PERGAMON

Phytochemistry 51 (1999) 903–908

PHYTOCHEMISTRY

A saponin with anti-ulcerogenic effect from the flowers of *Spartium junceum*

Erdem Yeşilada[a,*], Yoshihisa Takaishi[b]

[a]*Gazi University, Faculty of Pharmacy, Hipodrom 06330Ankara, Turkey*
[b]*Tokushima University, Faculty of Pharmaceutical Sciences, 770Tokushima, Japan*

Received 25 November 1998; received in revised form 23 March 1999; accepted 23 March 1999

Abstract

A new oleanene-type saponin with potent anti-ulcerogenic activity was isolated from the flowers of *Spartium junceum*. The various techniques of NMR spectral analysis, viz. ^{1}H, ^{13}C, DEPT, C–H COSY, H–H COSY, COLOC, NOESY, HMBC, HMQC, in conjunction with EI- and FAB-mass spectrometry, revealed that the structure of the isolated saponin was 3-*O*-[α-L-rhamnopyranosyl-(1 → 2)-*O*-β-D-glucopyranosyl-(1 → 2)-β-D-glucuronopyranosyl]-3β,16β,22β,24-tetrahydroxy-olean-12-ene and named as spartitrioside.

Keywords: *Spartium junceum* L; Fabaceae; Spartitrioside; Triterpenic saponin; Oleanene-type saponin

1. Introduction

Spartium junceum L. (Fabaceae), Spanish Broom, katır tırnağı or burçalak (in Turkish), is a small shrub indigenous in the Mediterranean countries and cultivated as an ornamental plant. The infusion of the flowers (1%) is used as mild sedative and diuretics in phytotherapy (Baytop, 1984). In our field surveys in Anatolia, we recorded the utilisation of the flower infusion for the treatment of gastric ulcers in southern Anatolia (Yeşilada, et al., 1993). In a following study, a potent anti-ulcerogenic activity was reported against water immersion and immobilization-induced stress ulcer in rats (Yeşilada, Sezik, Fujita, Tanaka, & Tabata, 1993).

For the evaluation of the anti-ulcerogenic activity and isolation of active principle(s), a study based on bioactivity-guided fractionation process was conducted. The methanolic extract from the flowers of *S. junceum* was first fractionated by solvent extraction, then by ion-exchange chromatography on Amberlite XAD-2 and finally by Sephadex LH-20. In consequence of this fractionation process, saponin fraction was determined as the active anti-ulcerogenic ingredient of the plant.

However, attempts for further purification of the main saponin remained unsuccessful. The crude saponin eluted from Sephadex LH-20 column with MeOH turned into a white mass, insoluble in any of the common organic solvents, i.e. H_2O, MeOH, *n*-BuOH, $CHCl_3$, etc. after the separation of flavonoids and some other compounds, which possibly solubilized saponins. In consequence, further fractionation of the saponin mixture for bioassay studies could not be conducted and the study was directed to the solution of this problem.

For this purpose, another separation technique (medium-pressure liquid chromatography) was employed for the isolation of the main saponin from the flowers of the plant. This study describes the details of this isolation procedure and the structure elucidation of the purified saponin.

