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Constituents of Egyptian *Astragalus tribuloides* Del.

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Abstract – Two soyasaponins were isolated from the aerial parts of *Astragalus tribuloides* Del. Their structures were established on the basis of spectroscopic and chemical methods. In addition, ursolic acid, β-sitosterol β-D-glucoside and isorhamnetin 3-*O*-glucoside were isolated and identified by comparing their mp, spectral and chromatographic data with those of authentic samples. This is the first report of screening and isolation of the chemical constituents of this species of genus *Astragalus*.
Key words – *Astragalus tribuloides*; Leguminosae; aerial parts; soyasapogenol B; azukisaponins II, V.

Introduction

The genus *Astragalus* (Leguminosae) is represented in the Egyptian flora by 37 species, including the annual silvery hairy herbs of *Astragalus tribuloides* Del. (Täckholm, 1974). Some *Astragalus* species have had considerable uses in ethnic medicine, notably in China and India, as antiprespirant, diuretic, tonic, antidiabetic and hypotensive agents (Hikino *et al.*, 1976; Tong and Eisenbrand, 1992). Many *Astragalus* species have shown cytotoxic activities in animals (McCracken *et al.*, 1970), and have been used for treatment of patients with leukemia and uterine cancer (Hartwell, 1970). Recently, we found that some cycloartane triterpene glycosides isolated from *Astragalus* species exhibit at high concentration, *in vitro*, antitumor activity against some human tumor cell lines and HIV antiviral activity (Abdallah *et al.*, 1990, 1993).

In continuation of our work on *Astragalus* species, we report herein the isolation and identification of the chemical constituents of the aerial parts of the Egyptian *Astragalus tribuloides* Del. Still, more research studies are needed to emphasize the pharmacological and biological activities of this plant and to explore its medicinal uses.

Experimental

Astragalus tribuloides Del. used in this study was collected from the flowering and fruiting plants growing in Marsa Matrouh, about 340 km. from Alexandria, Egypt.

The plant was previously identified by the late Prof. V. Täckholm, Faculty of Science, Cairo University. A voucher specimen has been deposited at the Herbarium of the Faculty of Science, University of Alexandria, Alexandria, Egypt.

General. Mp: uncorrected; ^{1}H-and ^{13}C-NMR spectra: 200, 50.2 MHz, samples were dissolved in pyridine-d_5. FAB-MS: samples were dissolved in a glycerol matrix and placed on a steel target prior to bombardment with Ar atoms at energy 7-8 kV. The TLC was carried out on silica gel G 60 plates (Merck); detection was achieved by spraying with anisaldehyde/ H_2SO_4 and heating at 105°C for 5 min. Authentic samples of ursolic acid and β-sitosterol β-D-glucoside were supplied by Merck.

Extraction and Isolation. The air-dried powdered aerial parts of *A. tribuloides* Del. (2 kg) were extracted exhaustively with 95% EtOH at room temp. The EtOH extract was concentrated *in vacuo* (200 mL), added slowly with continuous stirring to water (500 mL), left for 5 hr at room temp., and then filtered from the precipitated resin. The filtrate was partitioned into petrol, Et_2O, $CHCl_3$, EtOAc, and *n*-BuOH, successively. The $CHCl_3$ extract (3 g) was subjected to silica gel column chromatography. Elution was started with $CHCl_3$ to give ursolic acid (60 mg), mp 289-290°C, R_f 0.7 ($CHCl_3$-MeOH, 9:1) and 0.2 (petrol-EtOAc, 7:3), then with mixture of 1%

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MeOH in $CHCl_3$ to give β-sitosterol β-D-glucoside (120 mg), mp 286-288°C, R_f 0.5 ($CHCl_3$-MeOH, 9:1) and 0.2 ($CHCl_3$-MeOH, 20:1). The identity of these two compounds was confirmed by comparing their mp, spectral and TLC data with those of authentic ursolic and β-sitosterol β-D-glucoside. The EtOAc extract (8 g) was subjected to silica gel column chromatography, elution was started with $CHCl_3$ then $CHCl_3$-MeOH mixtures with gradual increase of MeOH contents. Fractions collected at 10% MeOH in $CHCl_3$ mixture were combined and subjected to preparative silica gel G plates, developing solvent $CHCl_3$-MeOH, 8:2 and detected by UV light, to give flavonoid 1. Flavonoid 1 (60 mg) : yellow needles, recrystallized from EtOH, mp 180°C, R_f 0.45 ($CHCl_3$-MeOH, 8:2), 0.62 (EtOAc-HOAc-H_2O, 5:1:1). The UV, ^{1}H-, and ^{13}C-NMR spectral data of flavonoid 1 are comparable with those reported for isorhamnetin 3-*O*-glucoside (Agrawal, 1989; Halim *et al.*, 1995; Mabry *et al.*, 1970).

