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Triterpenic Saponins from *Rubus sanctus* Schreber

Rubus sanctus Schreber' in Triterpenik Saponinleri

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Abstract

Six triterpenic saponins were isolated from *Rubus sanctus* Schreber known as ''Böğürtlen'' in Turkey. The isolation of 2β,3α,19α,23-tetrahydroxyurs-12-en-28-oic acid (I), pinfaensin (II), 2β,3α,19α,23-tetrahydroxyurs-12-en-28-oic acid 28-O-β-D-glucopyranosyl ester (III), tormentic acid glucosyl ester (IV), 2β,3α,19α-trihydroxyurs-12-en-28-oic acid 28-O-β-D-glucopyranosyl ester (V) and suavissimoside R_1 (VI) were achieved for the first time from *Rubus sanctus* in this study. Besides, these compounds were discussed chemotaxonomically.

Key words: Rosaceae, *Rubus sanctus*, triterpenic saponins, chemotaxonomy.

Introduction

Genus *Rubus* L. (Rosaceae) is represented by 17 species and 8 hybrids in Turkey (Davis,1972). *Rubus* species are mostly known as ''Böğürtlen'' in Turkish. *Rubus* species are used for treatment of different diseases. Among these species, the roots and leaves of *Rubus sanctus* Schreber are used to heal wounds, and abcess, to stop bleeding, to throw out renal calculi to treat breast cancer, hemorrhoids, eczema, stomach pain and bloating, and for sore throat. They also have hypoglycemic, antirheumatic, antidiarrheal and antidiuretic activities (Baytop,1984; Honda *et al.*,1996; Yeşilada *et al.*, 1993,1999).

In our previous studies, anti-inflammatory(Akcoş *et al.*,1998), antimicrobial (Akcoş *et al.*,1999), wound healing and gastroprotective (Batu *et al.*,1998) activities of different extracts of some *Rubus* species including *R. sanctus* were investigated. One flavonol aglycone and four flavonol glycosides were isolated from aerial parts of *R. sanctus*. (Ezer *et al.*,2001).

In this study, from the chloroform fraction which was obtained from the methanol extract of *R. sanctus*, a triterpenic saponin aglycon; 19α-hydroxyursolic acid derivative, five triterpenic saponins 28-*O*-β-D-glucosyl ester of 19α-hydroxyursolic acid derivatives from the ethyl acetate and n-butanol fraction were obtained. Structure elucidation of these compounds were established by spectroscopic methods

(UV, IR, ^{1}H-NMR, ^{13}C-NMR, APT, DEPT) and triterpenic saponins were confirmed as 2β,3α,19α,23-tetrahydroxyurs-12-en-28-oic acid 28-*O*-β-D-glucopyranosyl ester and its aglycone; 2β,3α,19α-trihydroxyurs-12-en-28-oic acid 28-*O*-β-D-glucopyranosyl ester, pinfaensin, tormentic acid glucosyl ester, suavissimoside R_1.

Material and Methods

General Procedure

For melting point determination, Buchi apparey was used. UV (λ_{max}) and IR (cm^{-1}) spectra were recorded by Schimadzu UV-160 A and Perkin-Elmer 257 spectrometers respectively. ^{1}H-NMR and ^{13}C-NMR spectra were recorded byBruker WM 300 spectrometer.

Kieselgel 60 (0.063-0.2 mm Merck), and Sephadex LH-20 were used for column chromatography, Sepralyte C-18 for vacuum-liquid chromatography, silica gel 60 F_{254} (0.2 mm Merck) commercial plates for thin layer chromatography. Compounds I-VI were detected by UV fluorescence and spraying vanilin/H_2SO_4 1% and 30%.

Plant Material

The aerial parts of *Rubus sanctus* Schreber were collected during flowering time, in July 1994, from Bolu, Akçakoca in Turkey. The voucher species are deposited in the herbarium of the Faculty of Pharmacy, Hacettepe University, Ankara (HUEF-94097).

