ISOLATION OF KAEMPFEROL-3-RUTINOSIDE FROM THE LEAF EXTRACT OF *Sideroxylon foetidissimum* SUBSP. *GAUMERI*

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ABSTRACT

Kaempferol-3-rutinoside (**1**), together with α-amyrin, β-amyrin, acetato de taraxasterilo and stigmastenol, were isolated from the organic crude extract of the leaves of *Sideroxylon foetidissimum* subsp. *gaumeri*. Identification of the various metabolites was carried out by analyzing their spectroscopic data and/or by comparing it with those reported in the literature.

Key words: *Sideroxylon foetidissimum* subsp. *gaumeri*, Sapotaceae, kaempferol-3-rutinoside, *amyrinas*, taraxasteryl acetate.

RESUMEN

Kaempferol-3-rutinósido (**1**), además de α-amirina, β-amirina, acetato de taraxasterol y estigmastenol, fueron aislados del extracto orgánico crudo de las hojas de *Sideroxylon foetidissimum*. La identificación de los diferentes metabolitos se llevó a cabo mediante el análisis de sus datos espectroscópicos y/o por comparación de los mismos con los reportados en la literatura.

Palabras clave: *Sideroxylon foetidissimum* subsp. *gaumeri*, Sapotaceae, kaempferol-3-rutinósido, amyrinas, acetato de taraxasterilo.

INTRODUCTION

Sideroxylon foetidissimum Jacq. subsp. *gaumeri* Pittier (T.D.Penn) is a tree of the Sapotaceae family that grows in the south-eastern areas of Mexico, particularly in Yucatan, where is known as "subul" or "caracolillo" (Argueta, 1994). This ornamental tree is commonly used for construction because its wood is hard, heavy, strong and durable (Argueta, 1994). Chemical studies of the leaves and roots of *S. foetidissimum* and other *Sideroxylon* species have revealed them to be a rich source of flavonoids and triterpenoid saponins (Narod, 2003; Jiang *et al.*, 1994; Nicola *et al.*, 1995; Sánchez-

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Medina *et al.*, 2009). Recently, as part of an ongoing investigation on biologically active secondary metabolites from the native flora of the Yucatan peninsula, the leaf extract of *S. foetidissimum* subsp. *gaumeri* showed DNA-interacting activity when tested using the DNA-methyl green assay (Fuentes-García, 2003). We wish to report herein on the isolation of secondary metabolites from the bioactive leaf extract of *S. foetidissimum* subsp. *gaumeri*.

MATERIAL AND METHODS

General experimental procedures

Flash and open-column chromatography separations were run using silica gel 60 (230-400 mesh, Merck). Sephadex LH-20 (GE Healthcare) was used for gel permeation column chromatography. TLC analyses were carried out using aluminium-backed silica gel 60 F_{254} (0.20 mm thickness) plates (Merck); chromatograms were first visualized by observing under a UV lamp (254 nm) and then spraying with 10% sulfuric acid, followed by heating at 100°C. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) were recorded at room temperature with a Bruker DRX 400 spectrometer; the spectra were determined in a mixture of $CDCl_3$ and CD_3OD and the solvent residual signals (δ_H7.26 and δ_C77.0, δ_H3.30 and δ_C49.0, respectively) were used as reference. The chemicals shifts (δ) are given in ppm and the coupling constants (*J*) in Hz. ESI-HRMS spectra were recorded in a Waters Q-TOF Micro system spectrometer, using H_3PO_4 for calibration and as internal standard.

Plant material

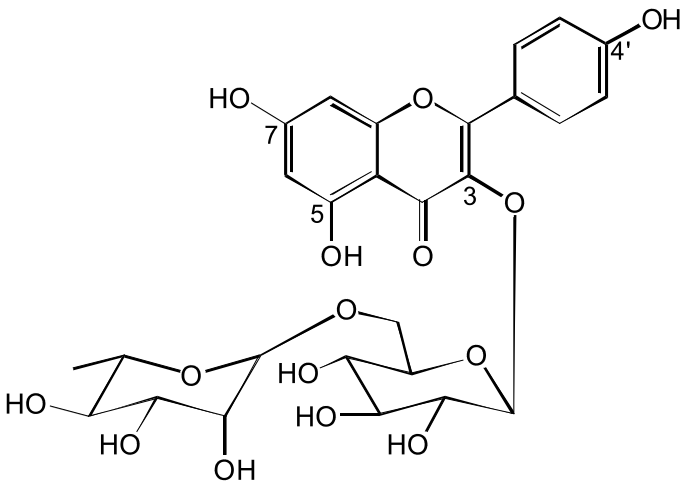
Leaves of *S. foetidissimum* Jacq. subsp. *gaumeri* were collected in July 2003 in Cenote Xtojil (Libre Unión), Yucatán, Mexico. A voucher specimen (PSimá 2661A) was deposited at the herbarium of the Unidad de Recursos Naturales of the Centro de Investigación Científica de Yucatán.

