

CONSTITUENTS OF *Hiraea reclinata* AND THEIR ANTI-HIV ACTIVITY

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ABSTRACT

From the methanolic extract of *Hiraea reclinata*, seven known compounds were isolated. Only 1,3,4,5-tetragalloyl quinic acid showed anti-HIV activity.

Key Words Index: *Hiraea reclinata*, Malpighiaceae, quinic acid tetragallate, flavonoid glycosides, NMR, anti-HIV.

INTRODUCTION

This work was carried out as part of an Ecologically Based Bioprospecting project in Panama funded by ICBG (International Cooperative Biodiversity Group, NIH). The genus *Hiraea* (Malpighiaceae) comprises approximately 40 species in the American tropics, occurring primarily in rain forests. *Hiraea reclinata* Jacq. (Malpighiaceae) is distributed from Mexico to Panama, Colombia, Venezuela, and Brazil. There is no phytochemical study, nor any ethnomedical

claims reported for this genus. The anti-HIV activity demonstrated by the total methanolic extract of the mature leaves of *H. reclinata*, prompted us to undertake a phytochemical investigation to isolate the anti-HIV active constituent(s) of this plant.

RESULTS AND DISCUSSION

Fractionation of the methanolic extract of *H. reclinata* using liquid-liquid partitions and column chromatography afforded kaempferol

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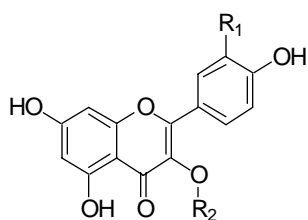
3-*O*-(6''-galloyl)- β -D-galactopyranoside (**1**) (Toshiya, *et al.*, 2001; Collins, *et al.*, 1975), hyperin 6''-gallate (**2**) (D'Agostino, *et al.*, 1992), 1,3,4,5-tetragalloylquinic acid (**3**) (Nishizawa, *et al.*, 1989), vitexin 2''-rhamnoside (**4**) (Jay, *et al.*, 1989), isovitexin 2''-rhamnoside (**5**) (Nikolov, *et al.*, 1982), orientin 2''-rhamnoside (**6**) (Nikolov, *et al.*, 1982), and isoorientin 2''-rhamnoside (**7**) (Sherwood, *et al.*, 1973). All the compounds were identified by spectroscopic means, hydrolysis and co-chromatography with authentic samples. Although the occurrence of compounds **4–7** has a wide taxonomic distribution, we could not find, except for the anomeric protons, any data for the chemical shifts of the glycosidic protons, (Escobar, L. K. *et al.*, 1983) and are thus reporting in Table 1 full ^1H and ^{13}C NMR data of the sugar part of compounds **4–7**. The ^1H and ^{13}C NMR were complicated due to duplication or broadening of some of the signals at room temperature as a result of the rotational barrier around the *C*-glycosidic linkage. This effect was observed for compounds **5** and **7**, which give rise to duplication of some proton signals (H-1'', H-2'' and Me-6'') and carbon signals (C-2'', C-3'', C-6'', C-1''' and C-6'''). Meanwhile, such effect was not observed for compounds **4** and **6**. It is of inter-

est to indicate that rhamnose protons showed high field shifts in ^1H NMR (Hatano, T. *et al.*, 1999) due to the shielding effect of ring A of the flavonoid unit (Kato, T. *et al.*, 1990, Cheng, G. *et al.*, 2002). The NOESY NMR spectra of **4** and **6** showed correlations for H-2''/H-2', H-6'', H-1''', H-5'''. In addition, **5** and **7** showed NOESY correlations of H-1''/H-3''; H-2''/5-OH, H-3'''. Compounds **1–7** were isolated for the first time from *H. reclinata*. The whole cell anti-HIV bioassay, showed that 1,3,4,5-tetragalloylquinic acid (**3**) was the only active compound with protection of 73.96 % and IC_{50} 0.103 $\mu\text{g}/\text{mL}$. The other compounds were inactive.

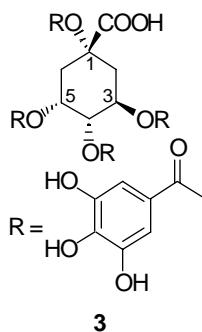
EXPERIMENTAL PART

General

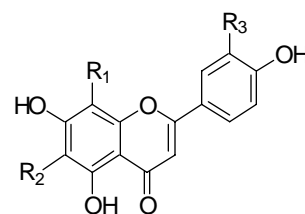
Melting points are uncorrected. Optical rotations were measured with a Perkin-Elmer 141 polarimeter. UV spectra was recorded on a Perkin Elmer UV/VIS Spectrophotometer, Lambda 2S. IR was recorded using a Perkin-Elmer 1310 spectrophotometer. NMR spectra were recorded using a Bruker Avance 300 spectrometer in $\text{DMSO}-d_6$ at 300 MHz for ^1H and 75.0 MHz for ^{13}C NMR. Mass spectra were obtained on a Kratos MS50TC mass spec-



- 1 R₁ = H, R₂ = O-Gal-6''-gallate
2 R₁ = OH, R₂ = O-Gal-6''-gallate



3



- 4 R₁ = C-Glc-2''-O-Rha, R₂ = H, R₃ = H
5 R₁ = H, R₂ = C-Glc-2''-O-Rha, R₃ = H
6 R₁ = C-Glc-2''-O-Rha, R₂ = H, R₃ = OH
7 R₁ = H, R₂ = C-Glc-2''-O-Rha, R₃ = OH

Table 1. ¹H- and ¹³C Data of the glycosidic part of compounds **4-7** (DMSO-*d*₆).

