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Artículo científico

Flavonoids from *Urena sinuata* L.

Adakarleny Sosa & Carmelo Rosquete*

Laboratorio de Productos Naturales, Departamento de Química, Facultad de Ciencias.
Universidad de los Andes, Mérida 5101, Venezuela.

(*) carmelor@ula.ve

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Resumen

En este manuscrito se reportan los resultados obtenidos del estudio fitoquímico de las hojas frescas de *Urena sinuata* L. (cadillo de perro). Los componentes mayoritarios identificados fueron los flavonoides: 3'-β-D-glucopiranosil-6,7-*O*-dimetilquercetagetina (**I**), 4'-β-D-glucopiranosil-6,7-*O*-dimetilquercetagetina (**II**) y 3-β-D-glucopiranosil-6,7-*O*-dimetil-quercetagetina (**III**). Los compuestos aislados fueron caracterizados mediante sus constantes físicas y el análisis de sus espectros ultravioleta, de masas y de resonancia magnética nuclear mono- y bi-dimensionales. También se muestran los resultados obtenidos para estos compuestos en el ensayo de citotoxicidad sobre *Artemia salina*.

Palabras claves: *Urena sinuata*; flavonoides; glicósidos de flavonoles; derivados de quercetagetina

Abstract

In this work it is exposed the obtained results of the phytochemical study of the fresh leaves of *Urena sinuata* L. (dog wart). The major components are the flavonoids: quercetagetin-6,7-*O*-dimethylether-3'-β-D-gluco-pyranoside (**I**), quercetagetin-6,7-*O*-dimethylether-4'-β-D-glucopyranoside (**II**), and quercetagetin-6,7-*O*-dimethylether-3-β-D-glucopyranoside (**III**). These products were characterized through their physical constants, UV, MS, and one- and two-dimensional NMR studies. By other hand, the obtained results of the *Artemia salina* cytotoxicity bioassay carried out to the isolated products are exposed.

Keywords: *Urena sinuata;* Flavonoids; Flavonol glycosides; Quercetagetin derivatives

Introduction

Urena L. (Malvaceae) is a genus composed by two species: *Urena lobata* L. and *Urena sinuata* L. although some Botanist suggest that *U. sinuata* is subspecie of *U. lobata*. This plant (*U. lobata*) has been phytochemically studied by some authors, and steroids (stigmasterol and β-sitosterol)[1], xanthones (mangiferin)[2], flavonoids (quercetina[2-4], kaempferol, hypolaetin, gossypetin, luteolin, apigenin and chrysoeriol[3]), sugars (glucose, mannose, xylose and fructose)[5], and vitamins (ascorbic acid)[5] has been reported. For *U. sinuata* only has been reported fatty acids: sterculic and malvalic acids[6].

In Venezuela, the infusion of foliage from *Urena sinuata* L. is used as anti-inflammatory, analgesic, and against kidney pain and gall stone[7]. For *U. lobata* antiparasitic[8,9], antibacterial[10-12], antidiarrheal[13], and immunomodulatory activity[14] have been mentioned.

Experimental

General Experimental Procedures

IR spectra were recorded as KBr disc on a Perkin Elmer FT-IR Spectrometer 1725X. UV spectra were recorded on an UV Varian Scan 3 using methanol as solvent. NMR spectra were run on Bruker Avance DRX 400 using $CDCl_3$ as solvent and TMS as internal standard. Mass spectra were recorded on a Hewlett-Packard Mass Spectrometer model 5930[a] (70 eV). Si gel 60 (Merck, 70-230 mesh) with dry assembly was used in CC and Si gel Merck HF 254 (10 – 40 μ) on glass sheet (0.25 and 0.5 mm thickness, respectively) was used as adsorbent for TLC and PTLC.

Plant Material

Urena sinuata L. (Malvaceae) was collected at San Cristóbal suburbs (Táchira State-Venezuela). Voucher specimens were stored at MERC Herbarium, Sciences Faculty, Universidad de los Andes-Venezuela.