Extraction and isolation

Dried-ground leaves (2.5 kg) were extracted with ethanol, three times at room temperature for one week. After filtration, the extracts were combined and the solvent was evaporated under reduced pressure to give 199.8 g of organic extract. The organic extract (75 g) was suspended in a mixture of water:methanol (9:1, v/v, 500 mL) and the resulting aqueous suspension was successively partitioned between petroleum ether (three times, 2:1, v/v), chloroform (three times, 2:1, v/v) and butanol (three times, 1:1, v/v), to yield the corresponding low (17.16 g), medium (7.04 g) and high polarity (28.01 g) fractions, respectively.

The low polarity fraction was purified by flash column chromatography using a gradient elution with mixtures of petroleum ether and ethyl acetate, to produce seven major fractions (A-G). Purification of fraction A (12.09 g) using Sephadex LH-20, eluting with chloroform/methanol (1:1, v/v), produced five new fractions (A1-E1). The metabolites in fractions E1 (11 mg) were identified as a mixture of α-amyrin and β-amyrin. Further purification of fraction C1 (70 mg), using flash column chromatography eluted with petroleum ether/ethyl acetate (95:5, v/v), produced 4 mg of taraxasteryl acetate. Successive purifications of fraction B (477 mg), using Sephadex LH-20 (chloroform/methanol 1:1, v/v) and crystallization (methanol), yielded 33.6 mg of stigmastenol in pure form.

Purification of the high polarity fraction (2.12 g) by Sephadex LH-20 (methanol) produced six major fractions (A2-F2). Fraction E2 (246 mg) was purified using silica gel open-column chromatography, eluting with chloroform/methanol (7:3, v/v), to produce nine fractions (A3-I3). Purification of fraction G3 (45 mg) by Sephadex LH-20 using methanol as eluant furnished 1.7 mg of kaempferol-3-rutinoside (**1**).



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RESULTS AND DISCUSSION

The ethanolic leaf extract of *S. foetidissimum* subsp. *gaumeri* was partitioned between petroleum ether, chloroform and butanol. Purification of the low polarity fraction yielded four components in a pure form, which were identified as α-amyrin, β-amyrin, taraxasteryl acetate and stigmastenol, by comparing their spectroscopic data with those previously reported in the literature (Lima *et al.*, 2004; Khalilov *et al.*, 2003; Rubinstein *et al.*, 1976; Forgo, 2004). It is interesting to point out that the triterpenes α-amyrin, β-amyrin, and taraxasteryl acetate are reported to have anti-inflammatory activity (Akihisa *et al.*, 1996; Sing *et al.*, 1991), while phytosterols such as stigmastenol have been suggested to reduce both serum cholesterol and low-density lipid cholesterol levels in normal and mildly hypercholesteraemic subjects (Honda *et al.*, 2000; Beveridge, 2002; Mallavadhani *et al.*, 2003). However, none of these metabolites showed DNA-interacting activity when tested in the DNA-methyl green assay.

Successive purification of the high-polarity fraction by silica gel and gel permeation (Sephadex LH-20) chromatography yielded a pure metabolite whose spectroscopic data coincided with those reported for kaempferol-3-rutinoside (**1**), a metabolite previously isolated from *Ficus pumila* (Moraceae) (Ning *et al.*, 2008; Jin *et al.*, 2007). The ESI-HRMS of the purified metabolite **1** showed a protonated molecular ion peak at *m/z* 595.1650, corresponding to a molecular formula of $C_{27}H_{30}O_{15}$, and the proton signals at δ8.05 (d, *J*=8.8 Hz) and δ6.88 (d, *J*=8.8 Hz), together with those at δ6.39 (d, *J*=1.8 Hz) and δ6.20 (d, *J*=1.8 Hz) confirmed the 1,4-disubstituted and 1,2,3,5-tetrasubstituted aromatic rings, respectively. Finally, the two anomeric protons at δ5.11 (d, *J*=7.2 Hz) and δ4.50 (d, *J*=1.6 Hz), together with a three-proton doublet at δ1.11 (d, *J*=6.4 Hz), confirmed a glycosilated flavonoid structure having a glucose and rhamnose units in the structure. Although kaempferol-3-rutinoside (**1**) has been reported to exhibit good antioxidant activity and a remarkable decrease in blood pressure (Ning *et al.*, 2008; Ahmad *et al.*, 1993), it proved inactive when tested in the DNA-methyl green assay.

CONCLUSIONS

The structural diversity of the five isolated secondary metabolites represents an important contribution to the chemotaxonomy of the *Sideroxylon* genus.

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