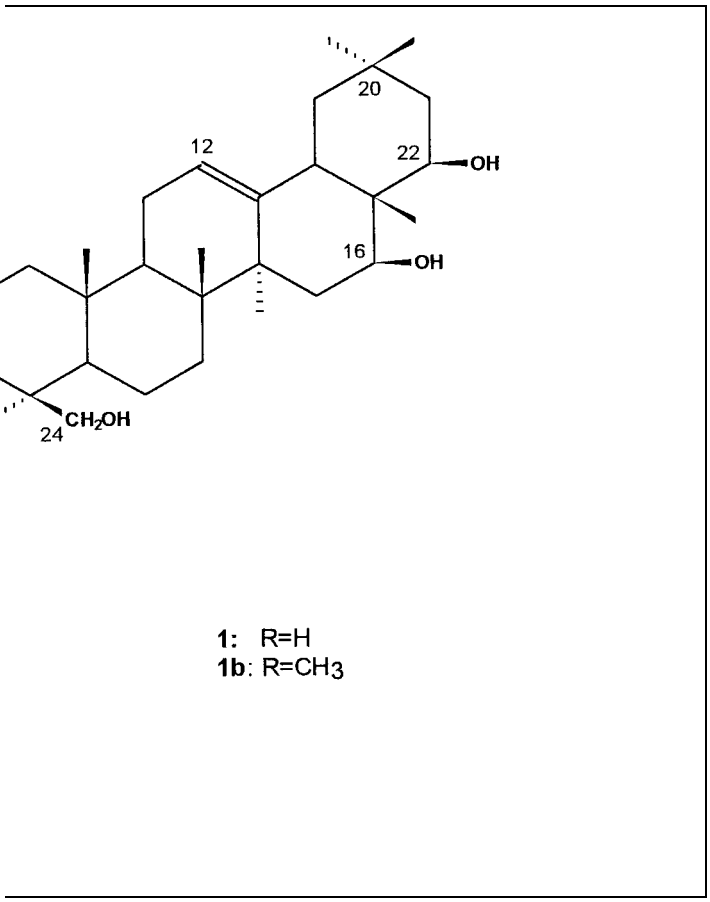
* Corresponding author. Fax: +90-312-223-5018.
E-mail address: yesilada@tr-net.net.tr (E. Yeşilada)

0031-9422/99/$ - see front matter
PII: S0031-9422(99)00198-3

2. Results and discussion

For the isolation of the main saponin, we decided to conduct the study using the raw fraction. The more polar components of the butanol fraction were first precipitated by adding into cold diethyl ether and the precipitate was subjected to chromatographic separation on a silica gel column using a gradient elution technique. Fractions containing the main saponin were combined and evaporated to dryness. Saponin started to precipitate even during the elution stage from the column with the decreasing ratio of $CHCl_3$ on exposure to air. Further purification of the main saponin was carried out by successive precipitation with MeOH in a refrigerator to obtain a white powder (**1**).

The positive FAB mass spectrum of **1** gave a strong molecular ion peak at m/z 981 $[M+Na]^+$ indicating a molecular mass of 958. The fragment ions at m/z 834 and 672 corresponded to $[M+Na\text{-deoxyhexose}+H]^+$ and $[M+Na\text{-deoxyhexose–hexose}+H]^+$ ions, respectively, indicating the sequential loss of sugar moieties. In addition, the electron impact mass spectrum (EI-MS) of the acetate derivative (**1a**) exhibited fragment ion peaks at m/z 561 $[(\text{deoxyhexose–hexosyl-})Ac_6]^+$ and 273 $[(\text{terminal deoxyhexosyl-})Ac_3]^+$, suggesting that saponin possessed a deoxyhexosyl–hexosyl moiety as terminal carbohydrate residue. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of the compound **1b** disclosed three anomeric proton signals at δ 4.96 (1H, d, $J=6.0$ Hz), 5.77 (1H, d, $J=7.6$ Hz) and



Because of the low solubility of saponin (**1**) in pyridine-d_5, we could not get satisfactory results from the NMR analysis. Peaks were not strong and clear enough to interpret the structure. The IR spectrum which evidenced the presence of hydroxyl (3400 cm^{-1}) and carbonyl (1720 cm^{-1}) units directed us to prepare the acetyl- and methyl ester-derivatives of the saponin. Acetylation (**1a**) was performed by Ac_2O in pyridine at room temperature, while the methyl ester (**1b**) was prepared by trimethylsilyldiazomethane and both derivatives solubilized easily.

6.28 (1H, s) which showed the existence of three sugar units. This conclusion was also supported by the presence of three anomeric signals at δ 105.5, 101.7 and 102.4 ppm in the ^{13}C-NMR spectrum.

