Flavonoids and Arbutin from *Turnera diffusa*

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The infusion of the aerial parts of *Turnera diffusa* was phytochemically examined. Chromatographic procedures led to the isolation of a new flavone glycoside, five known flavonoids and p-arbutin. Structures were determined by 1D- and 2D NMR experiments, as well as ES-MS and UV spectra.

Introduction

Turnera diffusa Wild. (Turneraceae) with the common name "damiana" is a small shrub that grows in North and South America and Africa. Aerial parts of *Turnera ssp* are popularly used for the treatment of diseases related to the gastric systems (Weniger *et al*, 1986). We have previously reported the anti-inflammatory and anti-ulcer activity of the aerial parts of *T. ulmifolia* L or "chanana", a species that presents similar morphology with *T. diffusa* (Antonio and Brito, 1998). Despite the infusion from the aerial parts of *T. diffusa* is used in the folk medicine as anti-ulcer, its chemical constituents were not described until now. Therefore, we decide to perform the phytochemical investigation of the infusion.

Material and Methods

General

Spectra were recorded using the following instruments: IR: Nicolet impact 400, KBr; UV: HP 8472-A spectrometer (MeOH, c = 1); NMR: Bruker DRX-spectrometer (600 MHz), Varian Inova 500 (500 MHz), solvents CD_3OD, DMSO-d_6, TMS as internal standard; ESMS (positive mode): Fisons Platform; GPC: Sephadex LH-20 (Pharmacia); preparative HPLC: Varian ProStar 210, Varian 350 refractive index detector, Luna C-18 Phenomenex column 250 cm × 10 mm i. d. × 10 μm, 50% MeOH, 2.0 ml min^{-1}.

Plant material

Turnera diffusa Wild. was collected at Quintana Roo, Mexico, in January, 2000. Voucher samples were deposited at the herbario of the Centro de Investigación Científica del Yucatan under number C. Chan 3773.
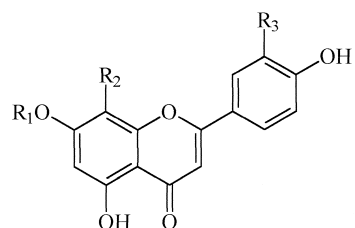
Preparation of the infusion, fractionation and purification of compounds

The aerial parts of *T. diffusa* were dried in an oven at 40 °C and milled. The powder (200 g) was extracted overnight with boiling water (2 l). The mixture was filtrated and lyophylized, affording ca. 7.5 g. An aliquot of 2 g of the lyophylized infusion was resuspended in MeOH, centifuged and chromatographed on a Sephadex LH-20 column eluted with pure MeOH at 0.5 ml min^{-1} flow rate. 100 fractions of 5 ml were collected. After TLC analysis (silica-gel, n-BuOH-HOAc-water 65:15:25 v/v/v) plates were sprayed with NP/PEG reagent (Wagner *et al.*, 1986), fractions with similar Rf's were recombined and further purified by reversed-

phase HPLC (μ-Bondapak C-18 Waters column 30 cm × 7.8 mm i. d., 50% MeOH, 2.0 ml min^{-1}) to give compound **1** (25 mg), **2** (35 mg), **3** (250 mg), **4** (25 mg), **5** (20 mg) and **6** (25 mg) and p-arbutin **7** (75 mg).

Results and Discussion

The infusion from the aerial parts of *T. diffusa* was chromatographed on a Sephadex LH-20 column. The fractions containing flavonoids, checked by TLC plates and visualized with NP-PEG reagent, were further purified by reversed phase HPLC to give compounds **1–6** (Fig. 1). The structures of all compounds were determined by 1D and 2D NMR experiments, positive ESMS spectra and UV data. Of the isolated metabolites, compound **1** is new. The known compounds **2–6** were identified by comparison of their spectroscopic data with those already reported (Agrawal, 1996; Harborne, 1996; Ahmed *et al.*, 2000).

Compound **1** was first identified as a flavonoid based on its orange spot on TLC plate visualized with NP-PEG reagent (Wagner *et al.*, 1986). The UV spectrum presented bands at 260 and 350 nm. The IR presented bands at 3300, 2912 and 1650 cm^{-1}. The ESMS spectrum of **1** exhibited protonated molecular ion [M+H]$^+$ at *m/z* 579 corresponding to molecular formula $C_{27}H_{30}O_{14}$. Loss of 146 mass units led to the fragment ion at *m/z* 433. The protonated aglycone fragment was not observed. The ^{1}H NMR spectrum of **1** (Table I) displayed signals corresponding to a trisubstituted B-ring of the flavonoid nucleus at δ 7.50 (1H, dd, *J* = 8.0, 2.0 Hz, H-6′), 7.46 (1H, d, *J* = 2.0 Hz, H-2′) and 6.89 (1H, d, *J* = 8.0 Hz, H-5′) (Table I). Two singlets appeared at δ 6.46 (1H, H-3) and 6.15 (1H, H-6). Two anomeric protons were observed at δ 5.11 (1H, d, *J* = 1.5 Hz) and 5.10 (1H, d, *J* = 7.5 Hz). Two doublets (*J* = 6.5 Hz) integrating for 3H each were visible at δ 1.42 and 0.72. The remaining sugar protons appeared in the range

Compound	R₁	R₂	R₃
1	H	qui(1→2)rha	OH
2	glc	H	H
3	glc(1→6)p-coum	H	H
4	H	glc(1→2)rha	H
5	H	glc(1→2)rha	OH
6	H	glc(1→6)glc	OCH₃
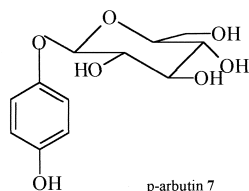
Fig. 1. Compounds isolated from the infusion of *Turnera diffusa*.

