

Isocordoin Derivatives From the Root Extract of *Lonchocarpus xuul*

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Abstract. Two natural isocordoin derivatives, dihydroisocordoin (**1**) and flemistricin B (**2**), were isolated from the root extract of *Lonchocarpus xuul*. Both metabolites were identified on the basis of their spectroscopic data and by comparing them with those reported in the literature.

Key words: *Lonchocarpus xuul*, Leguminosae, Chalcones, Dihydroisocordoin, Flemistricin B.

Resumen: Del extracto de la raíz de *Lonchocarpus xuul* fueron aislados dos derivados naturales de isocordoina, dihidroisocordoina (**1**) y flemistricina B (**2**). Ambos metabolitos fueron identificados con base en sus datos espectroscópicos y por comparación de los mismos con los reportados en la literatura.

Palabras clave: *Lonchocarpus xuul*, leguminosa, chalcones, dihidroisocordoina, flemistricina B.

Introduction

Lonchocarpus xuul Lundell (Leguminosae) is a tree endemic to the Yucatan Peninsula, which is known as “xuul”, “kan-xuul” or “yaax-xuul”. Previous phytochemical studies of *L. xuul* have resulted in the isolation and identification of a number of flavonoids (e.g. chalcones, flavones, flavans, pyranoflavans, flavanones), including the two novel flavans xuulanin and 3 β -methoxy-xuulanin, and the known flavanone spinoflavanone-B. [1-3] In our continuing search for novel secondary metabolites from Yucatecan native plants, we wish to report herein on the isolation and identification of two additional isocordoin derivatives from the root extract of *L. xuul* Lundell.

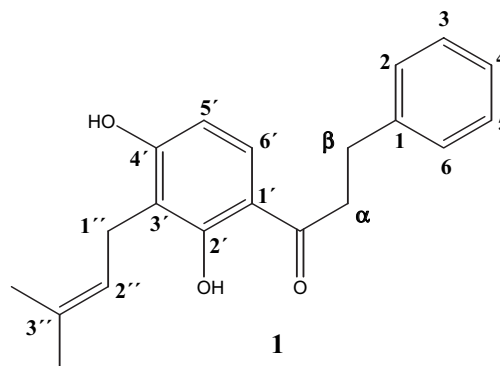
Results and Discussion

Successive chromatographic purifications of the hexane root extract of *L. xuul*, using a combination of VLC, gravity column chromatography and gel filtration on Sephadex LH-20, resulted in the isolation of metabolites **1** and **2** in pure form.

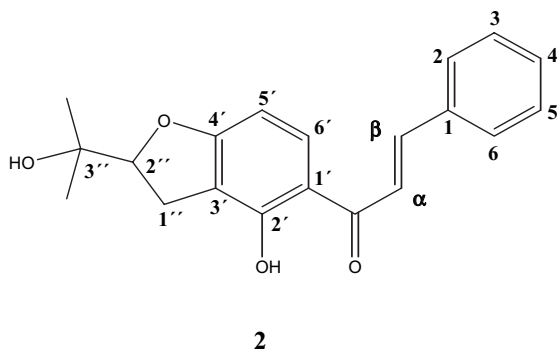
The less polar metabolite **1** was obtained as colorless oil. The parent ion peak at m/z 310 of its mass spectrum suggested a molecular formula of C₂₀H₂₂O₃, which in turn indicated the presence of ten unsaturation sites in the structure. The IR spectrum of **1** exhibited strong absorption bands for hydroxyl (3421 cm⁻¹) and carbonyl (1613 cm⁻¹) groups. The ¹H NMR spectrum of **1** showed a number of signals that suggested an isocordoin-type structure, namely two one-proton signals at 6.35 (H-5', d , 8.8) and 7.54 (H-6', d , 8.8), corresponding to the two *ortho*-coupled protons of a 1,2,3,4 tetrasubstituted aromatic ring, and two multiplets at 7.30 (2H) and 7.25 (3H), corresponding to the five protons of a second, monosubstituted, aromatic ring. While the monosubstituted nature of the aromatic ring was further confirmed by the fragment ion peak at m/z 77 in the mass spectrum of **1**, a chelated hydroxyl-proton

signal at 13.14, together with a chelated carbonyl-carbon resonance at δ 203.92, were consistent with a 2'-hydroxychalcone-type structure [4]. However, unlike isocordoin, the ¹H NMR spectrum of metabolite **1** did not show the two one-proton signals of the trans-olefinic protons assigned to α and β positions of the unsaturated ketone system; instead, two methylene signals at 3.04 (2H, t , 7.7) and 3.24 (2H, t , 7.7) were observed. Accordingly, the IR spectrum of **1** did not show an absorption band characteristic of a conjugated carbonyl system and the observed molecular ion peak at m/z 310 of **1**, indicated one less unsaturation site in the molecular structure when compared to that of isocordoin. Finally, the observed fragment ions at m/z 105 and 91 in the MS of **1** confirmed a dihydrochalcone-type structure [5]. On the basis of its spectroscopic data, metabolite **1** was identified as dihydroisocordoin, a dihydrochalcone previously reported as an intermediate in the semisynthesis of tetrahydroflemichapparin-A [6], and recently reported as erios-chalcone B, a novel bioactive (antimicrobial) natural product from *Eriosema glomerata* [7].

