ORIGINAL ARTICLE

*Rec. Nat. Prod.* 8:4 (2014) 354-359

records of natural products

# A New Ursane Type Sulfated Saponin from *Zygophyllum fabago* Linn.

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*(Received November 21, 2013; Revised January 14, 2014; Accepted February 14, 2014)*

**Abstract:** One new sulfated saponin 3*β*,23,30-trihydroxyurs-20-en-28-al-23-sulfate 3-*O*-*β*-D-xylopyranoside (Zygofaboside C; **1**) was purified from the water soluble fraction of ethanolic extract of the aerial parts of *Zygophyllum fabago* Linn. The structure of the compound was elucidated through spectral studies, especially 1D- and 2D-NMR, HR-FAB mass spectrometry, and comparison with literature data.

**Keywords:** Zygophyllaceae; *Zygophyllum fabago*; Sulfated saponin; Ursane.

## 1. Introduction

Zygophyllaceae is a family of flowering plants including about 22 genera and 285 species. The members of this family are small trees, shrubs, and herbs; often resinous and some are poisonous [1]. Trees are less common and usually the plants of this family require very less quantity of water [2]. The genus *Zygophyllum* is a leading genus in the family Zygophyllaceae. This genus usually contains tropical herbs or shrubs having ill-smelling flower buds. So far six species have been reported in Pakistan for the genus *Zygophyllum* [2]. *Zygophyllum fabago* is commonly known as Syrian bean-caper and is distributed in Pakistan, Iran, Afghanistan, Iraq, Arabia, North Africa, Spain, France, Italy, and Turkey. Many species belonging to the genus *Zygophyllum* have been shown to cure various diseases and are used in folk medicine in several regions of the world. *Z. coccineum* is active against rheumatism, gout, asthma, and hypertension and is also used as diuretic, anthelminthic, and antidiabetic agent [3, 4]. Extracts of *Z. coccineum* displayed significant antihypertensive properties in normotensive as well as in spontaneously hypertensive rats [5]. Compounds isolated from *Z. cornutum* have shown hypocholesterolemic and hypolipidemic activities [6] and those from *Z. simplex* have

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The article was published by Academy of Chemistry of Globe Publications
www.acgpubs.org/RNP © Published 07/03/2014 EISSN:1307-6167

shown antibiotic as well as laxative activities [7]. *Z. gaetulum* is used as anti-eczema, antispasmodic, hypoglycemiant, and in stomach and liver diseases [8]. *Z. album* is used as diuretic, local anaesthetic, antihistaminic, and antidiabetic agent [9]. *Z. fabago* is used as antitussive, expectorant, anti-inflammatory and for removing pains [10]. Aerial parts of the plant, collected at the flowering stages, have been used as anti-rheumatic, anthelminthic, cathartic, and anti-asthmatic [11]. The plant collected from Pakistan has shown a very strong activity against the human pathogen *Candida albicans* [12]. *Z. fabago* extracts may also contain antiviral photosensitizers but the activities have been seen only at high concentrations [13]. In an ongoing search for the bioactive compounds from *Z. fabago*, the EtOH extract of the aerial parts of this plant was selected for investigation and a new sulfated saponin trivially named as Zygofaboside C (**1**) (Figure 1) was isolated from it.

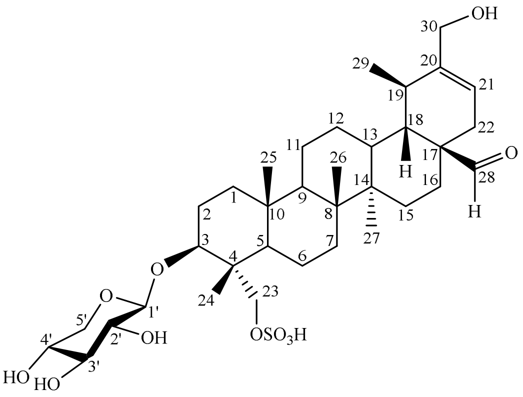

**Figure 1.** Structure of compound **1**

## 2. Materials and Methods

### *2.1. General*

Compound **1** was characterized by extensive 1D- (Bruker Avance-500 and Bruker Avance-AV-500 spectrometers) and 2D-NMR analysis (COSY, HSQC, HMBC, NOESY, Bruker Avance AV-500 and Bruker Avance AV-600 spectrometers), negative HR-FAB-MS (Jeol-JMS-HX-110), and IR (Vector-22) spectrometer. Optical rotations were measured on a Jasco-DIP-360 automatic digital polarimeter. Column chromatography (CC) was carried out on Sephadex LH-20 (25–100 mm; Sigma-Aldrich). MPLC separation was done using an Eyela-VSP-3050 instrument; column (200 mm × 25 mm i.d.; Eyela) filled with Polygroprep C-18 (15–25 mm, 10 nm; Macherey-Nagel). Analytical TLCs were carried out on RP-18 $F_{254s}$ plates (5 × 10 cm, Merck) or RP-18 $F_{254s}$ aluminum sheets (20 × 20 cm, Merck).

