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Anti-platelet stilbenes from aerial parts of *Rheum palaestinum*

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Abstract

A chemical investigation of biologically active compounds from the aerial parts of *Rheum palaestinum* has led to the isolation and identification of two stilbenes; *trans*-resveratrol-3-*O*-β-D-glucopyranosid (**I**) and rhaponticin (**II**). The structures of these stilbenes were established on the basis of spectroscopic data including MS and NMR. Compounds (**I**) and (**II**) have been shown to exhibit anti-platelet activity. Moreover, chrysophanol was also identified and characterized. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: *Rheum palaestinum*; Polygonaceae; Piceid; Rhaponticin; Chrysophanol; Anti-platelet

1. Introduction

R. palaestinum Feinbr. (Polygonaceae) is native in Jordan, a country rich in traditional medicine. This plant is a wild species locally known as Atrafan; it grows in the Syrian desert and Eastern desert of Jordan with a distribution extending to the southern part of Palaestine and Sinai (Zohary, 1966; Al-Eisawi, 1982). It is a perennial herb, 15–40 cm long, with underground woody stem. Leaves are rounded, huge, 20–50 cm in diameter, with wrinkled surface. Flowers greenish, not showy, producing triangular fruits with broad wings (Al-Eisawi, 1998).

Rhubarb remains in use as a laxative, especially as a powder (Bruneton, 1995). It produces a variety of secondary phenolic metabolites, i.e. anthraquinones, naphthalenes, stilbenes, chromones, flavonoids, and related compounds (Kashiwada and Nonaka, 1984; Bruneton, 1995). Since ancient times the aqueous extract of the rhizome of *R. palaestinum* is used as a coloring agent in the preparation of sheep butter among Bedouins (Al-Khalil and Al-Eisawi, 1993). Further information provided by the rural inhabitants and some herbalists indicated the efficacy of this plant to exhibit anti-platelet activity and to increase the time of clotting. This caused our interest in its components in order to isolate the compounds responsible for its pharmacological activity.

Early works on *R. palaestinum* reported the isolation of only chrysophanol and rheum-emodin from the roots and aerial parts of the plant (Al-Khalil and Al-Eisawi, 1993), which is not sufficient to explain its use in traditional therapy as anti-platelet aggregation agent.

Stilbenes isolated from different plants exhibited different pharmacological activity. Resveratrol-3-*O*-β-D-glucopyranoside and its corresponding aglycon, resveratrol has been reported as anti-platelet aggregation agents (Kimura et al., 1985; Chung et al., 1992; Orsini et al., 1997). Moreover, resveratrol has also shown coronary vasodilator (Fremont, 2000), anti-leukemia (Mannila et al., 1993), and anti-fungal activity (Langcake et al., 1979). Other stilbenes isolated from *Rheum palmatum* have reported as α-glucosidase inhibitory agent (Kubo et al., 1991).

The present work reports the isolation and identification of two stilbene derivatives: *trans*-resveratrol-3-*O*-β-D-glucopyranoside (Piceid) and rhaponticin (rhapontigenin-3-*O*-β-D-glucopyranoside), which have not been previously reported in this plant. The anti-platelet activity of these compounds on human platelet-rich plasma (PRP) aggregation induced by collagen and ADP are reported. Furthermore Chrysophanol which has previously been isolated from the roots of *R. palaestinum* (Al-Khalil and Al-Eisawi, 1993) is also identified and characterized.

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2. Experimental

2.1. Plant material

The aerial parts, comprising mainly leaves of *R. palaestinum* were collected from the Qatrana area, 90 km south of Amman, Jordan and a voucher specimen has been deposited in the author's research laboratory at the Faculty of Pharmacy, University of Jordan.

2.2. Extraction and fractionation

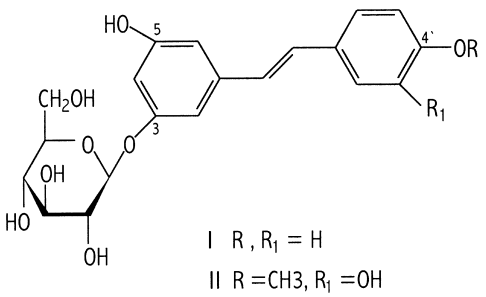
The aerial parts of the plant (1500 g) were dried at room temperature, powdered and then extracted by percolation with MeOH (6 l). After the solvent was evaporated, a syrupy residue (712 g) was obtained. The residue was suspended in H_2O (1 l) and extracted with $CHCl_3$ (750 ml ×3, fraction RPI, 78.9 g), and with ethylacetate (750 ml ×3, fraction RPII, 47 g) respectively.

