Flavonoids from *Gutierrezia repens* (*Asteraceae)*

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Abstract

7,3'-dimethylquercetin **1**, 7,3,3'-trimethylquercetin **2**, 7,3,4'-trimethylquercetin **3** and quercetin **4** were isolated from aerial parts of *Gutierrezia repens* (*Asteraceae*). The structures of **1**, **2** and **3** were determined mainly on the basis of 2D NMR data. Their ^{1}H NMR spectra in $CDCl_3$ and Me_2CO-d_6 are compared and discussed. The ^{13}C NMR spectra of these compounds are given here for the first time.

Resumen

7,3'-dimetilquercetina **1**, 7,3,3'-trimetilquercetina **2**, 7,3,4'-trimetilquercetina **3** y quercetina **4** fueron aislados de las partes aéreas de *Gutierrezia repens* (*Asteraceae*). Las estructuras de **1**, **2** y **3** fueron determinadas principalmente por espectroscopía 2D RMN. Sus espectros de RMN ^{1}H en $CDCl_3$ y Me_2CO-d_6 son comparados y discutidos. En este trabajo informamos por primera vez, los espectros de RMN ^{13}C de los flavonoides metilados.

Keywords: *Gutierrezia repens*, Asteraceae, phytochemistry, flavonoids.

Introduction

The *Asteraceae* is the second largest family in the Magnoliophyta Division with around 1100 genera and over 20000 recognized species. Cabrera, reported the occurrence of 197 genera and about 1400 species in Argentina [1].

As part of our phytochemical study on Asteraceae species growing in Argentina, we investigated the aerial parts of *Gutierrezia repens* Grisebach. There is no information about chemical and biological studies carried out on *G. repens*. Plant specimens were collected from their natural habitat in the northwest of Argentina, in Salta Province.

The genus *Gutierrezia* (tribe Eupatorieae) includes approximately 25 species which occur exclusively in the arid areas of America [1]. Earlier work on this genus revealed that diterpenes [2-8] and flavonoids [9-15] are the main classes of substances representative of the *Gutierrezia* genus.

In this paper, we report for the first time on a phytochemical investigation of *G. repens*.

Experimental

General

The NMR spectra were recorded on a Bruker AC 200 (^{1}H at 200 MHz and ^{13}C at 50 MHz) or a Bruker Ultrashield 400 (^{1}H at 400 MHz and ^{13}C at 100 MHz) spectrometer with TMS as internal reference. CC were performed on silica-gel 230-400 mesh, RPCC on C-18 silica gel, TLC was carried out on precoated Silica gel 60 F_{254} plates (Fluka). Detection was achieved by UV light and spraying with vainillin reagent followed by heating.

Plant Material

G. repens was collected during the flowering period in Valle Encantado, Province of Salta, Argentina, on February 2004. The identification was carried out by Ing. Julio Tolaba. A voucher specimen (nº 3464) is deposited at the Museo de la Facultad de Ciencias Naturales, Universidad Nacional de Salta.

Extraction and isolation

Air-dried and powdered aerial parts of *G. repens* (260 g) were macerated with EtOH at room temperature for 7 days to give 13.10 g of crude extract which was suspended in EtOH:H_2O (1:1) and extracted successively with hexane (3x150 mL), CH_2Cl_2 (3x150 mL) and EtOAc (3x100 mL). Evaporation of the CH_2Cl_2 extract in vacuo furnished 5.37 g of residue which was divided into 4 fractions by chromatography on reversed-phase

silica gel flash column, eluting with MeOH-H_2O (8:2), MeOH and Me_2CO. The fraction **1** (2.0 g) was chromatographed on a 230-400 mesh silica gel column using hexane containing increasing amounts of EtOAc (0-100 %), seven fractions being collected (F_1 to F_7). *Fraction F_5* (269 mg, hexane-EtOAc 3:7), was first purified by column chromatography on silica gel eluting with a gradient of hexane-Et_2O followed by preparative TLC (hexane-Me_2CO 7:3) affording 2.5 mg of 7,3`-dimethylquercetin **1** (Rf= 0.30) [16], 3.0 mg of 7,3,3`-trimethylquercetin **2** (Rf= 0.36) [16, 17] and 3.5 mg of 7,3,4`-trimethylquercetin **3** (Rf= 0.33) [18]. Column chromatography of *Fraction F_6* (230 mg, hexane-EtOAc 1:9) on silica gel and benzene-EtOAc gradient system followed by preparative TLC (hexane-Me_2CO, 1:1) afforded 7.0 mg of quercetin **4** (Rf= 0.48) [19].