Extraction and Isolation

1 Kg of fresh plant was extracted in a Soxhlet with *n*-hexane, dichloromethane, acetone and methanol successively. Hexane extract was percolated on Sephadex LH-20® column for eliminate chlorophylls and analyzed by GC/MS. None additional to previously reported interesting compounds was detected[6]. Dichloromethane extract (10.0 g) afforded before Si gel CC hexadecyl 4-

monoitaconate (500 mg) as mainly product. Acetone extract (8.9 g) was also percolated on Sephadex LH-20® column for eliminate chlorophylls using firstly a mixture of *n*-hexane/chloroform/methanol (1:1:1) and then methanol, as solvents. From methanol eluate (525 mg) were obtained, before PTLC using *n*-hexane/acetone (1:8) x 8 as eluent, quercetagetin-6,7-O-dimethylether-3'-β-D-glucopyranoside, **I** (63 mg), quercetagetin-6,7-O-dimethylether-4'-β-D-glucopyrano-side, **II** (72 mg), and quercetagetin-6,7-O-dimethylether-3-β-D-glucopyranoside, **III** (45 mg).

Biological Essay

Artemia salina cytotoxicity test was performed following the procedure described by Meyer *et al.*[16]

Quercetagetin-6,7-O-dimethylether-3'-β-D-glucopyrano-side, I: dark yellow prisms, mp > 300 ºC. IR (*KBr*) υ_{max}: 3377, 2923, 1654, 1554, 1130, 812 cm^{-1}. UV (MeOH): see Table 2. ^{1}H- and ^{13}C-NMR ($CDCl_3$): see table 1. MS *m/z*: $[M]^{+\cdot}$ 508 (< 1), $[M - Glu + H]^{+\cdot}$ 346 (100), $[M - Glu - H_2O + H]^{+\cdot}$ 328 (31), $[M - Glu - H_2C=C=O + H]^{+\cdot}$ 303 (66), $[M - Glu - H_2C=C=O - H_2O + H]^{+\cdot}$ 285 (16), $[M - Glu - H_2C=C=O - H_2O - CH_3 + H]^{+}$ 260 (9), $[A_1 - OCH_3 - CO]^{+}$ 137 (19).

Quercetagetin-6,7-O-dimethylether-4'-β-D-glucopyrano-side, II: dark yellow prisms, mp > 300 ºC. IR (*KBr*) υ_{max}: 3378, 2929, 1654, 1547, 1131, 811 cm^{-1}. UV (MeOH): see Table 2. ^{1}H- and ^{13}C-NMR ($CDCl_3$): see table 1. MS *m/z*: $[M]^{+\cdot}$ 508 (< 1), $[M - Glu + H]^{+\cdot}$ 346 (100), $[M - Glu - H_2O + H]^{+\cdot}$ 328 (34), $[M - Glu - H_2C=C=O + H]^{+\cdot}$ 303 (70), $[M - Glu - H_2C=C=O - H_2O + H]^{+\cdot}$ 285 (18), $[M - Glu - H_2C=C=O - H_2O - CH_3 + H]^{+}$ 260 (9), $[A_1 - OCH_3 - CO]^{+}$ 137 (19).

Quercetagetin-6,7-O-dimethylether-3-β-D-glucopyrano-side, III: pale yellow prisms, mp > 300 ºC. IR (*KBr*) υ_{max}: 3413, 2945, 1658, 1556, 1134, 806 cm^{-1}. UV (MeOH): see Table 2. ^{1}H- and ^{13}C-NMR ($CDCl_3$): see table 1. MS *m/z*: $[M]^{+\cdot}$ 508 (< 1), $[M - Glu + H]^{+\cdot}$ 346 (100), $[M - Glu - H_2O + H]^{+\cdot}$ 328 (31), $[M - Glu - H_2C=C=O + H]^{+\cdot}$ 303 (68), $[M - Glu - H_2C=C=O - H_2O + H]^{+\cdot}$ 285 (16), $[M - Glu - H_2C=C=O - H_2O - CH_3 + H]^{+}$ 260 (9), $[A_1 - OCH_3 - CO]^{+}$ 137 (19).

