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Flavanones from *Miconia prasina*

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Abstract

A glycosidic flavanone miconioside C (**1**) has been isolated from the methanolic extract of the stems of *Miconia prasina*, together with 7-*O*-β-D-apiofuranosyl-(1→6)-β-D-glucopyranosylmatteucinol (**2**), miconioside B (**3**), matteucinol (**4**), farrerol (**5**) and desmethoxymatteucinol (**6**). Their structures were mainly established by extensive NMR studies (^{1}H NMR, ^{13}C NMR, DEPT, ^{1}H-^{1}H COSY, HSQC, HMBC) and mass spectrometry. The compounds **1**- **3** were evaluated for *in vitro* binding assays using cannabinoid receptors (CB1 and CB2).

Keywords

Miconia prasina; Melastomataceae; flavanone; flavanone glycoside; cannabinoids receptors

1. Introduction

The *Miconia* genus (Melastomataceae) consists of approximately 700 species distributed throughout the tropical and subtropical regions of the Americas. Several *Miconia* species, as well as the compounds isolated from this genus, have shown biological activities, including genotoxic and mutagenic effects (Serpeloni et al., 2008). Moreover, *Miconia myriantha* has been reported as an enzyme inhibitor of secreted aspartic proteases (SAPs) from *Candida albicans* (Li et al., 2001).

As a part of our continuing search for novel biological agents (Wang et al., 2011; Gao et al., 2013; Tarawneh et al., 2013), *Miconia prasina* was evaluated for its biological properties. Previous phytochemical studies of the *Miconia* genus revealed the presence of several

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Appendix A. Supplementary data
Supplementary data associated with this article can be found, in the online version,

triterpenes (Peixoto et al., 2011), and flavanones (Zhang et al., 2003). This paper reports the fractionation, isolation and structural elucidation of a new flavanone glycoside, miconioside C **1** and five known flavanones **2**-**6**, (Figure 1) from the methanolic extract of the stems of *M. prasina*. Compounds **1**- **3** were evaluated for their *in vitro* binding affinity for cannabinoid receptors (CB1 and CB2). These compounds showed weak binding affinity for the cannabinoid receptors.

2. Results and discussion

Compound **1**, was isolated as a yellowish amorphous solid, and its molecular formula was determined to be $C_{28}H_{34}O_{13}$ by HRESIMS. The ^{1}H NMR showed six protons singlet signals at δ_H 2.14, suggesting two aromatic methyls groups, a non-substituted phenyl moiety at δ_H 7.53 (2H, d, *J* = 7.2 Hz), 7.42 (2H, t, *J* = 7.2 Hz) and 7.35 (1H, t, *J* = 7.2 Hz) related with the B-ring. A flavanone skeleton was evident from the coupling pattern of the C-ring protons δ_H 2.85 (1H, dd, *J* = 2.9, 17.0 Hz), 3.15 (1H, dd, *J* = 12.9, 17.0 Hz) and 5.47 (1H, dd, *J* = 2.9, 12.9 Hz). Analysis of the ^{13}C NMR spectrum and DEPT experiments, confirmed a flavanone moiety with the presence of a typical ketone carbonyl signal at δ_C 199.5, and the presence of sugar signals corresponding to one pentose and one hexose. The ^{1}H NMR and ^{13}C NMR spectroscopic data of **1** were found to be similar to those of miconioside B (Zhang et al., 2003) except for the absence of a hydroxyl group at C-4′. The nature and identity of this flavanone was deduced from the NMR experiments (COSY, NOESY, TOCSY, and HSQC). HMBC correlations allowed the complete assignments thus: the anomeric proton of the glucose at δ_H 4.69 (1H, d, *J* = 7.7 Hz, H-1″) showed correlation with the carbon at δ_C 162.7 (C-7), as well as, the correlation between the signal at δ_H 3.60 (1H, d,d *J* = 5.6 and 11.2 Hz, H_a-6″) and the signal at δ_C 110.9 (C-1‴), which together with published data (Zhang et al., 2003; Takahashi et al., 2001), identified **1** as 7-*O*-β-D-apiofuranosyl-(1→6)-β-D-glucopyranosyldemethoxymatteucinol. The absolute configuration at C-2 at the aglycone was established as *S* from the strong negative Cotton effect at 271 nm observed in the CD spectrum (Gaffield, 1970). Compound **1** is a newly reported flavanone glycoside 7-*O*-β-D-apiofuranosyl-(1→6)-β-D-glucopyranosyldemethoxymatteucinol, and is named miconioside C, the next consecutive designation in this nomenclature.

