Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas
ISSN: 0717-7917
editor.blacpma@usach.cl
Universidad de Santiago de Chile
Chile

SANCHEZ, Julie Andrea; MORENO-MURILLO, Bárbara; CUCA SUAREZ, Luis Enrique
Two new secoiridoids from Chelonanthus alatus (Aubl.) Pulle (Gentianaceae)
Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas, vol. 12, núm. 2, marzo-enero, 2013, pp. 186-195
Universidad de Santiago de Chile
Santiago, Chile

© 2013 Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas 12 (2): 186 - 195
ISSN 0717 7917
www.blacpma.usach.cl

Artículo Original | Original Article

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 Nº 45-03 Ed. 451, Ciudad Universitaria 111611, Bogotá DC, Colombia.
Contactos | Contacts: Bárbara MORENO-MURILLO - E-mail address: bdmorenom@unal.edu.co

Abstract
The species *Chelananthus 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 isobutirate, secoiridoids, *Chelonanthus alatus*, Gentianaceae

Resumen
La especie *Chelonanthus alatus* (Gentianaceae) es una hierba de aplicaciones ethnobotánicas 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

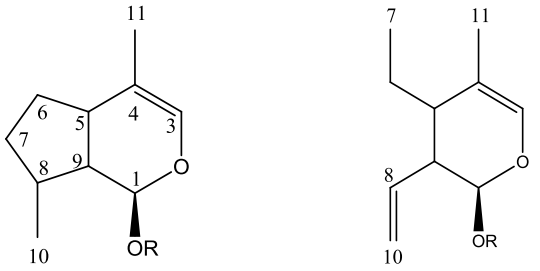
Recibido | Received: August 16, 2012.
Aceptado en versión corregida | Accepted in revised form: October 20, 2012.
Publicado en línea | Published online: March 30, 2013
Este artículo puede ser citado como / This article must be cited as: JA Sanchez, B Moreno-Murillo, LE Cuca-Suarez. 2013. Two new secoiridoids from *Chelonanthus alatus* (Aubl.)Pulle (Gentianaceae). **Bol Latinoam Caribe Plant Med Aromat** 12 (2): 186 – 195.
***Selected at the Third Congress of Chemistry of Natural Products Chilean, Argentine and Hispanic. Punta Arenas, Chile, April of 2012.**

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 Filipps *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 xantoquinones 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, tetrangle 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 pentamerical 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 tetrangle 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-glicosylflavonoids. The tribe Helieae is characterized by the presence of iridoids and secoiridoids, some biosynthetically primitive xanthones and an absence of mangiferine and C-glicosyl 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*, Oleacea) with a 3', 4'-dihydroxy-phenilethyl 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 Nº 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 Nº 2).



A B

Figure Nº 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 30º 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 LiChrocart 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_{254} 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.

