Revista Boliviana de Química, 2025, Vol. 42, N°2, 114-120
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https://doi.org/10.53287/rauu7065rq73r

Original article [1]

Pseudocypherellin A – A depside extracted from *Lobariella sipmanii* and *Everniastrum cirrhatum* lichens found in the Paramo de Sumapaz region, along with an updated analysis of its NMR spectral data

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Keys: *Pseudocypherellin A, Depside, Paramo de Sumapaz, Lobariella sipmanii, Everniastrum cirrhatum, NMR;* **Claves:** *Pseudociferelina A, Dépsido, Páramo de Supaz, Lobariella sipmanii, Everniastrum cirrhatum, RMN.*

ABSTRACT

Depside pseudocypherellin A was isolated for the first time as one of the metabolites from *Lobariella sipmanii* (Moncada, Betancourt & Lücking) and *Everniastrum cirrhatum* (E. Fr) Hale ex Sipman. This paper provides an update to the initial ^{1}H-NMR and ^{13}C-NMR assignments of this compound. Some previous assignments were corrected using 2D-NMR HSQC and HMBC experiments.

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RESUMEN

Pseudocipherelina A: un depsido extraído de los líquenes Lobariella sipmanii y Everniastrum cirrhatum encontrados en la región del Páramo de Sumapaz, junto con un análisis actualizado de sus datos espectrales de RMN. La pseudociferelina A, un compuesto dépsido, se identificó inicialmente como un metabolito producido por Lobariella sipmanii (Moncada, Betancourt y Lücking) y Everniastrum cirrhatum (E. Fr) Hale ex Sipman. Este estudio revisa las asignaciones espectrales originales de RMN de protón ^{1}H y carbono ^{13}C para el compuesto, incorporando correcciones basadas en técnicas de RMN bidimensional, específicamente análisis HSQC y HMBC.

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Revista Boliviana de Química, 2025, 42, 114-120
ISSN 0250-5460, Rev. Bol. Quim. ***Paper edition***
ISSN 2078-3949, Rev. boliv. quim. ***e-edition*****,** ***May-Nov***
30 noviembre 2025, https://doi.org/10.53287/rauu7065rq73r

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https://bolivianchemistryjournaliiq.umsa.bo

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[1] Received June 4, 2025, accepted October 23, 2025, published November 30, 2025. *Mail to: dgtorresv@unal.edu.co

INTRODUCTION

Lichenized fungi (LF) are symbiotic organisms composed of an association between at least one alga and a fungus. They inhabit almost all terrestrial environments on Earth, including extreme habitats, such as polar, mountain, and desert regions where isolated bionts cannot survive.[1 2 3 4 5 6] These organisms produce distinctive secondary metabolites in amounts ranging from 0.1 and 30 % thallus dry weight.[4 5 6] Paramo is a unique environment found in a high mountain regions above 3200 m in northern South America. Extending from the limits with Andean dry grasslands (puna) in northern Peru up to Venezuela, Ecuador and Colombia, the latter of which possesses more than 50 % of World's paramo surface area.[7 8 9] Paramos have defiant environmental conditions, such as persistent low temperatures, high humidity, intense solar irradiation, abrupt daily fluctuations in precipitation and temperature; although annual seasonality is minimal due to their low-latitude geographic location.[7 9] Like other high mountain environments worldwide, paramos are high biodiversity hotspots and are currently and increasingly threatened by human pressures, such as land-use shifts, and climate change.[7 8 9 10] LF are predominant organisms in paramos and can survive in various habitats such as on rocks, soil, or arbustive phorophytes.[11] These organisms can biosynthesise secondary metabolites in response to biotic and abiotic stressors.[4 5 6] Depsides such as pseudocypherellin A, an atranorin-like depside recently and for the first time isolated from *Lobariella sipmanii* and *Everniastrum cihrratum*, could be promising as antioxidant or photoprotective compounds;[12 13] so correct structural characterisation is essential for further activity studies or structure-activity research.

