

## Two new secoiridoids from *Chelonanthus alatus* (Aubl.) Pulle (Gentianaceae)\*

[Dos nuevos secoiridoides de *Chelonanthus alatus* (Aubl.) Pulle (Gentianaceae)]

Julie Andrea SANCHEZ, Bárbara MORENO-MURILLO & Luis Enrique CUCA SUAREZ

Departamento de Química, Facultad de Ciencias - Universidad Nacional de Colombia, sede Bogotá, Avenida 30 N° 45-03 Ed. 451, Ciudad Universitaria 111611, Bogotá DC, Colombia.

Contactos / Contacts: Bárbara MORENO-MURILLO - E-mail address: [bmorenom@unal.edu.co](mailto:bmorenom@unal.edu.co)

### Abstract

The species *Chelonanthus alatus* is an herbaceous plant with known ethno botanical and medicinal properties used in control of fever, especially those produced by malaria. From dried leaves (1.11 Kg), the crude alcoholic extract was fractionated by liquid-liquid partition with different polarity solvents. From the sec-butyl alcohol soluble fraction, by successive application of chromatographic methods, four compounds type iridoid were isolated and identified by spectroscopic techniques. Compound **1** is a new secoiridoid which was identified as sweroside 7-isobutyryloxy, and it is reported here for the first time in the Gentianaceae family; the other secoiridoids which were isolated are known as vogeloside (**2**), dihydro-chelonanthoside (**3**) and sweroside (**4**); vogeloside was identified for the first time in this plant (*C. alatus*). From the isopropyl acetate extract, in conjunction with the sweroside 7- isobutyryloxy (**1**), chelonanthoside (**5**) and sweroside (**4**), were identified, along with the sweroside 7-isovaleryloxy-(**6**) as a new side chain isomeric ester of dihydrochelonanthoside (**3**). This work presents the spectroscopic analysis of the new structures and some bioactivity data.

**Keywords:** 7-sweroside isobutirato, secoiridoids, *Chelonanthus alatus*, Gentianaceae

### Resumen

La especie *Chelonanthus alatus* (Gentianaceae) es una hierba de aplicaciones etnobotánicas reconocidas en medicina tradicional, especialmente en el control de la fiebre producida por la malaria. De las hojas secas (1,11 Kg) se realizó el extracto crudo en alcohol etílico, el cual se fraccionó por partición líquido-líquido (L-L) con disolventes de diferente polaridad. De la fracción soluble en alcohol sec-butílico, se aislaron cuatro compuestos tipo seco-iridoide por aplicación sucesiva de diversos métodos cromatográficos los cuales se identificaron por técnicas espectroscópicas. El compuesto **1** es un nuevo secoiridoide identificado como de 7- isobutiriloxi-swerosido, y se reporta por primera vez en la familia Gentianaceae; los otros tres secoiridoides aislados se conocen como vogelósido (**2**), dihidrochelonanthosido (**3**) y swerósido (**4**); el vogelósido se identificó por primera vez en *C. alatus*. De la fracción soluble en acetato de isopropilo además del 7-isobutiriloxi-swerosido (**1**) y el swerosido se aislaron e identificaron, el chelonanthosido (**5**) y el isovaleriloxi-swerosido (**6**), el cual es un nuevo isómero del dihidrochelonanthosido. En este trabajo se presenta el análisis espectroscópico que llevó a la elucidación estructural de los compuestos novedosos y algunos datos de bioactividad.