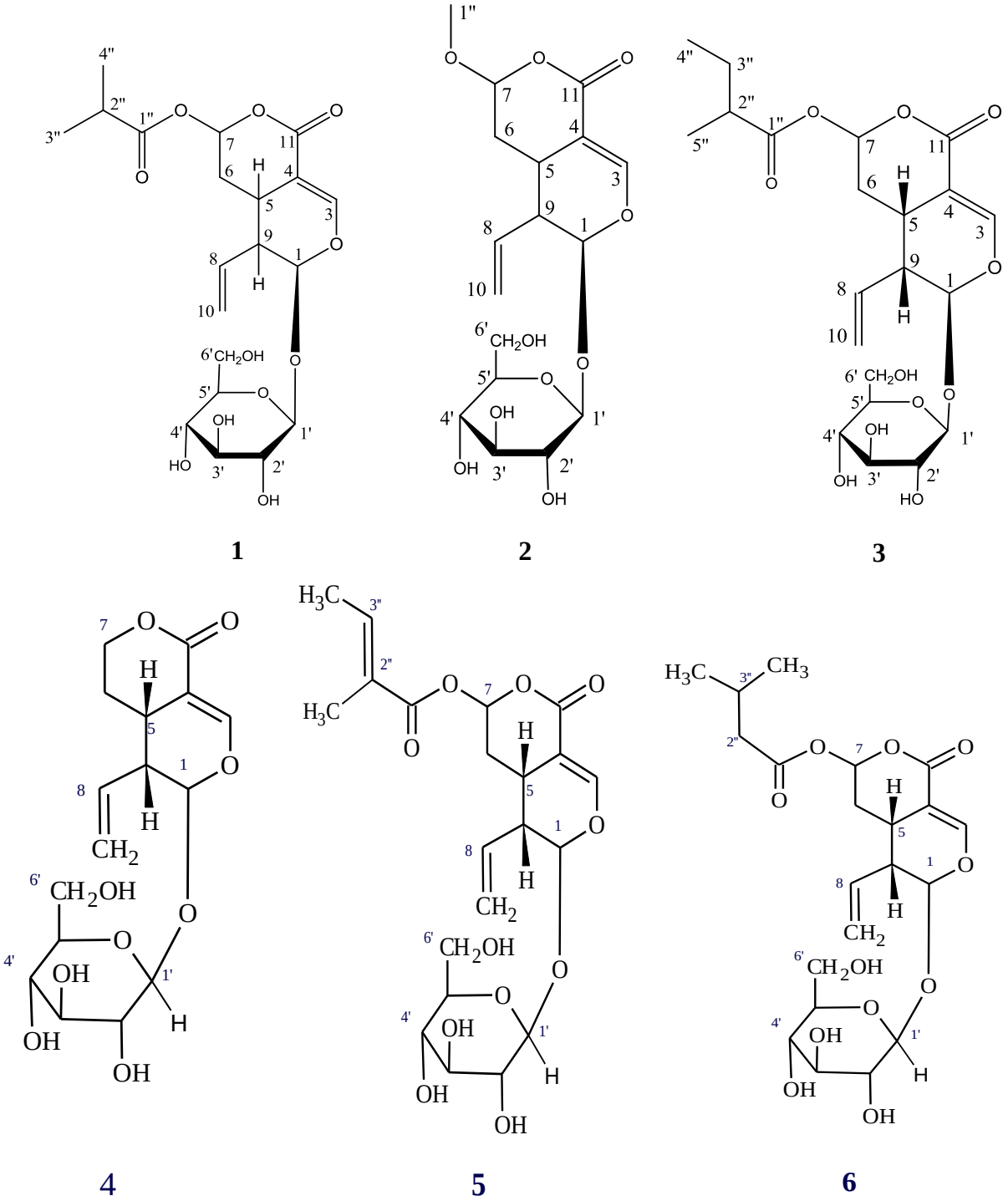


Figure Nº 2
Secoiridoids identified from leaves of *C. alatus*: (1) sweroside 7-isobutyryloxy; (2) vogeloside; (3) dihydrochelonanthoside; (4) sweroside; (5) chelonanthoside; (6) sweroside 7-isovaleryloxy.

The final purification of all compounds was made by CC over silica gel using different mixtures of $CHCl_3$–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-ibutyrate (**6**) were identified, the last as a new isomer derivative from the dihydro-chelanthoside.

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_{50} < 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_{50}) was evaluated (Finney, 1971; McLaughlin *et al.*, 1998).

RESULTS AND DISCUSSION

Biological activity

From results of BST all extracts and fractions gave EC_{50} 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. egypti*, show through the EC_{50} values, that the samples are not bioactive (Table Nº 1). The properties of these new compounds are currently under evaluation at the Pharmacology Department UNC, in Bogotá, Colombia.

Table Nº 1

Sample	*Artemia salina* EC_{50} (µg/mL)	*Aedes aegypti* EC_{50} (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_{50} (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 ºC; IR (film) *v* 3387 (OHws), 2924 (CH-), 1712 (C=O, enol-ester), 1620 (C=C), 1087 (C-O-C) and 1010 (C-OH) cm^{-1}. The UV spectrum showed absorption maxima at λ_{MeOH} = 245 nm. Positive HR-ESI-QTOF-MS showed a molecular ion peak at *m/z* = 444.1632 $[M+H]^+$, corresponding to a molecular formula $C_{20}H_{28}O_{11}$ with losses of glucose and one isobutyryl residue at *m/z* = 195.056 (El-Naggar and Beal, 1980). In its ^1H NMR spectrum, a downfield double signal at δ_H = 7.69 ppm

