Journal of Molecular Structure 1102 (2015) 57–62

Contents lists available at ScienceDirect

Journal of Molecular Structure

journal homepage: http://www.elsevier.com/locate/molstruc

FT-Raman spectroscopy of the *Candelaria* and *Pyxine* lichen species: A new molecular structural study

Rafaella F. Fernandes [a], Gilson R. Ferreira [a], Adriano A. Spielmann [b], Howell G.M. Edwards [c], Luiz Fernando C. de Oliveira [a, *]

[a] Núcleo de Espectroscopia e Estrutura Molecular, Departamento de Química, Universidade Federal de Juiz de Fora, 36036-330, Juiz de Fora, MG, Brazil
[b] Centro de Ciências Biológicas e da Saúde, Laboratório de Botânica, Universidade Federal de Mato Grosso do Sul, 79070-900, Campo Grande, MS, Brazil
[c] Centre for Astrobiology and Extremophile Research, School of Life Sciences, University of Bradford, Bradford, BD7 1DP, United Kingdom

ARTICLE INFO

Article history:
Received 18 June 2015
Received in revised form
11 August 2015
Accepted 18 August 2015
Available online 20 August 2015

Keywords:
Lichenized fungi
Raman spectroscopy
Carotenoids
Pulvinic acid derivatives
Chiodectonic acid
Quantum chemical calculations

ABSTRACT

In this work the chemistry of the lichens *Candelaria fibrosa* and *Pyxine coccifera* have been investigated for the first time using FT-Raman spectroscopy with the help of quantum mechanical DFT calculations to support spectral band assignments. The non-destructive spectral vibrational analysis provided evidence for the presence of pulvinic acid derivatives and conjugated polyenes, which probably belong to a carotenoid with characteristic signatures at *ca.* 1003, 1158 and 1525 cm^{-1} assigned respectively to δ(C$-CH_3$), ν(C−C) and ν(C=C) modes. The identification of features arising from chiodectonic acid in the *Pyxine* species and calycin and pulvinic dilactone pigments in *C. fibrosa* were assisted by the quantum mechanical DFT calculations. Raman spectroscopy can provide important spectroscopic data for the identification of the biomarker spectral signatures nondestructively for these lichen pigments without the need for chemical extraction processes.

1. Introduction

Lichens are considered to be symbiotic associations between a mycobiont (fungus) and one or more photobionts partners (algae) [1] and have a wide variety of protective and defensive mechanisms due to the production of key chemical protectants which act as spectral identifier biomarkers [2,3].

The association between the symbiotic partners in these organisms can contribute to the spatial size increase of the fungus [4,5] and also allows their photobiotic partners to grow under stressed environmental situations. These unique lichen characteristics lead to the development of protective strategies for their adaptation to damaging UVB (280–315 nm) and UVA (315–400 nm) radiation. Hence, the production of specific UV-radiation screening pigments such as pulvinic dilactone and calycin (Fig. 1) can be considered to fulfill an essential biological role for the absorption and filtering of solar radiation [6].

Another important group of molecules which act as screening agents for UV radiation are the carotenoid pigments which have a dual role in both light-harvesting and photoprotection [7]. Carotenoids are isoprenoids which are found in a wide variety of botanical and microbiological species; they can be divided into oxygenated carotenoids (xanthophylls) and into nonoxygenated molecules (carotenes), both of which are necessary for the survival strategies of lichens exposed to environmental photostress and oxidation [8,9]. Carotenoids have the ability to protect cellular organisms from photo-radiation damage through their antioxidant properties afforded by the conjugated C=C double bonds of the polyene chain [10] by absorbing excess energy from reactive oxygen species and the quenching of singlet oxygen [11].

Because of the effective strategies adopted by lichens for survival in extreme terrestrial conditions it has been suggested that these organisms could also be suited to survive extreme extraterrestrial conditions [12] and under some specific experiments undertaken to demonstrate this, lichen colonies exhibited the ability to maintain the germinative capacity of the symbionts and ascospores [13–15]. For this reason, lichens have recently received much attention in astrobiology and the search for life signatures on planetary surfaces [16–18].

The pigmentation of living organisms provides a primary role for the understanding of the evolution of life on Earth resulting

* Corresponding author.
E-mail address: luiz.oliveira@ufjf.edu.br (L.F.C. de Oliveira).

