Astertarone A: A Triterpenoid Ketone Isolated from the Roots of *Aster tataricus* L.

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Received July 13, 1998; accepted August 22, 1998

The structure of astertarone A isolated from the root extract of *Aster tataricus* L. (Compositae) was established to be D:A-friedoeuph-21-en-3-one (1) based on spectroscopic methods. This is the first example of a naturally occurring triterpenoid with a D:A-friedoeuphane skeleton. Isolation and identification of epishionol (2), shionone (3), friedelin, epifriedelinol, and ψ-taraxasterol are also described.

Key words *Aster tataricus*; Compositae; roots; triterpenoid ketone; astertarone A

The roots of *Aster 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 extract have been investigated and the presence of a triterpenoid ketone, shionone (shion-21-en-3-one; **3**),[2—4] seven triterpenoid glycosides, aster saponins A–G,[5—7] and three monoterpenoid glycosides, shionosides A–C,[7,8] have been reported. In this paper, we report the isolation and structure elucidation of a new triterpenoid ketone designated astertarone A (**1**) from the methanol extract of *A. tataricus* roots. Isolation and characterization of epishionol (shion-21-en-3β-ol; **2**), a known synthetic triterpene alcohol,[2—4, 9—11] which has not been isolated before as a natural product, is also described.

Column chromatography of the hexane soluble portion of the methanol extract of *A. tataricus* roots on silica gel afforded the triterpenoid ketone and triterpene alcohol fractions. Crystallization of the ketone fraction from acetone–methanol followed by preparative HPLC of the filtrate portion eventually yielded compound **1** in addition to **3**[2—4] and friedelin (D: A-friedoolean-3-one).[12]

Compound **1**, molecular formula $C_{30}H_{50O}$ determined from its high-resolution mass spectrum (HR-MS) ($[M]^+$, *m/z* 426.3848), gave IR absorptions at 1712 (ketone) and 824 cm^{-1} (trisubstituted double bond). Compound **1** displayed four methyl singlets at δ 0.72, 0.79, 0.86, and 0.87, two methyl doublets at δ 0.86 (J=6.5 Hz) and 0.88 (J=6.6 Hz), two olefinic methyl singlets at δ 1.61 and 1.69, with an olefinic methine signal at δ 5.09 (tt, J=1.4, 6.9 Hz) in the ^{1}H-NMR spectrum. These data, in combination with MS fragment ions having *m/z* 313 (base peak) [loss of side-chain (C_8H_{15}) with 2H transfer], 273 [loss of side-chain and ring D (C_3H_6)], and 69 $[CH_2CH{=}C(Me)_2]^+$ (C-20—C-22, and C-29 and C-30),[13] suggested that compound **1** has a C_8 side-chain containing an isopropylidene group, like euphol (eupha-8,24-dien-3β-ol) and tirucallol (tirucalla-8,24-dien-3β-ol),[14] and a tetracyclic triterpenoid skeleton with a keto group most probably located at C-3. Moreover, the presence as a secondary methyl group of one of the five skeletal methyl groups suggested it to possess a D:A-friedoeuphane or tirucallane skeleton. From the foregoing, compound **1** was assigned a D:A-friedoeuph/tirucall-21-en-3-one structure with as-yet-to-be determined stereochemistry. Analysis of the ^{13}C distortionless enhancement by polarization transfer (DEPT), ^{1}H–^{1}H correlation spectroscopy (COSY), ^{1}H detected multiple quantum coherence (HMQC), and heteronuclear multiple-bond correlation (HMBC) spectra, and the ^{13}C- and ^{1}H-NMR spectral comparison of **1** (Table 1) with **3** (see the Experimental section), friedelin,[12] and euphol and tirucallol acetates[14] confirmed the above assumption.

The stereochemistry of compound **1** was established by phase-sensitive nuclear Overhauser and exchange spectroscopy (NOESY). Compound **1** showed significant NOE correlations between [H-4α–H-10α] and [H-23 (4β-Me)–H-24 (5β-Me)–H-25 (9β-Me)–H-7β–H-26 (14β-Me)] (Fig. 1), which were observed also for compound **3** (see the Experimental section), demonstrating that **1** possessed the same stereochemistry as **3** as far as rings A, B and C and the C/D ring junction (13α, 14β) were concerned. In addition, compound **1** exhibited NOE correlations between [H-26 (14β-Me)–H-17–H-28], [H-28–H-16α,β], and [H-16α–H-18–H-27 (13α-Me)] which were consistent with those observed for euphol acetate.[13] From these data, it was concluded that **1** was D:A-friedoeuph-21-en-3-one with a 18*R*-chirality which