with *J* = 2.4 Hz, indicated the presence of an oxyolefinic hydrogen, typical of the secoirioids derived from the nucleus type sweroside. The signal at δ_H = 6.56ppm (1H, *t*, *J* = 2.2 H-7) was assigned to the proton of the C-7 by comparative analysis with reported data (Table Nº 2). Also there are two double doublets coupling between them, at δ_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 δ_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 δ_H = 2.7(1H, *ddd*, 9.9, 1.5 Hz, H-9) and with the H at δ_H = 5.52 assigned to H-1. Also the spectrum revealed a signal of an anomeric proton at δ_H = 4.76ppm (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 δ_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 δ_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 δ = 2,71 ppm (1H, septet, *J* = 07.0 Hz) which is coupled with two doublets assigned to two methyl groups at δ = 1.14 (3H, *d*, *J* = 7.0, H-3'') and δ = 1.13 (3H, *d*, *J* = 7.0, H-4'') (El-Naggar, and Beal, 1980; Jiang *et al.*, 2005)(Table Nº 2). Its ^{13}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 Nº 3).

Table Nº 2

Position	δ_H, *m*, *J*(Hz)	δ_C	HMBC
1	5.62, *d*, (1.5)	98.7	C-5, , C-3, C-1'
3	7.69, *d*, (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, *dt*, (9.6, 6.7, 2.6) 1.90, *ddd*, (9.6, 6.7, 2.6)	28.8	C-4, C-5, C-7,
7	6.61, *t*, (2.3)	93.2	C-5, C-11, C-1''
8	5.52, *dt*, (16.9, 9.9)	133.0	C-9, C-10
9	2.70, *ddd*, (9.9, 5.4, 1.5)	43.4	C-1, C-4, C-5, C-8, C-10
10	5.28, *dd*, (9.9, 2.1) 5.30, *dd*, (16.9, 2.1)	121.4	C-8, C-9
11	-	165.9	
1'	4.68, *d*, (8.0)	100.4	C-1
2'	3.18, dd, (9.9, 8.0)	74.7	
3'	3.23-3.40, m	78.1	
4'	3.23-3.40,m	71.4	
5'	3.23-3.40	78.3	
6'	3.69,*dd*, (12.0,5.7) 3.86, *dd*, (12.0, 2.0)	62.6	
1''	-	176.4	
2''	2.60, *septet*, (7.0)	34.9	C-1'', C-3'', C-4''
3''	1.14, *d*, (7.0)	19.1	C-1'', C-2'', C.4''
4''	1.13, *d* (7.0)	19.0	C-1'', C-2'', C-3''

^{1}H and ^{13}C-RMN spectroscopic data of compound 1 in CH_3OH-d_4 (400 MHz)

