Phytochemistry Letters 3 (2010) 9–12

Contents lists available at ScienceDirect

Phytochemistry Letters

journal homepage: www.elsevier.com/locate/phytol

Benzochromenes from the roots of *Bourreria pulchra*

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ARTICLE INFO

Article history:
Received 7 October 2009
Received in revised form 14 October 2009
Accepted 16 October 2009
Available online 30 October 2009

Keywords:
Bourreria pulchra
Boraginaceae
Benzochromene
Antiprotozoal
Leishmania mexicana
Trypanosoma cruzi

ABSTRACT

Two new benzochromenes, (6,6-dimethyl-2-methoxy-6*H*-benzo[c]chromen-9-yl)methanol (**1**), and 2-methoxy-6,6-dimethyl-6*H*-benzo[c]chromen-9-carbaldehyde (**2**), together with several already known metabolites, were isolated from the root extract of *Bourreria pulchra* (Boraginaceae). The structures of **1** and **2** were established on the basis of their spectroscopic data. Both were assayed for *in vitro* antiprotozoan activity, and especially **1** was found to possess significant activity against *Leishmania mexicana* and *Trypanosoma cruzi* parasites (IC_{50} 4.6 μg/mL and 7.5 μg/mL, respectively).

1. Introduction

Bourreria pulchra Millsp. (Boraginaceae), commonly known as "bakalche" and "azar del monte", is a medicinal plant used in Yucatecan traditional medicine as an antiviral and an antipyretic, and for the treatment of cutaneous diseases (Argueta et al., 1994). Although, to date, there are no reports on the isolation and characterization of secondary metabolites from *B. pulchra*, it has been reported that the root extract of *B. pulchra* shows DNA-interacting activity when tested using the DNA-methyl green assay (Fuentes-García, 2003). In the course of our continuing investigations into the chemistry of this genus, two new benzochromene derivatives together with twelve known compounds [didehydroconicol (Simon-Levert et al., 2005), 6-hydroxy-2,2-dimethyl-2*H*-1-benzopyran (Howard et al., 1979), oresbiusin A (Huang et al., 1996), rosmarinic acid methyl ester (Sawabe et al., 2006), 2,5-dihydroxybenzaldehyde (Pouchert and Behnke, 1993), 3-acetoxyolean-12-en-28-al (Kim et al., 2004), 3-acetoxyolean-12-en-28-oic acid (Ikuta and Itokawa, 1989), physcion (Danielsen et al., 1992), β-sitosterol (Garg and Nes, 1984), α- and β-amyrin (Lima et al., 2004), and 1,4-diphenyl-2,3-butanediol (Robbins et al., 1999)], were isolated from the organic extracts of the stem bark and roots of *B. pulchra*. This paper reports the structure elucidation of the new metabolites and the results of their evaluation for biological activity.

2. Results and discussion

The extract of the roots of *B. pulchra* was fractionated using a liquid–liquid partition procedure with solvents of increasing polarity. The heptane fraction was then purified using a combination of VLC, open column chromatography, and gel permeation (Sephadex LH-20), to produce the two new metabolites **1** and **2** in pure form.

The ESI-HRMS of **1** showed a protonated $[M+H]^+$ molecular ion peak at *m*/*z* 271.1350, indicating a molecular formula of $C_{17}H_{18}O_3$ and the presence of nine degrees of unsaturation. The ^1H NMR spectrum (all ^1D NMR data are given in Table 1) of **1** showed the presence of six aromatic protons, and their coupling constants as well as COSY couplings indicated that **1** had two 1,2,4-trisubstituted benzene rings. This was confirmed by the HMBC correlations from H-1 to C-2, C-3 and C-4a, from H-3 to C-1, C-2 and C-4a, from H-4 to C-2 and C-10b, from H-7 to C-9 and C-10a, from 8-H to C-6a and C-10, as well as from H-10 to C-6a and C-8. The two rings were connected at C-10a and C-10b, as shown by the HMBC correlations from H-1 to C-10a and from H-10 to C-10b. C-2

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doi:10.1016/j.phytol.2009.10.002

Table 1
^{1}H and ^{13}C NMR ($CDCl_3$) data for compounds **1** and **2**. The multiplicities of the ^{13}C signals were extracted from HMQC experiments.

No.	Compound **1**		Compound **2**	
	^{1}H (δ)	^{13}C (δ)	^{1}H (δ)	^{13}C (δ)
1	7.27, 1 H, d, J = 2.8	107.8 (d)	7.31, 1 H, d, J = 3.0	107.8 (d)
2	–	154.4 (s)	–	154.8 (s)
3	6.81, 1 H, dd, J = 2.8, 8.8	115.3 (d)	6.85, 1 H, dd, J = 3.0, 8.8	116.5 (d)
4	6.88, 1 H, d, J = 8.8	118.7 (d)	6.91, 1 H, d, J = 8.8	119.0 (d)
4a	–	146.7 (s)	–	146.7 (s)
6	–	77.2 (s)	–	77.3 (s)
6a	–	139.3 (s)	–	146.0 (s)
7	7.23, 1 H, d, J = 8	123.5 (d)	7.41, 1 H, d, J = 8	124.1 (d)
8	7.30, 1 H, dd, J = 1.6, 8	126.7 (d)	7.80, 1 H, dd, J = 1.6, 8	129.6 (d)
9	–	140.2 (s)	–	135.9 (s)
10	7.77, 1 H, d, J = 1.6	120.9 (d)	8.17, 1 H, d, J = 1.6	123.2 (d)
10a	–	128.9 (s)	–	129.9 (s)
10b	–	122.8 (s)	–	121.8 (s)
11	1.61, 3 H, s	27.3 (q)	1.64, 3 H, s	27.1 (q)
12	1.61, 3 H, s	27.3 (q)	1.64, 3 H, s	27.1 (q)
13	4.74, 2 H, s	65.2 (t)	10.06, 1 H, s	192.0 (d)
2-OMe	3.84, 3 H, s	55.8 (q)	3.87, 3 H, s	55.9 (q)

