

ISOLATION AND STRUCTURAL ELUCIDATION OF A NEW DEPSIDE OF LICHEN *Everniopsis trulla*

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ABSTRACT

In this research, a new depside of the lichen *Everniopsis trulla* has been isolated. The extraction was carried out to 400 g of dry sample and ground with ethanol for 3 repetitions, then, it was fractionated by applying column chromatography with the CHCl₃-MeOH system and purified by recrystallization with MeOH-Acetone (1: 1); Finally, white crystals in the form of needles (solid C) with a melting point of 198 ° C were obtained, whose structure was elucidated based on spectroscopic data (UV-Visible, IR, NMR-H¹, NMR-C¹³, mass spectrometry and single crystal X-ray diffraction). According to the Science Finder databases, it is a new depside, called trullarin, and it is observed that molecular packing is influenced by both intramolecular and intermolecular forces. Intermolecular hydrogen bonds of O - H ... O type binds neighboring molecules forming dimers.

Key words: Crystal structure; Depside; *Everniopsis trulla*; Trullarin

AISLAMIENTO Y ELUCIDACIÓN ESTRUCTURAL DE UN NUEVO DÉPSIDO DEL LIQUEN *Everniopsis trulla*

RESUMEN

En esta investigación se ha aislado a un nuevo dépsido del liquen *Everniopsis trulla*. Se realizó la extracción a 400 g de muestra seca y molida con etanol por 3 repeticiones, luego, se fraccionó aplicando cromatografía en columna con el sistema CHCl₃-MeOH y purificó por recristalización con MeOH-Acetona (1:1); finalmente se obtuvo unos cristales blancos en forma de agujas (sólido C) de punto de fusión de 198 °C, el cual se elucidó su estructura en base a los datos espectroscópicos (UV-Visible, IR, RMN-H¹, RMN-C¹³, espectrometría de masas y difracción de monocristales de rayos X). Según la base de datos de Science

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Finder, se trata de un nuevo dépsido, llamado trullarin y se observa que el empaquetamiento molecular está influenciado por fuerzas tanto intramoleculares como intermoleculares. Enlaces de hidrógeno intermoleculares del tipo O - H \cdots O enlaza las moléculas vecinas formando dímeros.

Palabras clave: Estructura cristalina; dépsido; *Everniopsis trulla*; trullarin.

INTRODUCTION

Biomass has become in recent years an alternative source of molecular scaffolds for the synthesis of the next generation of bioactive compounds (nutraceuticals, antioxidants, antimicrobials, insecticides, drugs or materials). The presence of one or more chiral atoms in these metabolites increases their utility in the development of new drugs. Moreover, biomass processing often generates by-products useful in other industries¹. A well-known example is the generation of 5-hydroxymethylfurfural (HMF) from the dehydration of fructose. The HMF can be transformed into different furan derivatives, such as 2,5-bis (hydroxymethyl) furan, which is an optimal substrate for the manufacturing of polyurethane foam with better flame hazards properties².

Lichens are organisms widespread in nature, and are of particular interest in medicinal chemistry due to the wide variety of metabolites associated to their biomass. The use of xanthenes has been reported for the synthesis of new molecules with better antifungal properties. Likewise, usnic acid, a major metabolite identified in many lichens, has been used to generate several derivatives with antimicrobial and tuberculostatic properties^{3,4}.

Depsides are polyphenolic compounds comprising two or more monocyclic aromatic units, linked by an ester bond. They are often found in lichens, but have also been isolated from plants from the *Ericaceae*, *Lamiaceae*, and *Papaveraceae* family. Depsides have antibiotic, anti-HIV, and antiproliferative activities. As inhibitors of prostaglandin biosynthesis and leukotriene B4 biosynthesis, depsides are considered potent nonsteroidal anti-inflammatory compounds⁵.

The *Parmeliaceae* is a large and diverse family of *Lecanoromycetes*. A botanical study of all genera of *Parmeliaceae* family includes information on basic morphological characteristics for each species, but up to date there is no specific information regarding their phytochemical and pharmacological characteristics⁶. Previous research performed on the lichen *Everniopsis trulla* (Ach.) Nyl, (family *Parmeliaceae*), identified atranorin and usnic acid contained in the thallus surface. Tri-terpenes and β -orcinol depsidones were also found in its medulla⁶. In another study, the compounds chloratranorin and atranorin were identified from two extracts of *E. trulla* by TLC. The presence of two additional unknown substances was also detected⁷.

In this paper, the chemical structure of a new depside isolated from lichen *E. trulla* (Ach.) Nyl is presented, which increases the knowledge about the metabolites present in this species.