The *n*-BuOH extract (25 g) was subjected to silica gel column chromatography; elution was started with EtOAc then EtOAc-MeOH mixture with gradual increase of MeOH contents. Fractions collected at 30-35% MeOH in EtOAc mixture were combined (0.3 g) and rechromatographed on silica gel column. Elution with $CHCl_3$- MeOH-H_2O (7:3:1, lower layer) afforded compounds 1 and 2:

Compound **1** (100 mg): colorless needles, recrystallized from MeOH, mp 285-287°C, R_f 0.19 (EtOAc-HOAc-H_2O, 5:1:1) and 0.31 ($CHCl_3$-MeOH-H_2O, 5:5:3). IR ν_{max}^{KBr} cm^{-1} : 3385 (OH), 2945 (CH_2), 3000-2500 (br, COOH), 1730 (O=C-OH), 1600 (C=C) and 1165 (O=C-OH). FAB-MS *m/z* 819 $[M+Na]^+$ corresponding to molecular weight of 796. ^{1}H-NMR of the methyl ester derivative (200 MHz, pyridine-d_5) δ ppm : 0.71, 0.93, 0.99, 1.21, 1.23, 1.30 and 1.39 (each 3H, each s, CH_3), 3.73 (3H, s, O-CH_3), 3.4-4.6 (O-CH), 4.93 (1H, d, J=7.2 Hz, H-1'), 5.28 (1H, br s, H-12) and 5.62 (1H, d, J=7.0 Hz, H-1''). Table 1 shows ^{13}C-NMR data (50.2 MHz, pyridine-d_5) of the methyl ester derivative of compound **1** as compared with those reported for 3-*O*-[β-D-glucopyranosyl (1→2)-β-D-glucuronopyranosyl] soyasapogenol B (azukisaponin II) and the aglycone, soyasapogenol B (Fukunaga *et al.*, 1987).

Compound **2** (120 mg): colorless needles, recrystallized from MeOH, mp 228-229°C, R_f 0.11 (EtOAc-HOAc-H_2O, 5:1:1), and 0.26 ($CHCl_3$-MeOH-H_2O, 5:5:3). IR ν_{max}^{KBr} cm^{-1}: 3386 (OH), 2948 (CH_2), 3000-

Table 1. ^{13}C-NMR data for methyl ester derivatives of compounds (1) and (2), 3-*O*-[β-D-glucopyranosyl (1→2)-β-D-glucuronopyranosyl] soyasapogenol B (3), and soyasapogenol B (4) (Fukunaga *et al.*, 1987); (in pyridine-d_5)

Carbon	(1)	(2)	(3)	(4)
1	38.5	38.0	38.6	39.0
2	26.5	26.4	26.5	28.5
3	90.8	91.8	90.9	80.2
4	43.6	43.8	43.7	43.2
5	56.0	56.3	56.3	56.2
6	18.5	19.1	18.7	19.2
7	33.1	33.3	33.3	33.6
8	39.8	39.9	40.0	40.1
9	47.6	47.8	47.7	48.2
10	36.3	36.5	36.5	37.1
11	23.9	24.1	24.1	24.1
12	122.9	123.2	122.5	122.5
13	144.9	144.9	144.9	144.9
14	42.2	42.4	42.4	42.4
15	26.3	26.4	26.4	26.5
16	28.6	28.7	28.7	28.7
17	37.9	38.0	38.0	38.1
18	45.2	45.4	45.4	45.4
19	46.7	46.8	46.8	46.9
20	30.8	30.9	30.9	30.9
21	42.2	42.4	42.4	42.4
22	75.3	75.5	75.6	75.6
23	22.4	22.8	22.8	23.6
24	63.4	63.3	63.4	64.6
25	15.5	15.7	15.8	16.3
26	16.9	17.1	17.1	17.1
27	25.6	25.7	25.8	25.8
28	28.6	28.7	28.7	28.7
29	33.1	33.3	33.4	33.3
30	21.0	21.2	21.2	21.2
3-O-glu				
1′	104.7	105.3	104.9	
2′	81.1	79.9	81.7	
3′	75.3	76.7	75.7	
4′	72.4	73.5	73.2	
5′	78.3	78.2	78.2	
6′	170.3	170.5	177.2	
O=C-O-CH_3		52.3	52.3	
3-O-glc				
1″	104.7	102.1	104.2	
2″	76.8	79.1	76.3	
3″	79.7	78.2	78.4	
4″	69.7	69.7	70.0	
5″	77.9	78.2	78.2	
6″	61.4	61.3	61.7	
3-O-rhm				
1‴	102.1			
2‴	72.3			
3‴	72.6			
4‴	73.5			
5‴	69.4			
6‴	18.6			

