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Ipolamiide and fulvoipolamiide from *Stachytarpheta glabra* (Verbenaceae): A structural and spectroscopic characterization

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Abstract

The phenylethanoid glycoside acteoside and the iridoids ipolamiide and 4-methoxycarbonyl-7-methylcyclopenta[*c*]pyran (fulvoipolamiide) were isolated from the leaves of *Stachytarpheta glabra*. The solid state structure of fulvoipolamiide was confirmed by X-ray diffraction studies. The molecules of fulvoipolamiide are displayed in layers parallel to the crystallographic axis ***a***. This molecule is planar with electron delocalization in the fused ring system and the pyran rings of adjacent layers in the solid state structure are involved in a π–π stacking interaction. Raman spectroscopy has also been used to characterize the most important bands present in the spectra of fulvoipolamiide and ipolamiide, and comparison made with literature allows the assignment of some key markers, specially the bands in the 1600–1700 cm^{-1} range.

Keywords: *Stachytarpheta glabra*; Verbenaceae; Ipolamiide; Iridoids; Raman; Crystal structure

1. Introduction

Verbenaceae is included in the order Lamiales and is widely found in practically every terrestrial ecosystem. It is one of the five most important families among the Eudicotyledonous of *Campus Rupestres*. This family includes 1035 species and 36 genera with a pantropical distribution few species are found in the temperate areas [1]. Several genera have being investigated due to their medicinal properties, of which *Lantana*, *Lippia* and *Stachytarpheta* [2] are noteworthy. The genus *Stachytarpheta* (Verbenaceae) includes nearly 90 species distributed in tropical and subtropical America [3]. Some species are traditionally used in folk medicine as a purgative, vermifuge, expectorant, diuretic, emmenagogue, sore-throat gargles and as a general tonic [4]. Previous studies have reported the isolation of the iridoids lamiide and ipolamiide in some species including *S. jamaicensis*, *S. cayennensis*, *S. indica*, *S. australis*, *S. mutabilis* and *S. urticifolia* [5–14]. These constituents have been shown to possess several biological activities such as antimicrobial, antitumoral, anti-inflammatory, antinociceptive, hepatoprotective and laxative. They are also used for inhibition of gastric secretion and for treatment of immunopathological diseases related to oxidative stress [11,14–19]. *Stachytarpheta glabra* Cham. is a shrub 0.5–1.0 m high which is widespread in tropical and subtropical America. In Brazil it can be found in Minas

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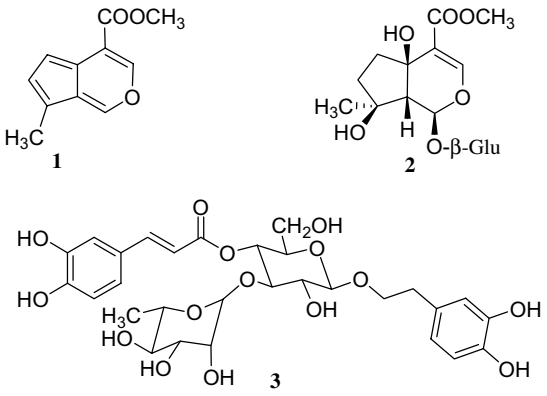


Fig. 1. Chemical structures of fulvoipolamiide (**1**), ipolamiide (**2**) and acteoside (**3**).

Gerais, Bahia and Rio de Janeiro states. Although the *Stachytarpheta* genus is significantly important in folk medicine, some species such as *S. glabra* have never been studied phytochemically hitherto. As part of an ongoing program to isolate compounds with biological activities from the *Stachytarpheta* genus and specifically *S. glabra*, in this paper we report the isolation and structural elucidation of fulvoipolamiide **1** and ipolamiide **2** (Fig. 1) which have been characterized in the solid state and in solution by means of Raman spectroscopy and X-ray diffraction techniques.

2. Experimental

2.1. Plant material

Samples of *S. glabra* Cham. were collected in Minas Gerais state, Brazil and identified by Dr. Fátima Regina Gonçalves Salimena (Dep. Botânica, UFJF). A voucher specimen (CESJ 42977) has been deposited at the Herbarium CESJ of the Universidade Federal de Juiz de Fora, Brazil.