Position	4		5			6		7	
	C	H	C	H	C	H	C	H	
1''	72.1	4.85 d(9.4)	71.7	4.70 d(10.3)	71.8	4.80 d(10.0)	71.8	4.65 d(10.1)	
2''	75.0	4.10 t(9.6)	76.2[74.9]	4.43[4.29] t(8.0)	75.4	4.09 t(8.2)	75.4[74.7]	4.37[4.22] t(9.5)	
3''	80.7	3.44 m*	80.3[79.9]	3.34 m*	80.3	3.35 m*	80.3[79.9]	3.26 m*	
4''	71.1	3.58 m*	70.9	3.60 m*	71.1	3.60 m*	71.2	3.61 m*	
5''	81.3	3.15 m*	81.3	3.13 m*	82.2	3.15 m*	81.7	3.07 m*	
6''	61.8	3.53 m*	62.1[61.5]	3.50 m*	61.8	3.50 m*	62.0[61.1]	3.50 m*	
		3.80 d(10.3)		3.73 d(10.0)		3.83 d(10.0)		3.75 d(10.0)	
1''''	102.3	5.01 s	101.0[100.6]	5.01[5.12] s	102.5	5.01 s	100.9[100.7]	5.01[5.10] s	
2''''	71.8	3.38 m*	70.9	3.12 m*	70.7	3.35 m*	70.4	3.25 m*	
3''''	70.8	3.16 dd(9.0, 9.2)	70.6	3.13 m*	71.5	3.13 d(9.3)	71.2	3.15 t(9.4)	
4''''	72.3	2.94 t(9.2)	70.9	2.96 t(9.2)	70.5	2.95 t(9.3)	71.8	2.91 t(9.4)	
5''''	68.7	2.26 dq(9.2, 5.0)	68.5	2.38 dq(9.2, 5.4)	68.5	2.16 dq(9.3, 5.2)	68.5	2.31 dq(9.4, 5.1)	
6''''	17.8	0.55 d(5.0)	17.9 [18.0]	0.58[0.51] d(5.4)	18.0	0.50 d(5.2)	17.8[18.1]	0.60[0.55] d(5.1)	

* Obscure signals by water.

trometer. Silica gel [Merck, Kieselgel 60 (0.063–0.200 mm) and (0.015–0.040 mm)], LiChroprep RP-18 (Merck, 9303), and Sephadex LH-20 (Sigma, 904-37-6) were used for column chromatography. Silica gel plates (Merck, Kieselgel 60 F_{254s}) were used for TLC.

Anti-HIV Bioassay

The assay was carried out according to the method described before (Kiser *et al.*, 1996). Subclone 1A2 was cultured on RPMI-1640 with L-glutamine, 10% FBS, and 0.3 mg/ml of G418. Cells are maintained in log phase of growth by passing cultures twice weekly. The assay protocol required 5000 cells/well to be plated into 96 well plates. Plant extracts or purified compounds were dissolved in DMSO. Virus Δ Tat/RevMC99 was added to appropriate wells at multiplicity of infection = 0.1. Control wells containing only cell (uninfected controls), cells with virus but without drug (infected controls), and a series of drug dilutions with uninfected cells (to assess toxicity controls). After incubation of the plates for 6 days at 37 °C in an atmosphere of 5% CO₂, plates were stained with 50 μ L/well of a solution of 1 mg/mL XTT-tetrazolium salt and 1% phenazine methosulfate. After a 4 h incubation, the optical densities were read at 450 nm/ 650 nm. The 3-azido-5'-thymidine (8.5 \times 10⁻⁸ M) was used as a standard reference compound.

Plant Material

The mature leaves of *H. reclinata* were collected from the Monumento Nacional de Barro Colorado-Barro Colorado Island. The voucher specimen 51364 (SCZ) has been deposited in the Herbarium of the Smithsonian Tropical Research Institute.

Extraction and Isolation of the constituents

The fresh mature leaves of *H. reclinata* (343 g) were extracted and partitioned according the method described before (Hussein *et al.*, 2003) to give four partitions (hexane, methanol, EtOAc, and H₂O). The activity was retained in the EtOAc and H₂O partitions. The EtOAc partition (13.0 g) was chromatographed over Sephadex LH-20 column, which was eluted with 10% aqueous methanol to give compound **1** (16 mg, 0.000046%), **2** (25 mg, 0.000072%) and **3** (1.0 g, 0.0029%). The water partition (20.0 g) was chromatographed on Sephadex LH-20 using 70% aqueous ethanol as eluent to give 3 fractions. Fraction 2 (1.5 g) was rechromatographed over C-18 column using a gradient of MeOH in water, followed by Sephadex LH-20 column using 10% aqueous MeOH as eluent, which afforded pure **3** (20 mg, 0.000058%), **4** (16 mg, 0.000046%), **5** (19 mg, 0.000055), **6** (20 mg, 0.000058%) and **7** (25 mg, 0.000075%).

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