2500 (br, COOH), 1730 (O=C-OH) and 1600 (C=C). FAB-MS of methyl ester derivative, *m/z* 979 $[M+Na]^+$ corresponding to molecular weight of 942. ^{1}H-NMR (200 MHz, pyridine-d_5) of methyl ester derivative, δ ppm: 0.71, 0.95, 1.01, 1.20, 1.25, 1.30 and 1.49 (each 3H, each s, CH_3), 1.80 (3H, d, J=5.8 Hz, CH_3-6'''), 3.73 (3H, s, O-CH_3), 4.97 (1H, d, J=7.2 Hz, H-1'), 5.30 (1H, br s, H-12), 5.84 (1H, d, J=7.0 Hz, H-1'') and 6.37 (1H, s, H-1'''). Table 1 shows ^{13}C-NMR (50.2 MHz, pyridine-d_5) spectral data of the methyl ester derivative of compound **2** as compared with those reported for 3-*O*-[β-D-glucopyranosyl(1→2)-β-D-glucuronopyranosyl] soyasapogenol B (azukisaponin II) and the aglycone, soyasapogenol B (Fukunaga *et al.* 1987).

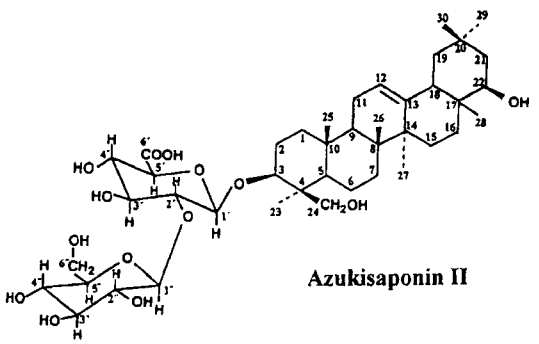
Results and Discussion

In continuation of our interest in the potential activity of *Astragalus* species, we investigated the aerial parts of *A.tribuloides* Del. From the $CHCl_3$ extract of the aerial parts, ursolic acid and β-sitosterol β-D-glucoside were isolated and identified through comparing their mp, spectral and TLC data with those of authentic samples.

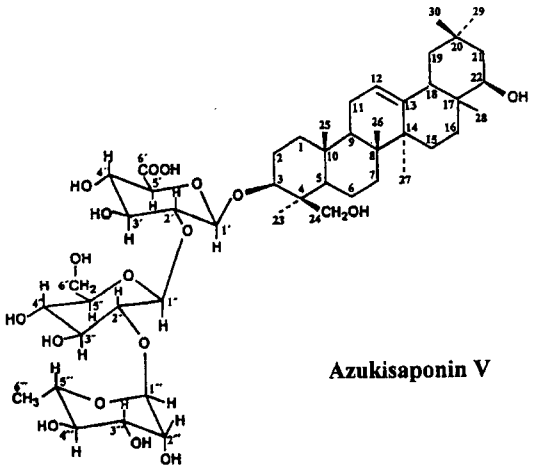
For a long time, these chemical constituents were considered to be pharmacologically inactive. However, ursolic acid was found to be medicinally active both topically and internally. It has anti-inflammatory (Manez *et al.*, 1997; Recio *et al.*, 1995; Ringbom *et al.*, 1998) and antitumor (Ahn *et al.*, 1998; Hsu *et al.*, 1997) activities, and it has the ability to decrease undesirable radiation damage to the hematopoietic tissue after radiotherapy (Hsu *et al.*, 1997). Furthermore, it is an effective inhibitor of tumor promotion in skin (Ohigashi *et al.*, 1986; Tokuda *et al.*, 1986) and inhibitor of HIV protease (Kashiwada *et al.*, 1998; Min *et al.*, 1999; Vlietinck *et al.*, 1998). On the other hand, β-sitosterol β-D-glucoside alone or in combination with similar plant sterols was found to reduce blood levels of cholesterol through blocking of cholesterol absorption (Pelletier *et al.*, 1995; Vanhanen and Miettinen, 1992). It has also been effective at reducing symptoms of prostatic hyperplasia (Berges *et al.*, 1995) and tumor yield of colon (Nair *et al.*, 1984) and prostate (Von Holtz *et al.*, 1998) cancers.