In the ^{1}H-NMR spectra of **1b** it was seen that there were nine methyl groups, one of which (a doublet at 1.78 ppm) must have an adjacent methine group, the other was attached to a carbonyl group, while the remaining seven were connected to quaternary carbons (Table 1). ^{13}C-NMR spectrum of **1b** showed 30 signals

due to the sapogenin part and 19 signals due to the sugar moiety, including three anomeric carbons (δ 105.5, 101.7 and 102.4 ppm) and one ester carbonyl carbon at δ 170.4 ppm. The presence of two sp^2 carbons at δ 122.3 and 144.8 ppm indicated that the aglycone possessed an olean-12-ene skeleton. In addition, there were four oxygenated sp^3 carbon resonance in the downfield region (δ 91.3, 69.4, 75.6, 61.6 ppm) and the remaining oxygenated carbon resonance accounted for the three sugars. The carbon resonance were as-

Table 1
^{1}H and ^{13}C-NMR spectra of **1b** and 2D-NMR correlations

C No.	^{13}C-NMR ((δ) ppm)	^{1}H-NMR ((δ) ppm	COLOC (C-) and H–H COSY (H-) correlations
Aglycone part			
1	38.6	1.40 m, 0.83 m	C-25
2	26.7	1.90, 2.08 m	H-3
3	91.3	3.38 dd (J = 3.6 and 12.7 Hz)	H-2
4	43.9	–	
5	56.2	0.88	C-23, C-25, C-7
6	18.5	1.26 m, 1.55 m	
7	33.3	1.47 m, 1.30 m	C-26
8	39.9	–	C-6, C-27, C-15
9	47.8	1.59	C-25, C-26, C-12
10	38.0	–	C-6
11	24.0	1.88	
12	122.3	5.31	C-18, H-11
13	144.8	–	C-15, C-27
14	42.4	–	C-26, C-7, C-12, C-18
15	26.4	1.86, 1.05	C-27
16	69.4	4.68	
17	36.4	–	
18	45.3	2.40 d (J = 13.0 Hz)	C-12, C-28, H-19
19	46.7	1.13 d (J = 13.0 Hz), 1.93	C-29, C-30
20	30.9	–	C-22
21	42.3	1.80, 1.64	C-30, H-22
22	75.6	3.76	H-21
23	23.0	1.44	
24	61.6	4.27 d (J = 4.4 Hz), 4.43 d (J = 4.4 Hz)	
25	15.8	0.72 (3H)	C-1, C-5
26	17.0	0.96 (3H)	
27	25.7	1.28 (3H)	
28	28.7	1.94 (3H)	
29	33.3	1.00	
30	21.2	1.23 (3H)	
Glucuronic acid			
1′	105.5	4.96 d (J = 6.0 Hz)	H-2′
2′	78.2	4.37	H-1′, H-3′
3′	76.6	4.10 dd (J = 3.2 and 9.6 Hz)	H-4′, H-2′
4′	74.3	4.35	
5′	77.0	4.55	H-4′
6′	170.4		
OMe	52.1	3.78	C-6′
Glucose			
1″	101.7	5.77 d (J = 7.6 Hz)	H-2″
2″	79.1	4.25	H-1″
3″	78.0	4.58	H-2″
4″	71.1	4.39	
5″	77.7	4.54	H-6″(b)
6″	63.5	3.25 d (J = 11.6 Hz), 4.26	H-6″ (a, b)
Rhamnose			
1‴	102.4	6.28 s	H-2‴
2‴	72.3	4.80	H-1‴, H-3‴
3‴	72.7	4.67	H-2‴, H-4‴
4‴	73.6	4.32	H-5‴
5‴	69.4	4.96	C-1‴, H-4‴, H-6‴
6‴	19.0	1.78 d (3H), (J = 6.4 Hz)	H-5‴

sociated with the corresponding proton signals using ^{1}H–^{1}H-COSY, ^{13}C–^{1}H-COSY, COLOC and DEPT experiments of **1b** and NOESY, HMBC and HMQC experiments of **1a** derivatives of saponin **1** and the assignments are provided in Tables 1 and 2.

Comparison of the ^{13}C NMR data of **1a** and **1b** with those of the aglycone obtained through methanolysis suggested that the sugar moiety was attached to the C-3 hydroxyl group of aglycone (Table 2). The C-3 signal (δ 89.9 ppm) of aglycone was shifted downfield by δ 8.4 (for **1a**) and 11.3 ppm (for **1b**) due to the glycosylation, indicating that **1** is a 3-*O*-monodesmosidic saponin. The sugar group obtained through methanolysis of saponin **1** revealed that it was composed of D-glucuronic acid, D-glucose and L-rhamnose on TLC examination with authentic samples.