was substituted by a methoxy group, demonstrated by the HMBC correlations from the methoxy protons to C-2 as well as by the chemical shift of C-2. The ^{1}H signal for H_2-13 appeared as a singlet integrating for two protons at δ 4.74, and H_2-13 gave HMBC correlations to C-8, C-9 and C-10. The ^{13}C chemical shift for C-13 was δ 65.2, suggesting that this was a hydroxymethyl group situated on C-9. In fact, when the ^{1}H spectrum of **1** was recorded in DMSO-d_6 the signal for OH-13 appeared as a doublet at δ 5.19, coupling with H_2-13 with the coupling constant of 5.8 Hz. The chemically equivalent methyl groups of C-11 and C-12 both gave HMBC correlations to each other, showing that they were geminal. In addition, H_3-11 and H_3-12 gave HMBC correlations to C-6 and C-6a. The ^{13}C chemical shift for C-6 showed that it was oxygenated, and with that all atoms available for the structure have been used. The remaining unsaturation must then be formed by an ether link between C-4a and C-6. Based on these observations, the structure of **1** was established as (6,6-dimethyl-2-methoxy-6*H*-benzo[c]-chromen-9-yl)methanol (Fig. 1).

The ESI-HRMS of the second metabolite (**2**) showed a protonated ($[M+H]^+$) molecular ion peak at m/z 269.1163, indicating the molecular formula $C_{17}H_{16}O_3$ and the presence of ten degrees of unsaturation. The NMR data for **2** were quite similar to those of **1**, except that the $^{1}H/^{13}C$ signals for the hydroxymethyl moiety of **1** had been substituted for signals for an aldehyde group in the spectra of **2**. The corresponding COSY and HMBC correlations were observed in the spectra of **2** as those discussed above for **1**, and the aldehyde proton H-13 gave HMBC correlations to C-8, C-9 and C-10. On the basis of this analysis, the new metabolite **2** was identified as 6,6-dimethyl-2-methoxy-6*H*-benzo[c]chromen-9-carbaldehyde.

An evaluation of the *in vitro* antiprotozoal activity of the new compounds was carried out, and the results are summarized in Table 2. Both compounds were weakly active against promastigotes of *Leishmania amazonensis* and *L. braziliensis*, but **1** had a significant activity against promastigotes of *L. mexicana* and epimastigotes of *T. cruzi* (IC_{50} values of 4.6 and 7.5 μg/mL, respectively). Due to the limited amounts of compound **2** available, its antiprotozoal activity could only be assayed towards the two former organisms. Even though the antiprotozoal activity of **1** can be considered moderate, the limited number of functionalities in its structure that could be associated with toxicity suggests that the antiprotozoal activity of the new metabolite is selective. Furthermore, the structural differences between **1** and pentamidine suggest different molecular targets in the parasites for each, and opens the possibility of increasing the potency of **1** through synthetic modification of its structure. An initiative to prepare analogues of **1** has been launched, and will hopefully shed light on this issue.

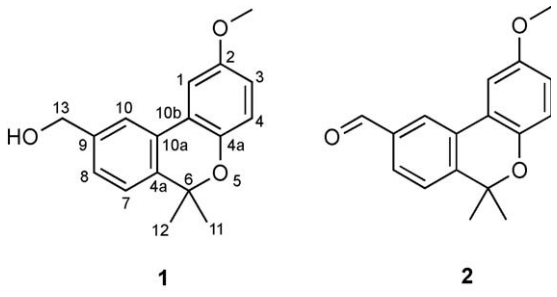

Fig. 1. Structures of the new metabolites **1** and **2**.

3. Experimental

3.1. General experimental procedures

Optical rotations were measured on a Perkin Elmer (Model 341) polarimeter. ESI-HRMS spectra were recorded on a Waters Q-TOF Micro-system spectrometer, using H_3PO_4 for calibration and as internal standard. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) were determined using a Bruker DRX 500 spectrometer; the spectra were recorded in $CDCl_3$ at 27 °C and the solvent residual signals (δ_H 7.26 and δ_C 77.0 ppm) were used as reference. The chemicals shifts (δ) are given in ppm, and the coupling constants (J) in Hz. Vacuum liquid chromatography (VLC) separations were carried out using TLC-grade silica gel (Merck), while flash and column chromatography separations were run using silica gel 60 (230–400 mesh, Merck). Sephadex LH-20 (GE Healthcare) was

Table 2
Antiprotozoal activity of compounds **1** and **2** (IC_{50} μg/mL). Pentamidine and amphotericin B were used as positive control.

	Compounds		Pentamidine	Amphotericin B
	1	**2**		
Organism				
Leishmania mexicana	4.6	NT	0.45	NT
Trypanosoma cruzi	7.5	NT	0.45	NT
Leishmania amazonensis	69	115	NT	1.75
Leishmania braziliensis	29	26	NT	1.75