From the EtOAc extract of *A. tribuloides* Del., isorhamnetin-3-*O*-glucoside was isolated and identified on the basis of comparing its mp, UV, ^{1}H, and ^{13}C-NMR spectra and chromatographic pattern with the data given in the literature (Agrawal, 1989; Halim *et al.*, 1995; Mabry, 1970). Isorhamnetin-3-*O*-glucoside was found to decrease platelets adhesiveness activity (Sempinska *et al.*, 1977); the activity which is related to various disease disorders, thus isorhamnetin-3-*O*-glucoside may be of value as a prophylactic agent against these disorders (Biggerstaff *et al.*, 1998; Evers and Grotemeyer, 1999).

The *n*-BuOH extract, after fractionation on silica gel, afforded compound **1** and compound **2.** Compound **1** is found to be 3-*O*-[β-D-glucopyranosyl (1→2)-β-D-glucuronopyranosyl] soyasapogenol B, which is previously isolated from the herb of goats rue (*Galega officinalis* L., Leguminosae) (Fukunaga *et al.*, 1987) and the seeds of *Vigna angularis*, Leguminosae (azuki beans) and it was named azukisaponin II (Kitagawa *et al.*, 1983a). The NMR of the methyl ester of compound **1** identified a pentacyclic triterpenoid glycoside. Its acid hydrolysis gave glucose, glucuronic acid and soyasapogenol B, identified by comparison of the physico-chemical characteristics with those reported in the literature (Fukunaga *et al.*, 1987; Kitagawa, *et al.*, 1983a). The ^{13}C-NMR of methyl ester of compound **2** showed 43 signals, which are comparable to those of the methyl ester of compound **1**, in addition to 6 more carbon signals indicating the presence of a third sugar moiety which was identified as α-L-rhamnopyranose based on TLC data compared with those of authentic sample, and ^{1}H-and ^{13}C-NMR data compared with those previously reported (Kitagawa *et al.*, 1983a). Therefore, the structure of compound **2** was found to be identical to 3-*O*-[α-L-rhamnopyranosyl (1→2)-β-D-glucopyranosyl (1→2)-β-D-glucuronopyranosyl] soyasapogenol B (azukisaponin V), previously isolated from *Vigna angularis* (Kitagawa *et al.*, 1983b) and *Astragalus trigonus* (Pelizzoni *et al.*, 1996; Toaima, S.M., 1996).



Azukisaponin II



Azukisaponin V

Some members of soyasaponins have shown hepatoprotective activities (Ikeda *et al.*, 1998; Kinjo *et al.*, 1998; Sasaki *et al.*, 1998) and hypocholesterolemic effect (Price *et al.*, 1987). They have antiasthmatic activity through activation of the calcium-dependent potassium channels which in turn regulates the tone of the airway smooth muscle and the release of broncho-mediating substances from nerves in the lung (Giangiacomo *et al.*, 1998; McManus *et al.*, 1993). The activation of calcium-dependent potassium channels may have unique clinical use of these natural products as antianginal and antihypertensive drugs. Moreover, some soyasaponins are found to inhibit some stages (virus adsorption) of HIV replication cycle (Vlietinck *et al.*, 1998).

More research studies on the pharmacological and biological activities of *A. tribuloides* Del. plant and its chemical constituents are required to explore its underlying medicinal uses.

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