Extraction and Isolation

The air-dried and powdered aerial parts of the plant (375 g) were extracted with methanol (3 x 3 l) and the combined extracts were evaporated under vacuum to dryness. The residue was dissolved in water and water soluble part was extracted with petroleum ether, chloroform, ethyl acetate and n-butanol, successively.

The chloroform extract was evaporated to dryness with a yield of 6.37 g and was chromatohraphed over silica gel eluting with petroleum ether:EtOAc (20:80) solvent system. The fraction including compound I was rechromatographed over silica gel eluting with CH_2Cl_2:MeOH (90:10) and EtOAc:MeOH (99:1). Compound I (76 mg) was isolated.

The ethyl acetate extract (12 g) was chromatographed over silica gel eluting with $CHCl_3$:MeOH (95:5) and $CHCl_3$:MeOH:H_2O (70:30:3) solvent systems. The fraction including compound II was rechromatographed by using silica gel column and CH_2Cl_2:MeOH (90:10) solvent system and compound II (48 mg) was isolated. The fraction including compound III was applied on silica gel column with $CHCl_3$:MeOH (80:20) solvent system and compound III yielded 40 mg. And the last fraction of the ethyl acetate extract was applied to Sephadex LH-20 column with MeOH and compound IV (65 mg) was isolated.

n-Butanol extract was chromatographed by using silica gel column with $CHCl_3$:MeOH:H_2O (80:20:2). The fraction including compound V was rechromatohraphed on silica gel column with $CHCl_3$:MeOH:H_2O (75:25:2.5) solvent system then purified by vacuum-liquid chromatography on Sepralyte C-18 with MeOH:H_2O 5-30% solvent system. Compound V (60 mg) was obtained. The fraction including compound VI was purified with silica gel column by using $CHCl_3$:MeOH:H_2O (70:30:3) solvent system and compound VI (23 mg) was yielded.

2β,3α,19α,23-tetrahydroxyurs-12-en-28-oic acid (I) mp: 283°C, UV λ_{max} (nm): 211.0 (MeOH), IR (1% KBr) (cm^{-1}): 3567 (O-H), 2923, 2851 (C-H), 1734 (C=O), 1029 (C-O-C). ^{1}H-NMR (CD_3OD, 300 MHz) Table 1 and ^{13}C-NMR (CD_3OD, 50.3 MHz) Table 2.

Table 1 ^{1}H-NMR Spectral Data of Triterpenic Saponins (CD_3OD, 300 MHz)