### *2.2. Plant Material*

The aerial parts of *Z. fabago* Linn. were collected from Ankara, Turkey in June 2002. The plant was identified by one of us (B. S.). A voucher specimen (GUE # 2312) was deposited in the herbarium of the Faculty of Pharmacy, Gazi University, Ankara, Turkey.

### *2.3. Extraction and Isolation*

The EtOH extract was prepared from aerial parts (12 kg) of *Z. fabago* by maceration. The dark-green residue (450 g) was dissolved in $H_2O$ and partitioned between *n*-Hexane (50 g), EtOAc (100 g), and $H_2O$ (300 g). An amount of 100 g from the $H_2O$ extract was dissolved in water and (25 mL × 4) was subjected to Sephadex LH-20 CC eluting with pure $H_2O$ (2.0 L), then polarity was decreased by the addition of MeOH in steps of 25% (2.0 L each) up to 100% MeOH (2.0 L). The fraction obtained (250 mg) with MeOH/$H_2O$ (1:3) was subjected to reverse-phase MPLC eluting with MeOH/$H_2O$ (1:1) which after repeated chromatography afforded 3$\beta$,23,30-trihydroxyurs-20-en-28-al-23-sulfate 3-*O*-$\beta$-D-xylopyranoside (Zygofaboside C; **1**) as a new natural compound. Purity of the compound was checked on RP-18 TLC (visualization by spraying with cerium (IV) sulfate reagent; followed by heating).

### *2.4. Zygofaboside C (**1**)*

White amorphous powder (10 mg); $[\alpha]_D^{25}$+ 43.2 (*c* = 0.086, $CH_3OH$); IR (KBr) $\nu_{max}$: 3345, 2925, 1710, 1662, 1122 cm$^{-1}$; $^1$H-NMR (500 MHz, $CD_3OD$): aglycone moiety: $\delta$ 9.42 (1H, s, H-28), 5.56 (1H, d, *J* = 6.0 Hz, H-21), 4.09 (1H, d, *J* = 10.0 Hz, $H_A$-23), 3.97 (2H, d, *J* = 13.0 Hz, $H_A$-30 and $H_B$-30), 3.72 (1H, d, *J* = 9.5 Hz, $H_B$-23), 3.65 (1H, dd, *J* = 11.5 Hz and 4.5 Hz, H-3), 2.34 (1H, t, *J* = 6.5 Hz, H-13), 2.16 (1H, m, H-18), 2.08 (2H, ovlp, $H_A$-7 and $H_B$-7), 1.79 (2H, ovlp, $H_A$-16 and $H_B$-16), 1.70 (2H, ovlp, $H_A$-2 and $H_B$-2), 1.69 (2H, ovlp, $H_A$-1 and $H_B$-1), 1.58 (2H, ovlp, $H_A$-11 and $H_B$-11), 1.42 (1H, ovlp, H-19), 1.41 (1H, ovlp, H-5), 1.39 (1H, ovlp, H-9), 1.35 (2H, ovlp, $H_A$-22 and $H_B$-22), 1.33 (2H, ovlp, $H_A$-6 and $H_B$-6), 1.08 (3H, d, *J* = 6.5 Hz, H-29), 1.01 (3H, s, H-27), 0.93 (3H, s, H-26), 0.91 (3H, s, H-25), 0.76 (3H, s, H-24), xylose moiety: $\delta$ 4.48 (1H, d, *J* = 8.0 Hz, H-1'), 3.81 (1H, dd, *J* = 11.5 Hz and 5.5 Hz, $H_A$-5'), 3.45 (1H, m, H-4'), 3.33 (1H, masked, H-3'), 3.29 (1H, dd, *J* = 10.5 Hz and 10.5 Hz, $H_B$-5'), 3.10 (1H, dd, *J* = 8.0 Hz and 8.0 Hz, H-2').$^{13}$C-NMR (125 MHz, $CD_3OD$): aglycone moiety: $\delta$ 39.7 (C-1), 26.5 (C-2), 82.7 (C-3), 43.1 (C-4), 48.3 (C-5), 18.6 (C-6), 36.1 (C-7), 42.1 (C-8), 51.8 (C-9), 37.9 (C-10), 22.6 (C-11), 29.3 (C-12), 33.6 (C-13), 43.4 (C-14), 28.7 (C-15), 30.7 (C-16), 51.9 (C-17), 41.3 (C-18), 48.2 (C-19), 148.1 (C-20), 118.3 (C-21), 34.9 (C-22), 69.5 (C-23), 13.1 (C-24), 17.4 (C-25), 16.5 (C-26), 14.8 (C-27), 209.0 (C-28), 23.8 (C-29), 64.8 (C-30), xylose moiety: $\delta$ 106.2 (C-1'), 75.8 (C-2'), 78.1 (C-3'), 71.3 (C-4'), 66.7 (C-5'); Negative mode HR-FAB-MS: *m/z* 683.3520 $[M - H]^-$ (Calcd. for $C_{35}H_{55}O_{11}S$, 683.3465).