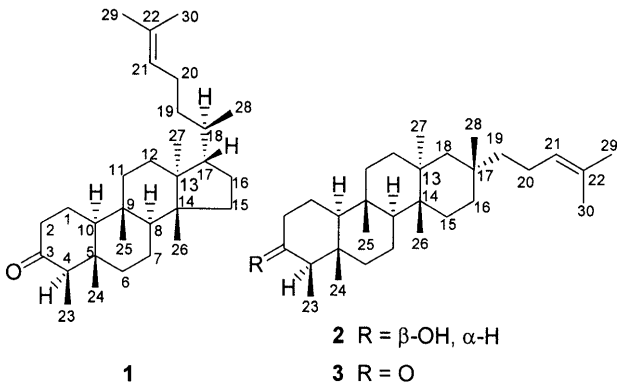

Chart 1. Structures of Triterpenoids from *Aster tataricus* Roots

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

C No.	δ_C		δ_H [a)]	Cross peaks (δ_C) in HMBC spectrum
1	22.8	CH_2	1.96 (α), 1.74 (β)	
2	41.6	CH_2	2.30 (α), 2.39 (β)	
3	213.2	C		
4	58.3	CH	2.28	6.8 (C-23), 14.7 (C-24), 42.4 (C-5), 213.2 (C-3)
5	42.4	C		
6	40.8	CH_2	1.32 (α), 1.72 (β)	
7	20.3	CH_2	1.29 (α), 1.57 (β)	
8	50.0	CH	1.58	16.0 (C-26), 18.5 (C-25), 20.3 (C-7), 37.9 (C-9), 59.1 (C-10)
9	37.9	C		
10	59.1	CH	1.60	18.5 (C-25), 22.8 (C-1), 36.8 (C-11), 41.6 (C-2), 42.4 (C-5)
11	36.8	CH_2	1.43 (2H)	
12	34.0	CH_2	1.22 (α), 1.14 (β)	
13	46.2	C		
14	48.2	C		
15	30.1	CH_2	1.76 (α), 1.56 (β)	
16	28.1	CH_2	1.30 (α), 1.89 (β)	
17	49.8	CH	1.46	19.3 (C-27), 35.3 (C-18), 46.2 (C-13)
18	35.3	CH	1.52	19.0 (C-28), 35.4 (C-19)
19	35.4	CH_2	1.11, 1.61	
20	24.7	CH_2	1.87, 2.04	
21	125.2	CH	5.09 (tt, 1.4, 6.9)	
22	131.0	C		
23	6.8	Me	0.88 (d, 6.6)	14.7 (C-24), 58.3 (C-4), 213.2 (C-3)
24	14.7	Me	0.72 (s)	40.8 (C-6), 42.4 (C-5), 58.3 (C-4), 59.1 (C-10)
25	18.5	Me	0.86 (s)	36.8 (C-11), 37.9 (C-9), 50.0 (C-8), 59.1 (C-10)
26	16.0	Me	0.87 (s)	30.1 (C-15), 42.4 (C-5), 46.2 (C-13), 48.2 (C-14)
27	19.3	Me	0.79 (s)	34.0 (C-12), 46.2 (C-13), 48.2 (C-14), 49.8 (C-17)
28	19.0	Me	0.86 (d, 6.5)	35.3 (C-18), 35.4 (C-19), 49.8 (C-17), 50.0 (C-8)
29	25.8	Me	1.69 (s)	17.7 (C-30), 125.2 (C-21), 131.0 (C-22)
30	17.7	Me	1.61 (s)	25.8 (C-29), 125.2 (C-21), 131.0 (C-22)

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

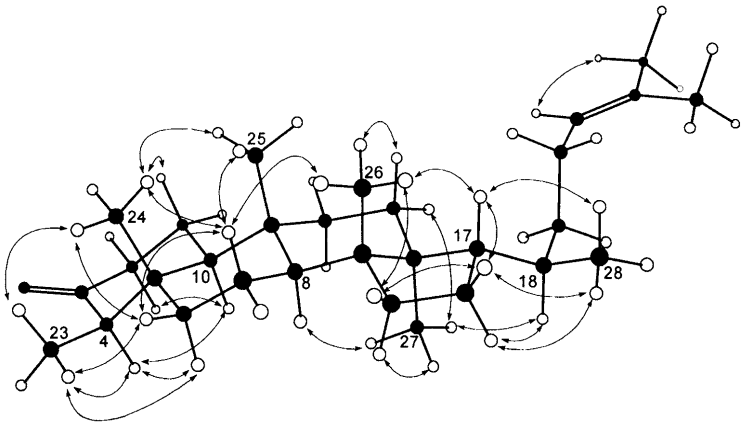

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Fig. 1. Energy-Minimized Conformations and Some Representative NOE Correlations (↔) for Astertarone A (**1**)

we named astertarone A.