EXPERIMENTAL PART

Sample. The lichen specimen *Everniopsis trulla* (400 g) was collected in “Canchas city”, Asunción (Huaraz, Peru), in 2011 at an altitude of 3427 meters (10,650 feet). A voucher specimen was deposited in the Museum of Natural History of National University of San Marcos, UNMSM, Lima, Peru.

Extraction and isolation

The powdered sample generated from *E. trulla*, was extracted successively with ethanol. The chloroform extract was run through a chromatographic column (silica gel, CHCl₃-MeOH gradient) isolating five compounds: solid A (usnic acid), solid B (atranorin), solid C (trullarin), 2,4-dihydroxy-3-formyl-6-methylbenzoate (ethyl haematommate) and a compound still unidentified). Trullarin was purified by crystallization. Colourless single crystals were obtained by crystallization from EtOH/H₂O (mp: 198-199 °C).

RESULTS AND DISCUSSION

Usnic acid, atranorin and 2,4-dihydroxy-3-formyl-6-methylbenzoate were identified by comparison of their spectroscopic data with the literature^{9, 13, 14}.

Trullarin chemical structure elucidation

Trullarin was identified as depside through qualitative chemical reactions. This compound reacted with NaOCl and Ca(OCl)₂ in solution to give red and orange colors; It also reacted with p-phenylenediamine to give red and orange colors. This reactivity is typical of a depside with an aromatic aldehyde moiety^{8,9}. The chemical structure of trullarin was elucidated using ¹H-NMR, ¹³C-NMR, UV and IR spectroscopies. The confirmation of the structure of trullarin was accomplished by single-crystal X-ray diffraction analysis.

¹H-NMR (benzene-d₆, 400MHz): δ (ppm) 12.70, 12.65, 12.44 (each 1H, s, Ar-OH), 10.24 (1H, s, -CHO), 6.28 (1H, s, Ar-H), 3.25 (3H, s, -CO₂Me), 2.33, 2.11 (each 3H, s, Ar-Me 5 and 6), 2.23 and 2.25 (each 3H, s, Ar-Me 3' and 5'). See figure 1.

^{13}C -NMR (benzene- d_6 , 100MHz): showed five methyl groups at δ 9.4, 23.7, 24.0, 25.1 in two aromatic rings and 51.4 for the methyl ester. It also showed two carbonyl esters at δ 163.5 and 172.2 and showed an aldehyde group at δ 193.4 ppm in C-3. See figure 2.

MS: HRMS m/z 389.1624 (calcd for $\text{C}_{20}\text{H}_{21}\text{O}_8$, 389.1622).

UV: λ_{max} in ethanol 219.3 nm and 269.5 nm.

IR (KBr, cm^{-1}): 3090, 3002, 1651, 1622, 1582, 1451, 1407, 1268, 1163, 1106, 820, 802, 610.

This compound called trullarin is new according to the PUCP BASE OF SCIENCE FINDER. Its chemical name is: (Methyl-2,6-dimethyl-4'- (3-formyl-2,4-dihydroxy-5,6-imethylbenzoyloxy) -3'-hydroxy benzoate). HRESIMS (negative mode): m/z 387.1071 [$\text{M}-\text{H}$] $^-$ (calculated for $\text{C}_{20}\text{H}_{19}\text{O}_8$: 387.1080). The title compound named trullarin, $\text{C}_{20}\text{H}_{20}\text{O}_8$, crystallizes with one molecule in the asymmetric unit in the monoclinic centrosymmetric space group $\text{P}2_1/\text{n}$ with the following unit cell parameters: $a = 11.0400(3)$ Å, $b = 11.2752(3)$ Å, $c = 14.7893(4)$ Å, $\alpha = \gamma = 90^\circ$, $\beta = 109.074(2)^\circ$, $V = 1739.87(8)$ Å 3 , $Z = 4$. Figure 3 shows the molecular structure in solid state, in which the individual conformation of trullarin can be observed. The crystallographic data is presented in Table 1. Selected bond lengths and angles are given in Table 2. All the bond lengths and angles are within the normal ranges.

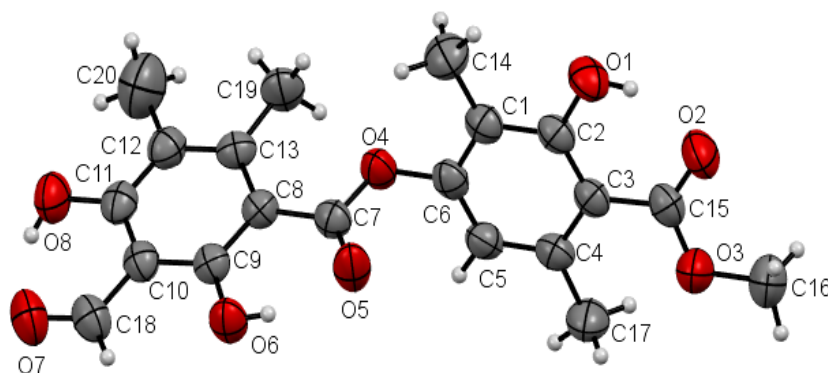


Figura 3. Molecular structure of trullarin.