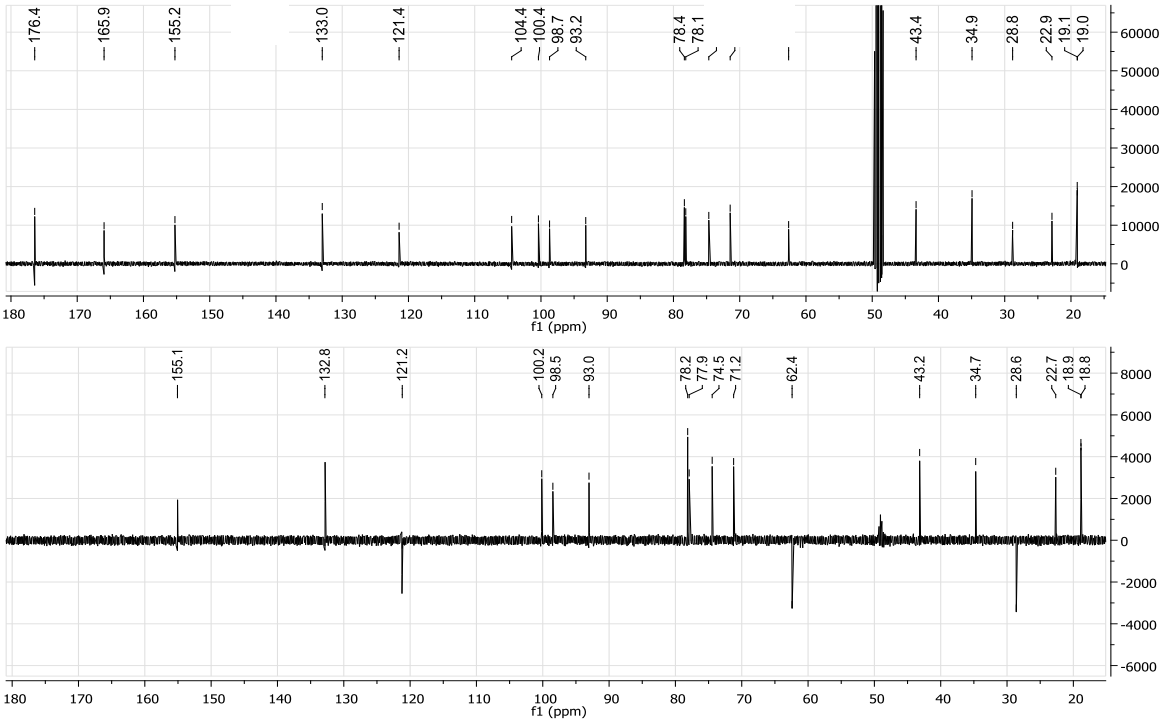


Figure Nº 3
^{13}C-NMR and DEPT-135 spectroscopic data of compound 1(100 MHz, CH_3OH-d_4)

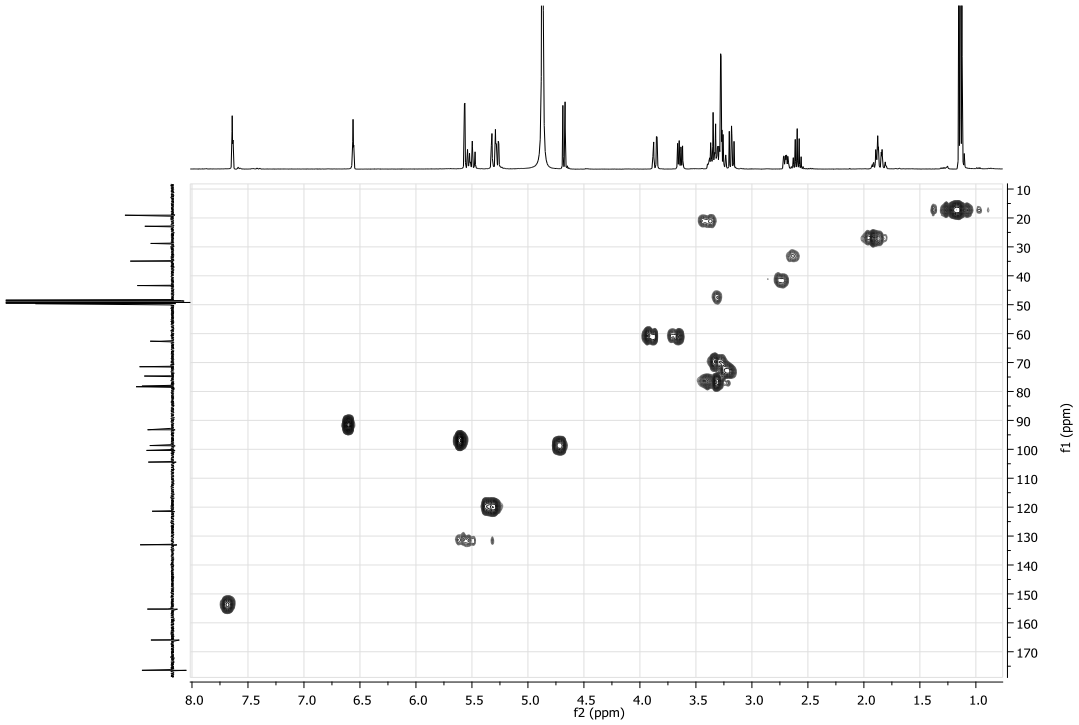
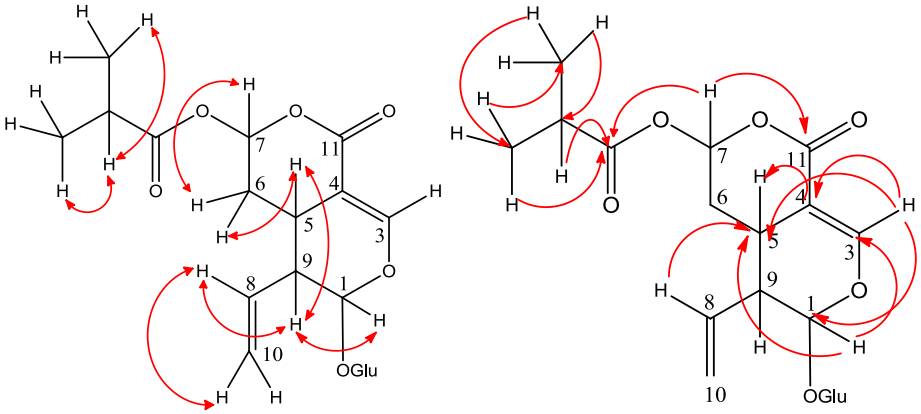