Compounds **2**-**6** were identified as the known compounds 7-*O*-β-D-apiofuranosyl-(1→6)-β-D-glucopyranosylmatteucinol **2** (Takahashi et al., 2001), miconioside B **3** (Zhang et al., 2003), matteucinol **4** (Wollenweber et al., 2000), farrerol **5** (Youssef et al., 1998) and demethoxymatteucinol **6** (Basnet et al., 1993). The structures of the known compounds were confirmed by comparison of their spectroscopic properties with published data. To date, this is the first chemical or biological studies dealing with *Miconia prasina*.

Compounds **1**-**3** were evaluated at a concentration of 10 μM for their affinity to bind with cannabinoid receptors (CB1 and CB2), following the methods described previously (Gao et al., 2011). The compounds **1**-**3** exhibited weak inhibition 26.5%, 13.1%, and 18.2% respectively for CB2, not activity for CB1. This is the first report of the evaluation of the binding affinity for the cannabinoid receptors of this kind of compounds.

3. Experimental

3.1. General experimental procedures

Optical rotations were recorded using a Rudolph Research Analytical Autopol V Polarimeter. UV was obtained using a Perkin-Elmer Lambda 3B UV/vis-spectrophotomer. CD spectra were recorder on an Aviv 202SF spectrometer. ^{1}H and ^{13}C NMR spectra were obtained on Bruker model AMX 500 NMR spectrometer with standard pulse sequences, operating at 500 MHz in ^{1}H and 125 MHz in ^{13}C. The chemical shift values were reported in parts per million units (ppm) from trimethylsilane (TMS) using known solvent chemical shifts. Coupling constants were recorded in Hertz (Hz). Standard pulse sequences were used for COSY, HMQC, HMBC, TOCSY, NOESY and DEPT. High-resolution mass spectra (HRMS) were measured on a Micromass Q-Tof Micro mass spectrometer with a lock spray source. Column chromatography was carried out on silica gel (70-230 mesh, Merck) and Sephadex LH-20 (Mitsubishi Kagaku, Tokyo, Japan). TLC (silica gel 60 F254) was used to monitor fractions from column chromatography. Visualization of the TLC plates was achieved with a UV lamp (λ = 254 and 365 nm) and anisaldehyde/acid spray reagent (MeOH-acetic acid-anisaldehyde-sulfuric acid, 85:9:1:5). All HPLC analyses were performed on a Waters LC Module I equipped with a UV detector 486 utilizing the Millenium 32 Chromatography Manager software (Waters). An ODS column (Phenomenex Luna C_{18}, 10 × 250 mm, 5 μm) was used. Solvents were HPLC grade, filtered through appropriate filters (water through 0.45 μm and organic solvents through 0.22 μm filters) and purged prior to and during analysis with nitrogen gas at a flow rate of 5 mL/min. All chemicals used were purchased from Sigma-Aldrich (St. Louis, Mo) with the following exceptions: for the binding experiments, [^{3}H]-CP-55,940 (144 Ci/mmol), was purchased from Perkin-Elmer Life Sciences Inc. (Boston, MA, U.S.A.). CP-55,940 was purchased from Tocris Bioscience (Ellisville, Missouri, U.S.A.).

3.2. Plant material

The plant *Miconia prasina* was collected in Puerto Rico near Caguas in March of 2006 and identified by Gregory Gust. A voucher specimen (Gust 1009 MO) has been deposited in the Missouri Botanical Garden.

3.3. Extraction and isolation

The dried and powered stems of *M. prasina* (140 g) were extracted with methanol after maceration for three days. Removal of the solvent afforded a viscous residue (7.5 g), which was fractionated by Si gel Vacuum Liquid Chromatography (VLC) stepwise from hexane to methanol, yielding nine fractions (hexane; 3:1 hexane-EtOAc; 1:1 hexane-EtOAc; 1:3 hexane-EtOAc; EtOAc; 3:1 EtOAc-MeOH; 2:2 EtOAc-MeOH; 1:3 EtOAc-MeOH and MeOH). Fractions eluted with hexane-EtOAc (1:1) and hexane-EtOAc (1:3) were combined and subsequently chromatographed by solid-phase extraction (SPE) column initially with hexane and stepwise elution to MeOH. The subfraction eluted with 60% EtOAc in hexane was rechromatographed using Sephadex LH-20 eluted with methanol to furnish compounds **4** (8 mg) and **6** (6 mg); subfraction eluted with 100 % EtOAc was purified by semipreparative HPLC (phenomenex C-18 Luna 5μm, 21 mm × 250 mm, step gradient elution with 30-70% MeOH/H_2O) to yield compound **5** (11 mg). Fractions eluted with

EtOAc; and 3:1 EtOAc-MeOH were combined and rechromatographed by SPE column initially with 3:1 hexane/EtOAc and stepwise elution to MeOH yielding eight subfractions (50 mL each) (3:1 hexane-EtOAc; 1:1 hexane-EtOAc; 1:3 hexane-EtOAc; EtOAc; 3:1 EtOAc-MeOH; 2:2 EtOAc-MeOH; 1:3 EtOAc-MeOH and MeOH). Subfraction 4 (3:1 EtOAc-MeOH) was purified by preparative HPLC (step gradient elution with 15-85% MeOH/H_2O) to furnish compounds **1** (3 mg) and **3** (5 mg). Compound **2** (8 mg) was purified with sephadex LH-20 eluted with CH_2Cl_2/MeOH (1:1) from subfraction 5 (2:2 EtOAc-MeOH).