**Palabras Clave:** isobutirato de 7-swerosido, secoiridoides, *Chelonanthus alatus*, Gentianaceae

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## INTRODUCTION

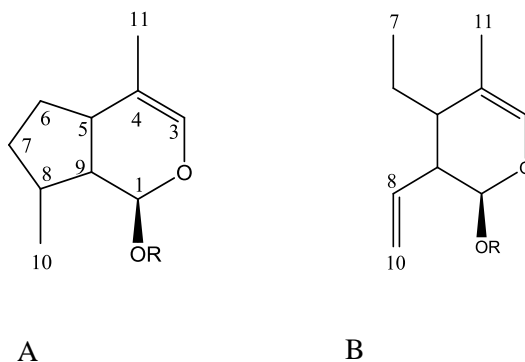
*Chelonanthus alatus* (Aubl.). Pulle (Gentianaceae), (Syn. *Irlbachia alata* (Aubl.), *Lisianthus chelonoides* L.) which is grown in Colombia, is commonly known as “wild tobacco”, “yuriballi” and “koeraja”. It is an herbaceous plant widely used as a remedy in the treatment of malaria and as a saline decoction to thin the bile; the whole plant is also used as purgative, for visceral obstructions, gastric disturbances and other tropical diseases. Stem sap is applied for itches and eczema in NW Guyana; an infusion of leaves was previously used to treat smallpox and is now used to bathe sores and is drunk to treat colds, jaundice and cleanse the blood; also it is known for its strong bitterness (De Filippis *et al.*, 2004). *C. alatus* belongs to the Gentianaceae family, Tribe Helieae. The Gentianaceae family is distributed worldwide with some 1600 species classified into six tribes and 87 genera: they grow mainly in tropical and subtropical areas and the presence of xanthones and bitter principles are common to all members of the genus. In Central and South America, 47 native genera with 36 endemic species have been described (Struwe and Albert, 2002; Filippa and Barboza, 2006). *C. alatus* is an annual herb, with erect stems, tetrahedral or sub-cylindrical, hairless, oval or elliptical leaves with acute apex and round base. Their inflorescences are terminal, sometimes axial, and ascendant. The flowers are pentamerous with bell form calyx; its corolla is yellow-greenish with stamens enclosed to slightly exert and oval ovary. Fruits are elliptical capsules and seeds are tetrahedral or irregular and of brown-reddish color. *C. alatus* is one of the few plants that is bat pollinated, because the bell form of their flowers fits with the form and size of the bat's face (Villarreal, 2001). *C. alatus* is a highly variable species having several subspecies from Mexico to Paraguay which are clearly differentiated (Pringle, 1995). Related to its distribution in Colombia, at the Herbario Nacional Colombiano, Instituto de Ciencias Naturales de la Universidad Nacional de Colombia, Bogotá (HNC-ICN-UNC-SB), there are samples from the provinces of Amazonas, Antioquia, Boyacá, Chocó, Caldas, Casanare, Cauca, Cundinamarca, Córdoba, Guainía, Huila, Magdalena, Santander, Tolima, Valle del Cauca, Meta, Caquetá, Vaupés and Brazilian border región. Despite their recognized therapeutically properties, this plant has not been deeply analyzed from a phytochemical point of view, with scant reports about their chemical composition.

In Gentianaceae family, some specific metabolites have been identified as chemotaxonomic markers; among these there are iridoids, xanthones such as mangiferine and C-glycosylflavonoids. The tribe Helieae is characterized by the presence of iridoids and secoiridoids, some biosynthetically primitive xanthones and an absence of mangiferine and C-glycosyl flavonoids (Bianco, 1990; Struwe and Albert, 2002).

Iridoids represent a large and still expanding group of cyclopentan-(c)-pyrane-mono terpenoids, described as iridane - (*cis* -2 - oxabicyclo - [4.3.0] - nonane formed by the alternative cyclisation of geranyl diphosphate, is classified into four different groups: iridoid glycosides, aglicone iridoids, secoiridoids and bisiridoids; they are found as natural constituents in a large number of plant families, usually but not invariably, such as glucosides (El-Naggar and Beal, 2004). Recently, they have been shown to have therapeutic properties and due to their bitter taste iridoids have been used by some plants and insects as a defensive constituent. Oleuropein is a secoiridoid from olives (*Olea europaea*, Oleaceae) with a 3', 4'-dihydroxy-phenylethyl ester unit which acts, as a feeding stimulant to the olive weevil *Dyscerus perforatus*; it also is a strong protein denaturant when hydrolysed by the enzymes in the plant, acting as a response to herbivore attacks (Nakajima *et al.*, 1995; Jensen *et al.*, 2002). The iridoid biosynthesis has been well investigated, and two main routes have been proposed; these derivatives have been used as chemotaxonomic markers for the super-orders Corniflorae, Gentianiflorae, Loasiflorae and Lamiiflorae (Jensen, 1991; Jensen *et al.*, 2002). The secoiridoids are formed by the oxidative breaking of the C7-C8 bond of the cyclopentan residue, the basic nucleus (Bianco, 1994) (Figure N° 1); the stereo chemical *cis* correlation of the protons H-5 and H-9 is one of the most important structural characteristics from the iridoids (Sampaio-Santos, 2001; Dinda *et al.*, 2007). There are only three previously published works on the chemical constituents of *C. alatus*. Firstly, (*S*)-dihydrochelonanthoside and sweroside were isolated from the polar fraction of the secoiridoids chelonanthoside, (Shiobara *et al.*, 1994); the sweroside 7-isovaleryloxi was isolated from the isopropyl acetate soluble fraction as a new isomer of the dihydro-chelonanthoside (Sánchez, 2008). Irlbacoline a bisphosphocoline derivative with potent

antifungal bioactivity against *Candida albicans*, *Cryptococcus neoformans*, *Aspergillus fumigatus* and *Trichopyton rubru* was identified from the roots; this compound was also reported in *Anthocleista djalonensis* (Loganiaceae) (Bierer *et al.*, 1995; Lu *et al.*, 1999). The present chemical study was conducted

as part of a Natural Products program of native species with biological activity; herein, we report the isolation and structure determination of two novel secoiridoids (**1** and **2**) from this plant, in conjunction with four known derivatives (**3-6**) (Figure N° 2).