http://dx.doi.org/10.1016/j.molstruc.2015.08.040
0022-2860/

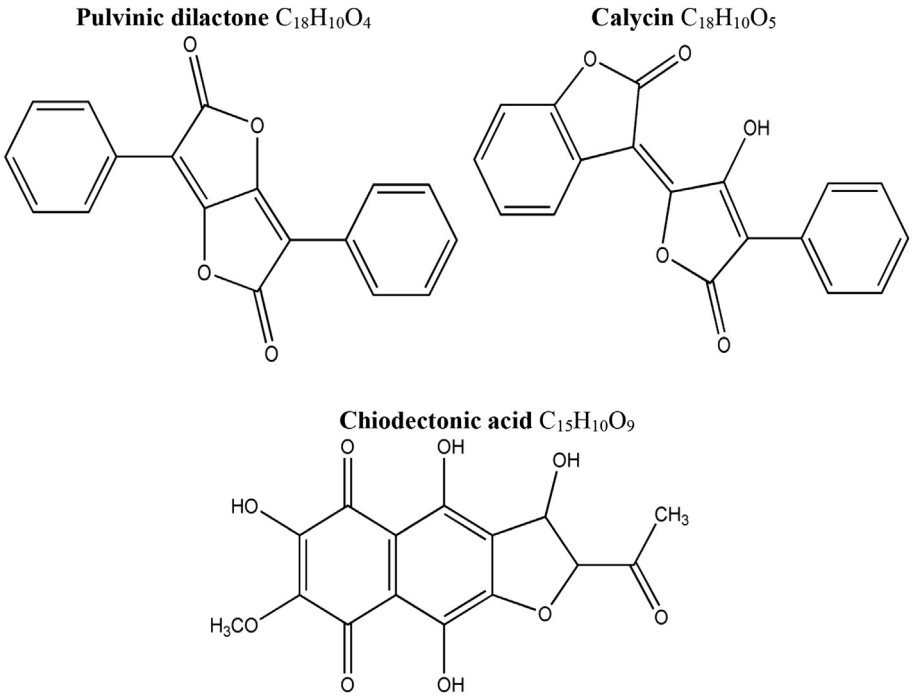

Fig. 1. Molecular structures of lichen protective chemicals.

from an aerobic atmosphere [6]; the current study seeks to provide the nondestructive identification of key lichen pigments using *in situ* Raman spectroscopy, which has already been confirmed as an analytical technique of choice in this role. In addition we have undertaken supportive quantum mechanical density functional theoretical calculations of these key protective pigments to assist their identification and molecular band assignments in Raman spectra; the lichens chosen for this study were *Candelaria fibrosa* (Fr.) Müll. Arg. and *Pyxine coccifera* (Fée) Nyl., which are native to Brazil and whose Raman spectra have not been recorded hitherto. An additional advantage of this study is that the lichen specimens were not chemically or mechanically treated for the spectral interrogation, which was therefore carried out *in situ* in the laboratory without any specimen preparation.

2. Experimental

The lichen species were identified and provided by A.A. Spielmann and the vouchers are deposited in the CGMS Herbarium of the Universidade Federal de Mato Grosso do Sul, Campo Grande, Brazil.

2.1. Samples

- *C. fibrosa* (Fr.) Müll. Arg.

Origin: Rio Grande do Sul State, Municipality of Piratini, Cerro do Sandi, 31.V.2013.
Coordinates: 31°28′58.9″S, 53°11′54.9″W. Altitude: 495 m.
Collectors: A.A. Spielmann, E.S Lacerda & J. Pedroso.

- *P. coccifera* (Fée) Nyl

Origin: Brazil, Mato Grosso do Sul State, Municipality of Campo Grande, 09.IV.2010.
Coordinates: 20º32′36.7″S, 54º23′57.8″W. Altitude: 555 m.
Collectors: A.A. Spielmann, G.A. Damasceno Junior, S.N Moreira & S.S Lima.

2.2. Raman measurements

Fourier transform Raman spectra were carried out using a Bruker RFS 100 instrument and a Nd:YAG laser operating at 1064 nm, equipped with a Ge detector cooled with liquid nitrogen and recorded using a spectral resolution of 4 cm^{-1}. Good signal-to-noise ratios were obtained with 1024 spectral scans using a laser power at the sample of 100 mW. The spectra were obtained directly from the specific parts of the lichen samples where the investigated pigments are clearly contained, which mean that the colored parts of the lichen structure were examined. For each spectrum, the experimental parameters were adjusted in order to obtain the best signal-to-noise ratio while the physical and chemical integrity of the samples was maintained, i.e., the position and intensity of each one of the bands in the spectrum was not changed.

2.3. Calculations

The structures for the all compounds were fully optimized in gas phase at B3LYP [19,20] level using the 6-311++G(d,p) [21] triple-zeta basis-set with inclusion of diffuse and polarization functions at heavy and hydrogen atoms (hereafter abbreviated as B3LYP/6-311++G(d,p)) (the optimized structures are shown in Fig. S1 as Supplementary Material). All of the geometries were considered as molecular neutral species. The final geometries were characterized as minima on the potential energy surface (PES) through harmonic frequencies calculation (all frequencies found real). The Raman intensities were also calculated and the band spectra simulated by fitting a Lorentzian type function [22], with parameters set to 15 cm^{-1} for the average width of the peaks at half height and 2×10^{-6} mol cm^{-3} for sample concentration. The spectra for all species were then assigned according to the normal-mode analysis (and are shown in Fig. S2 as Supplementary Materials). Frequency