Figure Nº 4
HETCOR NMR correlation contours observed for compound 1

There are two carbonyl signals, one for an ester at δ_C =165.9 (C-1''); the second was assigned to the C=O of the lactone of the sweroside nucleus at δ_C = 165.4 (C-11); four olefin carbons of the basic nucleus appear at δ_C = 133.0 y δ_C = 121.4, (C-8 and C-10) and the C-3 (δ_C = 155.2) and C-4 (δ_C = 104.4).The carbon atoms of the glucose residue were identified between δ_C 60 to δ_C 110 ppm (Table Nº 2). In the high field region there are signals attributed to a methine at δ_C = 34.9 and two methyl carbons observed at δ_C = 19.1and δ_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: (δ_H = 2.60- δ_C = 34.9, CH) and (δ_H = 1.13 - δ_C = 19.0 and δH = 1.14 - δC = 19.1, two methyl groups Figure Nº 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 δ_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 δ_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 (δ_H = 2.60 - δ_C = 34.9) and the two methyl groups (δ_H = 1.13 - δ_C = 19.0 and δ_H = 1.14 - δ_C = 19.1) that form the isopropyl unit were established along with the correlations coming from the sweroside nucleus (Figure Nº 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 Nº 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.



a b

Figure Nº 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 δ_H = 3.48 ppm as singlet for 3H, and a triplet signal assigned to the H-7 (δ_H = 5.31 ppm), which correlates with the protons H-6a and H-6b (Figure Nº 2b). From the HMBC register, the correlations among H-7 (δ_H = 5.31 ppm) and the carbons C-5 δ_C = 22,8 and C-11 δ_C = 167,4, was set; also the interaction between the methoxyl protons and C-7 (δ_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 Nº 2).

Compound 4: Sweroside

Was obtained as needles (110 mg) mp 169 – 170 ºC; $C_{16}H_{22}O_9$, 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.

REFERENCES

Bertani S, Bourdy G, Landau I, Robinson JC, Esterre PH, Deharo E. 2005. Evaluation of French Guiana traditional antimalarial remedies. **J Ethnopharmacol** 98: 45 - 54.

Bianco A. 1990. **The Chemistry of Iridoids**. In: "Studies in Natural Products Chemistry". Atta-ur-Rahman, Ed., Elsevier Science Publishers, Amsterdam, The Netherland.

Bianco A. 1994. Recent developments in Iridoids Chemistry. **Pure Appl Chem** 66: 2335 - 2338**.**

Bierer DE, Gerber RE, Jolad SD, Ubillas RP, Randle J, Nauka E, Latour J, Dener JM, Fort DM, Kuo J, Inman WD, Dubenko LG, Ayala F, Ozioko A, Obialor C, Elizabetsky E, Carlson T, Truong T, Bruening RC. 1995. Isolation, structure elucidation, and synthesis of Irlbacholine, 1,22-Bis[[[2-(trimethylam-monium) ethoxyl]-phosphinyl]-oxy]-docosa-ne: A novel antifungal plant metabolite from *Irlbachia alata and Anthocleista djalonensis***. J Org Chem** 60: 7022 - 7026.

De Filipps RA, Maina SL, Crepin J. 2004. **Medicinal plants of the Guainas (Guyana, Surinam, French Guiana).** Department of Botany, National Museum of Natural History. Smithsonian Institution Washington DC, USA.

Deharo E, Garcia R, Oporto P, Sauvain M, Gautret PH, Ginsburg H. 2002. A non-radiolabeled ferriprotoporphyrin IX biomineralization inhibition test (FBIT) for the high throughput screening of antimalarial compounds. **Expertl Parasitolog** 100: 252 - 256.

Desjardins RE, Canfield CJ, Haynes JD, Chulay JD. 1979. Quantitative assessment of antimalarial activity *in vitro* by a semi-automated microdilution technique. **Antimicrobial Agents Chem** 16: 710 - 718.

Dinda B, Choudhury DR, and Mohanta BC. 2009. Naturally occurring iridoids, secoiridoids and their bioactivity. An updated review, Part 3. **Chem Pharm Bull** 57: 765 - 796.

Dinda B, Debnath S, Harigaya Y. 2007. Naturally occurring iridoids. A review, Part 1. **Chem Pharm Bull** 55: 159 - 222.

