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ERGOSTEROL FROM THE MUSHROOM LAETIPORUS SP.; ISOLATION AND STRUCTURAL CHARACTERIZATION

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ABSTRACT

Laetiporus sp. a mushroom growing on species of *Eucalyptus* sp in Piribebuy city, Cordillera department, Paraguay, was submitted to extraction and crystallization procedures to obtain the main secondary metabolite, ergosterol. To the best of our knowledge, this is the first report on ergosterol in this genus. The structural characterization was achieved by means of NMR techniques, namely ^{1}H, ^{13}C, COSY H-H, HMQC and HMBC. ***Spanish title**: Aislamiento y caracterización estructural de ergosterol del hongo Laetiporus sp.*

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RESUMEN

Del hongo *Laetiporus* sp. se aisló ergosterol, que fue identificado a través de técnicas espectroscópicas de RMN-^{1}H, RMN-^{13}C, COSY H-H, HMQC y HMBC, esta especie fue colectada en la ciudad de de Piribebuy del Departamento de Cordillera de la República del Paraguay. Una investigación bibliográfica reveló que este el primer reporte de ergosterol en este género.

INTRODUCTION

The use and research of mushrooms has a recent history in the western civilization: Europe, North and South America; in contrast, in Asia, there is a long tradition of their use and of their investigative approach [1]. There are a grand number of reports on the secondary metabolites contents of mushrooms. The structural diversity of the metabolites of mushrooms has awakened a remarkable interest from the chemical and pharmacological stand point [1,2].

The mushrooms of the genus *Laetiporus* sp. grow in all type of forests. They can usually be found over the surface of dead trees. The mushroom studied and reported here is used as food; it has the reputation as cure for intestinal disorders [3].

Ergosterol is a common constituent of higher plants; however, it's present in lichens and fungi. The biological activities spectrum of ergosterol includes: anticancer [4], antioxidant [5], anticoagulant [6], and the involvement in the active expression of the specific defense gene [7]. In the present paper we report the isolation and identification of ergosterol from the mushroom *Laetiporus* sp. collected in in Piribebuy city.

RESULTS AND DISCUSSION

Laetiporus sp. is a mushroom growing on the trunk of species of *Eucalyptus* sp. It was collected in Piribebuy city, Cordillera department, Paraguay. The ethanolic crude extract (95%) was treated with acetone and methanol. This treatment gave rise to pure crystals of compound **1**.

Some 30 mg of 1 were dissolved in $CDCl_3$ and were submitted to 1D and 2D NMR analyses. The structure after its elucidation was confirmed by acetylation of **1** to give rise to the 3-OAc derivative (**2**) and by comparison of the ^1H and ^{13}C NMR spectra with data from the literature. Compound **1** showed in its ^1H NMR spectrum, signals of aliphatic protons, namely, methylenes and six fine singlets of methyl. The ^{13}C NMR spectrum showed 28 signals for 28 carbons, 6 methyls, 7 methylenes, 11 methines and 4 quaternaries. The HMBC spectrum shows correlation peaks for methyl protons at δ 0.83 with δ 33.1, δ 42.8 and the methyl at δ 19.6. Protons at δ 0.93 ppm correlate with methine carbons at δ 42.8, δ 33.1 and δ 132. The methine proton at δ 5.2 correlates with methine at δ 42.8 and δ 40.4. The methyl protons at δ 1.05 are remotely coupled with methines at δ 135.0, δ 40.4 and δ 55.7. The vinyl protons at δ 5.6 and δ 5.4 couple with quaternaries at 141.3 ppm y 139.8 ppm respectively. The protons of the methyl group at δ 0.96 correlate with methylene at δ 38.4, the methine at δ 45.2 with quaternaries at δ 37.0 and δ 139.8. The COSY H-H spectrum allows observing mainly the vicinal coupling of H_6 and H_7, and H_{22} and H_{23}. All these spectral features conduct to the structural proposal of compound **1** (Fig. 1). The carbon chemical shift values are in accordance to the published data for ergosterol [8].