Data obtained through NMR and MS analysis of the saponin indicated that glucuronic acid moiety was attached by its hemiacetalic group to the aglycone at C-3, whilst the disaccharide which was composed of one glucose and one rhamnose bound to the glucuronic acid by a glycosidic linkage. This was further proved by the heteronuclear multiple bond connectivity (HMBC) spectrum of **1a**, long range ^{1}H–^{13}C correlations were observed between the H-1 of glucuronosyl unit and the C-3 of the aglycone. The anomeric configuration of each glycosyl linkage was deduced from the coupling constants of each anomeric proton signal in the ^{1}H-NMR spectrum and supported by the downfield shift of relevant ^{13}C-NMR signal with those reported in the literature (Agrawal, 1992); C-2 of the inner glucose residue shifted δ 3.2 ppm downfield, while C-2 of glucuronic acid δ 5 ppm. From the ^{13}C-NMR data for **1b** (Table 2) the signals corresponding to the sugar moiety were superimposable on those previously reported from some other Fabaceae saponins (Mohammed, Ohtani, Kasai, & Yamasaki, 1993).

In conclusion, on the basis of the foregoing evidence and if glucose and glucuronic acid are assumed to be members of the commonly found D series, the structure of **1** was suggested to be 3-*O*-[α-L-rhamnopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucuronopyranosyl]-3β,16β,22β,24-tetrahydroxy-olean-12-ene. The structure of the aglycone was found to be identical with that of a known compound, junceogenol, which has previously been isolated and described from the twigs of the same plant by Bilia,

Table 2
^{13}C-NMR chemical shifts [(δ ppm] of aglycone (in $CDCl_3$) and sapogenol moieties of **1a** (in $CDCl_3$) and **1b** (in pyridine-d_5) and comparison with that reported for junceoside by Bilia et al. (1993)

C No.	Aglycone	Acetyl saponin **1a**	6′-Methyl ester of saponin **1b**	Junceoside
1	38.6	38.8	38.6	38.8
2	26.1	26.2	26.7	26.7
3	81.0	89.4	91.3	91.5
4	41.7	41.8	43.9	44.1
5	56.0	55.8	56.2	56.5
6	18.6	20.2	18.5	18.8
7	33.3	33.3	33.3	33.3
8	39.9	40.0	39.9	40.2
9	47.9	47.9	47.8	48.0
10	37.6	36.5	38.0	36.8
11	23.9	23.8	24.0	24.4
12	122.5	122.5	122.3	122.5
13	144.1	144.0	144.8	144.9
14	42.2	42.3	42.4	43.4
15	27.9	26.1	26.4	33.4
16	69.2	68.8	69.4	69.4
17	36.8	36.8	36.4	42.1
18	44.9	44.7	45.3	45.6
19	46.3	46.2	46.7	47.0
20	30.2	30.6	30.9	31.0
21	42.9	38.5	42.3	42.7
22	76.8	78.7	75.6	75.8
23	22.6	23.1	23.0	23.1
24	64.5	66.5	61.6	63.6
25	16.3	16.7	15.8	16.0
26	17.0	17.3	17.0	17.2
27	25.6	26.3	25.7	26.0
28	28.4	27.1	28.7	28.8
29	33.0	33.8	33.3	33.3
30	20.2	21.5	21.2	21.1

Table 3
Effect of saponin **1** (spartitrioside) against ethanol-induced ulcer model in rats.*$p < 0.05$, **$p < 0.01$ significant from the control group

Test compound	Dose[a] (mg/kg) per oral	Ulcer index (mean ± S.E.)	Prevention from ulcer[a]	Inibition (%)
Control		29.1 ± 8.9	–	
Spartitrioside	95	0.5 ± 0.5**	4/6	98.3
Famotidine	2	3.0 ± 0.5*	0/6	89.7

[a] Number of rats whose stomachs were completely prevented from bleeding.

Flammini, Flammini, Morelli, & Marsali (1993), but the sugar chain of the isolated saponin, namely junceoside, was reported to contain only D-glucose and L-rhamnose and the former was determined as the terminal sugar. Thus this is the first report for the isolation and structure elucidation of saponin **1** in nature and named as spartitrioside.