2.3. Chromatography of fraction RPI

Fr.RPI was chromatographed over silica gel (250 g, 70×4 cm) and eluted with varying proportions of $CHCl_3$ and MeOH. Elution with $CHCl_3$–MeOH (3:1, 500 ml) afforded a residue (21.1 mg) which was crystallized from EtOH to give chrysophanol, mp 196–198°C, spectral data consistent with authentic sample and with literature value (Al-Khalil and Al-Eisawi, 1993).

2.4. Chromatography of fraction RPII

The ethylacetate extract (47 g) was chromatographed over silica gel column (350 g, 90×4 cm), eluted with $CHCl_3$ containing increasing amount of MeOH (1:0–1:1) to give ten fractions. Fractions IV (300 mg) and VI (125 mg) consisted of a large amount of stilbene derivatives, which exhibited bluish-purple flourescent spots on UV illumination on the TLC plates. Thus, these fractions were separately rechromatographed using flash-chromatography (petroleum ether–ethylacetate 7:3 and 3:7) giving compounds **I** (230 mg) and **II** (97 mg). Compound II was not pure, and was further purified by preparative TLC ($CHCl_3$–MeOH 4:1) furnished a pure sample (69 mg).



I R, R_1 = H

II R = CH3, R_1 = OH

2.5. Resveraltrol 3-O-β-D-glucopyranoside (*I*)

This compound was crystallized from $CHCl_3$–MeOH 9:1; mp 219–223 [lit. mp. 220–225 (Orsini et al., 1997)]; $[\alpha]_D^{25} = -59.9$ (*c* 0.25, MeOH) [lit. −65.26) (Aritomi and Donnelly, 1976). FAB MS (positive) ($C_{20}H_{22}O_8$) *m*/*z*: 390[M]$^+$, 228 [M-162]$^+$. ^{1}H NMR (200 MHz, DMSO-d_6): 9.58 (1H,*bs*, phenolic OH), 9.43 (1H,*bs*, phenolic OH), 7.31 (2H,*d*, *J* = 8.6 Hz, H-2′ and H-6′), 7.15 (1H,*d*, *J* = 17.2 Hz, H-β), 6.85 (1H,*d*, *J* = 17.2 Hz, H-α), 6.79 (2H,*d*, *J* = 8.6 Hz, H-3′ and H-5′), 6.70 (1H, *dd*, *J* = 1.8, 1.8 Hz, H-2), 6.60 (1H, *dd*, *J* = 1.8, 1.8 Hz, H-6), 6.42 (1H,*dd*, *J* = 1.8, 1.8 Hz, H-4), 5.21 (1H, *d*, OH), 5.07 (1H,*d*,OH), 5.0 (1H,*d*,OH), 4.80 (1H,*d*, *J* = 7.0 Hz, H-1 Glc), 4.59 (1H,*t*,OH), 3.72 (1H, *dd*, *J* = 12.0, 1.8 Hz, H-6 Glc), 3.63–3.10 (5H,*m*).

2.6. Rhapontigenin 3-O-β-D-glucopyronoside (*II*)

Colorless needles (acetone), mp 245–247°C (lit. 246–248°C) (Kashiwada and Nonaka, 1984), $[\alpha]^{25} = -56$ [c 0.88, acetone–H_2O 1:1] (Lit. −56.3) (Kashiwada and Nonaka, 1984), FAB MS (Positive), 420 [M]$^+$,258 [M-162]$^+$, ^{1}H NMR (DMSO-d_6), 7.03 (1H,*d*, *J* = 2.1 Hz, H-2), 6.99 (1H,*dd*, *J* = 2.1, 8.4 Hz, H-6′), 6.83 (1H,*d*, *J* = 8.4 Hz, H-5′), 6.84, 7.04 (each 1H,*d*, *J* = 16 Hz, olefinic H), 6.60, 6.75 (each 1H, *brs*, H-2 and H-6), 6.37 (1H, *brs*, H-4), 4.85 (1H,d, *J* = 7 Hz, anomeric H), 3.78 (3H,*s*,OCH_3), 3.2–3.9 (6H,*m*, sugar-H).