3.4. Miconioside C (1)

Yellow gum. $[\alpha]^{25}_D$: −39.3 (*c* 0.03, MeOH); UV/Vis λ_{max} (MeOH) nm (log ε): 230 (3.83), 280 (4.82), 360 (3.29); IR (KBr): 3358, 2923, 1627, 1450, 1367, 1281, 1124, 1061, 668 cm^{-1}; CD (MeOH, *c* 0.14): $[\theta]_{205}$ + 22086 (max), $[\theta]_{231}$ - 206 (max), $[\theta]_{271}$ - 19680 (max), $[\theta]_{340}$- 617 (max); ^1H NMR (500 MHz, CD_3OD): see Table 1. ^{13}C NMR (125 MHz, CD_3OD): see Table 1. HRESIMS m/z $[M+Na]^+$ 601.1819 (calcd. for $C_{28}H_{34}NaO_{13}$, 601.1897).

4.4. Cell Culture

HEK293 cells (ATCC) were stably transfected via electroporation with full length human recombinant cDNA for cannabinoid receptor subtypes 1 and 2 (obtained from Origene). These cells were maintained at 37 °C and 5% CO_2 in a Dulbecco's Modified Eagles's medium (DMEM) nutrient mixture F-12 HAM supplemented with 29 mM sodium bicarbonate, 10% fetal bovine serum, penicillin-streptomycin, and G418 antibiotic solutions.

4.5. Radio-ligand Binding for Cannabinoid Receptor Subtypes

Compounds evaluated in the assay were run in competition binding against both the cannabinoid receptor subtypes, CB1 and CB2 (Ross et al., 1999). Cannabinoid receptor binding assays were performed following the methods described previously (Gao et al., 2011).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

Basnet P, Kadota S, Shimizu M, Xu HX, Namba T. 2′-Hydroxymatteucinol, a new C-methyl flavanone derivative from *Mattecia orientalis*; potent hypoglycemic activity in streptozotocin (STZ)-induces diabetic rat. Chem. Pharm. Bull. 1993; 41:1790–1795. [PubMed: 8281576]

Gaffield W. Circular dichroism, optical rotatory dispersion and absolute configuration of flavanones, 3-hydroxyflavanones and their glycosides. Tetrahedron. 1970; 26:4093–4108.

Gao J, Leon F, Radwan MM, Dale OR, Husni AS, Manly SP, Lupien S, Wang X, Hill RA, Dugan FM, Cutler HG, Cutler SJ. Benzyl derivatives with *in vitro* binding affinity for human opioids and cannabinoid receptors from the fungus *Eurotium repens*. J. Nat. Prod. 2011; 74:1636–1639. [PubMed: 21667972]

Gao J, Radwan MM, León F, Dale OR, Husni AS, Wu Y, Lupien S, Wang X, Manly SP, Hill RA, Dugan FM, Cutler HG, Cutler SJ. Neocosmospora sp.-derived resorcylic acid lactones with in vitro binding affinity for human opioid and cannabinoids receptors. J. Nat. Prod. 2013; 76:824–828. [PubMed: 23659286]

Li XC, Jacob MR, Pasco DS, ElSohly HN, Nimrod AC, Walker LA, Clark AM. Phenolic compounds from *Miconia myriantha* inhibiting Candida aspartic proteases. J. Nat. Prod. 2001; 64:1282–1285. [PubMed: 11678651]

Peixoto JA, Silva MLA, Crotti AEM, Veneziani RCS, Gimenez VMM, Januario AH, Groppo M, Magalhães LG, dos Santos FF, Alburquerque S, Filho AAS, Cunha WR. Antileishmanial activity of the hydroalcoholic extract of *Miconia langsdorffii*, isolated compounds and semi-synthetic derivatives. Molecules. 2011; 16:1825–1833. [PubMed: 21343887]

Ross RA, Gibson TM, Stevenson LA, Saha B, Crocker P, Razdan RK, Pertwee RG. Structural determinants of the partial agonist-inverse agonist properties of 6′-azidohex-2′-yne-Δ^8-tetrahydrocannabinol at cannabinoid receptors. Br. J. Pharmacol. 1999; 128:735–743. [PubMed: 10516656]