2.2. General experimental procedures

Dried leaves of *S. glabra* (72.0 g) were submitted to a hydrodistillation extraction process for 4 h using a Clevenger-type apparatus to give a crude red solid material after solvent removal. This crude residue was chromatographed on silica gel using hexane/ethyl acetate as an eluent to give 600 mg of **1** (mp from EtOH–H_2O 89–91 °C; lit. [20] mp from MeOH–H_2O 85–87 °C). Dried and pulverized leaves from the same source (16.1 g) were also exhaustively extracted with ethanol 99.3% in a Soxhlet apparatus. The ethanol extract was evaporated to yield 4.53 g of a syrupy residue. This material was applied to a column of silica gel and eluted with ethyl acetate containing increasing amounts of methanol to give 2 fractions. These fractions were further chromatographed, eluting with CH_2Cl_2:MeOH mixed solvent to furnish specimens **2** (710 mg) and **3** (253 mg).

2.3. X-ray diffraction data

Single crystal X-ray diffraction data of specimens **1** and **2** were obtained. Compound **2** has been previously described in the literature [13]. Single crystal X-ray data were collected in a Nonius Kappa CCD diffractometer with MoKα ($\lambda = 0.71073$ Å) at room temperature. Data collection and reduction were performed by DENZO and SCALEPACK programs [21]. The structure was solved and refined using SHELXL-97 [22]. The final R and R_w values were 0.0749 and 0.1661, respectively. CCDC 602636 contains the supplementary crystallographic data for this compound. These data can be obtained free of charge at www.ccdc.cam.ac.uk/conts/retrieving.html or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK [Fax: (internat.) 1 44-1223/336-033; E-mail: deposit@ccdc.cam.ac.uk].

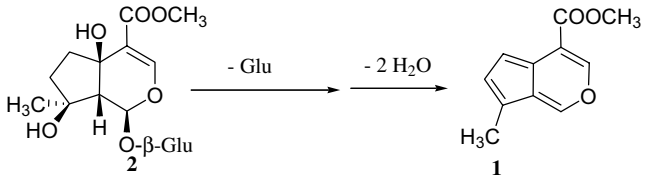
2.4. Raman measurements

Fourier-transform Raman spectra were obtained using a Bruker IFS66/FRA 106 instrument with Nd^{3+}/YAG laser operating at 1064 nm with 4 cm^{-1} spectral resolution and 500 spectral scans accumulated to improve the signal-to-noise ratio; laser powers were maintained at 50 mW or less at the samples to prevent possible damage to the biological materials. Wavenumbers of strong/sharp bands are accurate to ±1 cm^{-1} or better, and all spectra were recorded in triplicate for all samples to demonstrate that no thermal or photodecomposition had occurred during the spectroscopic analyses.

3. Results and discussion

The crude red material from the hydrodistillation process was chromatographed to furnish compound **1** as red crystals as reported previously [20]. A straightforward formation pathway giving rise to **1** can be envisaged starting from the known iridoid ipolamiide **2**, or some derivative, previously reported as a constituent of *Stachytarpheta* genus. As shown in Scheme 1, the loss of one molecule of glucose and two of water from **2** yields compound **1**.

Since the formation of compound **1** could have been accomplished during the Clevenger hydrodistillation process, a new extraction using ethanol 99.3% as solvent in a Soxhlet extractor was performed. Interestingly, in this crude extract, after solvent removal, the presence of compound **1** was not observed. This crude ethanolic extract



Scheme 1. Formation pathway of **1** from ipolamiide 2.

was subjected to column chromatography to afford the major components ipolamiide **2** and acteoside **3**. The structures of these three known compounds were identified by comparison of their ^{1}H and ^{13}C NMR spectroscopic data with those of reported values in the literature [20,23,24]. For example, in the ^{1}H NMR spectrum of compound **1** two signals can be observed at δ 2.39 and 3.96, corresponding to the hydrogen atoms of CH_3 groups. Signals at δ 6.85, 6.93, 8.10 and 8.15, attributable to the aromatic ring hydrogens, can also be observed. In the ^{13}C NMR spectrum of this compound, signals are observed at δ 12.2 and 52.1, corresponding to the CH_3 groups; and signals at δ 112.0–144.0 regions can be attributed to the aromatic carbons. A signal at δ 166.3 is also observed, corresponding to the carbonyl group.

