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Isolation of Saponin from Dried Roots of *Gypsophila simonii* Hub. Mor

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Abstract: In the present research, a saponin was isolated from the roots of *Gypsophila simoni.* The structure was characterized by means of ^{1}H NMR, ^{13}C NMR, FTIR and EIMS. The findings indicate that the proposed structure of that saponin was as a new Gypsogenin ester (C_{31} H_{51} O_3).

Key words: *Gypsophila simonii*, isolation, saponin structure

INTRODUCTION

Saponins (a group of phytoanticipins) are present constitutively in plants and play important roles in plant defense (Figen, 2006). Some saponins of *Gypsophila* species are broadly used as a drug with extensive medical importance such as an expectorant and diuretic (Ansari and Has, 1989) and are used in treatments of hepatitis, gastritis and bronchitis (Mizutani *et al*., 1984). A number of saponins were isolated from various *Gypsophila* species and investigated by many researchers (Henry *et al*., 1991; Frechet *et al*., 1991; Yaylı *et al*., 1998; Acebes *et al*., 1998; Bernadete and Parante, 2004).

Gypsophila simonii, local name Çöven, is an endemic growing plant at Çankırı province in Turkey (Davis, 1982). There is no study conducted on the gypsogenin ester saponins of this plant to date. In this study, we aim to present the isolation and structural elucidation of new unfamiliar saponin from the root of *Gypsophila simonii*.

MATERIALS AND METHODS

General: Chemical materials that are used in the experiments are in analytical grade. Infrared spectra of the compounds were recorded between 4000 and 400 cm^{-1} on Mattson 1000 FT-IR spectrometers which where calibrated using polystyrene bands. The resolution of IR spectrometers is 2 cm^{-1} and the number of scan is 20. The samples were prepared as a KBr disc.

The melting point was determined in a glass capillary tube. Mass spectrum was recorded on an electron impact mass spectrometer from Research Institute of Tübitak (Turkey). ^{1}H NMR (400 MHZ, DMSO-d_6) and ^{13}C NMR (100 MHZ, DMSO-d_6) were explored in Middle East Technical University (METU).

Plant material: Çöven (*Gypsophila simonii*) was collected around Çankırı province, Türkiye, in June 1997 and identified by Professor Zeki Aytaç, Department of Biology, Gazi University. The root material was dried in a cool dark place and powdered in the Faculty of Pharmacy of Gazi University.

Isolation and extraction: Collected plant's roots were removed and dried. Approximately 3.5 kg of dried materials were placed into a cartridge and then extracted with chloroform in Soxhlet apparatus for 24 h. The cartridge was re-extracted with ethanol for extra 8 h and dried completely at the room temperature (Baytop, 1991).

The extracts containing saponins were evaporated by using a rotary evaporator (Biby, Oklahoma) at 40 rpm, without solvent at the reduced pressure. The dried extracts were dissolved in ethanol and applied on thin-layer chromatograms (TLC) (20×20, silica gel G_{60} Art.7731) in the solvent system (1-Bu OH : 1-PrOH : HAc : H_2O) (40: 20:7,5:30). Spots on TLC were detected by spraying with 10% H_2SO_4 followed by heating at 110°C for 5-10 min. Spraying was done in order to identify the points of the separated compounds (Segal *et al*., 1978).

Acid hydrolysis of the saponins: Each of the separated saponins (*Gypsogenin ester*) was refluxed for 7 h with 5% HCl (Okawa *et al*., 2002). Third spot (R_f = 0.28) was run up for isolation and identification.

Sugar components were identified on Paper Chromatograms. The sugar in filtrate was identified as D-Glucose by comparison on PC (ethyl-acetate; pyridine; water, 12; 5; 4) with on authentic sugar (Boders, 1972).

RESULTS AND DISCUSSION

The ethanolic extract obtained from the dried roots of Çöven (*Gypsophila simonii*) was purified on TLC