Metabolite **2** was isolated as a yellow oil, which appeared as an intense deep brown spot when run on TLC and observed under UV light. The HR-EIMS of **2** indicated a molecular formula C₂₀H₂₀O₄, suggesting an isocordoin-type structure



with an extra oxygen atom. Accordingly, the IR spectrum of **2** showed the isocordoin-characteristic absorption bands at 3472 (hydroxyl) 1639 (carbonyl) and 1592 (aromatic) cm^{-1} , and its ^1H NMR showed the expected signals for a chalcone, including the protons corresponding to both a monosubstituted and a tetrasubstituted aromatic rings and those corresponding to the protons of a *trans* double bond [$\text{H}-\alpha$ at 7.57 ppm (*d*, 15.6) and $\text{H}-\beta$ at 7.86 ppm (*d*, 15.6)] and a hydrogen-bonded phenol group (13.40 ppm, *s*). The main difference between the ^1H NMR spectrum of **2** and that of isocordoin was the absence of the signals corresponding to the prenylated chain, and the presence of an oxygenated methine-proton signal at 4.78 ppm (*dd*, 8.4, 9.6). The presence of additional signals corresponding to a methylene (3.16 ppm, *m*) and to a *gem*-dimethyl group [1.24 (*s*) and 1.35 ppm (*s*)] in the ^1H NMR spectrum of **2**, together with a quaternary carbon signal at 71.91 ppm in its ^{13}C NMR spectrum, allowed the identification of an isopropoxy dihydrofuran moiety in the structure of **2** [8, 9]. The formation of a monoacetylated derivative, upon treatment of **2** with acetic anhydride-pyridine, confirmed the presence of a tertiary alcohol in the structure. Similarly, the correlation observed, in the HMBC experiment of **2**, between the oxymethine proton ($\text{H}2''$) and the phenolic carbon ($\text{C}-4'$), together with the hydrogen-bonded phenol group (13.40 ppm, *s*) in the ^1H NMR spectrum, confirmed the arrangement of the dihydrofuran ring. The spectroscopic data of metabolite **2** proved to be identical to those reported for flemistrictin B, a chalcone isolated from the leaves of *Flemingia stricta* [5] and the seeds of *Lonchocarpus sericeus* [10]. It is interesting to mention that, to date, the stereochemistry at $\text{C}-2''$ of flemistrictin B has not been reported; this is probably due to the fact that **2** appears to occur naturally as a racemic mixture, resulting from the non-stereospecific cyclization between the phenolic hydroxyl group at $\text{C}-4'$ and the epoxidated prenylated chain at $\text{C}-3'$, as suggested by the very small value (+3.3) of its optical rotation.



Experimental section

General Experimental Procedures

Vacuum Liquid Chromatography (VLC) and column chromatography purifications were performed using E.M. Merck

TLC-grade silica gel 60_{GF} and E.M. Merck silica gel (70-230 mesh), respectively. Gel permeation column chromatography purifications were carried out using Sephadex LH-20 (Sigma, size 25-100). Analytical TLC analyses were carried out using aluminum-backed silica gel (60F₂₅₄) plates (E.M. Merck, 0.2 mm thickness); the plates were first examined under UV light (λ 254 and 366 nm) and the various components in the chromatograms were visualized by dipping the plates in a solution of phosphomolybdic acid (20 g) and ceric sulfate (2.5 g) in 500 mL of sulfuric acid (5%), followed by drying and gentle heating. The optical rotation was measured in CHCl_3 using a Perkin Elmer 341 polarimeter. IR spectra were recorded in CHCl_3 (film) using an FT-Nicolet Magna Protégé 460 spectrophotometer. ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were obtained in CDCl_3 , on a Bruker Avance 400 spectrometer, using the residual CHCl_3 signal (7.26 and 77.00 ppm for ^1H and ^{13}C , respectively) as reference. GC analyses were run on a Hewlett Packard 5890 gas chromatograph [GC conditions: split injection of 1 μL of sample; Ultra 1 column (25 m \times 0.2 mm i.d.), flow rate 1.0 mL/min (Nitrogen); oven temperature program $T_1=180^\circ\text{C}$ (3 min), $T_2=280^\circ\text{C}$ (15 min), gradient $10^\circ\text{C}/\text{min}$, injector 300° and detector (FID) 300°C]. Mass spectra were performed with a JEOL-JMS-SX102 and ESI-HRMS (Electro-Spray Ionization Mass with the Waters Q-TOF microsystem) using 0.1% phosphoric acid in a 1:1 water/acetonitrile mixture as reference.