Additionally, the structures of **1** and **2** were also confirmed by X-ray analysis; here, the crystal structure of **1** is described for the first time but that of compound **2** was previously investigated by Roengsumran and co-workers [13]. Crystal data are displayed in Table 1 and the ortep [25] view of crystal structures is shown in Fig. 2. The molecules of compound **1** are displayed in layers parallel to the crystallographic axis ***a***, with layer distances of about 3.5 Å. An O···HC van der Waals interaction is observed between molecules of the same layer with an O···C distance of 3.251(5) Å. Although the molecules of the same layer present the same orientation with respect to each other, in adjacent layers the molecules are inverted in relation to each other. The molecules are planar and no methyl hydrogen atoms are located in special positions on the mirror plane *m* of space group $P2_1/m$. The average C–C ring and C–C bond distance are, respectively, 1.400(5) Å and 1.498(6) Å, and the largest C–C bond distance in the rings is about 1.467(5) Å, indicating the presence of electronic delocalization in the ring systems. The pyran rings of adjacent layers are involved in a π–π stacking interaction [26]

Table 1
Crystal data of compounds **1** and **2**

Compound	**1**	**2**
Formula	$C_{11}H_{10}O_3$	$C_{17}H_{28}O_{12}$
Formula weight	190.19	424.40
Crystal system	Monoclinic	Orthorhombic
Space group	$P2_1/m$	$P2_12_12_1$
a (Å)	8.248(2)	7.8831(2)
b (Å)	6.719(2)	10.3629(3)
c (Å)	8.662(2)	24.4899(6)
β (°)	95.08(1)	90.00
V (Å^3)	478.2(2)	2000.62(9)
Z	2	4
$d_{calc.}$ (g cm^{-3})	1.321	1.409
Refl. Meas./unique	4388/1137	31624/4210
Obs. refl. [$I_o > 2\,s(I_o)$]	449	3488
Parameters	86	334
R	0.075	0.029
w*R*	0.166	0.069
S	0.914	1.087
RMS peak (e$^-$Å^{-3})	0.044	0.032

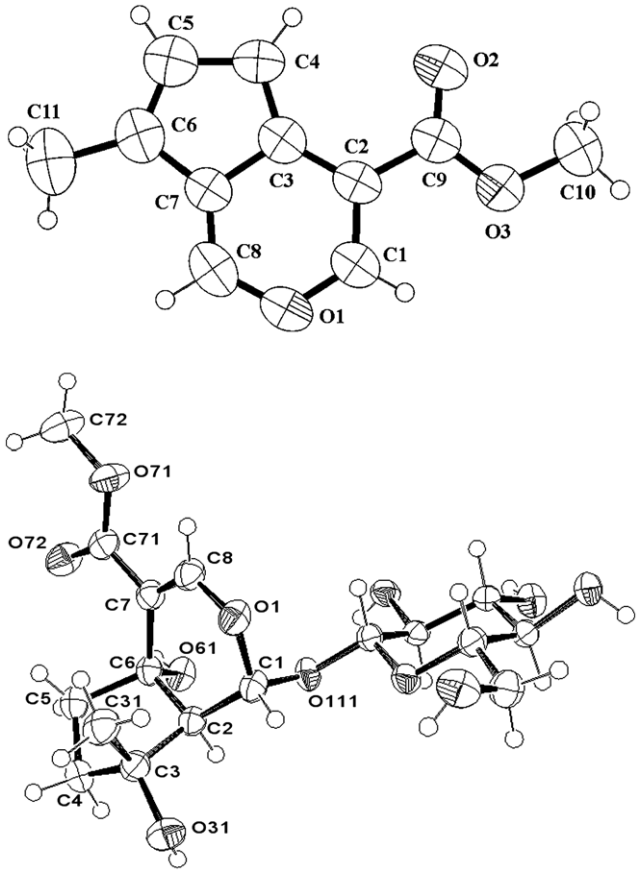


Fig. 2. Ortep [25] view of crystal structure of fulvoipolamiide (**1**, top) and ipolamiide (**2**, down). The ellipsoids are displayed with 50% probability level.

where the centroid–centroid and centroid–plane distances are, respectively, 3.54 and 3.36 Å, presenting a horizontal shift around of 1.00 Å.