## 3. Results and Discussion

### *3.1. Structure elucidation*

Compound **1** was isolated as a colorless amorphous powder. The HR-FAB-MS (–ve mode) of compound **1** showed the *quasi*-molecular-ion peak at *m/z* 683.3520 $[M–H]^-$ corresponding to a molecular formula of $C_{35}H_{56}O_{11}S$. This formula was also supported by IR and NMR spectra. Acid hydrolysis of **1** followed by treatment with $BaCl_2$ established the presence of a sulfate moiety in **1** [14]. In the IR spectrum of compound **1**, typical absorption bands were found at 3345 (OH groups), 2925 (C–H stretch), 1710 (aldehydic C=O), 1662 (C=C bond), and 1122 cm$^{-1}$ (sulfate stretch). The $^1$H-NMR spectrum of compound **1** exhibited signals for four tertiary methyl groups at $\delta$ 0.76 (s, $H_3$-24), 0.90 (s, $H_3$-25), 0.93 (s, $H_3$-26), and 1.01 (s, $H_3$-27), one secondary methyl group at $\delta$ 1.08 (d, *J* = 6.5 Hz, $H_3$-29), and a hydroxymethylene group at $\delta$ 3.97 (ovlp, $H_{A,B}$-30) indicating a pentacyclic triterpenoid skeleton. The $^1$H-NMR spectrum also displayed one olefinic proton signal at $\delta$ 5.56 (d, *J* = 6.0 Hz, H-21). The $^{13}$C-NMR spectra indicated signals for four tertiary methyls at $\delta$ 13.1, 17.4, 16.5, and 14.8 and one secondary methyl at $\delta$ 23.8, respectively. Accordingly, compound **1** was suggested to contain an urs-20-ene skeleton [15]. Furthermore, the $^{13}$C-NMR spectra displayed one oxygenated methine signal at $\delta$ 82.7 (C-3) and two oxygenated methylene signals at $\delta$ 64.8 (C-30) and 69.5 (C-23). The $^{13}$C-NMR spectra exhibited signals for a total of thirty five carbons, of which thirty were assigned to the aglycone while the remaining five were due to the sugar moiety. From the $^1$H- and $^{13}$C-NMR data and comparison with the literature the basic skeleton of compound **1** was found to be 3$\beta$,23,30-trihydroxyurs-20-en-28-al-23-sulfate [15]. A downfield signal in the $^1$H-NMR spectrum at $\delta$ 9.42 (s) with the corresponding $^{13}$C-NMR signal at $\delta$ 209.0 was assigned to an aldehyde group in the molecule. The point of attachment of aldehyde group was suggested at C-17 from the HMBC interactions of H-28 ($\delta$ 9.42) with C-17 ($\delta$ 51.9), C-18 ($\delta$ 41.3), and C-22 ($\delta$ 34.9) (Figure 2). The H-18 ($\delta$ 2.16) also showed HMBC interactions with C-28 ($\delta$ 209.0). The positions of the sulfate and the alcohol groups in **1** were determined from the chemical shifts of C-3, C-30, and C-23. Hydroxylated C-3 signals resonate near $\delta$ 72.0 and hydroxylated methylenes attached to a six-membered ring resonate near $\delta$ 62.0 [16]. Although, in compound **1** C-3 was observed at $\delta$ 82.7 (10.7 ppm downfield) but this downfield shift was not due to the sulfate group rather it was due to the glycosylation effect as confirmed by HMBC interactions of anomeric proton of the sugar unit with the C-3 of aglycone and vice versa. The C-30 in compound **1** was observed at $\delta$ 64.8 but the C-23 appeared at $\delta$ 69.6 (7.5 ppm downfield) indicating that the sulfate group was attached at C-23. These assignments were further supported by comparison of the $^{13}$C chemical shifts to the related triterpenoids [17, 18]. The attachments of the hydroxyl and the sulfated groups at these positions were also augmented by HMBC spectra. Thus, HMBC interactions were observed between H-21 ($\delta$ 5.56) and C-30 ($\delta$ 64.8). The H-30 ($\delta$ 3.97) in turn also showed HMBC interactions with C-20 ($\delta$ 148.1) and C-21 ($\delta$ 118.3), thus confirming the point of attachment of the hydroxyl group at C-30. Furthermore, H-18 ($\delta$ 2.16) showed HMBC interactions with C-20.