H Atom	I δ (ppm) *J* (Hz)	II δ (ppm) *J* (Hz)	III δ (ppm) *J* (Hz)	IV δ (ppm) *J* (Hz)	V δ (ppm) *J* (Hz)	VI δ (ppm) *J* (Hz)
Aglycon						
2	3.96 ddd (14.0/10.2/1.4)	3.86 ddd (12.3/9.6/4.0)	3.97 ddd (15.9/11.1/1.5)	3.85 ddd (11.1/9.0/4.2)	3.96 ddd (15.0/9.0/1.8)	3.95 ddd (10.8/9.6/4.9)
3	3.71 d (10.2)	3.10 (9.6)	3.69 d (11.1)	2.94 d (9.0)	3.80 d (9.0)	3.38 d (9.6)
12	5.04 m	5.03 br.s	5.08 br.s	5.02 m	5.02 m	5.02 br.s
18	3.00 s	2.70 s	2.70 s	2.70 s	2.71 s	2.70 s
23		10.05 s		1.48 s		-
23a	3.84 d (11.2)		3.86 d (11.4)		1.30 s	
23b	3.84 d (-)		3.91 d (11.4)			
24	1.05 s	0.96 s	0.89 s	1.00 s	0.95 s	0.99 s
25	1.07 s	1.12 s	1.11 s	1.13 s	1.22 s	1.13 s
26	1.11 s	1.20 s	1.22 s	1.20 s	1.22 s	1.23 s
27	1.40 s	1.39 s	1.39 s	1.39 s	1.39 s	1.39 s
29	1.63 s	1.52 s	1.56 s	1.53 s	1.53 s	1.53 s
30	1.05 d (6.0)	0.99 d (6.0)	0.97 d (6.3)	1.12 d (6.0)	1.12 d (6.0)	1.10 d (6.0)
β-D-Glucose						
1'	-	5.50 d (7.8)	5.51 d (7.8)	5.51 d (7.8) -	5.51 d (7.8)	5.52 d (7.9)
2'	-	3.57 dd (9.3/7.8)	3.54 dd (9.6/7.8)	-	3.57 d (9.7/-)	3.57 dd (9.3/8.1)
3'	-	3.45-3.60 =	3.42-3.60=	3.45-3.60 =	3.45-3.64 =	3.38-3.62=
4'	-	3.45-3.60 =	3.42-3.60=	3.45-3.60 =	3.45-3.64 =	3.38-3.62=
5'	-	3.51 m	3.50 m	-	3.51 m	3.50 m
6'A	-	3.84 dd (11.7/2.1)	3.85 dd (11.7/2.0)	3.86 dd (11.1/2.4)	3.81 dd (12.4/2.4)	3.85 dd (11.7/3.9)
6'B	-	3.88 dd (-/4.6)	3.88 dd (-/4.0)	3.88 dd (11.4/4.2)	3.83 dd (12.7/4.5)	3.88 dd (11.7/4.2)

Table 2 ^{13}C-NMR Spectral Data of Triterpenic Saponins (CD_3OD, 50.3 MHz)

C Atom	I δ (ppm)	II δ (ppm)	III δ (ppm)	IV δ (ppm)	V δ (ppm)	VI δ (ppm)
Aglycon						
1	47.90 d	48.14 d	47.73 t	48.57 t	48.14 t	48.14 t
2	68.84 d	69.60 d	69.73 d	69.42 d	69.35 d	70.57 d
3	78.27 d	84.63 d	78.60 d	84.55 d	81.04 d	79.09 d
4	43.56 s	48.71 s	44.12 s	42.83 s	42.58 s	65.16 s
5	49.59 d	56.75 d	50.29 d	57.80 d	52.63 d	56.73 d
6	18.61 t	19.69 t	19.27 t	21.56 t	21.75 t	21.59 t
7	33.13 t	34.15 t	33.56 t	34.40 t	33.58 t	34.14 t
8	40.40 s	40.47 s	41.29 s	39.80 s	39.12 s	39.28 s
9	47.70 d	45.11 d	45.10 d	47.72 d	47.71 d	47.84 d
10	38.32 s	39.25 s	39.02 s	39.62 s	38.15 s	38.24 s
11	24.09 t	24.80 t	24.75 t	25.00 t	25.00 t	25.10 t
12	127.90 d	129.60 d	129.53 d	129.66 d	129.28 d	129.95 d
13	139.93 s	139.68 s	139.76 s	139.89 s	139.57 s	139.64 s
14	42.11 s	41.37 s	42.81 s	41.23 s	41.22 s	41.27 s
15	29.90 t	29.65 t	29.65 t	29.65 t	29.50 t	29.64 t
16	26.32 t	27.22 t	26.56 t	26.60 t	26.43 t	26.72 t
17	48.22 s	48.43 s	48.16 s	48.15 s	50.26 s	48.43 s
18	54.53 d	55.00 d	54.99 d	55.06 d	54.82 d	55.11 d
19	72.65 s	73.71 s	73.67 s	73.63 s	73.62 s	73.70 s
20	42.29 d	42.92 d	42.95 d	42.94 d	42.80 d	42.92 d
21	29.20 t	26.60 t	27.23 t	27.25 t	26.50 t	27.24 t
22	38.32 t	38.27 t	38.32 t	38.35 t	38.15 t	38.24 t
23	66.55 t	208.51 d	66.58 t	25.57 q	29.50 q	181.84 s
24	14.25 q	16.52 q	13.85 q	25.57 q	12.76 q	16.23 q
25	17.31 q	17.12 q	17.71 q	15.65 q	16.52 q	16.53 q
26	17.25 q	17.72 q	17.61 q	16.57 q	16.52 q	16.53 q
27	24.60 q	24.67 q	25.04 q	24.53 q	24.66 q	24.57 q
28	180.60 s	178.54 s	178.57 s	178.55 s	178.51 s	178.64 s
29	27.02 q	27.13 q	27.13 q	27.09 q	27.10 q	27.15 q
30	16.70 q	17.39 q	16.59 q	17.57 q	17.46 q	17.63 q
***β*-D-Glucose**						
1'	-	95.83 d	95.81 d	95.80 d	95.75 d	95.87 d
2'	-	73.95 d	73.90 d	73.90 d	73.84 d	73.98 d
3'	-	78.39 d	78.35 d	78.33 d	78.43 d	78.34 d
4'	-	71.30 d	71.20 d	71.19 d	71.12 d	71.34 d
5'	-	78.56 d	78.59 d	78.57 d	78.51 d	78.52 d
6'	-	62.61 t	62.50 t	62.50 t	62.49 t	62.63 t