Serpeloni JM, dosReis MB, Rodrigues J, dosSantos LC, Vilegas W, Varanda EA, Dokkedal AL, Cólus IMS. *In vivo* assessment of DNA damage and protective effects of extracts from *Miconia* species using the comet assay and micronucleus test. Mutagenesis. 2008; 23:501–507. [PubMed: 18765422]

Takahashi H, Hirata S, Minami H, Fukuyama Y. Triterpene and flavanone glycoside from *Rhododendron simsii*. Phytochemistry. 2001; 56:875–879. [PubMed: 11324921]

Tarawneh A, León F, Radwan MM, Rosa LH, Cutler SJ. Secondary Metabolites from the Fungus *Emericella nidulans*. Nat. Prod. Commun. 2013; 8:1285–1288. [PubMed: 24273867]

Wang X, Habib E, León F, Radwan MM, Tabanca N, Gao J, Wedge DE, Cutler SJ. Antifungal metabolites from the roots of *Diospyros virginiana* by overpressure layer chromatography. Chem. Biodiversity. 2011; 8:2331–2340.

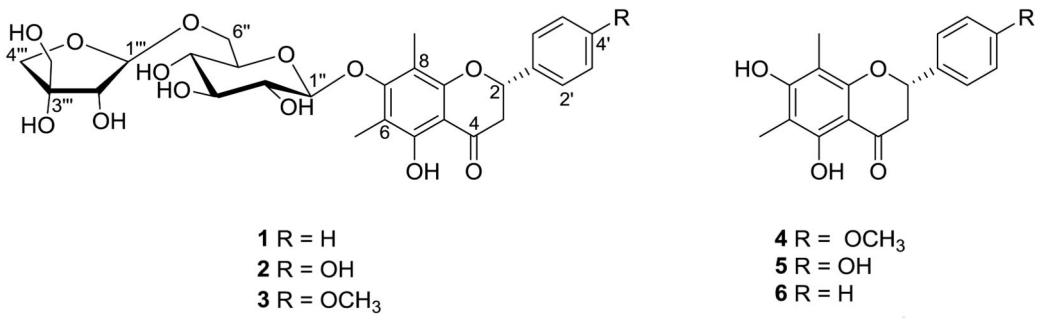
Wollenweber E, Wehde R, Dörr M, Lang G, Stevens JF. C-Methyl-flavonoids from the leaf waxes of some Myrtaceae. Phytochemistry. 2000; 55:965–970. [PubMed: 11140533]

Youssef DTA, Ramadan MA, Khalifa AA. Acetophenones, a chalcone, a chromone and flavonoids from *Pancratium maritimum*. Phytochemistry. 1998; 49:2579–2583.

Zhang Z, ElSohly HN, Li XC, Khan SI, Broedel SE, Raulli RE, Cihlar RL, Walker LA. Flavanone glycosides from *Miconia trailii*. J. Nat. Prod. 2003; 66:39–41. [PubMed: 12542342]

Highlights

- Phytochemical study of *Miconia prasina*.
- One new flavanone glycoside and five known flavanones were isolated.
- *in vitro* binding assays using cannabinoid receptors (CB1 and CB2) were evaluated.



1 R = H
2 R = OH
3 R = OCH_3

4 R = OCH_3
5 R = OH
6 R = H

Figure 1.
Flavanones isolated from *Miconia prasina*

Table 1

^{1}H (500 MHz) and ^{13}C NMR (125 MHz) spectral data for 1 in CD_3OD.

Position	Miconioside C (1)				
	δ_c	δ_h		δ_c	δ_h
Aglycone			Glc		
2	80.2	5.47 dd (3.0, 12.9)	1″	105.3	4.69 d (7.7)
3	44.5	2.85 dd (3.0, 17.0) 3.15 dd (12.9, 17.0)	2″	75.7	3.51 t br (8.7)
4	199.5		3″	78.0	3.41 t (8.5)
5	159.9		4″	71.7	3.25-3.35*
6	113.2		5″	77.1	3.25-3.35*
7	162.7		6″	68.6	3.60 dd (5.6, 11.2) 3.84 dd (1.7, 11.2)
8	112.0		Api		
9	159.0		1‴	110.9	4.89 d (2.0)
10	106.4		2‴	77.9	3.80 d (2.0)
1′	140.6		3‴	80.5	
2′, 6′	127.2	7.53 d (7.2)	4‴	74.9	3.67 d (9.6) 3.74 d (9.6)
3′, 5′	129.7	7.42 t (7.2)	5‴	65.9	3.49 d (3.1)
4'	129.5	7.35 t (7.2)			
6-Me	9.8	2.14 s br			
8-Me	9.2	2.14 s br			

* overlapped signals.