Plant material

The roots of *L. xuul* were collected in September 2007 from plants growing in a field located at km 6 of the Libre Unión-Yaxcabá highway in Yucatán, Mexico. A voucher specimen has been deposited at the herbarium of "Unidad de Recursos Naturales CICY", under the collection number 1089. The plant material was washed with tap water and dried, first for a week at room temperature, and then for 72 h in an oven at 55°C .

Extraction and isolation

The dry and ground roots (1.8 kg) were extracted four times with hexane at room temperature; the solution was filtered and evaporated to produce 11.5 g of crude hexane extract, which was subjected to VLC purification, eluting with increasing amounts of acetone in hexane to produce four main fractions. Fraction 3 was further purified by VLC, again using a gradient elution with hexane-acetone mixtures. Successive purifications using gravity column chromatography (benzene 100%, followed by hexane/acetone 9:1 and 8:2) and Sephadex LH-20 (MeOH), resulted in the isolation of **1** (10 mg) and **2** (40 mg) in pure form.

Dihydroisocordoin (**1**)

Colorless oil; soluble in CHCl_3 and EtOAc. R_f 0.35 in hexane-acetone 9:1. t_R (GC) = 12.89 min. IR (CHCl_3 , film): 3421 cm^{-1} (OH), 2924 cm^{-1} (C-H), and 1613 cm^{-1} (C=O). MS m/z 310.2

[M⁺]. ¹H NMR (CDCl₃, 400 MHz) δ 7.30 (2H, m, H-2/H-6), 7.25 (3H, m, H-3,4,5), 6.35 (1H, d, *J*= 8.8, H-5'), 7.54 (1H, d, *J*= 8.8, H-6'), 3.44 (2H, da, *J*= 7.1, H-1''), 5.26 (1H, tt, *J*= 1.3, 6.1, H-2''), 1.76 (3H, s, CH₃), 1.82 (3H, s, CH₃), 3.24 (2H, t, *J*= 7.7, Hα), 3.04 (2H, t, *J*= 7.7, Hβ), 6.06 (1H, s, OH-4'), 13.14 (1H, s, OH-2'); ¹³C NMR (CDCl₃, 100MHz) δ 141.1 (C-1), 128.5 (C-2/C-6), 128.7 (C-3/C-5), 126.4 (C-4), 113.4 (C-1'), 162.8 (C-2'), 114.1 (C-3'), 161.5 (C-4'), 107.9 (C-5'), 129.6 (C-6'), 21.8 (C-1''), 121.1 (C-2''), 136.1 (C-3''), 26.0 (CH₃), 18.1 (CH₃), 39.8 (Cα), 30.6 (Cβ), 203.9 (C=O).

Flemistricin B (2)

Yellow oil, soluble in CHCl₃ and EtOAc. *R*_f 0.17 in hexane-CH₂Cl₂-EtOAc 6:3:1. [α]_D²⁷ +3.3 (*c* 0.0083, CHCl₃). *t*_R (GC) = 15.33 min. IR (CHCl₃, film): 3472 cm⁻¹ (OH), 2939 cm⁻¹, 2975 cm⁻¹ (C-H), 1639 cm⁻¹ (C=O) and 1592 (C-H, aromatic ring). HRESIMS *m/z* 325.1440 [M⁺ + H] (calcd for C₂₀H₂₁O₄: 325.1439). ¹H NMR (CDCl₃, 400 MHz) δ 7.64 (2H, m, H-2/H-6), 7.42 (3H, m, H-3,4,5), 6.44 (1H, d, *J*= 8.8, H-5'), 7.80 (1H, d, *J*= 8.8, H-6'), 4.78 (1H, dd, *J*= 8.4, 9.6, H-2''), 3.16 (2H, m, H-1''), 1.24 (3H, s, CH₃), 1.35 (3H, s, CH₃), 7.57 (1H, d, *J*= 15.6, Hα), 7.86 (1H, d, *J*= 15.6, Hβ), 13.40 (1H, s, OH-2'); ¹³C NMR (CDCl₃, 100MHz) δ 134.8 (C-1), 128.5 (C-2/C-6), 128.9 (C-3/C-5), 130.6 (C-4), 113.8 (C-1'), 161.5 (C-2'), 114.9 (C-3'), 166.7 (C-4'), 101.8 (C-5'), 131.9 (C-6'), 91.7 (C-2''), 27.3 (C-1''), 71.9 (C-3''), 23.8 (CH₃), 25.9 (CH₃), 120.5 (Cα), 144.2 (Cβ), 192.0 (C=O).

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