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Short communication

Flavonol glycosides from the leaves of *Boldoa purpurascens* and their anti-inflammatory properties

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ABSTRACT

Boldoa purpurascens is used in Latin America and the Caribbean as a potent diurectic. Phytochemical analysis has shown the presence of flavonoids and other active compounds. In the present work, three flavonol glycosides were isolated from the leaves of the plant. Their structures have been determined by mass spectrometry and by 1D and 2D NMR analysis as 6-methoxykaempferol-3-*O*-[α-L-rhamnopyranosyl-(1‴ → 2″)]-β-D-xylopyranoside (**1**); 3,4′,5-trihydroxy-6,7-methylenedioxyflavone-3-*O*-[α-L-rhamnopyranosyl-(1‴ → 2″)]-β-D-glucopyranoside (**2**); and 3,4′,5′,5-tetrahydroxy-6,7-methylenedioxyflavone-3-*O*-[α-L-rhamnopyranosyl-(1‴ → 2″)]-β-D-xylopyranoside (**3**). Compounds **1** and **3** are reported for the first time from nature. The NF-κB luciferase assay showed that these compounds have a partial inhibitory effect on NF-κB activation, compound **2** being the most potent one. In the carrageenan induced paw oedema assay in rats, the flavonoid fraction showed acute anti-inflammatory activity, with the highest percentage of inhibition (75.8%) at a dose of 40 mg/kg.

1. Introduction

Boldoa purpurascens Cav. ex Lag (Nyctaginaceae) is a plant species distributed from northern Mexico and the West Indies to northern South America, Central America and South America. Since it can thrive in disturbed environments it is not considered vulnerable to extinction. In Cuba its leaves are used in a decoction as a diuretic (Roig, 1998) and to treat other kidney disorders. In a previous study we have reported some new flavonoids from the aqueous extract of leaves of *B. purpurascens* (González et al., 2008), as well as anti-inflammatory and antihyperglycemic activities (González et al., 2011, 2013). Herein we describe the isolation of three flavonoid glycosides from the aqueous extract of the leaves of *B. purpurascens* (compounds **1**–**3**), two of which are new, and the evaluation of the anti-inflammatory activity of the flavonoid fraction, one of the isolated constituents (compound **2**), the major flavonoid of *B. purpurascens* isolated before [3,4′,5-trihydroxy-6,7-methylenedioxyflavone 3-*O*-α-L-rhamnopyranosyl-(1‴ → 2″)-β-D-xylopyranoside] (compound **4**), and the aglycone 3,4′,5-trihydroxy-6,7-methylenedioxyflavone obtained after hydrolysis of the major compound.

2. Experimental

2.1. General experimental procedures

1D and 2D NMR spectra were recorded in DMSO-*d* 6 (compound **3**) and CD_3OD (compounds **1** and **2**) using a Bruker DRX-400 instrument (Rheinstetten, Germany), operating at 400 MHz for ^1H and at 100 MHz for ^{13}C. Chemical shifts are expressed in ppm and coupling constants (*J*) in Hz.

Mass spectra were obtained using an LXQ linear ion trap mass spectrometer (Thermo Fisher, Bremen, Germany) equipped with an ESI source operated in the negative ion mode. Mass spectra of the isolated compounds (**1**-**3**) were obtained using direct infusion. All data were acquired and processed using Xcalibur software, version 2.0. (Thermo Fisher). Accurate mass measurements were carried out using a QTOF 6530 mass spectrometer (Agilent technologies) equipped with an electrospray ionisation (ESI)

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source operated in the positive ion mode. Accurate mass measurements were obtained using external calibration. MS spectra of the isolated compounds (**1**-**3**) were obtained using direct infusion. All data were acquired and processed using MassHunter software, version B.06.

Specific rotation was determined using a Jasco P-2000 polarimeter (VA Ijsselstein, The Netherlands).

2.2. Plant material

Boldoa purpurascens Cav. ex Lag. was collected in November 2014 at the experimental station "Las Antillas" of the Las Villas University Cuba and was identified by Cristobal Rios Albuernes, agronomical engineer and specialist of the Botanical Garden of this University. A voucher specimen (No. 3012) is deposited at the Herbarium of the Botanical Garden of this University.

2.3. Isolation of flavonoids

The flavonoid fraction was obtained according to the procedure described by González et al., 2008. The fraction (874 mg) was separated by repeated semi-preparative HPLC with an Autopurification™ system with Quattro Micro-mass detector (Waters) together with a Luna C18 Column (Phenomenex) (250 × 10 mm, 5 μm). The injection volume of a 10 mg/mL 70% methanol solution of the flavonoid mixture was 500 μL and the solvent program was as follows: solvent A: water + 0.1% formic acid; solvent B: acetonitrile + 0.1% formic acid. The gradient conditions were as follows: 0–24 min, 22% B; 25–33 min, 95% B; 34–41 min, 22% B. A flow of 4.75 mL/min was used. Mass spectra were obtained in positive ion mode with following settings: capillary voltage 3.5 KV, cone voltage 50 V, source temperature 140 °C, desolvation temperature 400 °C, desolvation gas flow 800 L/Hr, cone gas flow 50 L/h. Compounds were collected based on the *m/z* value of the ions of the aglycone moiety of the flavonoids i.e. *m/z* 317, *m/z* 315, *m/z* 331.