23-formyl-2α,3β,19α-trihydroxyurs-12-en-28-oic acid 28-*O*-β-D-glucopyranosyl ester (Pinfaensin) (**II**) Mp: 224°C, UV λ_{max} (nm): 206.4 (MeOH), IR (1% KBr) (cm^{-1}): 3402 (O-H), 2926, 2880 (C-H), 1734, 1720 (C=O), 1072 (C-O-C). ^{1}H-NMR (CD_3OD, 300 MHz) Table 1 and ^{13}C-NMR (CD_3OD, 50.3 MHz) Table 2.

2β,3α,19α,23-tetrahydroxyurs-12-en-28-oic acid 28-*O*-β-D-glucopyranosyl ester (**III**) mp: 235°C, UV λ_{max} (nm): 206.2 (MeOH), IR (1% KBr) (cm^{-1}): 3401 (O-H), 2924, 2860 (C-H), 1734 (C=O), 1052 (C-O-C). ^{1}H-NMR (CD_3OD, 300 MHz) Table 1 and ^{13}C-NMR (CD_3OD, 50.3 MHz) Table 2.

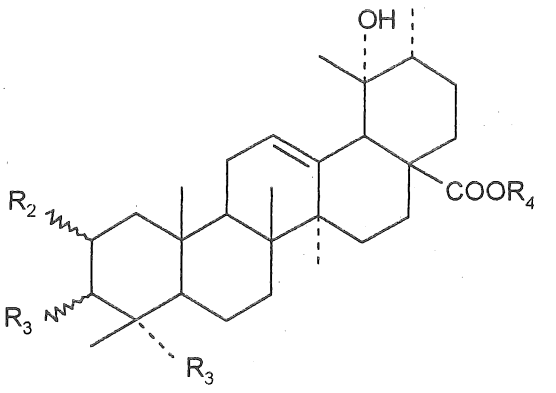
2α,3β,19α-trihydroxyurs-12-en-28-oic acid 28-*O*-β-D-glucopyranosyl ester (Tormentic acid glucosyl ester) (**IV**) mp: 190°C, UV λ_{max} (nm): 207.4 (MeOH), IR (1% KBr) (cm^{-1}): 3395 (O-H), 2925, 2860 (C-H), 1734 (C=O), 1070 (C-O-C). ^{1}H-NMR (CD_3OD, 300 MHz) Table 1 and ^{13}C-NMR (CD_3OD, 50.3 MHz) Table 2.

