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Short Communication

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An Orientin Derivative Isolated from *Passiflora tripartita* var. *mollissima*

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***SUMMARY. Passiflora tripartita* var. *mollisima* (banana passion fruit) is an edible fruit widespread in the Andean highlands of Colombia and Ecuador. The fruit is used for juices as well as for the sedative properties of the leaves. As a contribution to the chemical characterization of this species, a new compound, 4'-methoxyluteolin-8-*C*-6"acetylglucopyranoside, was isolated from the ethanolic extract of *Passiflora tripartita* var. *mollisima* leaves and identified by spectroscopical data (NMR, MS, UV).**

INTRODUCTION

Passifloraceae is a wide spread family of plants that comprises more than 500 different species. From these, almost 450 species belong to *Passiflora,* a genus originary from America; some of these species as *Passiflora edulis* f. *edulis* and *P. edulis* f. *flavicarpa,* the purple and yellow passion fruits, respectively, are cultivated by their edible fruits. In addition, the leaves of several other *Passiflora* species, as *P. alata, P. incarnata,* and *P. edulis* f. *flavicarpa* have been used by their therapeutics properties as anxiolytics, sedatives, diuretics, analgesics and anti-inflammatories [1,2]. Several chemical studies showed that plants of this genus contain mainly triterpenoid saponins as cycloartane and oleane, steroid saponins, harman alkaloids, and cyanogenic glycosides, as well as *O*- and *C*-glycosides of flavonoids [1,3]. Despite none of these compounds have been identified as the responsible for the biological activities observed in the extracts of these plants, there is some evidence suggesting that the flavonoid fraction can play a key role as antiinflamatory and anxyolitic agents [1,2].

The "banana passion fruit" (*Passiflora tripartita* var. *mollissima)* also known as *Passiflora mollissima* is a species that grows in Ecuador and Colombia between 2000 and 3000 m above the sea level, where the fruit is commonly known as "curuba" and consumed mainly in juices [4]. Leaves of *P. tripartita* var. *mollissima* are commonly used as sedative and are accepted by the Foods and Drugs Colombian Agency ("Invima") as sedative [5]. Previous studies on this species deal with the glycosilated aroma components in the fruit [6], cyanogenic glycosides [7-9], as well as the anti-hyperglycemic activity [10]. The main constituents detected in this plant were alkaloids, saponins, flavonoids, triterpenoids and proteins [11]. In this study, we present the isolation and structural elucidation of a new orientin derivative (**1**), obtained from the aerial parts of *P. tripartita* var. *mollissima.*

MATERIALS AND METHODS

General Experimental Procedures

The UV-Vis spectra were measured on a Perkin Elmer UV-Vis Lambda 25 Spectrometer; while NMR spectra were measured on a Bruker AVANCE 400 spectrometer (400 MHz for 1H, 100 MHz for ^{13}C, Karlsruhe, Germany). High resolution electrospray ionization mass spectra (HRESIMS) was obtained on Shimadzu LC-IT-TOF instrument by direct injection. The HPLC was a Hitachi-Merck 6000 equipment equipped with a L-6000 pump and a L-4250 UV-VIS detector (at 330 nm). The solid supports for column

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chromatography and gel permeation chromatography (GPC) were: silica gel 60 (0.60-0.200 mm, Merck), Lichroprep RP-18 (0.040-0.063 mm, Merck), Sephadex LH-20 (25-100 µm, Sigma-Aldrich). The packed column used was a Luna-Phenomenex Silica (250 x 10.00 x 5 µm).

Plant Material

P. tripartita var. *mollisima* leaves were collected from a live specimen at the Botanical Garden of Bogotá "José Celestino Mutis" (G. Morales voucher number 3039).

Extraction and Isolation

The collected leaves were dried at 40 °C and crushed, then a sample (60.5 g) of dried leaves were extracted three times with ethanol 90% at 65 °C. The extract was filtered and concentrated under vacuum to obtain 10.5 g of dried extract. This raw extract was partitioned between CH_2Cl_2, BuOH and water. The butanolic fraction (684 mg) was chromatographed on a Sephadex LH-20 column and eluted with methanol to obtain 13 fractions (F1 to F13), were F8 and F10 showed to be rich in flavonoids by TLC sprayed $AlCl_3$ [12]. Fraction F8 was separated in a RP-18 column using MeOH-water (1:1) as eluent, to obtain 8 fractions F8.1 to F8.8. In addition F10 was chromatographed in a silica gel column and eluted isocratically with ethyl acetate-formic acid-water (8:1:1) to obtain 8 fractions F10.1 to F10.8. Fractions F8.6, F8.7, F10.7 and F10.8 showed the presence of the same main component by TLC, so these fractions were pooled obtaining 81 mg. Further separation of this sample by HPLC, using as eluent a solution of formic acid 0.1% in a mixture of EtOAc-MeOH (92:8, 1 mL/min), yielded 15 mg of compound **1**.

RESULTS AND DISCUSSION

The ethanolic extract of *P. tripartita* var. *mollissima* was fractionated by LC on a Sephadex LH-20, silica gel and RP-18 with a discontinuous gradient of MeOH-Water, obtaining four main fractions (F8.6, F8.7, F10.7 and F10.8), which were then pooled according their TLC profiles. This new fraction was isolated by preparative HPLC yielding 15 mg of a yellow powder designed as compound **1**.