Table 1. Crystallographic data for C₂₀H₂₀O₈.

CCDC	1508526
Empirical formula	C ₂₀ H ₂₀ O ₈
Formula weight	388.36
Temperature (K)	298(2)
Wavelength (Å)	1.54178
Crystal system	Monoclinic
Space group	P2 ₁ /n
Unit cell dimensions	
a (Å)	11.0400(3)
b (Å)	11.2752(3)
c (Å)	14.7893(4)
α [°], β [°], γ [°]	90, 109.074(2), 90
Volume (Å ³), Z	1739.87(8), 4
Density (calculated (Mgm ⁻³))	1.483
Absorption coefficient (mm ⁻¹)	0.975
F(000)	816
Crystal size (mm)	0.15 x 0.10 x 0.08
Range for data collection θ (°)	4.38 to 66.90
Limiting indices h, k, l	-13 ≤ h ≤ 13, -12 ≤ k ≤ 13, -17 ≤ l ≤ 14
Reflections collected	11153
Independent reflections	2934 (R _(int) = 0.0312)
Refinement method	Full-matrix least-squares on F ²
Data / restraints / parameters	2934 / 0 / 262
Goodness-of-fit on F ²	1.065
Final R indices [I > 2σ(I)]	R ₁ = 0.0678, wR ₂ = 0.2309
R indices (all data)	R ₁ = 0.0819, wR ₂ = 0.2556
Largest diff. peak and hole	0.270 y -0.765

Tabla 2. Selected bond lengths (Å) and bond angles (°) for C₂₀H₂₀O₈.

Bond	Length (Å)	Diedral angles	Angles (°)
O(1)-C(2)	1.353(3)	C(15)-O(3)-C(16)	117.3(3)
O(2)-C(15)	1.227(4)	C(7)-O(4)-C(6)	117.8(2)
O(3)-C(15)	1.308(4)	O(1)-C(2)-C(3)	122.6(3)
O(3)-C(16)	1.449(4)	O(1)-C(2)-C(1)	115.0(2)
O(4)-C(7)	1.345(3)	C(1)-C(6)-O(4)	117.4(3)
O(4)-C(6)	1.414(3)	C(5)-C(6)-O(4)	118.5(3)
O(5)-C(7)	1.212(3)	O(5)-C(7)-O(4)	120.7(3)
O(6)-C(9)	1.328(3)	O(5)-C(7)-C(8)	124.3(2)
O(7)-C(18)	1.227(4)	O(4)-C(7)-C(8)	115.0(2)
O(8)-C(11)	1.336(3)	O(6)-C(9)-C(10)	115.3(2)
C(7)-C(8)	1.468(4)	O(6)-C(9)-C(8)	123.5(3)
		O(8)-C(11)-C(12)	118.9(3)
		O(8)-C(11)-C(10)	120.8(3)
		O(2)-C(15)-O(3)	121.7(3)
		O(2)-C(15)-C(3)	122.7(3)
		O(3)-C(15)-C(3)	115.6(2)
		O(7)-C(18)-C(10)	123.4(3)

The planar conformation of the ester groups is established from the torsion angles O3-C15-O2-C3 = -178.5(5) and O4-C7-O5-C8 = 179.3(5). The hydroxyl groups are coplanar with the aromatic rings; the O1—C2—C1—C6, O6—C9—C10—C11 and O8—C11—C12—C13 torsion angles are -179.9 (3)°, 179.5 (3)° and -179.6 (3)°, respectively.

In the crystal structure of trullarin, there are two benzene rings in the molecule. C(1), C(2), C(3), C(4), C(5) and C(6) form the first plane, with a mean deviation of 0.0167 Å, defined as plane I. Similarly, C(8), C(9), C(10), C(11), C(12) and C(13) forms the second plane with a mean deviation of 0.0048 Å, defined as plane II. The dihedral angle between plane I and plane II is 64.4(9)°. In addition, the ester group C(7), C(8), O(4) and O(5) forms a third plane, defined as plane III. The dihedral angle between plane II and plane III is 4.3(1)°, which suggests that both are nearly co-planar. This indicates that there is a π - π conjugation between the C(=O) group and the benzene ring of plane II^{15, 16}.