El-Naggar LJ, Beal JL. 1980. Iridoids. A review. **J Nat Prod** 43: 649 - 707.

Filippa E, Barboza G. 2006. Novedades en Gentianaceae para América del Sur. **Brit Org/Sida** 22: 129 - 143.

Finney DJ. 1971. **Probit Analysis**, 3rd Eds, Cambridge University Press, Cambridge, UK.

Handjieva N, Saadi H, Popov S, Baranovska I. 1991. Separation of iridoids by vacuum liquid chromatography. **Phytochem Anal** 2: 130 - 133.

Herbario Nacional Colombiano – Instituto de Ciencias Naturales – Universidad Nacional de Colombia sede Bogota. http://www.biovirtual.unal.edu.co/ICN/?controlador=QuickSearch&accion=searchByOther&keyword=Chelonanthus+alatus&stype=searchByGender&keyword1=Chelonanthus (Consulted February 1, 2013)

Jensen SR, Franzyk H, Wallander E. 2002. Chemotaxonomy of the Oleaceae: iridoids as taxonomic markers. **Phytochemistry** 60: 213 - 231.

Jensen SR. 1991. **Plant iridoids, their biosynthesis and distribution in angiosperms.** In: Harborne, JB, Tomas-Barberan FA, Eds. Ecological Chemistry and Biochemistry of Plant Terpenoids, Clarendon Press, Oxford, UK.

Jiang RW, Wong KL, Chan YM, Xu HX, But PPH, Shaw PC. 2005. Isolation of iridoid and secoiridoid glycosides and comparative study on *Radix gentianae* and related adulterants by HPLC analysis. **Phytochemistry** 66: 2674 - 2680.

Kawai H, Kuroyanagi M, Ueno A. 1988. Iridoid Glycosides from *Lonicera japonica* Thunb. **Chem Pharm Bull** 36: 3664 - 3666.

Lu Q, Ubillas RP, Zhou Y, Dubenko LG, Dener JM, Litvak J, Phuan PW, Flores M, Ye ZJ, Gerber RE, Truong T, Bierer DE. 1999. Synthetic analogues of irlbacholine: A novel antifungal plant metabolite isolated from *Irlbachia alata***. J Nat Prod** 62: 824 - 828.

McLaughlin JL, Rogers, LL, Anderson J. 1998.The use of biological assays to evaluate botanicals. **Drug Information J** 32: 513 - 524.

Meyer BN, Ferrigni NR, Putnam JE, Jacobson LB, Nicholas DE, McLaughlin JL. 1982. Brine shrimp: a convenient general bioassay for active plant constituents. **Planta Med** 45: 31 - 34.

Nakajima S, Kitamura T, Baba N, Iwasa J, Ichikawa T. 1995. Oleuropein, a secoiridoid from olive as a feeding stimulant to the olive weevil (*Dyscerus perforatus*). **Bioscience Biotech Biochem** 59: 769 - 770.

Pringle JS. 1995. **Gentianaceae - Flora of Ecuador**. Part 159A. Series Flora de Ecuador. Pringle JS Ed. Botanical Institute, Goteborg University, Sweden.

Sampaio-Santos MI. 2001. Biosynthesis: significance of Iridoids in chemosystematics. **J Braz Chem Soc** 12: 144 - 153

Sánchez JA. 2008. **Estudio fitoquímico preliminar de *Chelonanthus alatus* (Gentianaceae)**. Tesis de pregrado. Universidad Nacional de Colombia, Bogotá, Colombia.

Shiobara Y, Kato K, UedaY, Taniue K, Syoha E, Nishimoto N, De Oliveira F, Akisue G, Kubota-Akisue M, Hashimoto G.1994. Secoiridoid glucosides from *Chelonanthus chelonoides*. **Phytochemistry** 37: 1649 - 1652.

Struwe L, Albert V. 2002. **Gentianaceae: systematic and natural history.** First Ed.; Cambridge University Press, Cambridge, UK.

Van Beek TA, Lankhorst PP, Verpoorte R, Svenden AB. 1982. Isolation of the secoiridoid sweroside from *Tabernaemontana psoroapa.* **Planta Med** 44: 30 - 31.

Villarreal JA. 2001. **Gentianaceae. Flora de Veracruz.** Ed. Instituto de Ecología, Veracruz, Univ. California, Riverside, CA, USA.