Spectroscopic characterization of compound 1

Compound 1, was obtained as a yellow powder and presented the following spectroscopical data: UV λ max (log ε), 343 (3.94), 295 (3.70), 269 (3.95), 212 (4.56). ^{1}H NMR (CD_3OD) and ^{13}C NMR (CD_3OD) results are shown in Table 1. Electron spray ionization mass spectra (HRESIMS) presented *m/z* 505.1343 [M + H] as pseudomolecular ion, corresponding to $C_{24}H_{25}O_{12}$.

No.	δH	δC
2	-	166.2
3	6.59 (1H, s)	104.9
4	-	184.1
5	-	158.2
6	6.28 (1H, s)	99.5
7	-	164.7
8	-	104.9
9	-	162.8
10	-	104.3
1'	-	125.3
2'	7.50 (1H, s)	114.6
3'	-	148.4
4'	-	152.7
5'	7.07 (1H, d, *J* = 8.4Hz)	112.6
6'	7.55 (1H, d, *J* = 8.4Hz)	120.4
1''	4.98 (1H, d, *J* = 9.6Hz)	75.5
2''	4.18 (1H, t, *J* = 9.6Hz)	73.7
3''	3.56 (1H, m)	80.3
4''	3.80 (1H, t, *J* = 9.2Hz)	72.8
5''	3.66 (1H, m)	80.1
6''	4.47 (1H,d, *J* = 12Hz) 4.33 (1H,m)	65.4
CH_3-O	3.94 (1H)	56.6
CH_3COO	1.91 (1H)	20.7
CH_3COO	–	173.1

Table 1. NMR data (^{1}H-NMR, 400 MHz; ^{13}C-NMR, 100 MHz) for compound **1** measured in CD_3OD.

The ^{1}H-NMR spectra (400MHz, CD_3OD, Table 1) presented signals for a 1,3,4-trisubstituted aromatic ring with two of these protons at *orto*- position (δ_H 7.55, 1H, d, *J* = 8.4 Hz; δ_H 7.50, 1H, s; δ_H 7.07, 1H, d, *J* = 8.4 Hz.), signals for a penta-substituted aromatic ring (δ_H 6.28, 1H, s) and signals for an olefinic proton (δ_H 6.59, 1H, s). Additionally, signals for 5 hydroxymetines and an oxymethylene between δ_H 4.98 and δ_H 3.56, suggesting the presence of a sugar moiety, as well as the signals for an aromatic methoxyl (δ_H 3.94) and a methyl probably due to an acetyl group (δ_H 1.91, 3H, s) were observed.

The ^{13}C-NMR (100 MHz, CD_3OD, Table 1)

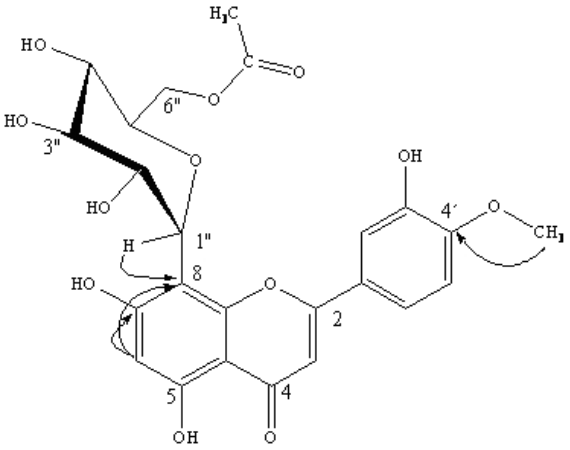

Figure 1. Structure of compound **1** (relevant HMBC correlations →).

presented signals for an α,β-unsaturated carbonyl (δ_C181.7), together with signals for other 14 carbons (δ_C 166.2 - 99.5) corresponding to one olefin and two aromatic rings, signals for a sugar moiety (δ_C 80.3 - 65.4) and finally signals for an acetyl group (δ_C173.1 y 20.7) and a metoxyl group (δ_C 56.6). These signals have a close resemblance with those published for orientin (luteolin-8-*C*-glucopyranoside), a C-glycoside flavone commonly isolated from other *Passiflora species* [13,14]. However, some differences in the NMR data of compound **1** can be observed, specially the presence of the metoxyl (δ_H 3.94, 3H, s; δ_C 56.6) and the acetyl groups (δ_H 1.91, 3H, s; δ_C 20.7 y δ_C 173.1). Assignation of the protons to the carbons (Table 1) was completed by HSQC correlations and some correlations observed in HMBC.

The sugar was identified as β-glucose due to the coupling constants for H-2" (δ_H 4.18, 1H, t, *J* = 9,6 Hz) and H-4" (δ_H 3.80, 1H, t, 9.2) that suggest the equatorial positions of the four hydroxyls at C-1, C-2, C-3, C-4 and the hydroxymethylene bonded to C-5. Despite of the absence of HMBC correlations, the acetyl group was proposed to be bonded to the sugar at position C-6" due to the chemical shift for this carbon at δ_C 65.4, and its comparison with those of orientin [13]. The glycosidation at C-8 is proposed by the HMBC correlation between the proton C-1" at δ_H 4.99 and the carbon C-8 at δ_C 104.9. The signal for the methoxyl at δ_H 3.94 showed a strong HMBC correlation with carbon at C-4' (δ_C 152.7) suggesting the methoxilation of compound 1 at C-4'. These data allowed identification of compound 1 as 4'-methoxyluteolin-8-C-6"acetylglucopyranoside (Fig. 1), a flavonoid not previously reported in the literature.

Orientin has proved to be active as anxiolytic, antioxidant [14], vasorelaxant [15] and as antiviral against parainfluenza type 3 virus [16], but so far, in the case of orientin derivatives, there are not biological activities reported. Further research in the flavonoid composition and biological activities for this plant must be done.

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