RESULTS AND DISCUSSION

The Mid-IR spectrum is normally useful for the structural elucidation of organic substances and natural products, as it can identify functional groups present in the compounds. Mid-IR spectrum presented several coupled signals from phenolic hydroxyl O-H stretching at 3377 cm^{-1}, which extend to 2500 cm^{-1}. Similar signals have been found in other polyhydroxy aromatic compounds like flavonoids.[14] In pseudocypherellin A, the bands of symmetric and asymmetric C-H bond stretching from methyl groups (2932 and 2953 cm^{-1}) are partially covered by the phenolic signals. Carbonyl C=O stretching was observed with a band at 1 617 cm-1, and the C-O flexions were observed with several bands between 1000 and 1350 cm^{-1}.[12]

The ^1H NMR spectrum ($CDCl_3$) of pseudocypherellin A exhibited ten signals (Figure 1A and Table 1). Six of these signals corresponded to methyl groups; five were methyl groups linked to aromatic rings, and one was a methoxycarbonyl group (δ_H 4.01 C7'-$COOCH_3$).[12 15 16 17] The assignment of the methyl groups was performed using 2D NMR HSQC experiments (Figure 1B). Two methyl groups (δ_H = 2.21 and 2.73) are assigned to aromatic ring A and are correlated to C-9 (δ_C/δ_H 10.8/2.21) and C-10 (δ_C/δ_H 20.5/2.73). H-9 (δ_H = 2.73) is deshielded because this methyl group is located *meta* respect to two aromatic hydroxyl groups (OH-2 and OH-4) and *ortho* to an ester carbonyl group, which is electron withdrawing. The three remaining methyl signals are in aromatic ring B (δ = 2.11, 2.09 and 2.50). Using HSQC these signals were correlated with their respective carbon atoms, obtaining C-8' (δ_C/δ_H 9.8/2.11), C-9' (δ_C/δ_H 13.3/2.09) and C-10' (δ_C/δ_H 18.9/2.50).[12] These signals have been newly assigned in the present paper, contrasting with the previous Hüneck signal assignments.[15 16 17]

Additionally, the hydrogen formyl group signal from carbonyl group of aromatic ring A (δ= 10.40 C3-CHO). The remaining hydrogen signals originate from the phenolic hydroxyl groups: two from ring A (δ = 12.41 OH-2 and 13.10 OH-4) and one from ring B (δ = 11.14 OH-2'). Phenolic hydroxyl signals were not reported in previous studies.[15 16 17]

Whereas ^{13}C NMR ($CDCl_3$) one-dimension spectrum showed 21 carbon signals (Table 1). The chemical shifts confirmed the presence of five methyl groups on aromatic rings. Besides, twelve signals corresponded to aromatic rings (δ_C 102.8 to 166.9).[12 15 16] Four of these signals were carbon atoms linked to oxygen according to their chemical shift values, the first signal (δ_C 151.5 C-4') corresponds to an ester link between both aromatic rings. Another three signals resonate from carbon atoms of phenolic oxygens, one on ring B (δ_C 158.9) and the other two on ring A (δ_C 166.1 C-4 and 166.9 C-2). Three signals were assigned to carbon atoms linked to carbonyl groups (δ_C 102.8 C-1, 107.9 C-3 and 111.9 C-1'). The remaining three carbon signals are carbonyl groups, two from ester groups (δ_C 169.8 C-7 and 172.1 C-7') and the last from ring A aldehyde group (δ_C 194.8 C-8).