**Figure N° 1**  
Basic nucleus of iridoids (A) and secoiridoids (B)

## MATERIALS AND METHODS

### *Plant Material*

Whole plants of *C. alatus* were collected in the airport neighborhood of the city of Florencia, Departamento de Caquetá, Colombia, at 800 msnm and average temperature of 30° C, in June of 2009. One voucher specimen is deposited in the HNC-ICN-UNC-Bogotá, under the code COL 520461.

### *General experimental procedures*

Melting points were determined in a Koffler instrument and were uncorrected. IR spectra were recorded as film on FTIR Shimadzu IR Prestige-21 equipment. UV spectra were registered on a spectrophotometer UV-Vis- Thermo SCIENTIFIC Evolution 300. RMN experiments were performed on Bruker Avance 400 spectrometer using TMS as internal standard; chemical shifts are in ppm and the *J* values in Hz. HR mass spectra were registered on a Liquid Chromatograph with a mass detector IT-TOF Shimadzu with ESI interphase in positive mode. The HPLC preparative analysis were made in a liquid chromatograph Hitachi L6000A with UV-Vis detector, and a semi preparative column LiChrospher 250 - 100 LiChrospher 100 RP-18 (10 µm) was used and the detection was made at 244 nm, isocratic mode. CC Silica gel 60 - 120 mesh Merck (Germany) and TLC silica gel 60 F<sub>254</sub> plates were used; other chemicals were of LiChrospher, analytical or

synthesis grade. For GPC, Sephadex LH-20 was applied; further purification of the compounds by HPLC was achieved with mobile phases of decreasing polarity gradient, mainly with acetonitrile-methanol-water mixtures selected in each case (Jiang *et al.*, 2005).

### *Extraction and chromatographic Separation*

A sample of dried and milled aerial parts of *C. alatus* (1.11 Kg) were extracted with ethylic alcohol 96% at room temperature percolation, assisted with ultrasonic bath (USE), changing the solvent continuously. The filtered extract (F-1) was concentrated at reduced pressure in a rotating evaporator (Heidolph VV2000). A suspension of the crude extract was fractionated by L-L-partition between water and hexane (F-2), chloroform (F-3), isopropyl acetate (F-4), and sec-butyl alcohol (F-5); the aqueous residue was denominated (F-6). After biological assessment and successive chromatographic analysis by TLC in different mobile phases, F-5 was selected to continue the chemical composition study. This extract was fractionated by vacuum liquid chromatography (Handjieva *et al.*, 1991; Coll and Bowden, 1986), gel permeation chromatography (with Sephadex LH-20), which allows a quick, effective and inexpensive separation of complex mixtures of organic compounds.