Saponin **1** exerted a potent anti-ulcerogenic effect against ethanol-induced gastric lesions in rats (Table 3). The effect was found more prominent than that of the reference compound, famotidine. Effects of the extracts and the fractions obtained through bioassay-guided fractionation as well as saponin fraction from the flowers of the plant on various ulcer models will be reported elsewhere.

3. Experimental

3.1. General

All mps are uncorr. ^{1}H-NMR (400.1 MHz) and ^{13}C-NMR (100.6 MHz) analysis were carried out using TMS as internal standard. FAB-MS spectra was obtained in a thioglycerol matrix, in the positive ion mode. IR Spectra (cm^{-1}) were obtained on Perkin Elmer 2000 FT-IR spectrophotometer in KBr pellets. In MPLC column, Kieselgel 0.040–0.063 mμ (Merck) was used as adsorbent. TLC analysis were performed on precoated Kieselgel $60F_{254}$ plates (Merck, Art.5719). Spots were visualised by spraying 4% α-naphtol/EtOH 96° + 5% H_2SO_4 reagent for sugars and 5% H_2SO_4/MeOH reagent for saponins and then plates were heated at 110°C.

3.2. Plant material

Flowers of *S. junceum* were collected in FoÇa (Izmir, Turkey) in May 1997 and dried in shade. A voucher specimen is deposited in the Herbarium of Faculty of Pharmacy, Gazi University (Ankara).

3.3. Extraction

Dried flowers (2 kg) were extracted with MeOH (60 L) at room temperature, then the solvent was evaporated under reduced pressure. The extract was dissolved in H_2O (1 L) and fractionated by successive solvent extractions with $CHCl_3$ (3 L) and then with *n*-BuOH satd. with H_2O (10 × 0.5 L). The butanolic layer was evaporated to dryness under reduced pressure to give a gummy residue (111 g). The butanolic extract was dissolved in MeOH (450 ml) and added in portionwise into cold Et_2O (2.5 L). The precipitate was removed by filtration on a Buchner funnel, washed with cold Et_2O, and dried under reduced pressure (76 g).

3.4. Isolation of saponin

4.0 g of precipitate was concentrated on 7 g of silica gel and applied to a dry silica gel column (200 g; Kieselgel 0.040–0.063 μm, Merck) and chromatographed in a MPLC column using $CHCL_3$–MeOH–H_2O (10:3:0.3)(532 ml), (9:3:0.5) (480 ml) and (7:4.2:1) (1830 ml) solvent systems, successively, 8 ml/min (each fraction was collected in 20 ml volume). The fractions were combined according to TLC control using the solvent systems $CHCL_3$–MeOH–H_2O (7:4.2:1) and *n*-BuOH–*i*-PrOH–HOAc–H_2O (4:2:1:3). Eluents from 60 to75, which contain the main saponin were combined and evaporated to dryness under reduced pressure. Further purification of the main saponin was carried out by successive precipitation. Saponin started precipitation even during the elution stage from the column by the decreasing ratio of $CHCl_3$ due to the evaporation on exposure to air. The precipitate was removed by decantation of the upper layer and dissolved in hot MeOH and kept in refrigerator to obtain a precipitate. This process was repeated several times to obtain a white powder; saponin **1**, $[\alpha]_D^{24}$: −5.950 (23 mg/ml in pyridine). IR ν_{max}^{KBr} cm^{-1}: 3400, 2944, 1720, 1075, 1040. FAB-MS (positive mode) *m/z*: 981 $[M + Na]^+$ $[C_{48}H_{78}O_{19}]$, 834 $[M + Na\text{-rhamnose}]^+$, 672 $[M + Na\text{-rhamnose–glucose}]^+$.

