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Original Article

Coumarins of *Loricaria ferruginea*

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ABSTRACT

In the presented research we isolated and characterized compounds from *Loricaria ferruginea* (Ruiz & Pav.) Wedd., Asteraceae. To the best of our knowledge no data on any compounds from *L. ferruginea* have been published to this day. As main compounds of the hexane extract we found four known coumarins: 5,7-dimethoxycoumarin; 5,7,8-trimethoxycoumarin; 5-hydroxyobliquine and 5-methoxyobliquine. All the structures were determined by spectroscopic and spectrometric methods.

Introduction

The Asteraceae are the second most diverse family in the Peruvian flora, with approximately 250 genera and 1590 species (Dillon and Hensold, 1993; Ulloa Ulloa et al., 2004). Most Peruvian Asteraceae are herbs, shrubs and subshrubs (Beltrán et al., 2006). The genus *Loricaria* contains currently 22 mostly rhizomatous perennial and a few annual species, with conspicuous flat leaves and laterally compressed stems, glabrous or pubescent achenes with glandular-stipitate biseriate hairs (Hind, 2011). Most species of *Loricaria* are restricted to high altitude grasslands.

De Feo (1992) reported two species of *Loricaria* being used in the divination practices in the northern Peruvian Andes. Other authors described the traditional use of *Loricaria ferruginea* (Ruiz & Pav.) Wedd. for menstrual delay, blood circulation, and ritual purposes (spiritual flowering, protection, good health, good fortune, good business, fragrance, success, good travels, becoming sociable and good relations with others) (Bussmann and Sharon, 2007). However, a toxicological brain shrimp bio-assays showed a LC_{50} 15 μg/ml in ethanol (Bussmann et al., 2011).

Up to 2009 more than 1300 coumarins were isolated from plants, bacteria, and fungi (Iranshahi et al., 2009). Coumarins consist of a large class of phenolic substances biosynthesized by medicinal plants. These type of secondary metabolites are known for their pharmacological properties such as anti-inflammatory, anticoagulant, antibacterial, antifungal, antiviral, anticancer, antihypertensive, antitubercular, anticonvulsant, antiadipogenic, antihyperglycemic, antioxidant, and neuroprotective properties (Iranshahi et al., 2009; Venugopala et al., 2013).

In this work, we report the isolation and characterization of four known coumarin derivatives (Basnet et al., 1993; Bohlmann and Zdero, 1980): 5,7-dimethoxycoumarin (**1**); 5,7,8-trimethoxycoumarin (**2**); 5-hydroxyobliquine (**3**) and 5-methoxyobliquine (**4**).

To the best of our knowledge no constituents from *L. ferruginea* have been reported so far. The structures of these coumarins were elucidated by spectroscopic methods.

Materials and methods

NMR and MS infrastructure and methods

NMR spectra (^{1}H, ^{13}C, APT, NOESY1D, H,H-COSY, edited HSQC, and HMBC) were recorded on a Varian Mercury 400 plus (400 MHz for ^{1}H, 100 MHz for ^{13}C) and a Varian Mercury 300 plus (300 MHz for ^{1}H, 75 MHz for ^{13}C) spectrometer, respectively, at 26 °C and with $CDCl_3$ as a solvent. The chemical shifts were reported relative to the residual solvent peak, used as an internal reference (^{1}H: 7.26 ppm, ^{13}C: 77.16 ppm). Chemical shifts are given in δ values, coupling constants *J* in Hz. All separations were carried out in carefully purified and dried solvents and were monitored by thin-layer chromatography (TLC) on plates of Silufol UV/VIS 254 nm. Preparative column chromatography was carried out on silica gel (MERCK 70–230 mesh) in gradient regime.

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Plant material

Bulk material of *Loricaria ferruginea* (Ruiz & Pav.) Wedd., Asteraceae, was collected in March 2009 from Huamachuco, Sanchez Carrion Province-Peru and identified by Botanist Eric F. Rodríguez Rodríguez at Herbarium Truxillense (HUT), National University of Trujillo, Peru. A voucher specimen under No.50003 (HUT) documenting the collection was deposited at Herbarium Truxillense (HUT) in Peru.

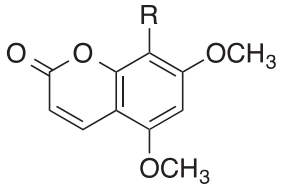
Preparation of plant extracts

The crude extract of the powder plant material was obtained by maceration using hexane (200 g/3 l) for five days, followed by filtration and evaporation under reduce pressure, with a final yield of 5.2 g (2.60%).

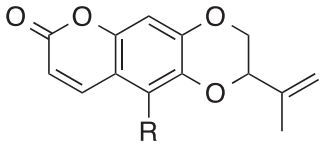
Isolation

The hexane extract (5.2 g) was purified by CC using an eluent system of hexane/ethyl acetate (5:3) and the resulting fractions were combined according to TLC profiles. Six fractions were obtained; the first fraction (468 mg) was eluted with hexane/acetone (10:1) to yield 40 mg of 5-methoxyobliquine (**4**) (R_f 0.71) and the second fraction (1.1 g) containing 5,7-dimethoxycoumarin (**1**) was purified again with hexane/ethyl acetate (2:1) and yielded 28.1 mg of (**1**) (R_f 0.61).

