bond). Compound **1** displayed two olefinic methyl singlets at δ 1.61 and 1.69, with an olefinic methine signal at δ 5.10 (br t, J=7.0 Hz), which correspond to one isopropylidene group, and six tertiary methyl singlets at δ 0.82, 0.89, 0.90, 0.93, 1.15, and 1.39 in the ^{1}H-NMR spectrum. These data, in combination with the mass fragmentation observed at *m/z* 359 (loss of C_6H_{11} side-chain; fragment **a** in Fig. 1), suggested that compound **1** possessed a tetracyclic-ring system with all six-membered rings. Four of the six tertiary methyl ^{1}H singlets (δ 0.89, 0.90, 0.93, 1.15) agreed well with those of the methyls H-26, H-28, H-25, and H-27, respectively, which are located at rings B—D, of shionone,[9] indicating that compound **1** has a shionone structure with a hydroxylated ring A. A highly deshielded methyl singlet at δ 1.39, assignable to H-23, attached to a hydroxyl bearing carbon adjacent to a ketonic carbon[10] which, along with the absence of a methyl doublet, suggested that **1** possesses a 4-hydroxyshionone structure. Further diagnostic mass fragmentations at *m/z* 341 (fragment **c** in Fig. 1), 273 (**d**), 263 (**e**), 218 (**f**), and 205 (**g**) supported the proposed structure.[11] From the foregoing, compound **1** was assigned a 4-hydroxy-shion-21-en-3-one structure with a yet-to-be-determined stereochemistry. Analysis of the ^{13}C distortionless enhancement by polarization transfer (DEPT), ^{1}H–^{1}H corelation spectroscopy (COSY), ^{1}H detected multiple quantum coherence (HMQC) and heteronuclear multiple-bond correlation (HMBC) spectra (Table 1), and the ^{13}C- and ^{1}H-NMR spectral comparison of **1** with shionone[9] confirmed the above assumption.

The stereochemistry of compound **1** was established by phase-sensitive nuclear Overhauser and exchange spectroscopy (NOESY) and difference nuclear Overhause effect (NOE) spectroscopy. Compound **1** showed strong NOE correlations between [H-24 (5β-Me)–H-25 (9β-Me)–H-26 (14β-Me)–H-16β, H-18β–H-28 (17β-Me)] and [H-10α–H-8α–H-27 (13α-Me)] (Fig. 2), which were also observed for shionone,[9] demonstrating that **1** possesses the same stereochemistry for the ring-system as shionone. In addition, com-

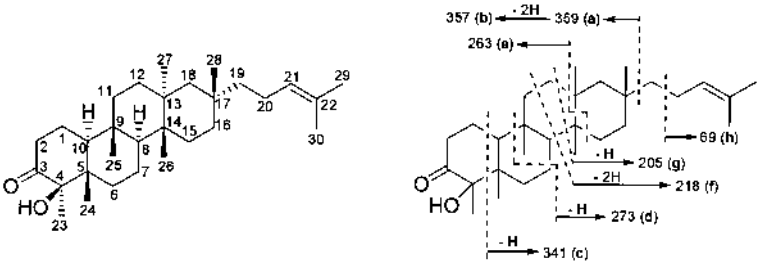

Fig. 1. Structure and Mass Spectral Fragments (*m/z*) of Astertarone B (**1**)

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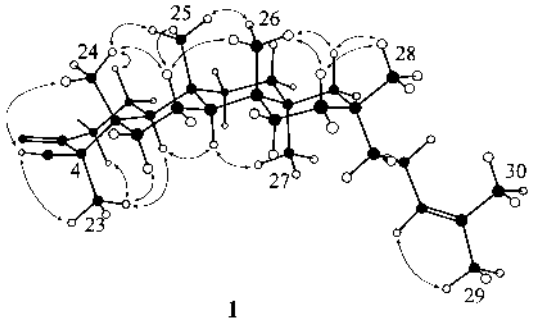

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Fig. 2. Most Stable Conformation and Some Representative NOE Correlations (↔) for Astertarone B (**1**)

Table 1. ^{13}C- (125 MHz) and ^{1}H- (500 MHz) NMR Spectral Data (δ Values; $CDCl_3$) and ^{1}H–^{13}C Long-Range Correlations of Astertarone B (**1**) Obtained by DEPT, ^{1}H–^{1}H COSY, HMQC, and HMBC