The most stable conformation of **1** was simulated using MacroModel. The results of the calculations[15,16] are shown in Fig. 1 together with the significant NOE's (↔). The conformer of simulated **1** shows C-19 of the side chain at C-17 oriented into a "left-handed" conformation (C-19 *trans*-oriented to C-13) similar to that of butyrospermol (eupha-7,24-dien-3β-ol) acetate[17] and the crystal structure of euphol acetate.[18] This was consistent with the NOE experiment done in solution, thus confirming the proposed structure of **1**.

Preparative HPLC of the triterpene alcohol fraction yielded epishionol (**2**),[2—4, 9—11] along with epifriedelinol (D:A-friedoolean-3β-ol)[2,10,11] and ψ-taraxasterol (taraxast-20-en-3β-ol).[19]

Astertarone A (**1**) is the first naturally occurring triterpenoid possessing a D:A-friedoeuphane skeleton. A triterpenoid hydrocarbon possessing a closely related structure with **1**, named aonena-3,21-diene with a (17ξ, 18S)-D:A-friedotirucallane-type skeleton, has been isolated from the rhizomes of *Polypodiodes niponica*.[20] Although epishionol (**2**) has previously been synthesized from shionone (**3**) by reduction,[2—4, 9—11] this is the first report for its detection as a natural product.

Experimental

Crystallizations were performed from acetone–MeOH. Melting points measured were uncorrected. Reverse-phase HPLC was carried out on an octadecyl silica column (Superiorex ODS S-5 μm column, 10 mm i.d.×25 cm; Shiseido Co., Ltd., Tokyo) at 25 °C with MeOH (4 ml/min) as mobile phase. GLC was performed using a DB-17 fused-silica capillary column (30 m×0.3 mm i.d., column temp., 275 °C). In both HPLC and GLC, cholesterol (cholest-5-en-3β-ol) was the standard for the determination of Rt_R of triterpenoids. Electron-impact MS and HR-MS were recorded at 70 eV. NMR spectra were recorded at 400 MHz (^{1}H-NMR) and 100.6 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. IR spectra were recorded in KBr. Instrumental details were the same as described previously.[21] The dried roots of *A. tataricus* were purchased from Tochimoto Co., Ltd. (Osaka). Identification of the plant material was performed by Prof. Norio Sahashi (Department of Biology, School of Pharmaceutical Sciences, Toho University). Friedelin[12] and ψ-taraxasterol[19] were used as reference compounds.

Isolation Procedures Dried and ground *A. tataricus* roots (2.5 kg) were extracted 3× for 3 d with MeOH (6 l each) at room temperature to give an extract (600 g) which was partitioned between EtOAc–H_2O to afford an EtOAc fraction (34.5 g). This extract was partitioned between hexane–MeOH–H_2O (19 : 19 : 2). The hexane fraction (19.9 g) was subjected to column chromatography (CC) on silica gel (600 g) using hexane and a hexane–EtOAc gradient of 50 : 1—1 : 1, which yielded triterpenoid ketone (A) and triterpene alcohol (B) fractions. Repeated CC of the fractions using the same solvent system eventually yielded purified fractions A (1.8 g) and B (0.5 g). Recrystallization of the fraction A from acetone–MeOH gave crystallized (1.2 g), constituted with shionone (**3**), and filtrate portions (360 mg). Preparative HPLC of the filtrate portion yielded astertarone A [**1**; 5 mg; amorphous solid; Rt_R: 1.09 (HPLC), 2.28 (GLC)], **3** [227 mg; mp 162—164 °C (colorless needles) (lit.,[2] mp 158.5—159.5 °C; lit.,[8] mp 155—156 °C); Rt_R: 1.19 (HPLC), 2.54 (GLC)], and friedelin [32 mg; mp 266—269 °C (colorless plates) (lit.,[12] mp 268—269 °C); Rt_R: 1.48 (HPLC), 3.05 (GLC)]. On the other hand, preparative HPLC of the fraction B afforded epishionol [**2**; 10 mg; mp 116—119 °C (colorless needles) (lit.,[2] mp 117.5—118.5 °C); Rt_R: 1.15 (HPLC), 2.32 (GLC)], epifriedelinol [35 mg; mp 275—279 °C (colorless plates) (lit.,[2] mp 279—283 °C; lit.,[8] mp 299 °C); Rt_R: 1.54 (HPLC), 2.76 (GLC)], and ψ-taraxasterol [67 mg; mp 290—292 °C (colorless plates); Rt_R: 1.23 (HPLC), 2.72 (GLC)]. Identification of **2**,[10,11] **3**,[10,11] and epifriedelinol[10,11] was based on spectroscopic (MS and ^{1}H-NMR) comparison with data in the literature, whereas that of friedelin and ψ-taraxasterol was done by chromatographic (HPLC, GLC) and spectroscopic (MS, ^{1}H-NMR) comparison with reference compounds. Since the fully assigned ^{1}H- and ^{13}C-NMR spectral data for **2** and **3** were unavailable in the literature, these also are shown below accompanied by some representative NOE correlations. The NMR assignments were aided by ^{13}C DEPT, ^{1}H–^{1}H COSY, HMQC, HMBC, and phase-sensitive NOESY spectroscopy.