Results and Discussion

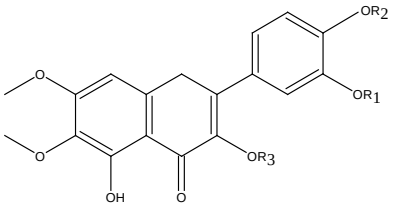
All compounds, **I** to **III,** showed very similar IR, mass and ^{13}C-NMR spectra and only slight differences at ^{1}H-NMR spectra (see table 1). The whole analysis of 1D spectroscopic data allowed us to determine that **I** to **III** correspond to flavone-type compounds, *O*-substituted at 3, 5, 6, 7, 3', and 4' positions, with one β-D-glucopyranosyl (mainly identified by ^{13}C-NMR chemical shift), two methoxyl, and three hydroxyl groups as substituents. HMBC experiments confirmed us that methoxyl groups were placed at 6 and 7 positions for all three compounds, remain therefore to insert the β-D-glucopyranosyl group between 3, 5, 3' and 4' positions, which was made using shift reagent for UV spectra.

During UV spectra analysis of the compounds (see table 2) were observed: For compound **I**, the bathochromic shift for band I (+ 42 nm) showing a low intensity decrease with in time (hypochromic effect) observed when UV spectrum was recorded in methanol + sodium methoxyde can let us to place hydroxyl group on positions 3 and 4'. When UV spectrum was recorded in methanol + $AlCl_3$, a + 53 nm bathochromic shift was observed; that shift remain unchanged after hydrochloric acid addition, which place hydroxyl group on positions 3 and 5. In consequence, the β-D-glucopyranosyl moiety should be located on 3' carbon, and compound **I** should be quercetagetin-6,7-*O*-dimethylether-3'-β-D-glucopyranoside.

Similar to **I**, compound **II** showed for band I, a bathochromic shift (+ 54 nm) in presence of $AlCl_3$ and $AlCl_3$ + HCl, which place hydroxyl groups on positions 3 and 5 too. The bathochromic displacement (+ 42 nm) of band I observed after sodium methoxyde addition without in time intensity decrease, together with the existence of C3 hydroxyl group previously established, place in this case, the β-D-glucopyranosyl moiety on C4', and compound **II** should be quercetagetin-6,7-*O*-dimethylether-4'-β-D-glucopyranoside.

Different to previously described compounds, band I of UV spectrum in methanol + $AlCl_3$ of compound **III** was affected after HCl addition, underwent a hypsochromic shift of -37 nm (respect to methanol + $AlCl_3$) which indicated the existence of an *orto*-dihydroxyl moiety on B-ring (3' and 4' positions), which was corroborated by band I hypsochromic displacement (-32 nm) observed on methanol + sodium acetate UV spectrum after boric acid addition. The stability in time of the methanol + sodium methoxyde, together with the 12 nm bathochromic shift remanent respect to methanol UV spectrum observed on band I of methanol + $AlCl_3$ + HCl, place the third hydroxyl group on C5 carbon, and therefore compound **III** should be quercetagetin-6,7-*O*-dimethylether-3-β-D-glucopyranoside. Although this compound has been reported from *Brickellia dentata*[15], none spectroscopic data has been published in reference 15 and references cited therein.

The UV β-D-glucopyranosyl moieties locations for **I-III** were confirmed by HMBC interactions observed between the hydrogen on anomeric carbon from each saccharide moiety and it flavonoid moiety carbon (3', 4', and 3, for **I**, **II**, and **III** respectively)



	I	II	III
R_1	Glu	H	H
R_2	H	Glu	H
R_3	H	H	Glu

Table 1: NMR data for compounds I – III.