6-Methoxykaempferol-3-O-[*α*-L-rhamnopyranosyl-(1‴ → *2″)]-β-D-xylopyranoside* [1]*:* light yellow solid; $[\alpha]_D^{20}$ −84.57° (DMSO, c 0.36 g/100 mL); ^{1}H- and ^{13}CNMR: see Tables 1 and 2; ESI–MS (neg. ion mode): *m/z* 593 [M − H]−; HR-MS (pos. ion mode): *m/z* 617.1430 $[M + Na]^+$ (calculated for $C_{27}H_{30}NaO_{15}$ 617.1477).

3,4′,5-Trihydroxy-6,7-methylenedioxyflavone-3-O-[*α*-L-rhamnopyranosyl-(1‴ → *2″)]-β-D-glucopyranoside* (**2**)*:* light yellow solid; $[\alpha]_D^{20}$-110.33° (DMSO, c 0. 66 g/100 mL); ^{1}H- and ^{13}C NMR: see Tables 1 and 2; ESI–MS (neg. ion mode): *m/z* 621 [M − H]−; HR-MS (pos. ion mode): *m/z* 645.1272 $[M + Na]^+$ (calculated for $C_{24}H_{30}NaO_{19}$ 645.1273).

3,4′,5′,5-Tetrahydroxy-6,7-methylenedioxyflavone-3-O-[*α*-L-rhamnopyranosyl-(1‴ → *2″)]-β-D-xylopyranoside* (**3**)*:* yellow powder; $[\alpha]_D^{20}$-32.52°(DMSO,c 0. 34 g/100 mL); ^{1}H- and ^{13}C NMR: see Tables 1 and 2; ESI–MS (neg. ion mode): *m/z* = 607 [M − H]−; HR-MS (pos. ion mode): *m/z* 631.1252 $[M + Na]^+$ (calculated for $C_{27}H_{27}NaO_{16}$ 631.1270).

2.4. Hydrolysis

The major compound isolated before from *B. purpurascens* [3,4′,5-trihydroxy-6,7-methylenedioxyflavone-3-*O*-α-L-rhamnopyranosyl-(1‴ → 2″)-β-D- xylopyranoside] (**4**) (González et al., 2008) (18.3 mg) was dissolved in 2 N HCl (H_2O-MeOH 1:1, 18.3 mL) and hydrolysed by heating at 100 °C during 3 h. After evaporation of the solvent under vacuum, the residue was suspended in H_2O (36.6 mL) and the mixture extracted with ethyl acetate

Table 1
^{1}H NMR data for compounds **1**, **2** and **3** (400 MHz).

	Proton	1 (CD_3OD)	2 (CD_3OD)	3 (DMSO-d_6)
Aglycone	Position	δ_H	δ_H	δ_H
	2′	8.02 (d, *J* = 8.97 Hz)	8.06 (d, *J* = 9.06 Hz)	6.84 (d, *J* = 8,81 Hz)
	3′′	6.88 (d, *J* = 8.82 Hz)	6.89 (d, *J* = 8.91 Hz)	
	5′	6.88 (d, *J* = 8.82 Hz)	6.89 (d, *J* = 8.91 Hz)	7.56 (t,[a])
	6′	8.02 (d, *J* = 8.97 Hz)	8.06 (d, *J* = 9.06 Hz)	7.57 (t,[a])
	4′-OH			
	5-OH			
	8	6.49 (s)	6.67 (s)	6.91 (s)
	CH_3O	3.86 (s)		
	OCH_2O		6.08 (s)	6.17 (s)
β-D-Xylose	Position			
	1	5.61 (d, *J* = 7.0 Hz)		5.52 (d, *J* = 7.4 Hz)
	2	3.63 (t)		3.57 (t)
	3	3.49 (m)		3.34 (m)
	4	3.48 (m)		3.35 (m)
	5	3.7 (dd, *J* = 4.83 Hz, 11.73 Hz)		3.60 (m,[a])
		3.07 (dd, *J* = 8.59 Hz, 11.17 Hz)		2.95 (dd, *J* = 8.94 Hz, 11.35 Hz)
α-L- Rhamnose	Position			
	1	5.19 (d, *J* = 1.16 Hz)	5.26 (d, *J* = 1.23)	5.08 (brs)
	2	3.99 (m)	4.02 (m)	3.73 (m)
	3	3.77 (m)	3.78 (m)	3.48 (m)
	4	3.35 (t)	3.35 (m)	3.14 (m)
	5	4.05 (m)	4.06 (m)	3.78 (m)
	6	1.04 (d, *J* = 6.39 Hz)	0.98 (d, *J* = 6.25 Hz)	0.87 (d, *J* = 6.22 Hz)
β-D-Glucose	Position			
	1		5.75 (d, *J* = 7.33)	
	2		3.63 (m)	
	3		3.59 (m)	
	4		3.27 (m)	
	5		3.29 (m)	
	6		3.72 (dd, *J* = 2.02 Hz, 11.82 Hz)	

[a] Could not be determined due to overlap.