CALYCIN AND PHYSCION FROM THE LICHEN *CANDELARIA CONCOLOR*

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Received: 11/04/12 Accepted: 16/06/12 Published: 30/06/12

Keywords: *Calycin; Mannitol; Candelaria concolor; 2D-NMR; Physcion.*

ABSTRACT

Calycin, Physcion and Mannitol were isolated from *Candelaria concolor,* lichen specie. Molecular structures, were elucidated by 2D- NMR spectroscopy techniques.

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RESUMEN

Calycin, Physcion y el Manitol fueron aislados de la especie liquenica *Candelaria concolor*. La elucidación estructural se baso en el análisis espectroscópico de RMN-2D.

INTRODUCTION

Lichens species have a great variety of metabolic products, most of them occur naturally only in lichens and the others metabolites are also present in higher plants and fungi. Characteristics secondary metabolites of lichens species are depsides, depsidones, benzoxazine, benzofurane, usnic acid and antraquinone derivatives [1–3]. Thanks to their metabolites, lichens are widely used in perfumery and drug industry [4–5]; about sixty lichen species are present in some different types of commercial drugs, such as antimicrobial, anticancer, antiallergen, immunugical and expectoral.

The lichens are organisms that cover about 8% of the earth's surface. They often live on trees, rocks and soil. Lichens are symbiotic organisms, and members of three evolutionary lines: fungi, algae, and *cyanobacteria* (formerly called "blue-green algae"), but they behave as a particular organism. Lichens are commonly thought to be moss, with which they sometimes grow, and which they often resemble in size, shape, or color.

The color of the lichen body, known as a *thallus*, is very important organ for a botanic identification. The most conspicuous species of lichens on trees tend to be gray-green or yellow-green in color, but some may be orange, bright yellow, brown, slate blue, or black. The lichens are found growing as three primary life forms: *foliose*, *fruticose*, and *crustose*.

The lichens are able to colonize environments that have extremes of humidity, temperature and light, and they often occur in places where few other living things are not be able to survive.

It is estimated that de Bolivian lichen flora consists of about 2500 species, now a day's approximately 150 species are known. Most of the lichen collection at National Herbarium has been collected by botanists who were mainly interested in phanerogams or other cryptogamic groups. *Candelaria concolor* lichen specie grows round Andean region. [6-7].

Lichen material, of *Candelaria concolor*, was collected from the Pongo area, 3,800 m.a.s.l. (La Paz, Bolivia). This paper describes the isolation and structure characterization of the Pulvinic acid derivative (Calycin **1**), Anthraquinone derivative (Physcion **2**) and the carbohydrate (Mannitol **3**).

RESULTS AND DISCUSSION

The EIMS **1** showed a molecular ion at *m/z* 306, while the ^{1}H NMR and ^{13}C NMR spectra indicated that the molecular formula should be $C_{18}H_{10}O_5$. The ^{1}H NMR ($CDCl_3$) spectrum revealed the presence of seven signals

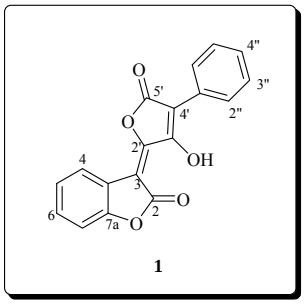
between at δ 7.10 and δ 8.20 ppm [δ 7.15 (1H, *d*, *J*=8.2 Hz), δ 7.25 (1H, *dd*, *J*= 7.6 Hz, *J*=7.6 Hz), δ 7.32 (1H, *dd*, *J*= 7.1 Hz, *J*=7.1 Hz), δ 7.37 (1H, *d*, *J*=8.2 Hz), δ 7.40 (2H, *dd*, *J*=7.7 Hz, *J*=7.7 Hz), δ 7.90 (1H, *d*, *J*=7.7), δ 8.11 (2H, *d*, *J*=7.1 Hz)] and one signal at δ 12.95 *s* for one hydroxyl proton engaged in proton bond. The ^{13}C NMR and DEPT-135 spectra indicated the presence of eighteen carbons, nine tertiary and nine quaternary. Important HMBC correlation peaks were observed between the following protons and carbons: δ 7.15 (1H, *d*, *J*=8.2 Hz) and C-3a (δ 121.9), C-5 (δ 126.2) and C-7a (δ 154.1); δ 7.25 (1H, *dd*, *J*= 7.6 Hz, *J*=7.6 Hz) and C-7 (δ 111.6) and C-3a (δ 121.9); δ 7.37 (1H, *d*, *J*=8.2 Hz) and C-4 (δ 126.2) and C-7a (δ 154.1); δ 7.90 (1H, *d*, *J*=7.7) and C-6 (δ 131.9), C-7a (δ 154.1) and C-3 (δ 128.9); δ 8.11 (2H, *d*, *J*=7.1 Hz) and C-4" (δ 129.6), C-4' (δ 106.1), C-5"/C-3" (δ 129.1) and C-2"/C-6" (δ 128.5); δ 7.32 (1H, *dd*, *J*= 7.1 Hz, *J*=7.1 Hz) and C-2"/C-6" (δ 128.5) and between δ 7.40 (2H, *dd*, *J*=7.7 Hz, *J*=7.7 Hz) and C-4" (δ 129.6) and C-3"/C-5" (δ 129.1). The structure was established to be calycin, the assignment of the ^{13}C to compound **1** (Table 1).