Table I. ^{1}H- and ^{13}C-NMR spectral data for compound **1** (600 and 175 MHz in CD$_3$OD, *J* in Hz).

Position	δ C	δ H
2	166.5	
3	103.1	6.46 s
4	183.0	
5	157.1	
6	101.8	6.15 s
7	163.9	
8	105.9	
9	158.5	
10	104.2	
1′	123.9	
2′	114.2	7.46 δ (2.0)
3′	147.3	
4′	151.2	
5′	116.7	6.89 δ (8.0)
6′	121.1	7.50 dd (8.0, 2.0)
Quinovose		
1″	73.9	5.10 δ (7.0)
2″	78.4	4.33 dd (7.0, 9.0)
3″	81.4	3.60 dd (9.0, 9.0)
4″	78.0	3.45 dd (9.0, 9.0)
5″	78.1	3.53 m
6″	17.7	1.42 δ (6.5)
Rhamnose		
1‴	102.7	5.11 δ (1.5)
2‴	72.3	3.86 dd (1.5, 3.0)
3‴	71.6	3.49 dd (3.0, 9.0)
4‴	73.9	3.11 dd (9.0, 9.0)
5‴	69.9	2.66 m
6‴	17.3	0.72 δ (6.5)

δ 4.33–2.56. The ^{13}C NMR spectrum of **1** (Table I) revealed 27 carbon signals, 15 of which were assigned to a luteolin unit. The anomeric carbons absorbed at δ 102.7 and 73.9, while the methyl carbons appeared at δ 17.3 and 17.7. 1D-TOCSY combined to DQF-COSY experiments afforded the spin sequences of the two sugar moieties. Irradiation of the methyl signal at δ 0.72 led to the set of resonances at δ 2.66, 3.11, 3.49 and 3.86. On the basis of the coupling constants this spin system was identified as a rhamnose unit. Irradiation of the methyl signal at δ 1.42 showed the sequence 3.45, 3.53, 3.60, 4.33, 5.10 which, on the basis of the coupling constants, allowed the identification of a quinovose unit (Table I). The recognition of all proton resonances for the sugar moieties allowed to assign the resonances of the linked carbons by HSQC (Table I) and substantiated the presence of luteolin, rhamnose and quinovose unitis. The HMBC spectrum showed correlations between the proton at δ 5.10 (H-1″-qui) with C-8 of the aglycone (δ 105.9), and between the proton

signal at δ 5.11 (H-1‴-rha) with C-2 of the quinovose unit (δ 78.4). The coupling constants of the anomeric protons (Table I) indicated the α-configuration of the rhamnose unit and the β-configuration of the quinovose moiety. On the basis of these findings, compound **1** was deduced to be the new luteolin-8-C-α-L-rhamnopyranosyl(1→2)quinovopyranoside.

The antiulcerogenic activity of many plant species has already been related to their flavonoid content (Yesilada *et al.*, 2000; Lewis and Hanson, 1991). Therefore, the folk usage of the infusion of *T. diffusa* may be supported by these compounds. Further investigations are under way to determine the role of these flavonoids.

Agrawal P. K. (1989), Carbon-13 NMR of Flavonoids. Elsevier, New York, 564 pp.

Ahmed A. S., Nakamura N., Meselhy M. R., Makhboul M. A., El-Emary N. and Hattori M. (2000), Phenolic constituents from *Grevillea robusta*. Phytochemistry **53**, 149–54.

Antonio M. A. and Souza Brito A. R. M. (1998), Oral anti-inflammatory and anti-ulcerogenic activities of a hydroalcoholic extract and partitioned fractions of *Turnera ulmifolia* (Turneraceae). J. Ethnopharmacol. **61**, 215–228.

Harborne J. B. (1996), The Flavonoids. Advances in Research since 1986. Chapman and Hall, London, 675 pp.

Lewis D. A. and Hanson P. J. (1991), Anti-ulcer drugs of plant origin. Progr. Med. Chem. **28**, 201–231.

Wagner H. M., Bladt S. and Zgainski E. M. (1986), Plant Drug Analysis. Springer, Berlin, p. 303.

Weniger B., Rouzier M., Daguilh R., Henrys D., Henrys J. H. and Anton T. (1986), Popular medicine of the Central Plateau of Haiti 2. J. Ethnopharmacol. **171**, 13–30.

Yesilada E., Tsuchiya K., Takaishi Y. and Kazuyoshi K. (2000), Isolation and characterization of free radical scavenging flavonoid glycosides from the flowers of *Spartium junceum* by activity-guided fractionation. J. Ethnopharmacol. **73**, 471–478.