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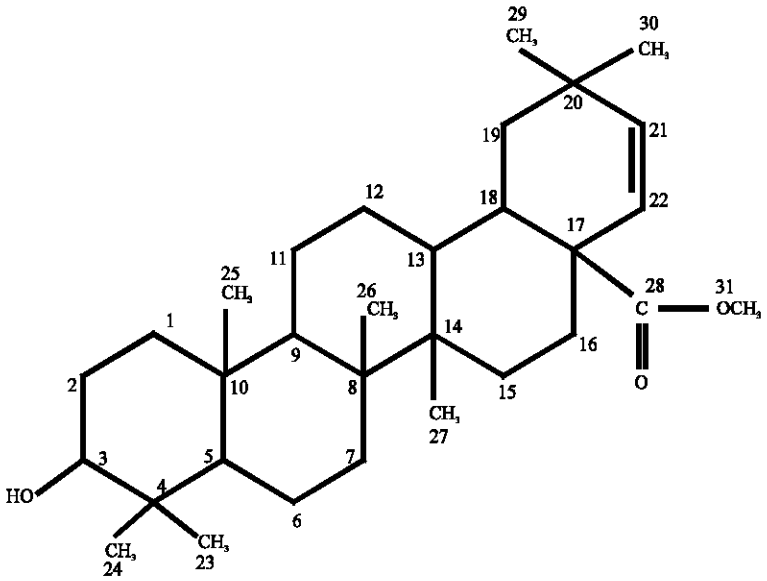

Fig. 1: Gypsogenin ester saponin

Table 1: ^{13}C and ^{1}H NMR data (δ ppm), DMSO-d_6

^{13}C δ (ppm)	^{1}H δ (ppm)
37.440	1.60/1.62
26.208	2.33/2.02
79.047	4.17
37.440	-
45.392	1.49
20.107	2.11/1.85
30.454	0.98
36.450	-
45.602	1.73
45.392	-
25.337	1.93/1.86
37.440	1.14
45.000	1.49
45.392	-
36.100	1.86-1.45
73.481	2.11-1.85
48.581	-
30.440	3.06
34.450	1.75/1.23
30.000	-
137.244	5.44
133.533	5.57
25.300	1.93
20.100	1.50
19.500	0.93
20.250	0.99
25.450	1.28
168.000	-
35.450	0.98
25.337	0.93
69.648	1.70

(R_f = 0.28). Structure of the isolated Gypsogenin ester was characterized by ^{1}H NMR, ^{13}C NMR, IR and EIMS. The results were compared with the similar studies (Malaviya *et al*., 1991; Zhao *et al*., 1990; Delay *et al*., 1997; Zhao *et al*., 1997). It was concluded that the structure of Gypsogenin ester may have the form presented in Fig. 1.

Table 2: FTIR spectral data (KBr, cm^{-1})

Wave numbers (cm^{-1})	Assignment
3400	-OH stretching
2967-2877	Aliphatic C-H stretching
1714	C = O stretching (for ester)
1656-1618	C = C stretching
1450	C-H bending
1190	-OCH_3 stretching

Data of IR spectrum (KBr, cm^{-1}) exhibited absorptions at 3400 (-OH), 1656-1618 (C = C), 2967-2877 aliphatic C-H stretching and an ester (1714 carbonyl stretching (-C (O)-OH), 1190 asymmetric stretching).

The proton nuclear magnetic resonance, ^{1}H NMR (400 MHZ, DMSO-d_6 δ ppm), IR spectral data of compound (Fig. 1) showed the doubled vinylic proton [6.50-7.00].

The carbon-13 nuclear magnetic resonance, ^{13}C NMR (400 MHZ, DMSO-d_6 δ ppm), spectral of compound (Fig. 1) and its derivatives are shown in Table 1.

Gypsogenin ester saponin (Fig. 1); mp: 235°C (uncorrupted); IR spectrum of studied compound (Fig. 1); and its derivatives are shown in Table 2. Molecular ion peak was also observed at EIMS; m/z, [M^+]: 472. All these results confirm that the proposed structure of that saponin appears as a new one and is called as Gypsogenin ester ($C_{31}H_{51}O_3$).

PC results showed the presence of D-Glucose by comparing their retention times with those of authentic sugar (R_G = 1.00, mp = 204°C).

CONCLUSIONS

In recent years, although technology and medicine have developed extensively due to the decrease in natural richness and other drawbacks, some countries have made it obligatory to use natural products for many goals (Ertürk *et al*., 2003). For these reason *Gypsophila* species are used for the treatment of various diseases.

The above studies the saponins were extracted from *Gypsophila simonii* (Çöven) dried roots and then separated its components by thin layer chromatography. NMR, FTIR and EIMS were carried out to investigate unknown saponin presents in this plant. The comparision of this investigation results with similar studies shows that the proposed structure of that saponin is a new type and could be named as Gypsogenin ester (C_{31} H_{51} O_3). However little is known about sugar presence and sugar linkage pattern in saponins of *Gypsophila simonii*. Paper chromotograms showed that, in *Gypsophila simonii* (Çöven), D-Glucose was identified by comparison with on authentic sugar.

In conclusion, the studies on the saponins highlight the necessity for a comprehensive detailed investigation on the other gypsogenins saponin types which have a broad medical and industrial potential.

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