1,8-dihydroxy-3-methoxy-6-methylanthraquinone (Physcion) **2** was obtained as orange crystals. The molecular formula $C_{16}H_{12}O_5$ was deduced from IEMS and NMR data. The ^{1}H NMR (C_6D_6) spectrum revealed the presence of two methyl groups at δ 1.8 *s* and δ 3.0 *s*. The ^{1}H NMR spectrum also indicated the presence of four aromatic proton at δ 7.5 (H-4, *d*, *J=2*.2 Hz), δ 6.5 (H-2, *d*, *J*=2.2 Hz), δ 6.8 (H-5, *d*, *J=2*.2 Hz), δ 7.6 (H-7, *d*, *J*=2.2 Hz), and two signals at δ 12.4 *s* and δ 12.3 *s* for two hydroxyl protons engaged in proton bonds. In the ^{13}C NMR spectrum, the presence of two carbonyls groups at δ 191.3 suggested an anthraquinone. The 2D experiments, COSY, HMQC and HMBC, complete the elucidation of the structure 1,8-dihydroxy-3-methoxy-6-methylanthraquinone (Table 2 and 3).

Mannitol **3** was obtained as white crystals, mp 188-189 °C from, a MeOH-toluene mixture. The structure was established as mannitol by comparation of melting point and NMR data reported in the literature [8].

Tables 1 and 2. *^{1}H NMR and ^{13}C NMR shift values for Calycin **1** ($CDCl_3$) and Physcion **2** ($CDCl_3$)*

1			**2**		
Position	δ_H (J_{HH} [Hz])	δ_C	Position	δ_H (J_{HH} [Hz])	δ_C
C-2		165.7	**C-1**		165.4
C-3		128.9	**C-2**	6.7 *d* (*J*=2.0)	107.0
C-4	7.90 (1H, *d*, *J*=7.7)	126.2	**C-3**		166.8
C-5	7.25 (1H, *dd*, *J*= 7.6, *J*=7.6)	126.2	**C-4**	7.4 *d* (*J*=2.0)	108.4
C-6	7.37 (1H, *d*, *J*=8.2)	131.9	**C-5**	7.6 *d* (*J*=2.0)	121.5
C-7	7.15 (1H, *d*, *J*=8.2)	111.4	**C-6**		148.7
C-2'		153.4	**C-7**	7.1 *d* (*J*=2.0)	124.7
C-3'		160.3	**C-8**		162.7
C-4'		106.1	**C-9**		191.3
C-5'		173.3	**C-10**		182.2
C-1"		128.9	**C-11**		110.4
C-2"	8.11 (1H, *d*, *J*=7.1)	128.5	**C-12**		135.3
C-3"	7.40 (1H, *dd*, *J*=7.7, *J*=7.7)	129.1	**C-13**		132.2
C-4"	7.32 (1H, *dd*, *J*= 7.1,	129.6	C-14		113.9
C-5"	*J*=7.19)	129.1	CH_3-6	1.8 *s*	21.7
C-6"	7.40 (1H, *dd*, *J*=7.7, *J*=7.7)	128.5	OCH_3-3	3.0 *s*	55.4
C-3a	8.11 (1H, *d*, *J*=7.1)	129.1	OH-1	12.1 *s*	
C-7a		154.1	OH-8	12.3 *s*	



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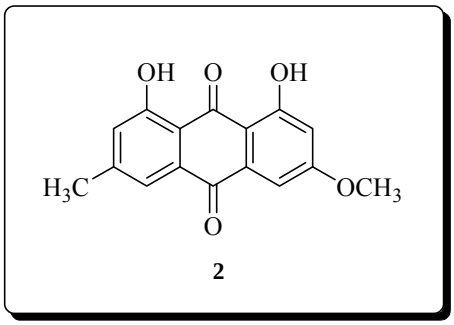

2

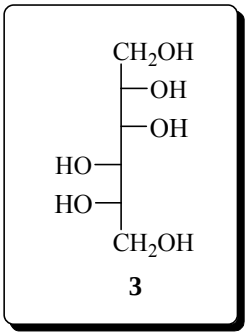

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Table 3. *^{1}H –COSY and HMBC data of* ***2***

	2	
Proton	^{1}H -COSY	HMBC
H-2	H-4	C-4, C-11, C-1, C-3
H-4	H-2	C-3, C-11, C-10
H-5	H-7	CH_3-6, C-14, C-7, C-10
H-7	H-5	CH_3-6, C-14, C-5, C-8
OCH_3-3		C-3
CH_3-6		C-5, C-6, C-7
$OCOCH_3$-1		
$OCOCH_3$-8		

EXPERIMENTAL

General

The mass spectra were recorded with a Jeol SX 102 spectrometer, ^{1}H NMR (300 MHz), on a Bruker DRX 300 MHz instrument. Column chromathography was run on Merk silica gel 60 and TLC was carried out on Silica gel GF_{254}.

Plant Material

The lichen *Candelaria Concolor* were collected from Pongo-La Paz Bolivia at 3800 m.a.s.l. in June 2010.

Extraction and Isolation

Candelaria concolor grow over rocks. The lichen material was dried and powdered 2.5 g and were extracted with acetone. The solution was concentrated and the residue (0.5 g) was subjected to CC on silica gel (G-250 mesh) eluted with hexane-$CHCl_3$-MeOH mixtures of increasing polarity. Three compounds were obtained, 0.20 g of physcion (**2**), 0.16 g of calycin (**1**) and 0.10 g of mannitol (**3**).

ACKNOWLEDGEMENTS

The authors would like to express their gratitude to the Swedish International Development Cooperation Agency (Sida) and IDH-2010 for supporting this work.

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