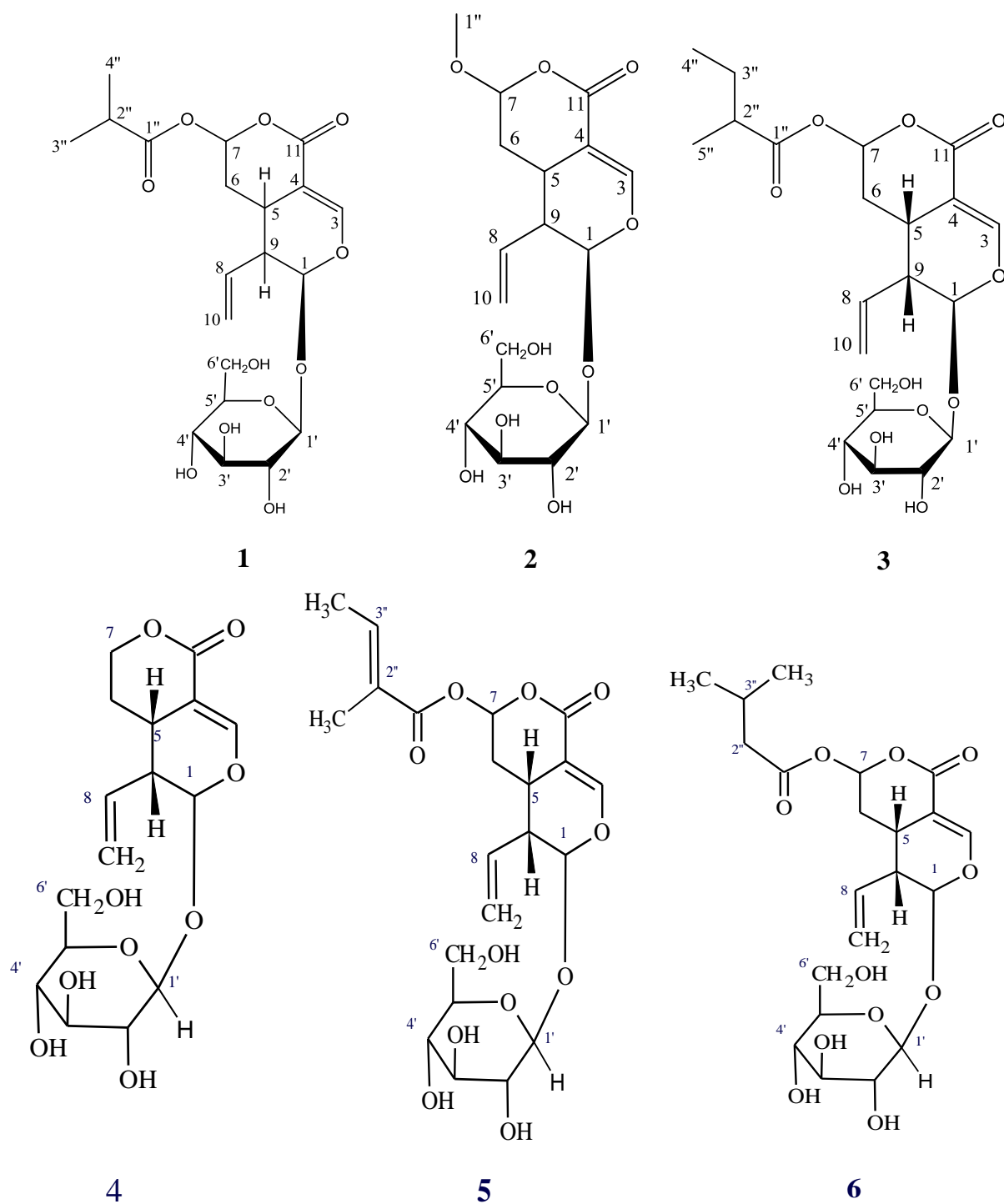


Figure N° 2

Secoiridoids identified from leaves of *C. alatus*: (1) sweroside 7-isobutyryloxy; (2) vogeloside; (3) dihydrochelonanthoside; (4) sweroside; (5) chelonanthoside; (6) sweroside 7-isovaleryloxy.

The final purification of all compounds was made by CC over silica gel using different mixtures of CHCl<sub>3</sub>-MeOH and analytical and semi-preparative HPLC that further led the isolation of the mentioned compounds (1-4). From the F-4, besides the new compound 7-sweroside-isobutyrate (1), the known sweroside (4), chelanonthoside (5), and 7-sweroside 3-methyl-isobutyrate (6) were identified, the last as a new isomer derivative from the dihydro-chelanonthoside.

### Biological activity

In a previous work the anti-malarial potential of traditional remedies was assessed, as they are currently used, instead of plant alcoholic extracts, as it is generally the rule in screening procedures. In fact, it can be assumed that chemical contents vary according to the condition of extraction of the plant. In this way, the anti-malarial activity of traditionally prepared remedies was tested through classical *in vitro* and *in vivo* tests on chloroquine resistant *Plasmodium falciparum* strain, and on *Plasmodium yoelii* rodent malaria. They also, tested the capacity of these remedies to inhibit the formation of hemozoin, the formation of which is a specific process of *Plasmodium*, as hemozoin derives from the digestion of ingested haemoglobin, being highly toxic for the parasite. This specific function is a good target for anti-malarial chemotherapy. Using two

recipes with leaves and roots *C. alatus* gave a CI<sub>50</sub> < 5µg/mL and a 52% inhibition of *P. yoelii* growth in mice, value considered as good and this result justifies further investigation into this species (Desjardins *et al.*, 1979; Deharo *et al.*, 2002). The method of Meyer *et al.* (1982) known as brine shrimp test (BST) was adopted to study the general toxicity of extracts and major fractions obtained from the partition process applied. Also the larvicidal activity bioassay was applied (LAB), with third instar of yellow fever mosquito *Aedes aegypti* [Diptera: Culicidae] in multiple 96 well plates, with samples at concentrations of 2000, 200 and 20 ppm; after 24 h the dead larvae were counted and with the Probit system the effective concentration to 50% (EC<sub>50</sub>) was evaluated (Finney, 1971; McLaughlin *et al.*, 1998).

## RESULTS AND DISCUSSION

### Biological activity

From results of BST all extracts and fractions gave EC<sub>50</sub> with values less than 1000 µg/mL considered of interest to continuing the search for promising bioactive compounds. The results observed, after application of the specific larvicidal tests with third instar of *A. aegypti*, show through the EC<sub>50</sub> values, that the samples are not bioactive (Table N° 1). The properties of these new compounds are currently under evaluation at the Pharmacology Department UNC, in Bogotá, Colombia.

Table N° 1

Sample	<i>Artemia salina</i> EC <sub>50</sub> (µg/mL)	<i>Aedes aegypti</i> EC <sub>50</sub> (µg/mL)
F-1 (ethanol)	186	943
F-2 (hexane)	82	750
F-3 (chloroform)	243	790
F-4 isopropyl acetate	225	1000
F-5 sec-butyl alcohol	198	960

### General and specific bioassay results as EC<sub>50</sub> (µg/mL) of main extracts from leaves of *C. alatus*

#### Compound 1: Sweroside 7-isobutyryloxy

Compound 1 was isolated as a white amorphous powder. Melting point 102-103 °C; IR (film) ν 3387 (OHs), 2924 (CH-), 1712 (C=O, enol-ester), 1620 (C=C), 1087 (C-O-C) and 1010 (C-OH) cm<sup>-1</sup>. The UV spectrum showed absorption maxima at λ<sub>MeOH</sub> =