	I		II		III	
	δ_C	δ_H, m (*J* Hz)	δ_C	δ_H, m (*J* Hz)	δ_C	δ_H, m (*J* Hz)
2	156.9		156.8		156.6	
3	133.5		133.5		133.3	
4	177.8		177.9		177.7	
5	151.9		151.9		151.7	
6	131.9		131.8		131.6	
7	158.8		158.8		158.3	
8	91.4	6.85, s	91.3	6.82, s	91.2	6.83, s
9	151.9		151.8		151.6	
10	105.5		105.5		105.3	
1'	121.3		121.3		121.1	
2'	116.5	7.62, d (2.0)	116.5	7.60, d (2.0)	116.3	7.61, d (2.0)
3'	144.9		150.0		144.8	
4'	148.7		148.8		148.5	
5'	115.4	6.84, d (8.0)	115.4	6.83, d (8.0)	115.1	6.84, d (8.0)
6'	121.8	7.61, dd (8.0, 2.0)	121.8	7.57, dd (8.0, 2.0)	121.6	7.58, dd (8.0, 2.0)
1"	100.9	5.46, d (7.3)	100.9	5.44, d (7.3)	100.7	5.48, d (7.3)
2"	76.6	3.23, dd (8.0, 7.3)	76.6	3.24, dd (8.0, 7.3)	76.5	3.19, dd (8.0, 7.3)
3"	70.1	3.26, dd (8.0, 8.0)	70.0	3.25, dd (8.0, 8.0)	69.9	3.27, dd (8.0, 8.0)
4"	74.3	3.08, dd (8.0, 8.0)	74.2	3.08, dd (8.0, 8.0)	74.1	3.08, dd (8.0, 8.0)
5"	77.6	3.08, ddd (8.0, 5.5, 1.5)	77.6	3.07, ddd (8.0, 5.5, 1.5)	77.5	3.07, ddd (8.0, 5.5, 1.5)
6"a	61.1	3.37, dd (11.5, 1.5)	61.1	3.34, dd (11.5, 1.5)	61.8	3.55, dd (11.5, 1.5)
6"b		3.30, dd (11.5, 5.5)		3.31, dd (11.5, 5.5)		3.29, dd (11.5, 5.5)
6-OMe	60.3	3.71, s	60.3	3.73, s	60.3	3.71, s
7-OMe	56.6	3.90, s	56.6	3.97, s	56.5	3.90, s
5-OH		12.58, s		12.55, s		12.60, s

Table 2: UV data for compounds I – III.

	UV λ_{max} (nm)					
	I		II		III	
	Band I	Band II	Band I	Band II	Band I	Band II
MeOH	352	256	352	259	353	258
MeOH+NaOMe	394↓	272↓	405	270	393	270
MeOH+NaOAc	404	258	376	266	407	256
NaOAc+H_3BO_3	404	258	376	266	375	258
MeOH+$AlCl_3$	405	271	406	276	402	273
MeOH+$AlCl_3$+HCl	408	272	406	274	365	266

↓ Intensities decrease in time.

Due to the frequently ingestion of *U. sinuata* leaves infusion by Andean peoples and, by other hand, by the possibility of use of the three flavonoids in pharmacological essays, the cytotoxicity of these compounds was tested. Compounds **I** to **III** showed similar values for $DL_{50} \approx$ 1000 ppm, which point out the low cytotoxicity showed for the three compounds to *Artemia salina*[16].

Hexadecyl 4-monoitaconate isolated from dichloro-methane extract probably arises from fungus of the *Aspergillus* genus which colonized the plant material during the storage.

Conclusions

From *Urena sinuata* leaves, three quercetagetin glucosides were isolated and identified; two of them are new natural products. The presence of **I-III** in *U. sinuata* leaves difference chemically to this plant from *U. lobata*, from the which only flavonoid aglycones were isolated; this sentence support the location of these *taxa* in different species.

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