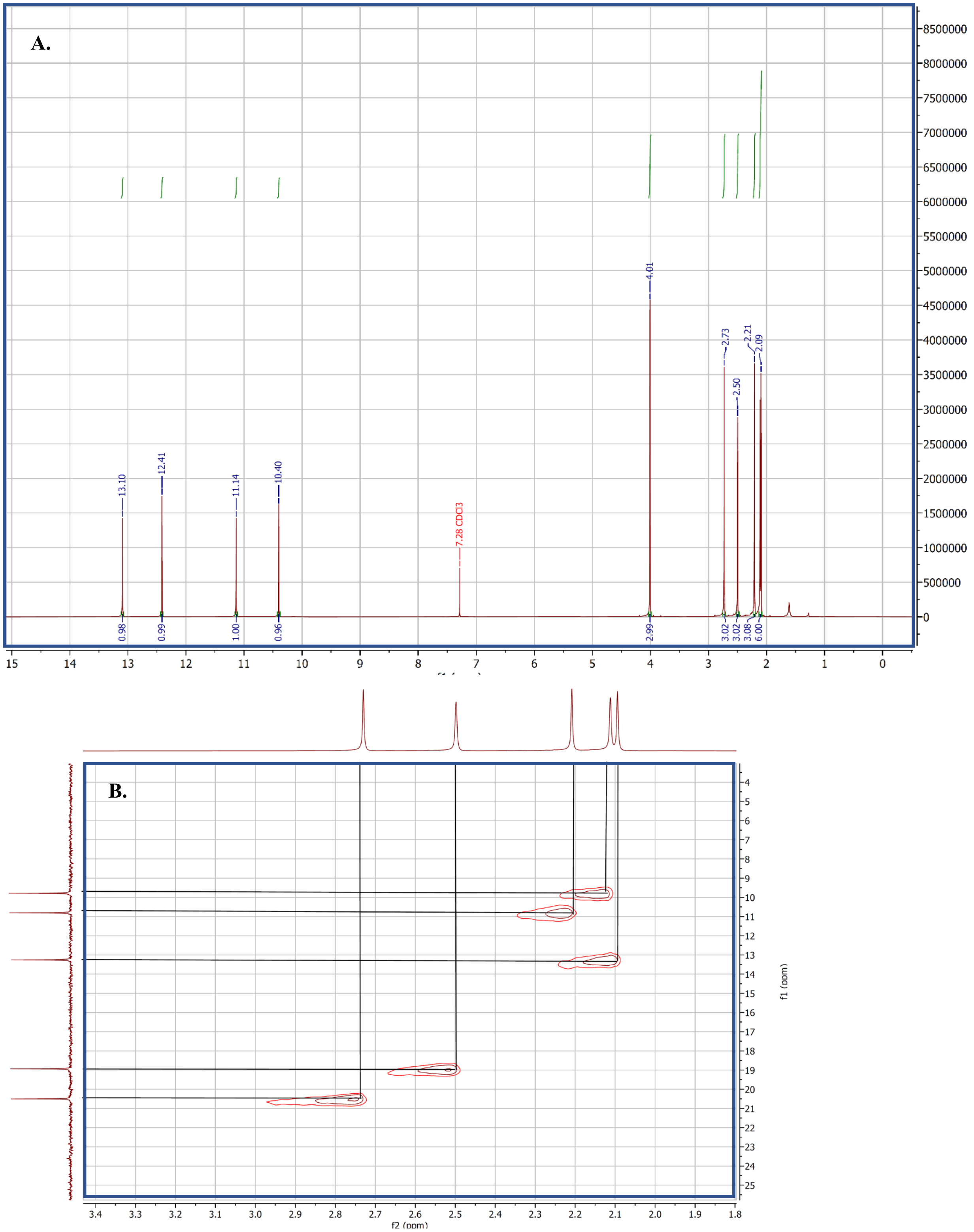

Fig. 1. A. ^{1}H NMR spectrum; B. HSQC spectrum, pseudocypherellin A

Table 1. ^{1}H and ^{13}C NMR data for pseudocypherellin A

	Experimental			Previously Reported[15–17]	
Position	δ (^{13}C) 100 MHz	δ (^{1}H) 400 MHz	HMBC Results	δ (^{13}C) 50 MHz	δ (^{1}H) 270 MHz
C-1	102.8	-	-	102.9	-
C-2	166.9	**12.41 (s, 1H, OH)**	C-1, C-2, C-3	167.0	-
C-3	107.9	-	-	108.0	-
C-4	166.1	**13.10 (s, 1H, OH)**	C-3, C-4, C-5	166.1	-
C-5	118.2	-	-	118.2	-
C-6	**150.1**	-	-	**151.5**	-
C-7	169.8	-	-	169.7	-
C-8	194.0	10.41 (s, 1H, CHO)	C-4	194.0	10.25 (s, 1H)
C-9	10.8	**2.21 (s, 3H)**	C-4, C-5, C-6	10.7	**2.38 (s, 3H)**
C-10	**20.5**	**2.73 (s, 3H)**	C-1, C-5, C-6	**18.8**	**2.08 (s, 3H)**