C No.	δ_C	δ_H [a)]	Cross peaks in HMBC spectrum
1 CH_2	22.0	1.95 (α), 1.72 (β)	
2 CH_2	35.6	2.55 (α, ddd, 7.3, 12.3, 14.0) 2.45 (β, ddd, 1.4, 4.2, 14.0)	
3 C	215.3		
4 C	81.3		
5 C	45.8		
6 CH_2	33.2	1.63 (α), 1.57 (β)	
7 CH_2	17.2	1.51 (α), 1.33 (β)	
8 CH	50.1	1.31	C-7, C-9, C-14
9 C	38.7		
10 CH	52.9	1.76 (dd, 3.1, 12.8)	C-1, C-5, C-9
11 CH_2	35.9	1.52 (α), 1.39 (β)	
12 CH_2	32.3	0.88 (α), 1.58 (β)	
13 C	36.9		
14 C	38.5		
15 CH_2	29.3	1.35 (α), 1.28 (β)	
16 CH_2	34.6	1.37 (α), 1.59 (β)	
17 C	31.7		
18 CH_2	44.5	1.14 (α, d, 14.3), 1.22 (β; d, 14.0)	
19 CH_2	43.6	1.17, 1.67	
20 CH_2	23.2	1.86, 2.00	
21 CH	125.2	5.10 (br t, 7.0)	
22 C	130.9		
23 Me	21.8	1.39 (s)	C-3, C-4, C-5, C-10
24 Me	14.9	0.82 (s)	C-4, C-5, C-6, C-10
25 Me	19.6	0.93 (s)	C-8, C-9, C-10, C-11
26 Me	15.3	0.89 (s)	C-8, C-13, C-14, C-15
27 Me	20.7	1.15 (s)	C-12, C-13, C-14, C-17, C-18
28 Me	33.0	0.90 (s)	C-16, C-17, C-18
29 Me	25.8	1.69 (s)	C-21, C-22, C-30
30 Me	17.6	1.61 (s)	C-21, C-22, C-29
4 OH		3.81 (s)	

a) Figures in parentheses in the ^{1}H chemical shift column denote *J* values (Hz).

pound **1** exhibited a diagnostic NOE correlation between [H-24 (5β-Me)–OH-4β–H-23 (H-4α)–H-10α], indicating that H-23 at C-4 is oriented to the α-face of the ring-system. From these data, it was concluded that **1** was 4β-hydroxy-4-epishion-21-en-3-one (4β-hydroxy-4-epishionone), which we named astertarone B. The most stable conformation of **1** was simulated using Macro Model. The results of the calculations[12] are shown in Fig. 2, together with the significant NOE's. This conformation of **1** was fairly consistent with the results from the NOE experiment carried out in solution. The assigned ^{13}C- and ^{1}H-NMR spectral data are shown in Table 1.

Astertarone B (**1**) is the first example of a naturally occurring triterpenoid possessing a C-4 hydroxylated shionane skeleton. Spinasterone has previously been identified in tea seed oil.[13]

Experimental

NMR spectra were recorded by a JEOL JNM LA-500 spectrometer at 500 MHz (^{1}H-NMR) and 125 MHz (^{13}C-NMR) in $CDCl_3$ with tetramethylsilane (TMS) (^{1}H-NMR) and $CDCl_3$ at δ 77.0 (^{13}C-NMR) as internal standard and chemical shifts were recorded in δ values. Optical rotation was measured on a JASCO DIP-370 polarimeter at 25 °C in $CHCl_3$. The other instrumental details and the source of the plant material were described previously.[9] Spinasterone[13] was used as a reference compound.