245 nm. Positive HR-ESI-QTOF-MS showed a molecular ion peak at *m/z* = 444.1632 [M+H]<sup>+</sup>, corresponding to a molecular formula C<sub>20</sub>H<sub>28</sub>O<sub>11</sub> with losses of glucose and one isobutyryl residue at *m/z* = 195.056 (El-Naggar and Beal, 1980). In its <sup>1</sup>H NMR spectrum, a downfield double signal at δ<sub>H</sub> = 7.69 ppm

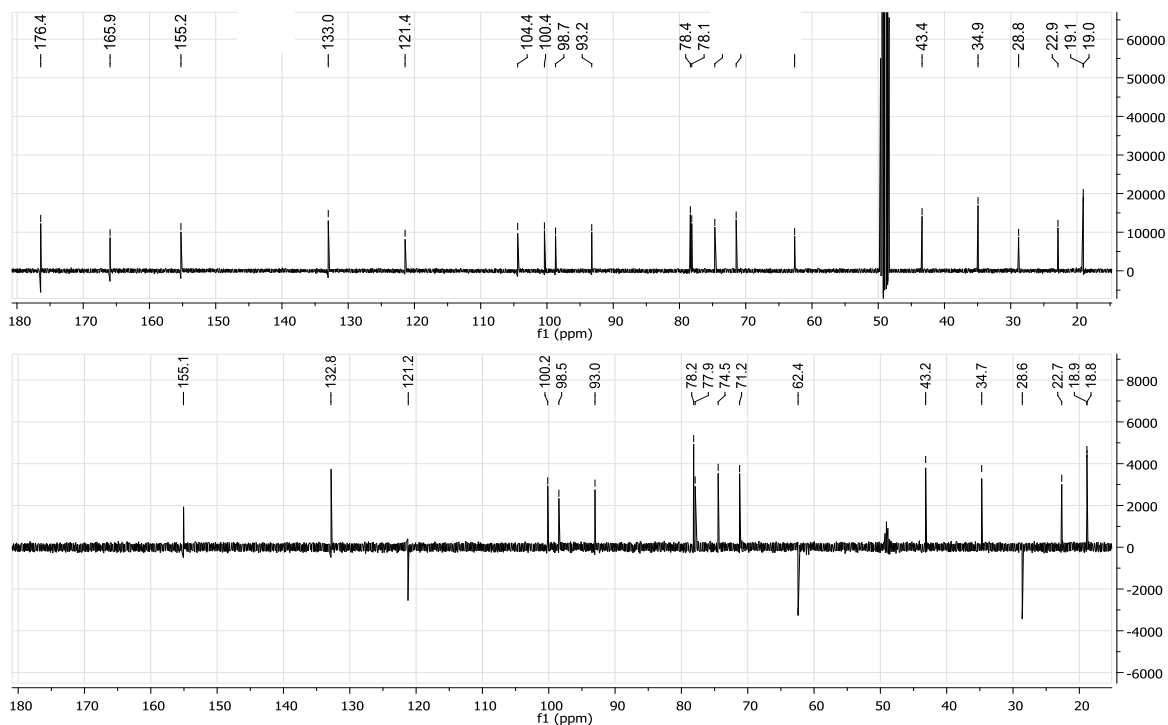
with  $J = 2.4$  Hz, indicated the presence of an oxyolefinic hydrogen, typical of the secoiridoids derived from the nucleus type sweroside. The signal at  $\delta_{\text{H}} = 6.56$  ppm (1H, *t*,  $J = 2.2$  Hz) was assigned to the proton of the C-7 by comparative analysis with reported data (Table N° 2). Also there are two double doublets coupling between them, at  $\delta_{\text{H}} = 5.28$  ppm (1H, *dd*,  $J = 9.9, 2.1$ , H 10a, *cis*) and 5.30 (1H, *dd*,  $J = 16.9, 2.1$ , H10b *trans*), that were assigned to two protons of a  $sp^2$  methylene group, which is coupled with another proton observed at  $\delta_{\text{H}} = 5.52$  ppm as a double triplet with  $J = 16.9$  and 9.9 Hz, assigned to H-8, forming a vinilic fragment, which interacts with the H observed at  $\delta_{\text{H}} = 2.7$  (1H, *ddd*, 9.9, 1.5 Hz, H-9) and with the H at  $\delta_{\text{H}} = 5.52$  assigned to H-1. Also the spectrum revealed a signal of an anomeric proton at  $\delta_{\text{H}} = 4.76$  ppm (1H, *d*,  $J = 8.0$ , H-1') and its  $J$  value indicated a *trans* biaxial configuration with the proton denominated as H-2', which is found in a