C-1’	**111.9**	-	-	**116.2**	-
C-2’	158.9	**11,14 (s, 1H, OH)**	C-2’, C-3’	159.0	-
C-3’	**116.2**	-	-	**111.9**	-
C-4’	**151.5**	-	-	**150.1**	-
C-5’	120.5	-	-	120.5	-
C-6’	137.6	-	-	137.6	-
C-7’	172.1	-	-	172.1	-
C-8’	**18.9**	**2.11 (s, 3H)**	C-2’, C-3’	**20.4**	**2.45; 2.05 (s, 6H)**
C-9’	13.3	**2.09 (s, 3H)**	C-4’, C-5’, C-6’	13.2	**2.70; 2.13 (s, 3H)**
C-10’	9.8	**2.50 (s, 3H)**	C-1’, C-5’, C-6’	9.7	**2.15; 2.05 (s, 6H)**
C7’-COOMe	52.3	4.01 (s, 3H, OMe)	C-7’	52.3	3.82 (s,3H)

Signals in bold are the new assignments and signals in dark grey are the former assignments

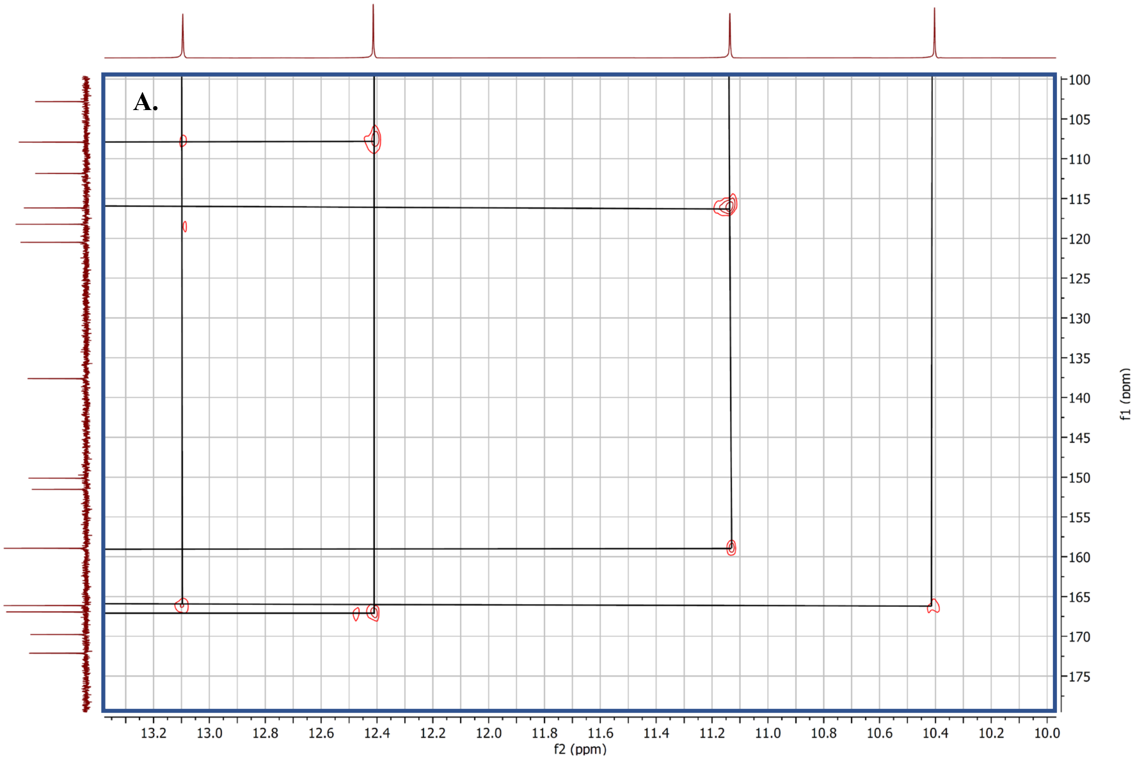

Fig. 2. A. HMBC expansion (δ_C 100 - 180; δ_H 1.6 - 3.4), pseudocypherellin A.