2.7. Platelet aggregation

The samples were dissolved in DMSO at a final concentration of 0.5%. Platelet-rich plasma (PRP) were obtained from human blood taken from the forearm vein of volunteers. Blood was then collected by free flow along the side of plastic tube containing 3.8% sodium citrate (1:9) and was then centrifuged at room temperature at 1300 rpm for 15 min. Platelets were counted under microscope, and the platelet count was adjusted to 300 000 platelets/μl with platelet-poor plasma (PPP) obtained by centrifugation of the PRP at 13000 rpm for 3 min.

Aggregation was measured by the aggregometric method (Beretz and Cazenave, 1991). The aggregometer was calibrated so that the PRP gave 10% of light transmission while the PPP gave 90% of light transmission. The aggregation was measured by an aggregometer (Elvilogos 840) connected to dual channel recorders. The platelet suspension was stirred at 1000 rpm. Platelets were preincubated with different concentrations of the tested compounds (3.2–256 μg/ml) or DMSO for 3 min before the addition of aggregation inducer.

2.8. General experimental procedures

Melting points were determined on a Stuart Scientific melting point apparatus and are uncorrected and UV

spectra on a Unicam-810 Kontron spectrophotometer. NMR spectra were obtained with Varian L-200 and Bruker AC-200 and AC-300 instruments. Mass spectra were recorded with a VG 707 E9 spectrometer and optical rotations on a Perkin–Elmer 241 polarimeter.

Flash-chromatography was performed on Si gel Merck Kieselgel 60 (230–400 mesh ASTM), and CC on Merck Kieselgel (70–230 me). Thin layer chromatography (TLC) was carried out on Si gel plates (60 F_{254}, Merck).

Anhydrous Na_2SO_4 was routinely used for drying organic solvents, and all solvents were evaporated under reduced pressure at 40°C. Spots were detected visually by ultraviolet absorption (254 nm) or by spraying with MeOH–H_2SO_4, 9:1 followed by heating at 100°C.

3. Results and discussion

Many anthraquinone and stilbene derivatives have been isolated from different *Rheum* species (Kashiwada and Nonaka, 1984; Al-Khalil and Al-Eisawi, 1993; Kubo et al., 1991). A literature survey showed that *R. palaestinum* has received chemically little attention. However the studies carried out on *R. palaestinum* reported the presence of two anthraquinones in this plant namely rheum-emodin and chrysophanol (Al-Khalil and Al-Eisawi, 1993).

Resveratrol-3-*O*-β-D-glucopyranoside showed bluish-purple fluorescence on UV irradiation. The FAB MS showed $[M]^+$ at *m/z* 390 corresponding to the molecular formula $C_{20}H_{22}O_8$ and 228 corresponding to the loss of glucose. The UV spectrum suggested the presence of a highly conjugated system λ_{max}^{EtOH} nm = 219,316).

Table 1
^{13}C NMR data of compounds **I** and **II** (DMSO-d_6)

Carbon	I	II
C-1	139.3	138.9
C-2	102.7	104.3
C-3	158.8	158.0
C-4	104.7	101.5
C-5	158.3	158.4
C-6	107.2	105.9
C-1[1]	130.0	129.2
C-2[1]	127.9	112.0
C-3[1]	116.5	146.1
C-4[1]	157.3	147.3
C-5[1]	116.5	112.0
C-6[1]	127.9	119.0
C-β	128.5	127.7
C-α	125.2	126.5
Glc-1	100.7	100.2
Glc-2	73.3	73.0
Glc-3	76.7	76.5
Glc-4	69.7	69.6
Glc-5	77.1	76.2
Glc-6	60.7	60.7
-OCH_3	–	55.1