The crystal structure of compound **2** has been described earlier [13]. This compound does not have electronic delocalization in the rings due to its non-planarity, unlike compound **1**. The average C–C ring bond distance here is 1.534(2) Å, which is significantly larger than that observed for compound **1**. This compound has a single water of hydration molecule involved in 3 medium-strength hydrogen bonds, for which the average of O···O distance is 2.752(4) Å. The ester group is also involved in medium-strength hydrogen bonding to the hydroxyl group of neighboring molecules where the O···O distance is 2.791(4) Å. Selected bond distances of both compounds are listed in Table 2.

Raman spectra of compounds **1** and **2**, obtained with 1064 nm excitation, can be seen in Fig. 3a and b. The assignments of the main vibrational bands have been carried out, based on similar molecules [27–29] in the literature. The Raman spectra of both compounds are quite different, arising from the structural differences between fulvoipolamiide (**1**) and ipolamiide (**2**); this can be attributed to the electronic delocalization in compound **1** which also gives rise to the red colour of this compound. In the

Table 2
Selected bond distances (Å) and bond angles (°) of compounds **1** and **2**

	(**1**)	(**2**)		(**1**)	(**2**)
C1–C2	1.362(6)	1.512(2)	C1–O1	1.346(5)	1.436(2)
C2–C3	1.419(6)	1.549(2)	C8–O1	1.364(5)	1.355(2)
C3–C4	1.359(6)	1.527(2)	C9–O2	1.208(5)	
C4–C5	1.442(5)	1.523(2)	C9–O3	1.307(5)	
C5–C6	1.337(7)	1.557(2)	C71–O71		1.338(2)
C6–C7	1.436(6)	1.514(2)	C72–O71		1.440(2)
C7–C8	1.315(6)	1.331(2)	C71–O72		1.213(2)
C1–O1–C8	118.9(6)	116.2(1)	C4–C5–C6	111.5(9)	106.0(1)
O1–C1–C2	123.6(8)	114.0(1)	C5–C6–C7	106.0(8)	
C1–C2–C3	118.4(8)	116.6(1)	C3–C7–C6	107.5(7)	
C2–C3–C7	116.3(7)		C3–C7–C8	120.5(7)	
C4–C3–C7	107.1(7)		C7–C8–O1	122.2(8)	126.8(2)
C3–C4–C5	107.9(8)	103.8(1)			