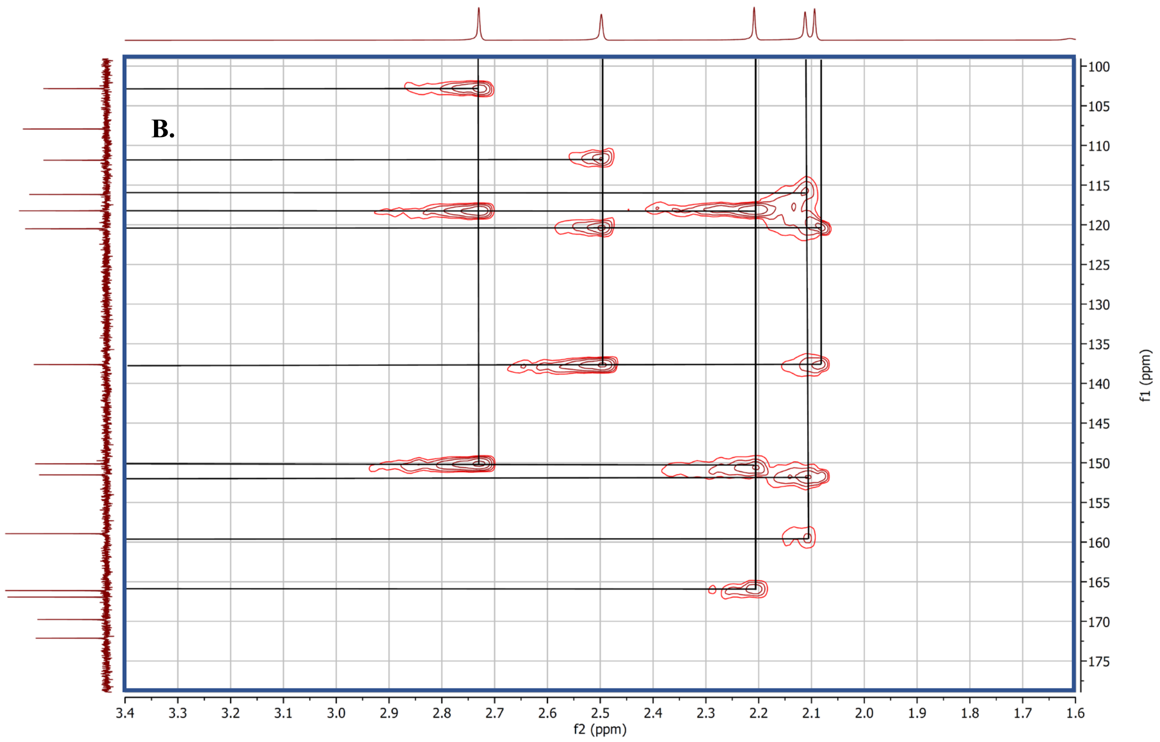

Fig. 2. B. HMBC expansion (δ_C 100 - 180; δ_H 10.0 - 13.5), pseudocypherellin A.