multiple signal observed at  $\delta_{\text{H}} = 3.18$  (1H, *dd*,  $J = 9.0, 8.0$ ) as is reported in other sweroside derived compounds. Also there are observed signals attributed to the methylene group named as H-6' of a residue of a monosaccharide that in this case was assigned as glucose, that appear at  $\delta_{\text{H}} = 3.65$  ppm (1H, *dd*,  $J = 12.0, 5.6$ ) and 3.86 (1H, *dd*,  $J = 12.0, 1.8$ ). In addition, in the high field region there are signals corresponding to a isopropyl unit formed by a septet of one methine at  $\delta = 2.71$  ppm (1H, septet,  $J = 07.0$  Hz) which is coupled with two doublets assigned to two methyl groups at  $\delta = 1.14$  (3H, *d*,  $J = 7.0$ , H-3'') and  $\delta = 1.13$  (3H, *d*,  $J = 7.0$ , H-4'') (El-Naggar, and Beal, 1980; Jiang *et al.*, 2005) (Table N° 2). Its  $^{13}\text{C}$ -NMR spectra showed twenty signals according its molecular formula, classified by DEPT 135 as 14 C with positive phase, three methylene groups and four quaternary carbons (Figure N° 3).

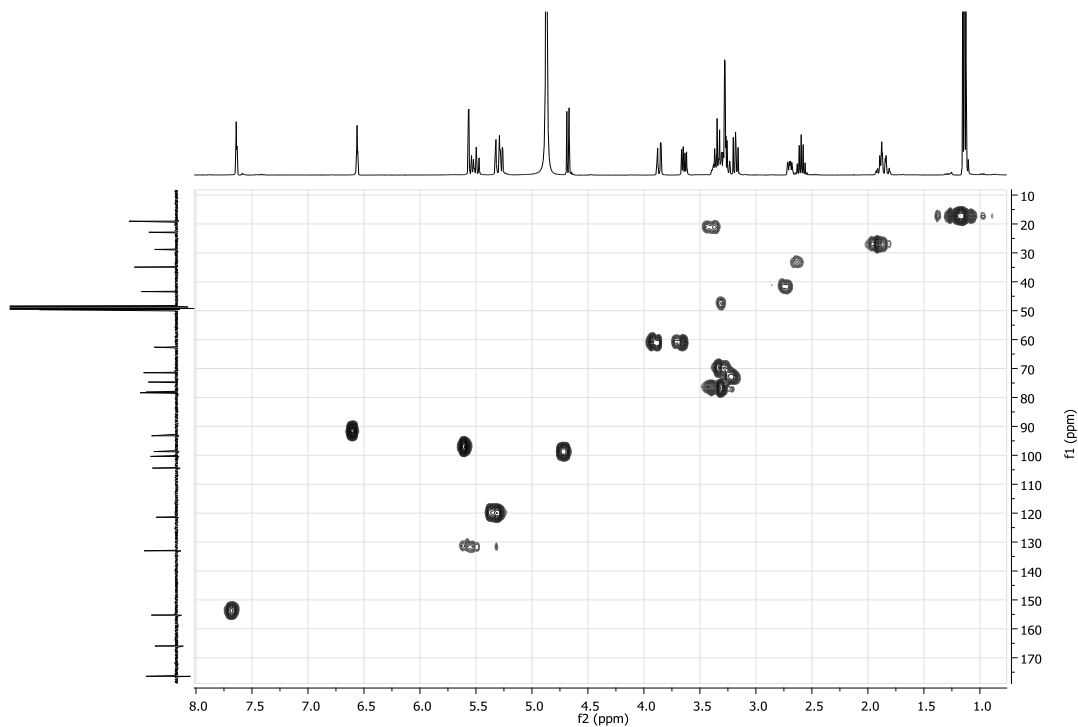
Table N° 2

Position	$\delta_{\text{H}}$ , <i>m</i> , $J$ (Hz)	$\delta_{\text{C}}$	HMBC
1	5.62, <i>d</i> , (1.5)	98.7	C-5, , C-3, C-1'
3	7.69, <i>d</i> , (2.4)	155.2	C-1, C-4, C-5, C-11
4	-	104.4	
5	3.35-3.40	22.9	
6	1.84, <i>dt</i> , (9.6, 6.7, 2.6) 1.90, <i>ddd</i> , (9.6, 6.7, 2.6)	28.8	C-4, C-5, C-7,
7	6.61, <i>t</i> , (2.3)	93.2	C-5, C-11, C-1''
8	5.52, <i>dt</i> , (16.9, 9.9)	133.0	C-9, C-10
9	2.70, <i>ddd</i> , (9.9, 5.4, 1.5)	43.4	C-1, C-4, C-5, C-8, C-10
10	5.28, <i>dd</i> , (9.9, 2.1) 5.30, <i>dd</i> , (16.9, 2.1)	121.4	C-8, C-9
11	-	165.9	
1'	4.68, <i>d</i> , (8.0)	100.4	C-1
2'	3.18, <i>dd</i> , (9.9, 8.0)	74.7	
3'	3.23-3.40, <i>m</i>	78.1	
4'	3.23-3.40, <i>m</i>	71.4	
5'	3.23-3.40	78.3	
6'	3.69, <i>dd</i> , (12.0, 5.7) 3.86, <i>dd</i> , (12.0, 2.0)	62.6	
1''	-	176.4	
2''	2.60, <i>septet</i> , (7.0)	34.9	C-1'', C-3'', C-4''
3''	1.14, <i>d</i> , (7.0)	19.1	C-1'', C-2'', C-4''
4''	1.13, <i>d</i> (7.0)	19.0	C-1'', C-2'', C-3''