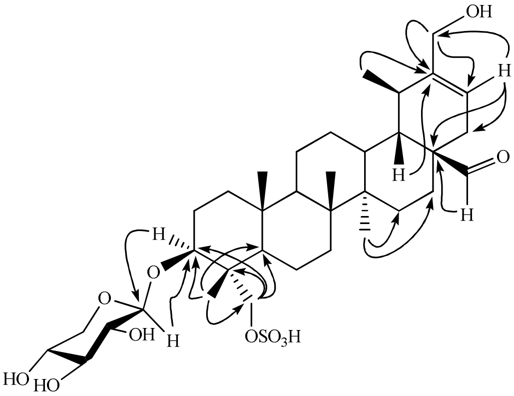

**Figure 2.** Important HMBC interactions of compound **1**

The point of attachment of the OH group at C-30 was further supported by the NOESY interactions (Figure 3) between H-30 ($\delta$ 3.97) and H-21 ($\delta$ 5.56). The point of attachment of the sulfate group at C-23 was also authenticated by the HMBC spectra, where H-24 ($\delta$ 0.76) gave HMBC interactions with C-23 ($\delta$ 69.5). The H-23 ($\delta$ 4.09 and 3.72) showed inverse HMBC correlations with C-3 ($\delta$ 82.7), C-4 ($\delta$ 43.1), and C-5 ($\delta$ 48.3), thus establishing the presence of a sulfate moiety at C-23. Based on the above facts and comparison of the chemical shift data of C-23 ($\delta$ 69.5) and C-24 ($\delta$ 13.1) with those of hydroxylated C-23 and non-hydroxylated C-24 [19-21] as well as hydroxylated C-24 and non-hydroxylated C-23 in the literature [19, 22-25] it was established that the sulfate group was attached to C-23 of the aglycone. The OH group at C-3 of aglycone was disposed $\beta$ and equatorial on the basis of coupling constant of H-3 (dd, $J$ = 11.5, 4.5 Hz). This point of attachment and $\beta$ disposition was also supported by the cross peaks in NOESY plot. Thus, $CH_3$-24 ($\delta$ 0.76) showed NOESY interactions with $CH_3$-25 ($\delta$ 0.91) while the $CH_2$-23 ($\delta$ 4.09 and 3.72) showed interactions with H-3 ($\delta$ 3.66).

The anomeric proton signal of the sugar moiety was observed at $\delta$ 4.48 (d, $J$ = 8.0 Hz). The spin constitution of the sugar moiety was observed from the $^1$H–$^1$H-COSY correlations between the sugar protons. For this purpose, the anomeric proton signal was selected as a starting point. From the coupling constant data the anomeric configuration of the sugar unit was found to be $\beta$-xylose. The absolute configuration of the sugar unit was determined as $\beta$-D-xylose after acid hydrolysis of compound **1** followed by finding the optical rotation of the sugar moiety. The point of attachment of the xylopyranoside moiety to the aglycone was determined from the chemical shift data of the glycosylated position as well as from the HMBC interactions. The C-3 of the aglycone was shifted downfield to $\delta$ 82.6 due to glycosylation. The point of attachment of the sugar unit at this position was also supported by the HMBC interactions of the anomeric proton of the sugar unit to the C-3 of aglycone. Thus, the proton signal at $\delta$ 4.48 (H-1′) showed HMBC correlations with the carbon signal at $\delta$ 82.6 (C-3). The H-3 of aglycone ($\delta$ 3.66), in turn, also showed HMBC interactions with the anomeric carbon signal at $\delta$ 106.2. This connectivity was also supported by NOESY interactions between anomeric proton of the sugar unit ($\delta$ 4.48) and proton of the aglycone at the glycosylated carbon ($\delta$ 3.66). On the basis of above observations the structure of compound **1** was established as

3*β*,23,30-trihydroxyurs-20-en-28-al-23-sulfate 3-*O*-*β*-D-xylopyranoside (trivially named as Zygofaboside C) which is a new compound.

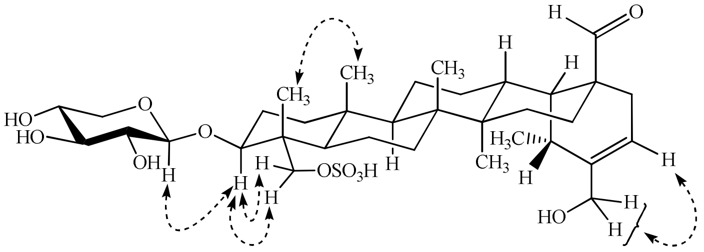

**Figure 3.** Important NOESY interactions of compound **1**

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