The third to the fifth fraction (520.7 mg) was eluted with hexane/acetone (2:1) to give 43.1 mg of 5,7,8-trimethoxycoumarin (**2**) (R_f 0.91). The sixth fraction (406.1 mg) was eluted with dichloromethane/ethyl acetate (10:0.5) yielding 61.3 mg of 5-hydroxyobliquine (**3**) (R_f 0.5).



1 R=H
2 R=OCH_3



3 R=OH
4 R=OCH_3

Results and discussion

Spot tests were used for the qualitative determination of secondary metabolites present in *L. ferruginea* (Dominguez, 1973; Harborne, 1984). We identified coumarins by NaOH. The filter paper was then examined under UV light, with yellow fluorescence indicating the presence of coumarins. Steroids and triterpenoids (dark green) were identified by the Liebermann–Burchard's test, flavonoids (red) by the Shinoda test (both only in small amounts), and no alkaloids were detected using Dragendorff's test.

Previously, 5,7-dimethoxycoumarin (**1**) was reported with cytotoxic activity against different types of cancer cells, and as potent inhibitor of iNOS expression (Nakamura et al., 2009; Riveiro et al., 2009), and 5,7,8-trimethoxycoumarin (**2**) was reported as anti-HIV active (Cheng et al., 2005).

The spectra of all coumarin derivatives are in agreement with literature results (Bohlmann and Zdero, 1980; Jaensch et al., 1989; Osborne, 1989; Maes et al., 2008).

5,7-Dimethoxycoumarin (**1**) white solid; 28.1 mg (0.5%); R_f = 0.61 (hexane/EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.86 (s, 3H, C7-OCH_3), 3.89 (s, 3H, C5-OCH_3), 6.16 (d, J = 9.6 Hz, 1H, C3-H), 6.29 (d, J = 2.29 Hz, 1H, Ar–H), 6.42 (d, J = 2.29 Hz, 1H, Ar–H), 7.97 (d, J = 9.6 Hz, 1H, C4-H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 55.73, 55.91, 93.00, 94.87, 103.87, 110.72, 138.67, 156.69, 156.92, 161.39, 163.67.

5,7,8-Trimethoxycoumarin (**2**) white solid; 43.1 mg (0.82%); R_f = 0.91 (hexane/acetone); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.91 (s, 3H, C5-OCH_3), 3.91 (s, 3H, C7-OCH_3), 3.96 (s, 3H, C8-OCH_3), 6.16 (d, J = 9.7 Hz, 1H, C3-H), 6.34 (s, 1H, C6-H), 7.99 (d, J = 9.7 Hz, 1H, C4-H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 56.26, 56.71, 61.82, 91.67, 104.27, 111.48, 130.52, 139.02, 148.98, 152.58, 156.35, 161.07.

5-Hydroxyobliquine (**3**) Yellow solid; 61.3 mg (1.17%); R_f = 0.50 (DCM/EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.84 (s, br, 13-H), 3.98 (dd, J = 9.6, 1.2 Hz, 9′-H), 4.34 (dd, 9.6, 1.2 Hz, 9-H), 4.51 (dd, br, J = 9.6, 1.2 Hz, 10-H), 5.12 (s, br, 12-H), 5.17 (s, br, 12′-H), 6.17 (d, J = 9.6 Hz, 3-H), 6.56 (s, 8-H), 7.88 (d, J = 9.6 Hz, 4-H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 18.94, 67.68, 75.69, 99.69, 107.68, 112.92, 114.71, 133.38, 138.70, 139.12, 144.39, 147.60, 148.65, 161.31.

5-Methoxyobliquine (**4**) Yellow solid; 40 mg (0.77%); R_f = 0.71 (hexane/acetone); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.87 (s, br, 13-H), 4.00 (s, OCH_3), 4.04 (dd, 9.6, 1.2 Hz, 9′-H), 4.38 (dd, 9.6, 1.2 Hz, 9-H), 4.54 (dd, br, 9.6, 1.2 Hz, 10-H), 5.13 (s, br, 12-H), 5.19 (s, br, 12′-H), 6.22 (d, J = 9.6 Hz, 3-H), 6.61 (s, 8-H), 7.92 (d, J = 9.6 Hz, 4-H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 18.94, 61.81, 67.67, 75.69, 99.67, 107.67, 112.91, 114.70, 133.36, 138.68, 139.11, 144.37, 147.58, 148.63, 161.30.

Conclusions

The present paper describes the isolation and characterization of four constituents of *L. ferruginea*: 5,7-dimethoxycoumarin (**1**); 5,7,8-trimethoxycoumarin (**2**); 5-hydroxyobliquine (**3**) and 5-methoxyobliquine (**4**). The results may be helpful in further investigations of the biological activity of these natural compounds.

Authors' contributions

GRMG (PhD student) contributed running the laboratory work, and drafted the paper; LH did the NMR investigations; EFRR has identified the species and revised the paper and RWB contributed in collecting plant samples and revised the paper.

All the authors have read the final manuscript and approved the submission.

Conflicts of interest

The authors declare no conflicts interest.

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