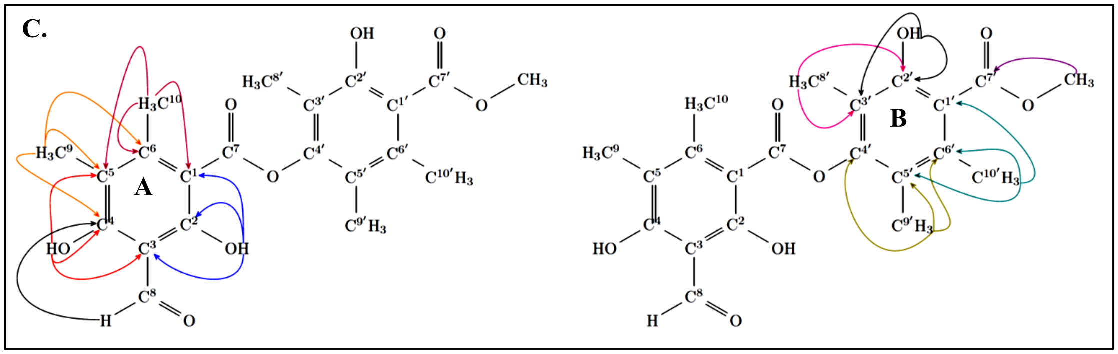

Fig. 2. C. HMBC, H/C assignments, rings A and B, pseudocypherellin A.

Two-dimensional HMBC correlations showed a β-orcinol pattern for both pseudocypherellin A rings A and B (Figure 2 and Table 1). For ring A, correlations were observed between the hydroxyl hydrogen atom 2 (δ_H = 12.41) and C-1 (δ_C = 102.8), C-3 (δ_C = 107.9) and C-2 (δ_C = 166.9). This hydrogen atom exhibited a minor chemical shift possibly due to intramolecular hydrogen bonding with the oxygen atoms of the ester group, which locks its position and partially shields the hydrogen atom; this has been evidenced in X-ray crystallography of lobariellin, the monoaromatic compound that composes the ring A structure.[18] Conversely, the phenolic hydrogen atom 4 (δ_H = 13.10) cannot form intramolecular hydrogen bonds, so it is fully deshielded. This hydrogen atom showed correlations with C-3 (δ_C = 107.9), C-4 (δ_C = 166.1) and C-5 (δ_C = 118.2). The hydrogen atoms from the methyl groups confirmed correlations for ring A. H-9 (δ_H = 2.21) was correlated with C-4 (δ_C = 166.1), C-5 (δ_C =118.2) and C-6 (δ_C =150.1); whereas H-10 (δ_H = 2.73) had correlations in HMBC with C-1 (δ_C = 102.8), C-5 (δ_C = 118.2) and C-6 (δ_C = 150.1). Besides, the HMBC correlations of ring B came mainly from the hydrogen atoms of the methyl groups, H-8' (δ_H = 2.11) is correlated with C-3' (δ_C = 116.2) and C-2' (δ_C = 158.9); H-9' (δ_H = 2.09) obtained correlations with C-5' (δ_C = 120.5), C-6 (δ_C = 137.6) and C-4 (δ_C = 151.4); H-10' (δ_H = 2.50) was correlated with C-

1' (δ_C = 111.9), C-5' (δ_C = 120.5) and C-6' (δ_C = 137.6). Finally, the methyl hydrogen atoms from methoxyl group (δ_H = 4.01) showed correlations with C-7' (δ_C = 172.1).

When the obtained assignments were compared with previous report s,[15] [16] [17] some differences were evident, particularly regarding to the phenolic hydrogen signals appearing in our spectra (Table 1 and Figure 1). These phenolic signals are here reported for the first time for this compound due to the absence of solvent effects. Our spectra were taken in chloroform, a solvent that does not form hydrogen bonds with organic compounds. Conversely, the reported spectra were taken in DMSO-*d6*, a solvent that acts as a hydrogen bond acceptor. In the present paper, HSQC and HMBC experiments enabled the correct assignment of the phenolic and methyl carbon signals. These signals were difficult to properly assign using one-dimensional spectra alone, and some NMR experiments such as DEPT cannot solve this issue because all aromatic carbons in pseudocypherellin A are quaternary and all substituents are methyl.