$^1\text{H}$  and  $^{13}\text{C}$ -RMN spectroscopic data of compound 1 in  $\text{CH}_3\text{OH}-d_4$  (400 MHz)



**Figure N° 3**  
<sup>13</sup>C-NMR and DEPT-135 spectroscopic data of compound 1(100 MHz, CH<sub>3</sub>OH-*d*<sub>4</sub>)



**Figure N° 4**  
 HETCOR NMR correlation contours observed for compound 1

There are two carbonyl signals, one for an ester at  $\delta_C=165.9$  (C-1''); the second was assigned to the C=O of the lactone of the sweroside nucleus at  $\delta_C = 165.4$  (C-11); four olefin carbons of the basic nucleus appear at  $\delta_C = 133.0$  y  $\delta_C = 121.4$ , (C-8 and C-10) and the C-3 ( $\delta_C = 155.2$ ) and C-4 ( $\delta_C = 104.4$ ). The carbon atoms of the glucose residue were identified between  $\delta_C$  60 to  $\delta_C$  110 ppm (Table N° 2). In the high field region there are signals attributed to a methine at  $\delta_C = 34.9$  and two methyl carbons observed at  $\delta_C = 19.1$  and  $\delta_C = 19.0$ , of the isopropyl unit. In the HETCOR spectrum, the direct attachment between carbons and protons were revealed for the nucleus sweroside and the isopropyl residue: ( $\delta_H = 2.60$ -  $\delta_C = 34.9$ , CH) and ( $\delta_H = 1.13$  -  $\delta_C = 19.0$  and  $\delta_H = 1.14$  -  $\delta_C = 19.1$ , two methyl groups Figure N° 4). The correlation between H-1 and C-1 confirm that the glucose is located in C-1 position of the aglucone.

Moreover the scalar interactions of the sweroside nucleus, the COSY spectrum of **1** showed correlation contours between H-3'' at 1.14 (3H, *d*, *J* = 7.0) and H-4'' at 1.13 (3H, *d*, *J* = 7.0); the methine H-2'' at 2.60 (1H, *septet*, *J* = 7.0) of the isopropyl unit. The complex multiple signal between  $\delta_H = 3.23$  - 3.40 ppm integrating for 6H, included the glucose protons: H-3', H-4' and H-5'; according to the observed correlations of the H-6 and H-9 in this region in the HETCOR register, it could be possible to assign it to the proton H-5 at  $\delta_H = 3.38$  m, as it has been described for other secoiridoids of this type

previously identified; from this register, also the connectivity of the methine C-H ( $\delta_H = 2.60$  -  $\delta_C = 34.9$ ) and the two methyl groups ( $\delta_H = 1.13$  -  $\delta_C = 19.0$  and  $\delta_H = 1.14$  -  $\delta_C = 19.1$ ) that form the isopropyl unit were established along with the correlations coming from the sweroside nucleus (Figure N° 4). (El-Naggar and Beal, 1980; Shiobara *et al.*, 1994). From the HMBC spectrum the long range correlations between the H-3'' with the carbonyl group, confirm that the isopropyl fragment form part of the isobutyryloxy residue; besides the correlations of the sweroside nucleus between the H-3 and C-1, C-4 and C-5 and those of the proton H-9 with C-1, C-4 and C-5 can be observed, and they led to the establishment of one ring of the structure. H-8 presents interactions with C-9 and C-10 showing that the vinyl residue is joined with C-9. The HMBC correlation between the protons H-6 and C-4, C-5 and C-7 and that between H-7 and the carbonyl carbons C-11 and C-1'' are observed and confirmed that the isobutyryloxy fragment is bonded to C-7 (Figure N° 5). Moreover, according to the bibliographical review, the chemical shifts and coupling constant analysis, the relative stereochemistry of the new compound was assigned like similar to the other known identified compounds, fact to be proved with the NOESY spectra. In conclusion, to the best of our knowledge, compound **1** was assigned as sweroside 7-isobutyryloxy herein reported in *C. alatus* by the first time.