7,3`-dimethylquercetin **1.** Amorphous solid, UV (MeOH) λ_{max} nm: 260, 270, 370; +NaOMe: 270, 300, 330, 430 (dec); +NaOAc: 260, 335, 375; +$AlCl_3$: 275, 430; +$AlCl_3$/HCl: 275, 430.

7,3,3`-trimethylquercetin **2.** Amorphous solid, UV (MeOH) λ_{max} nm: 270, 348, 355; +NaOMe: 265, 405; +NaOAc: 270, 355; +$AlCl_3$: 270, 302, 406; +$AlCl_3$/HCl: 348, 406.

7,3,4`-trimethylquercetin **3.** Amorphous solid, UV (MeOH) λ_{max} nm: 270, 300, 348; +NaOMe: 265, 375; +NaOAc: 265, 355; +$AlCl_3$: 270, 300, 362, 400; +$AlCl_3$/HCl: 270, 300, 362, 400.

quercetin **4.** Yellow solid, UV (MeOH) λ_{max} nm: 255, 300, 370, 385; +NaOMe: 330 sh; +NaOAc: 274, 394; +$AlCl_3$: 266, 300, 358, 430; +$AlCl_3$/HCl: 266, 300, 358, 430.

Discussion

The CH_2Cl_2 soluble extract of the aerial parts of *G. repens* Griseb. yielded four known flavonoids 7,3'-dimethylquercetin **1** [16], 7,3,3'-trimethylquercetin **2** [16, 17], 7,3,4'-trimethylquercetin **3** [18] and quercetin **4** [19].

Bathocromic shifts upon addition of $AlCl_3$ and $AlCl_3$/HCl (see experimental) together with the presence of a chelated hydroxyl group in the ^{1}H NMR spectrum (Table 1 and Table 2), indicated 5-hydroxy substitution for all four compounds.

In the ^{1}H NMR spectra of all the compounds three aromatic protons formed the characteristic pattern for a 3',4'-disubstituted B ring. Additionally, the UV spectra recorded with NaOMe indicated 4'-hydroxy substitution for **1**, **2** and **4**.

Flavonoids **1**, **2** and **3** also showed ^{1}H NMR signals indicative of O-methyl substituents. Their UV spectra were unchanged upon addition of NaOAc, indicating that one of the methoxyl groups was at the C-7 position. The structures of these compounds were deduced on the basis of their HSQC, HMBC and NOESY spectra.

The ^{1}H NMR data of known compounds **1**, **2** and **3** were previously measured using low resolution instrument. As far as we know, the ^{13} C NMR spectra of flavonoids **1**, **2** and **3** have not been described in the literature so far (Table 1 and 2).

The ^{1}H NMR data of **3,** indicate that 4`-O-methylation induces a downfield shift of ca. 0.15 ppm in the signal of H-5', in the spectrum measured in Me_2CO-d_6 (Table 2). In the spectrum measured in $CDCl_3$ this effect is clearly smaller (less than 0.1 ppm) (Table 2). On the other hand, in all the compounds with 7-O-methylation (**1, 2** and **3**), we always observed a downfield shift of 0.15-0.30 ppm in the signal of H-8, in spectra measured in Me_2CO-d_6 (Tables 1 and 2).

The A-Ring carbon signals are similar in **1**, **2** and **3**. B-ring signals show that 4'-O-methylation in **3** induces an upfield shift of ca. 4.0 ppm in the chemical resonance of C-5' (Table 2).

Conclusions

7,3'-dimethylquercetin **1** and 7,3,3'-trimehtylquercetin **2** are now reported for the first time in the genus, while 7,3,4'-trimethylquercetin **3** was isolated before from *G. alamanii* [13] and quercetin **4** from *G. grandis* [12], *G. alamanii* [13] , *G. wrightii* [14] and *G. microcephala* [15].

The isolation of compounds **1**-**4** from *G. repens* is completely in accordance with the typical chemical profile of the *Gutierrezia* genus.