Ultimately, we highlighted that pseudocypherellin A and other two monoaromatic compounds were obtained as major metabolites from *Lobariella sipmanii*.[12] While in *Everniastrum cirrhatum* from the Paramo de Sumapaz, atranorin, salazinic, usnic, and atraric acid were obtained as major metabolites, with pseudocypherellin A being obtained as a minor metabolite.[13] For *Everniastrum cirrhatum* from high mountains in Bolivia only atranorin has been reported as major metabolite, with salazinic acid being reported as a minor metabolite.[19]

EXPERIMENTAL

General

NMR spectra were obtained in Bruker Avance 400 located in Physics department at the Universidad Nacional de Colombia in Bogota. The spectra were processed using MestReNova v 14.0.0-26256. Mid-IR spectrum was taken using an IR-FT Thermo Scientific Nicolet iS10 spectrophotometer in the Pharmacy Department at Universidad Nacional de Colombia in Bogota.

Lichen material

Samples of *Lobariella sipmanii* and *Everniastrum cirrahtum* were collected in Andabobos, Chisacá, and Alto Caicedo areas of the Sumapaz National Natural Park (3700 - 3800 m), which is located in southern Bogotá DC, Colombia. The lichens were cleaned in fresh state using a stereoscope to remove insects, mosses, liverworts, and other plant material. A portion of the material was prepared and sent to the Colombian National Herbarium (COL) for deposit as a voucher specimen under reference numbers 609185 and 609190.

Extraction and isolation

Lobariella sipmanii was firstly fractionated by using ethyl ether (sol/liq extraction), resulting in a white solid phase (892.8 mg). It was placed in an open silica gel chromatography column (41.54 g) and eluted using a gradient of dissolvents ranging from pure hexane to dichloromethane (DCM), and then DCM to acetone. Pseudocypherellin A (219.9 mg) was obtained in the fractions corresponding to the mixtures 6:4 to 4:6 hexane:DCM mixtures. The white solid obtained was washed several times with acetone and recrystalised using a methanol:acetone (1:1) mixture to obtain the pure compound (68.0 mg). In the case of *Everniastrum cirrhatum* pseudocypherellin A (18.4 mg) was obtained from the preparative thin-layer chromatography of the mother liquor residue (58.9 mg) from a washing of a grey solid fraction (3884.6 mg), whose main constituent was salazinic acid.

Pseudocypherellin A*:* m.p. (◦C): 174-175. IR (KBr) ν_{max} (cm^{-1}): 3377, 3367, 3208, 2916. (O-H); 2953 (C-H), 2932 (C-H); 2704 (CHO Overtone); 1647 (C=O); 1577 (C=C); 1261 (C-O); 1198 (C-O); 1076 (C-O). ^{1}H NMR ($CDCl_3$ - 400 MHz): 2,09 (3H, s, CH3-9'); 2,11 (3H, s, CH3-10'); 2,21 (3H, s, CH3-9); 2,50 (3H, s, CH3-8'); 2,73 (3H, s, CH3-10); 4,01 (3H, s, COOCH3); 10,40 (1H, s, CHO); 11,14 (1H, s, OH-2'); 12,41 (1H, s, OH-4); 13,10 (1H, s, OH-2). ^{13}C NMR ($CDCl_3$): C-1: 102,8; C-2: 166,9; C-3: 107,9; C-4: 166,1; C-5: 118,2; C-6: 150,1; C-7: 169,8; C-8; 194,0; C-9; 10,8; C-10: 20,5; C-1': 111,9; C-2': 158,9; C-3': 116,2; C-4': 151,5; C-5': 120,5; C-6': 137,6; C-7': 172,1; C-8': 18,9; C-9': 13,3; C-10': 9,8; C7'-COOMe: 52,3. Solubility: soluble in $CHCl_3$, DCM, MeOH, partially soluble in acetone.

ACKNOWLEDGEMENTS

We acknowledge to Colombian Environment and Sustainable Development Ministry for the Licence Nº 004 of 21st March 2018 added to the Contract Nº 121 of 2016, which covered the Project entitled "*Bioprospection of lichens* *from Paramo de Sumapaz as original natural source of dual substances with antioxidant and photoprotective activity for preventing sun-related skin diseases*".

We appreciate the accompaniment of Natural National Park Service during biological sampling. Special thanks to MSc Roberto Dávila Villamizar from Colombian Biodiversity Foundation for the taxonomic determination of lichen material.

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