3.5. Acetylsaponin (*1a*)

Thirty mg of saponin **1** was acetylated with Ac_2O/pyridine (1:1) at room temperature overnight; acetylsaponin (**1a**), $[\alpha]_D^{24}$ 4.20 (25 mg/ml in $CHCl_3$), mp. 152–3°C. TLC solvent system for **1a**, hexane–Me_2CO (3:1). ^{1}H-NMR (in $CDCl_3$): 0.93, 1.61 (H-1), 1.08, 1.74 (H-

2), 3.30 dd ($J=5.2$ and 12.3 Hz) (H-3), 0.83 (H-5), 0.75 (H-6), 1.29, 1.37 (H-7), 1.48 (H-9), 1.81 (H-11), 5.20 (H-12), 1.60, 1.70 (H-15), 5.19 (H-16, 2.14 (H-18), 1.03, 1.72 (H-19), 1.44 (H-21), 4.58 (H-22), 1.02 (H-23), 4.14 (H-24), 0.89 (H-25), 1.03 (H-26), 1.07 (H-27), 0.93 (H-28), 0.83 (H-29), 0.75 (H-30), 6.22 d ($J=1$ Hz) (H-1‴, rhamnose), 4.72 d ($J=8.0$ Hz)(H-1″, glucose), 5.31 d ($J=5.5$ Hz) (H-1′, glucuronic acid) and ^{13}C-NMR (in $CDCl_3$): see Table 2. EIMS: 273 (terminal rhamnose–Ac_3), 561 ($C_{24}H_{33}O_{15}$; rhamnose–glucose–Ac_6), retro-Diels Alder fragments:244 $[C_{14}H_{21}O_2]^+$, 255 $[C_{16}H_{24}O_2]^+$.

3.6. Saponin-6′-methyl ester (*1b*)

Sixty mg saponin **1** was dissolved in 10 ml MeOH and treated with trimethylsilyldiazomethane (TCI, T1146) to methylate the carbonyl function at 6′ position of glucuronic acid; 6′-methyl ester of saponin (**1b**), $[\alpha]_D^{24}$ −6.45 (21.0 mg/ml in MeOH). ^{1}H and ^{13}C-NMR in pyridine-d_5: see Table 1 and 2.

3.7. Methanolysis of saponin *1*

The saponin **1** was added into 9% HCl in dry MeOH and refluxed at 100°C for 6 h. The hydrolysate was then neutralised passing through Dowex (Cl^- form) and processed as usual to obtain aglycone and sugars. Aglycone, mp 278–280 °C, $[\alpha]_D^{24}$ 6.10 (20 mg/ml in $CHCl_3$). ^{13}C-NMR in $CDCl_3$: see Table 2. Solvent system for TLC analysis of sugars: $CHCl_3$–MeOH–Me_2CO–H_2O (3:3:3:1) and EtOAc–MeOH–HOAc–H_2O (13:3:4:3).

3.8. Pharmacological test: ulceration induced by ethanol

Fifteen min after the oral administration of a test sample to a group of six rats weighing 132–148 g, lesions were induced by an oral administration of 1 ml absolute ethanol by means of a gavage needle. One h later, the animals were killed by an overdose of ether. The abdomen was immediately opened to remove the stomach which was ligated both at the oesophagus and the pylorus after inflation with saline solution. The ulcer index for each stomach was calculated as described in the relevant reference (Yeşilada et al., 1993). Statistical evaluation of the results was performed according to the Student's *t*-test.

Acknowledgements

The first author is grateful to Fujii-Otsuka Research Fund for the award of a scholarship to study in Japan. The authors are also grateful to Dr. Kazuyoshi Kawazoe and Dr. Kiyoshi Kida from the Tokushima University, Faculty of Pharmaceutical Sciences for NMR measurements.

References

Agrawal P, K. (1992). *Phytochemistry*, *31*, 3307.

Baytop, T. (1984). *Phytotherapy in Turkey, past and present*. Istanbul: Istanbul University Press.

Bilia, A. R., Flammini, F., Flammini, G., Morelli, I., & Marsili, A. (1993). *Phytochemistry*, *34*, 847.

Mohammed, K. M., Ohtani, K., Kasai, R., & Yamasaki, K. (1995). *Phytochemistry*, *40*, 1237.

Yeşilada, E., Honda, G., Sezik, E., Tabata, M., Goto, K., & Ikeshiro, Y. (1993). *Journal of Ethnopharmacology*, *39*, 31.

Yeşilada, E., Sezik, E., Fujita, T., Tanaka, S., & Tabata, M. (1993). *Phytotherapy Research*, *7*, 263.