Conformational Analysis 1000-Step systematic Monte Carlo conformation searches were carried out with the MM3* force field, as implemented in MacroModel Ver. 6.0, to predict the fully optimized lowest energy structure.[12] Energies were minimized with the PR conjugate gradient minimizer, and convergence was obtained when the gradient root mean square was less than 0.001 kJ Å^{-1} M. The MM calculations assumed a dielectric constant of 1.0. The MD simulations were carried out with MacroModel beginning with the lowest energy structures obtained by the Monte Carlo conformation search. The following options were used in the MD calculations: time step: 1.5 fs, equilibration time period: 10 ps, and production run time period: 100 ps. Initial kinetic energy was added to all atoms as random velocities. Translational and rotational momentum was reset to zero every 0.1 ps. To maintain a constant temperature, the system was coupled to an external temperature bath set at 300 K. Coupling between bath and molecule was updated every 0.2 ps. In the production run time at 300 K the conformers were sampled every 1 ps, followed by energy minimizations using the MM3* force field. The final MM calculations provided the fully optimized lowest energy structure. Calculations were performed on an SGI O_2 computer.

Isolation Procedures Crystallization of a triterpenoid ketone fraction (1.8 g), separated from the MeOH extract of *A. tataricus* roots (2.5 kg), as described previously,[9] from acetone–MeOH to give crystallized (1.2 g), constituted with shionone, and filtrate portions (360 mg). Preparative HPLC of the filtrate portion yielded astertarone B (**1**, 2.2 mg) and spinasterone (2.4 mg), in addition to previously reported astertarone A and several other triterpenoid ketones.[9] Since the fully assigned ^{13}C- and ^{1}H-NMR spectral data for spinasterone were unavailable in the literature, these also are shown below.

Astertarone B (**1**): Amorphous gum, $[\alpha]_D$ −2.5° (*c*=0.10). IR ν_{max} cm^{-1}: 3473, 1712, 833. MS *m/z*: 442 (M^+, 30), 427 (1), 359 (2), 357 (1), 341 (4), 273 (7), 263 (2), 218 (5), 205 (18), 69 (100). HR-MS *m/z*: 442.3836 [Calcd for $C_{30}H_{50}O_2$ (M^+): 442.3808], 359.2938 (Calcd for $C_{24}H_{39}O_2$: 359.2947), 341.3114 (Calcd for $C_{25}H_{41}$: 341.3206), 273.2571 (Calcd for $C_{20}H_{33}$: 273.2579), 263.1999 (Calcd for $C_{17}H_{27}O_2$: 263.2009), 218.1933 (Calcd for $C_{16}H_{26}$: 218.2032), 205.1836 (Calcd for $C_{15}H_{25}$: 205.1954).

Spinasterone: Colorless needles, mp 173—175 °C. MS *m/z*: 410 (M^+). ^{13}C- and ^{1}H-NMR: C-1 [δ_C 38.8; δ_H 1.47, 2.13 (ddd, *J*=6.1, 14.6, 14.6 Hz)], C-2 [38.1; 2.28 (br d, *J*=15.0 Hz), 2.43 (ddd, *J*=6.1, 14.6, 14.6 Hz)], C-3 (212.0), C-4 [44.3; 2.23 (2H)], C-5 (42.9; 1.81), C-6 [30.1; 1.82 (2H)], C-7 (117.0; 5.18), C-8 (139.5), C-9 (48.9; 1.76), C-10 (34.4), C-11 (21.7; 1.55, 1.75), C-12 (39.3; 1.27, 2.04), C-13 (43.3), C-14 (55.0; 1.83), C-15 (23.0; 1.40, 1.52), C-16 (28.5; 1.29, 1.77), C-17 (55.9; 1.30), C-18 [12.1; 0.58 (s)], C-19 [12.5; 1.02 (s)], C-20 (40.8; 2.05), C-21 [21.4; 1.04 (d, *J*=6.7 Hz)], C-22 [138.1; 5.16 (dd, *J*=8.5, 15.2 Hz)], C-23 [129.6; 5.02 (dd, *J*=8.8, 15.3 Hz)], C-24 (51.3; 1.56), C-25 (31.9; 1.57), C-26 [19.0; 0.80 (d, *J*=6.1 Hz)], C-27 [21.1; 0.85 (d, *J*=6.7 Hz)], C-24^1 (25.4; 1.18, 1.41), C-24^2 [12.3; 0.81 (t, *J*=7.3 Hz)]. The NMR assignments were aided by comparison with literature data of relevant compounds,[14] and by using the results of ^{13}C DEPT, ^{1}H–^{1}H COSY, HMQC, and HMBC spectroscopy.

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