2β,3α,19α-trihydroxyurs-12-en-28-oic acid 28-*O*-β-D-glucopyranosyl ester (**V**). mp: 200°C, UV λ_{max} (nm): 206.0 (MeOH), IR (1% KBr) (cm^{-1}): 3575 (O-H), 2925, 2860 (C-H), 1734 (C=O), 1074 (C-O-C). ^{1}H-NMR (CD_3OD, 300 MHz) Table 1 and ^{13}C-NMR (CD_3OD, 50.3 MHz) Table 2.

2α,3β,19α-trihydroxyurs-12-en-23,28-dioic acid 28-*O*-β-D-glucopyranosyl ester (Suavissimoside R_1) (**VI**) mp: 260°C, UV λ_{max} (nm): 206.0 (MeOH), IR (1% KBr) (cm^{-1}): 3393 (O-H), 2925, 2860 (C-H), 1734 (C=O), 1073 (C-O-C). ^{1}H-NMR (CD_3OD, 300 MHz) Table 1 and ^{13}C-NMR (CD_3OD, 50.3 MHz) Table 2.

Results and Discussion

From the methanolic extract of the aerial parts of *R. sanctus*, six triterpenic saponins were first isolated by using chromatographic methods and the structures of these compounds were idendified by using physical (mp det.) and spectroscopic methods (UV, IR, ^{1}H-NMR, ^{13}C-NMR, APT, DEPT). Results were compared with the reported data. 2β,3α,19α,23-tetrahydroxyurs-12-en-28-oic acid (**I**), pinfaensin (**II**), 2β,3α,19α,23-tetrahydroxyurs-12-en-28-oic acid 28-*O*-β-D-glucopyranosyl ester (**III**), tormentic acid glucosyl ester (**IV**), 2β,3α,19α-trihydroxyurs-12-en-28-oic acid 28-*O*-β-D-glucopyranosyl ester (**V**) and suavissimoside R_1 (**VI**) were determined (zhou *et al.*, 1992;Geo *et al.*,1985;Kojima *et al.*, 1989; Seto *et al.*,1984). Compounds (**I**), (**III**) and (**V**) were first reported from the genus of *Rubus*, in this study. The chemical investigations on *Rubus* species in identification of diterpene glycosides and triterpenic saponins suggesting two groups chemotaxonomically. The plant groups were named as A and B, respectively (Ohtani *et al.*, 1990; Seto *et al.*,1984). According to the result of our study, *R. sanctus* contains triterpenic saponins so this plant may be included to B group chemotaxonomically.



	R_1	R_2	R_3	R_4
I	-β-OH	-α-OH	CH_2OH	H
II	-α-OH	-β-OH	CHO	-β-D-Glucose
III	-β-OH	-α-OH	CH_2OH	-β-D-Glucose
IV	-α-OH	-β-OH	CH_3	-β-D-Glucose
V	-β-OH	-α-OH	CH_3	-β-D-Glucose
VI	-α-OH	-β-OH	COOH	-β-D-Glucose

Özet

Türkiye' de ''Böğürtlen'' olarak bilinen *Rubus sanctus* Schreber' den altı triterpenik saponin izole edildi. 2β,3α,19α,23-tetrahidroksiurs-12-en-28-oik asit **(I)**, pinfensin **(II)**, 2β,3α,19α,23-tetrahidroksiurs-12-en-28-oik asit 28-O-β-D-glukopiranozit esteri **(III)**, tormentik asit glukozil esteri **(IV)**, 2β,3α,19α-trihidroksiurs-12-en-28-oik asit 28-O-β-D-glukopiranozit esteri **(V)** and suavisimozit R_1 **(VI)** ilk kez bu çalışmada *Rubus sanctus'* dan elde edildi. Ayrıca bu bileşikler kemotaksonomik olarak tartışıldı.

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