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Table 1. Spectroscopic data of flavonoid **1** and **4*** (Me_2CO-d_6, TMS as internal standard)

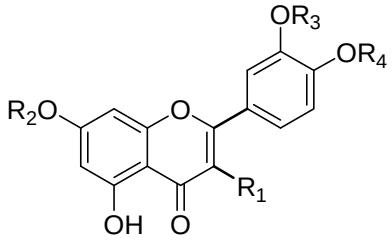
Position	**1**				**4**
	δ (C)*	δ (H)*	δ (H)†	HMBC*	δ (H)*
2	146.5	-	-	-	-
3	135.3	-	-	-	-
4	175.0	-	-	-	-
5	161.1	-	-	-	-
6	97.4	6.33 *d* (2)	6.36 *d* (2.2)	C-5, C-7, C-10	6.27 *d* (2.2)
7	165.8	-	-		-
8	92.0	6.80 *d* (2)	6.48 *d* (2.2)	C-7, C-9, C-10	6.53 *d* (2.2)
9	156.9	-	-	-	-
10	103.9	-	-	-	-
1'	121.9	-	-	-	-
2'	111.2	7.90 *d* (2.2)	7.7-7.8 *m*	C-1', C-4'	7.82 *d* (2.0)
3'	147.5	-	-	-	-
4'	148.9	-	-	-	-
5'	116.0	7.02 *d* (8.5)	7.02 *d* (8.8)	C-6',C-3'	7.00 *d* (8.4)
6'	122.6	7.86 *dd* (8.5, 2.2)	7.7-7.8 *m*	C-4', C-2'	7.70 *dd* (8.4, 2.0)
3'-OCH_3	55.6	3.95 *s*	3.97 *s*	C-3'	-
7-OCH_3	55.5	3.94 *s*	3.86 *s*	C-7	-
5-OH		12.14 *s*	12.62 *s*		12.60 *s*

* At 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR.
† At 200 MHz in $CDCl_3$.
δ (H) values are followed by multiplicity and below, in parentheses, coupling constants in Hz.

Table 2. Spectroscopic data of flavonoid **2** and **3*** (Me_2CO-d_6, TMS as internal standard)

Position	**2**				**3**			
	δ (C)*	δ (H)*	δ (H)†	HMBC*	δ (C)*	δ (H)*	δ (H)†	HMBC*
2	155.9	-	-	-	155.9	-	-	-
3	138.6	-	-	-	138.9	-	-	-
4	178.7	-	-	-	178.5	-	-	-
5	162.0	-	-	-	162.0	-	-	-
6	97.3	6.33 *d* (2)	6.37 *d* (2)	C-5, C-7, C-10	97.7	6.33 *d* (2)	6.36 *d* (2.2)	C-5, C-7, C-10
7	165.7	-	-	-	165.9	-	-	-
8	91.8	6.68 *d* (2)	6.45 *d* (2)	C-9	92.0	6.70 *d* (2)	6.45 *d* (2.2)	C-7, C-9, C-10
9	156.8	-	-	-	156.9	-	-	-
10	105.7	-	-	-	105.7	-	-	-
1'	121.9	-	-	-	123.2	-	-	-
2'	111.8	7.80 *d* (2.0)	7.71 *s, br* ‡	C-3'	114.9	7.67 *d* ‡ (2.2 Hz)	7.70 *d* ‡ (2.0 Hz)	C-3', C-4', C-6'
3'	147.4	-	-	-	146.4	-	-	-
4'	149.7	-	-	-	150.1	-	-	-
5'	115.2	7.02 *d* (8.5)	7.05 *d* (8.3)	C-4'	111.2	7.15 *d* (8)	6.97 *d* (8.3)	C-4', C-1'
6'	122.5	7.72 *dd* (8.5, 2.2)	7.67 *dd* ‡ (8.3, 2.2)	C-4'	121.0	7.72 *dd* ‡ (8.0, 2.2)	7.72 *dd* ‡ (8.0, 2.2)	-
OCH_3	55.5	3.95 *s*	3.98 *s*	C-3'	55.4	3.97 *s*	3.99 *s*	C-4'
7-OCH_3	55.5	3.93 *s*	3.87 *s*	C-7	55.4	3.94 *s*	3.88 *s*	C-7
3-OCH_3	59.1	3.90 *s*	3.86 *s*	C-3	59.3	3.90 *s*	3.88 *s*	C-3
5-OH		12.73 *s*	12.63 *s*	C-5		12.70 *s*	12.70 *s*	C-5

* At 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR.
† At 200 MHz in $CDCl_3$.
δ (H) values are followed by multiplicity and below, in parentheses, coupling constants in Hz.
‡ Overlapped signals



	R_1	R_2	R_3	R_4
1	OH	CH_3	CH_3	H
2	OCH_3	CH_3	CH_3	H
3	OCH_3	CH_3	H	CH_3
4	OH	H	H	H