Astertarone A (**1**): IR ν_{max} cm^{-1}: 1712, 824. MS *m/z* (%): 426 (M^+, 13), 411 (21), 341 (19), 313 (100), 273 (3), 245 (3), 218 (14), 203 (7), 191 (10), 189 (6), 69 (97). HR-MS: *m/z* 426.3848 [Calcd for $C_{30}H_{50}O$ (M^+): 426.3859]; 313.2506 [Calcd for $C_{22}H_{33}O$: 313.2528]; 273.2243 [Calcd for $C_{19}H_{29}O$: 273.2217]; 69.0697 [Calcd for C_5H_9: 69.0703]. See Table 1 for the ^{13}C- and ^{1}H-NMR data.

Epishionol (**2**): ^{13}C- and ^{1}H-NMR: C-1 [δ_C 15.8; δ_H 1.46 (α), 1.56 (β)], C-2 [35.2; 1.57 (α), 1.90 (β)], C-3 [72.8; 3.73, dt, J=2.5, 3.1 Hz], C-4 [49.2; 1.25], C-5 [37.9], C-6 [41.7; 0.95 (α), 1.72 (β)], C-7 [17.3; 1.26 (α), 1.37 (β)], C-8 [50.0; 1.22], C-9 [38.2], C-10 [61.1; 0.96], C-11 [35.3; 1.39 (2H)], C-12 [32.5; 0.89 (α), 1.53 (β)], C-13 [36.9], C-14 [38.7], C-15 [29.2; 1.30 (α), 1.24 (β)], C-16 [34.8; 1.33 (α), 1.60 (β)], C-17 [31.8], C-18 [44.7; 1.13 (α), 1.19 (β)], C-19 [43.6; 1.16, 1.67], C-20 [23.6; 1.85, 1.99], C-21 [125.4; 5.10, tt, J=1.5, 7.3 Hz], C-22 [130.7], C-23 [11.6; 0.93, d, J=7.0 Hz], C-24 [16.4; 0.95, s], C-25 [20.0; 0.92, s], C-26 [15.1; 0.87, s], C-27 [20.7; 1.09, s], C-28 [33.0; 0.89, s], C-29 [25.7; 1.68, s], C-30 [17.6; 1.60, s]. Significant NOE correlations observed were between [H-3α–H-4α–H-10α –H-8α –H-27 (13α-Me)], [H-23 (4β-Me)–H-6β–H-24 (5β-Me)], [H-6β–H-7β–H-25 (9β-Me)], and [H-7β–H-26 (14β-Me)–H-18β–H-28 (17β-Me)].

Shionone (**3**): ^{13}C- and ^{1}H-NMR: C-1 [δ_C 22.3; δ_H 1.97 (α), 1.70 (β)], C-2 [41.5; 2.40 (α), 2.30 (β)], C-3 [213.1], C-4 [58.2; 2.25, q, J=6.2 Hz], C-5 [42.2], C-6 [41.2; 1.24 (α), 1.73 (β)], C-7 [17.9; 1.47 (α), 1.35 (β)], C-8 [50.0; 1.32], C-9 [38.5], C-10 [59.7; 1.58], C-11 [35.3; 1.40, 1.52], C-12 [32.3; 0.92 (α), 1.58 (β)], C-13 [36.9], C-14 [38.6], C-15 [29.3; 1.34 (α), 1.27 (β)], C-16 [34.7; 1.36 (α), 1.59 (β)], C-17 [31.7], C-18 [44.6; 1.12 (α), 1.22 (β)], C-19 [43.6; 1.17, 1.67], C-20 [23.2; 1.83, 2.00], C-21 [125.3; 5.10, ttt, J=1.3, 1.5, 7.2 Hz], C-22 [130.8], C-23 [6.8; 0.88, d, J=6.6 Hz], C-24 [14.6; 0.72, s], C-25 [19.6; 0.93, s], C-26 [15.2; 0.90, s], C-27 [20.6; 1.14, s], C-28 [33.0; 0.91, s], C-29 [25.7; 1.69, s], C-30 [17.6; 1.61, s]. The following diagnostic NOE correlations were observed between [H-4α–H-10α–H-8α, H-11α–H-27 (13α-Me)] and [H-23 (4β-Me)–H-24 (5β-Me)–H-25 (9β-Me)–H-7β–H-26 (14β-Me)–H-18β–H-28 (17β-Me)].

Acknowledgements The authors are indebted to Prof. Norio Sahashi (Toho University) for the identification of the plant material.

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