The molecular packing of trullarin is influenced by both intramolecular and intermolecular forces. Intramolecular forces determine molecular shape, which in turn play an important

role in determining the most effective ways of packing the molecules in the crystal [13]. In the structure, there are three intramolecular hydrogen bonds of O—H···O type. The O1···O2, O5···O6 and O7···O8 intramolecular hydrogen bonds generate a six-membered rings (Table 3 and Fig. 4). Furthermore, one intermolecular hydrogen bond of O—H···O type, O5···O6, links adjacent molecules into dimers (Table 3 and Fig. 4). The dimers form twelve-membered rings and present an anti-parallel orientation along an axis. The packing diagram in Fig. 5 shows the stacking pattern of trullarin, viewed along the [010] direction. The chemical structure obtained by single crystal XRD agrees very well with NMR spectroscopy data.

Tabla 3. Intra- and Intermolecular hydrogen bonds geometry (Å, °) for C₂₀H₂₀O₈.

D—H···A	D—H	H···A	D···A	D—H···A
O1—H1···O2 ⁱ	0.82	1.81	2.540(3)	147
O6—H6···O5 ⁱ	0.82	1.83	2.541(3)	144
O8—H8···O7 ⁱ	0.82	1.83	2.554(4)	146
O6—H6···O5 ⁱⁱ	0.82	2.52	3.088(3)	127

Symmetry codes: (i) = x, y, z (ii) = 1-x, 1-y, -z

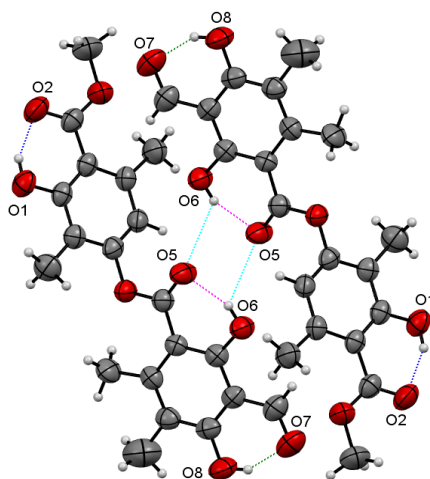


Figura 4. Intermolecular hydrogen bonding in trullarin.

The chemical structure obtained by single crystal XRD aligns very well with NMR spectroscopy data. Figure 6, shows the chemical structure of the isolated new compound from E. trulla.

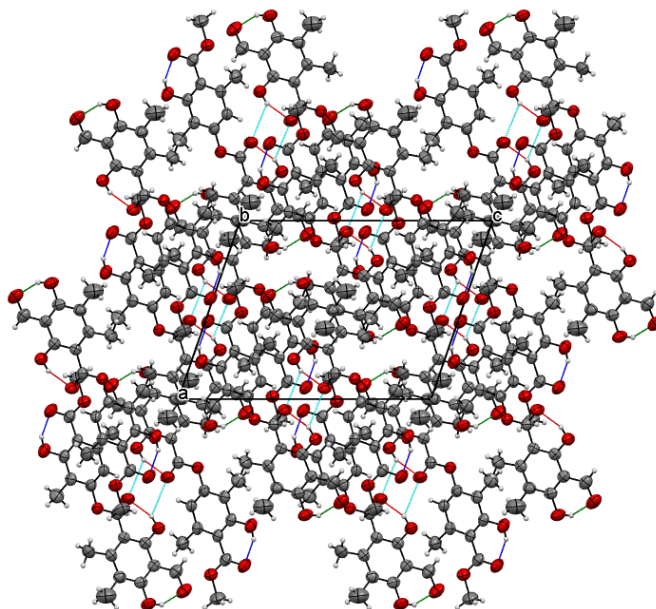


Figura 5. Stacking pattern in trullarin.

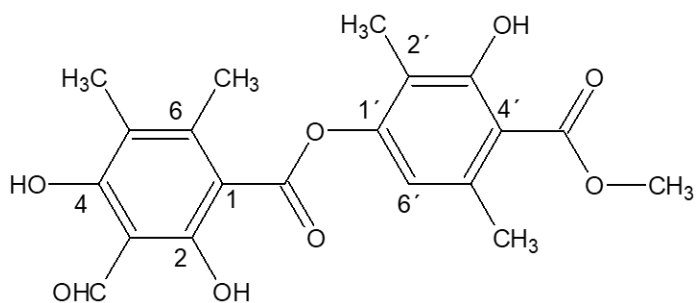


Figura 6. Chemical structure of trullarin.

CONCLUSIONS

Four compounds were isolated from the Peruvian Lichen *E. trulla*. One of them, compound C (trullarin), is reported for the first time. Has melting point of 198 °C were obtained, whose structure was elucidated based on spectroscopic data (UV-Visible, IR, NMR-H¹, NMR-C¹³, mass spectrometry and single crystal X-ray diffraction) and its chemical name is: (Methyl-2,6-dimethyl-4'-(3-formyl-2,4-dihydroxy-5,6-imethylbenzoyloxy)-3'-hydroxy benzoate).

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