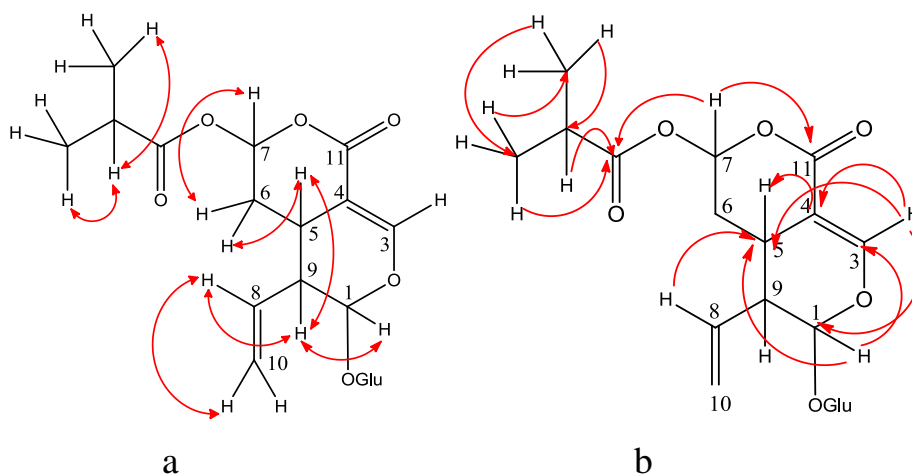


Figure N° 5

Long range correlations observed in COSY (a) and HMBC (b) spectra for compound **1**



**Compound 2: Vogeloside**

Compound **2** was isolated as a colorless oil. Analyzing their LREIMS the molecular formula of  $C_{17}H_{24}O_{10}$  and the molecular weight of 388 amu (calculated 388.1920), determined from their pseudo-molecular ion at  $m/z = 389.17$ , and also the fragment ion at  $m/z = 227$ , it was concluded the presence of a glucose residue, which lost at  $([M+H]^+ - 162)$  was observed. All the NMR spectra are similar to the sweroside (**4**), concluding that this compound has this basic nucleus. The main difference is the presence of a methoxyl residue at  $\delta_H = 3.48$  ppm as singlet for 3H, and a triplet signal assigned to the H-7 ( $\delta_H = 5.31$  ppm), which correlates with the protons H-6a and H-6b (Figure N° 2b). From the HMBC register, the correlations among H-7 ( $\delta_H = 5.31$  ppm) and the carbons C-5  $\delta_C = 22,8$  and C-11  $\delta_C = 167,4$ , was set; also the interaction between the methoxyl protons and C-7 ( $\delta_C = 103,3$ ) was determined. In this way, the presence of a methine in C-7 and the bonding of the methoxyl to this carbon were confirmed. Comparative analysis with the literature data lead to the structure of 7-methoxy-sweroside, known as vogeloside, firstly reported in species *Anthocleista vogelii* (Gentianaceae), and found in *C. alatus* for the first time (El-Naggar and Beal, 1980; Kawai *et al.*, 1988).

**Compound 3: Dihydrochelonanthoside**

By HRMS, the molecular formula for compound **3** was established as  $C_{21}H_{30}O_{11}$  with mw of 458.1788, from their pseudo-molecular ion  $m/z = 459.1821$   $[M+H]^+$ , adduct ions at  $m/z = 481.1682$ ,  $[M+Na]^+$  and  $m/z = 497.1423$   $[M+K]^+$ . By tandem MS/MS over the ion  $m/z = 481$ , fragment ions at  $m/z = 379$  ( $[M+Na]^+ - 102$ ),  $m/z = 319$  ( $[M+Na]^+ - 162$ ) corresponding to a glucose lost and the simultaneous losing of the two mentioned ions at  $m/z = 217$  ( $[M+Na]^+ - (102 + 162)$ ) were observed. Detailed analysis of NMR spectra of 1D and 2D afforded for this compound the structure of the dihydrochelonanthoside, previously identified in *C. alatus* (Shiobara *et al.*, 1994) (Figure N° 2).

**Compound 4: Sweroside**

Was obtained as needles (110 mg) mp 169 – 170 °C;  $C_{16}H_{22}O_9$ , from MS pseudo molecular ion at ( $m/z = 359$   $[M+H]^+$ ). NMR data identical with reported data (Van Beek *et al.*, 1982).

**Compound 5: Chelonanthoside**

White powder. HR ESI MS positive mode  $m/z$   $[M+H]^+ = 457.1727$  (calculated for  $C_{21}H_{31}O_{11} = 457.1710$ ) (NMR 400 MHz, in agreement with reports in the literature (Shiobara *et al.*, 1994).

**Compound 6: Sweroside 7-isovaleryloxy**

It was obtained as a white powder; its mw is the same of compound **3** with the same molecular formula. The difference was centered in the position of a methyl group, that formed part of an isopropyl residue and led to the establishment of the structure of an ester, named as sweroside 7-isovaleryloxy (Dinda *et al.*, 2007; Dinda *et al.*, 2009).

In conclusion, here we report the structural analysis of six compounds isolated and identified from the leaves extract of the species *C. alatus*, growing in Colombia, which were named as the new compounds sweroside 7-isobutytyloxy and vogeloside and the known compounds sweroside, chelonanthoside, dihydrochelonanthoside and sweroside 7-isovaleryloxy, in agreement with the literature reports.

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