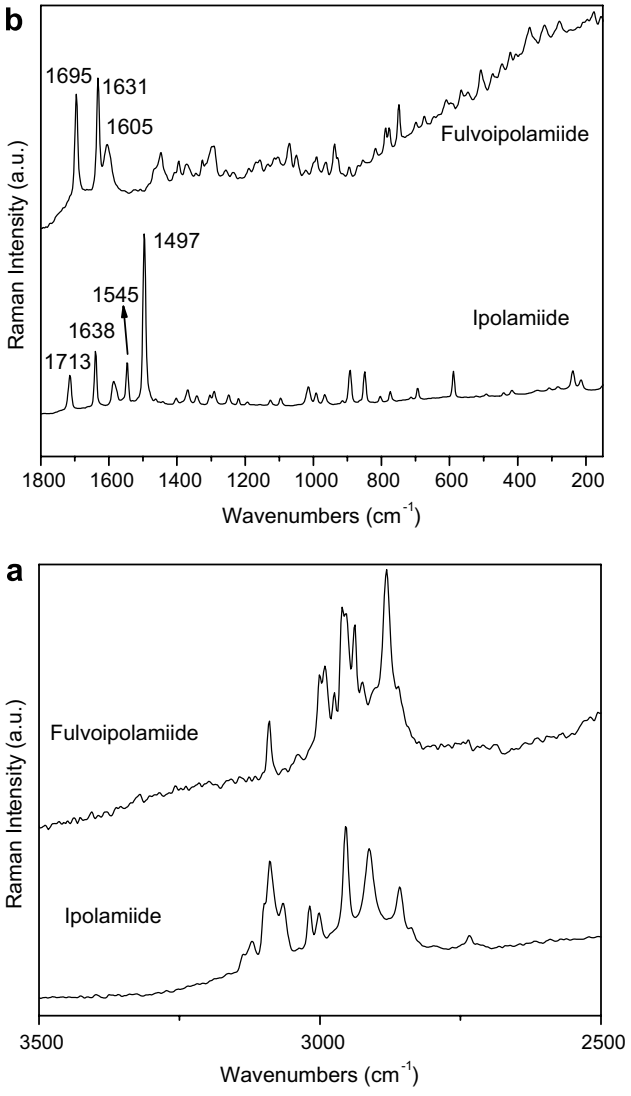


Fig. 3. Fourier transform Raman spectra of fulvoipolamiide (**1**) and ipolamiide (**2**), excited at 1064 nm, splitted in two regions: 3500–2500 cm^{-1} (a) and 1800–150 cm^{-1} (b).

high wavenumber region (Fig. 3a), compound **1** presents some medium intense bands related to CH_3 mode stretching, whereas in compound **2**, in addition there are also present CH_2 groups and carbohydrate species. However, the biggest differences between these two spectra can be seen in the 1400–1700 cm^{-1} regions, for which the Raman spectra of benzofuran [28] provides clarification. The bands observed at 1631 and 1605 cm^{-1} in the Raman spectrum of fulvoipolamiide (**1**) are clearly present in the spectrum of benzofuran, and they can be assigned as carbon–carbon stretching modes, termed ν_7 and ν_8 modes in the Klots and Collier investigation [28]. The intense band at 1695 cm^{-1} of benzofuran is not present in the Raman spectrum, and seems to be related to the carboxyl moiety stretching mode. Fig. 3 depicts the Raman spectrum of ipolamiide, which contains the substituted iridoid ring and also a glucose unit. The most intense band, that at 1497 cm^{-1}, can be assigned to a coupled C=O and C=C stretching vibration, as well some contribution from the CH_2 bending mode of a β-glucose species which has a medium intensity band in the Raman spectrum of glucose around 1470 cm^{-1} [30]. The observed bands in the wavenumber region between 1600–1700 cm^{-1} can be interpreted as key bands of such a compound, according to Schulz et al. [31,32], who characterized the iridoid glycoside (harpagoside) by means of Raman spectroscopy and assigned them to C=C and C=O modes of the iridoid structure. It is worth noting that in this region (around 1600 cm^{-1}) the C=O stretching vibration mode must have a higher contribution to the mode description than the C=C vibration. The band at 1713 cm^{-1} can be assigned to the almost pure carbonyl stretching mode of this compound and some of the low intensity bands observed in the spectrum can be attributed to the carbohydrate modes, as for instance those at 1368 and 1290 cm^{-1} related to COH and CCH stretching modes, and those at 1094 and 1016 cm^{-1} related to CC stretching from the cyclic ring and HCO modes [33].

4. Conclusions

Fulvoipolamiide (**1**) and ipolamiide (**2**), isolated from leaves of *S. glabra*, have been characterized by means of X-ray diffraction and Raman spectroscopy. The crystal structure of **1** is described here for the first time, and the data show that the molecule is planar and is arranged in layers parallel to the crystallographic axis ***a***. The molecules in the same layer have the same orientation, however in adjacent layers the molecules are inverted in relation to each other. The C–C ring bond distances are very similar to each other, indicating the electronic delocalization in these rings. The pyran rings of adjacent layers are involved in a π–π stacking interaction [26] where centroid–centroid and centroid–plane distances are, respectively, 3.54 and 3.36 Å. Compound **2** does not exhibit electronic delocalization in the rings and from this, is ascribed the colour difference between the two compounds, compound **2** being colourless although **1** is red. Fulvoipolamiide contains one hydration water molecule that is involved in three medium-strength hydrogen bonds, for which the average O···O distance is 2.752(4) Å. The ester group is also involved in

medium strength hydrogen bonding to the hydroxyl groups of neighboring molecule, where the O···O distance is 2.791(4) Å. Raman spectroscopic measurements for compounds **1** and **2** indicate some key bands that can be used for reliable analysis and discrimination for both compounds, mainly those at 1695 and 1631 cm^{-1} for compound **1** and 1713 and 1497 cm^{-1} for compound **2**, assigned as ν(C═O) and ν(C═O) and ν(C═C) for both compounds, respectively.

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