The proton nuclear magnetic resonance (^1H NMR) spectrum showed the presence of six hydroxyl from which two are phenolics ($\delta = 9.58$ and 9.43) and four alcoholic ($\delta = 5.21$–4.59). It showed also the presence of two independent aromatic rings. One is para disubstituted ring ($\delta = 7.31$, *d*, $J = 8.6$ Hz, H-2′ and H-6′; $\delta = 6.79$,d,$J = 8.6$ Hz, H-3′ and H-5′), while the other is meta trisubstituted ring ($\delta = 6.70$ and 6.60, both *t*, $J = 1.8$, 1.8 Hz, H-2 and H-6; $\delta = 6.42$, *t*, $J = 1.8$, 1.8 Hz,H-4). Moreover, it showed trans-olefinic protons ($\delta = 7.15$ and $\delta = 6.85$, *d*, $J = 17.2$ Hz, H-β and H-α). The ^1H NMR spectra also exhibited a sugar anomeric proton doublet ($\delta = 4.80$, $J = 7$ Hz). The ^{13}C NMR (Table 1) confirmed the presence of glucose moiety which displayed signal patterns analogous to that of β-D-glucose. Considering that the ^1H and ^{13}C NMR spectra, this compound was assumed to be resveratrol-3-O-β-D-glucopyranoside. This conclusion was confirmed by comparison with literature data (Chung et al., 1992; Orsini et al., 1997).

The acid hydrolysis of resveratrol-3-O-β-D-glucopyranoside confirm the presence of glucose by direct comparison with authentic sample.

The UV spectrum of compound II suggested the presence of a highly conjugated system (λ_{max} nm = 300, 320). The ^1H NMR spectra of II showed the presence of a methoxyl group ($\delta = 3.78$), a trans olefinic group ($\delta = 6.84$, 7.04, each 1H,d,$J = 16$ Hz) and two aromatic rings with 1,3,4- and 1,3,5-trisubstitution systems. The ^1H NMR spectra also exhibited a sugar anomeric proton doublet ($\delta = 4.85$,d,$J = 7$ Hz). Other sugar signals appeared in the range of $\delta = 3.2$–3.9. The presence of a glucose moiety was deduced from the examination of the ^{13}C NMR spectra. From these spectral data this compound was assumed to be rhaponticin (rhapontigenin 3-*O*-β-D-glucopyranoside). On acid hydrolysis rhaponticin afforded glucose as detected by TLC.

The anti-platelet effects of piceid (**I**), rhaponticin (**II**) and *trans*-resveratrol were studied on the aggregation of human platelet-rich plasma (PRP) induced by collagen (2.5 μg/ml) and ADP (6 μM), and the results are shown in (Table 2).

Resveratrol-3-*O*-β-D-glucopyranoside (**I**) tested in vitro on human platelet rich plasma (PRP) has shown significant inhibitory effect on platelet aggregation induced by collagen (Table 2) and to a minor extent, by ADP (Table 2). Rhaponticin (**II**) has also shown significant inhibitory effect on platelet aggregation induced by collagen (Table 2) and, to a minor extent by ADP (Table 2).

To obtain further insight on the structure–activity relationship and to compare our results with a known anti-platelet agent, *trans*-resveratrol has been tested under the same conditions and the results are shown in (Table 2). The inhibitory effect of both compounds **I** and **II** when platelet aggregation induced by collagen or ADP is less than that of *trans*-resveratrol. When platelet aggregation was induced by collagen the activity of

Table 2
Effects of piceid (**I**), rhaponticin (**II**) and *trans*-resveratrol on the platelet aggregation induced by collagen and ADP[a]

	IC_{50}(μM)		
Inducer	Piceid (**I**)	Rhaponticin (**II**)	*trans*-resveratrol (**III**)
Collagen	41.75±2.2[b]	52.34±4.1[c]	11.60±2.1[b]
ADP	91.91±6.7[b]	112.07±16.93[c]	17.75±3.3[b]

[a] Platelets were preincubated with different concentrations of the various agents or DMSO (0.5%, control) at 37°C for 3 min then collagen (2.5 μg /ml) or ADP (6 μM) was added. Results are expressed as means ±SE of five replicates experiments.
[b] $P < 0.01$ as compared with control values.
[c] $P < 0.05$ as compared with control values.

these compounds appeared to be depressed by glucosylation as observed by comparing the IC_{50} of *trans*-resveratrol and piceid. Similar observation can be made for platelet aggregation induced by ADP.

Compounds **I** and **II** were isolated from *Erythrophleum lasianthum* (Orsini et al., 1997), *Rhei rizoma* (Bruneton, 1995) and *Polygonum cuspidatum* (Kimura et al., 1985), and this is the first report of their occurrence in *R. Palaestinum*.

In conclusion, the phytochemical analysis of *R. palaestinum* revealed the presence of two stilbenes: piceid and rhaponticin which may explain the use of this plant in traditional medicine for its anti-platelet activity as these compounds exhibit significant anti-platelet aggregation activity.

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