Compound **1** was acetylated to afford compound **2**. The downfield shift of the signal of H-3 (δ 3.65) until (δ 4.88, see Fig. 2), confirms the hydroxyl-bearing condition of C-3 in **1**. Compound **2** shows in the ^{13}C NMR spectrum, the existente of a carbonyl at 170.6 ppm, as well as the acyl methyl at δ 21.4. The ^1H NMR spectrum of **2** exhibits the signal at δ 2.1 ppm corresponding to a mehtyl whose protons correlate to the C=0 (170.6 ppm) in the HMBC. This correlation permits corroborating the presence of the acetyl group at C-3 in **2** and thus the OH group at C-3 in **1** (Fig. 3). See Table 1 for the ^{13}C chemical shifts.

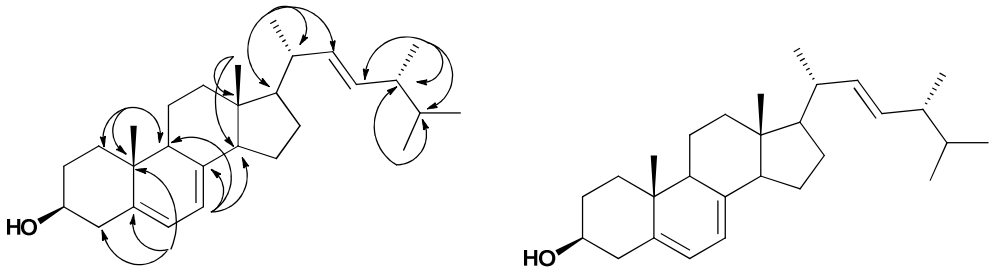

Fig. 1. *HMBC correlations and structure of compound **1**.*

EXPERIMENTAL

General

Solvents for crystallization were acetone and methanol p.a. Monitoring of compounds was done through TLC Silica gel 60 F_{254},TLC Merck. NMR spectra were run in a Bruker 300 MHz, AV300 using $CDCl_3$ and TMS as internal standard.

Vegetal material

The mushroom *Laetiporus sp*., was collected in Piribebuy city, Cordillera department, Paraguay, coordinates were latitude 25°,29'49.3"S y longitude 56°57'25.0"O on April 30, 2015 at 200 m.a.s.l. The total amount of collected material was 7 kg. The source was trunk of trees of the species *Eucalyptus* sp. The classification of the genus was done in handmade slices for the visualization of microscopic features like: hyfal system, esperes and espores; and macroscopic characteristics like size, color, context, hymenia. These observations were compared to bibliographic reported data for the genus. A voucher specimen is deposited at the FACEN-Herbarium, Paraguay, under the code HFACEN45.

Extraction and isolation

Tabla 1. *Datos de espectros de RMN^{13}C del compuesto 1 y 2*

C	Tipo de Carbono	**1** (δ ppm)	**2** (δ ppm)
1	CH_2	38.4	37.9
2	CH_2	32.0	28.1
3	CH	70.4	72.8
4	CH_2	40.8	37.08
5	C	139.8	138.5
6	CH	119.6	120.2
7	CH	116.3	116.3
8	C	141.3	141.5
9	CH	46.2	46.0
10	C	37.0	36.6
11	CH_2	21.1	21.1
12	CH_2	39.1	39.0
13	C	42.8	42.8
14	CH	54,6	54.5
15	CH_2	23.0	23.0
16	CH_2	28.3	28.3
17	CH	55.7	55.7
18	CH_3	12.0	12.1
19	CH_3	17.6	17.6
20	CH	40.4	40.5
21	CH_3	21.1	21.0
22	CH	135,6	135.6
23	CH	132.0	132.0
24	CH	42.8	40.5
25	CH	33.1	33.1
26	CH_3	19.6	19.7
27	CH_3	19.9	20.0
28	CH_3	16.2	16.2
	CO	-	170.6
	CH_3CO	-	21.4

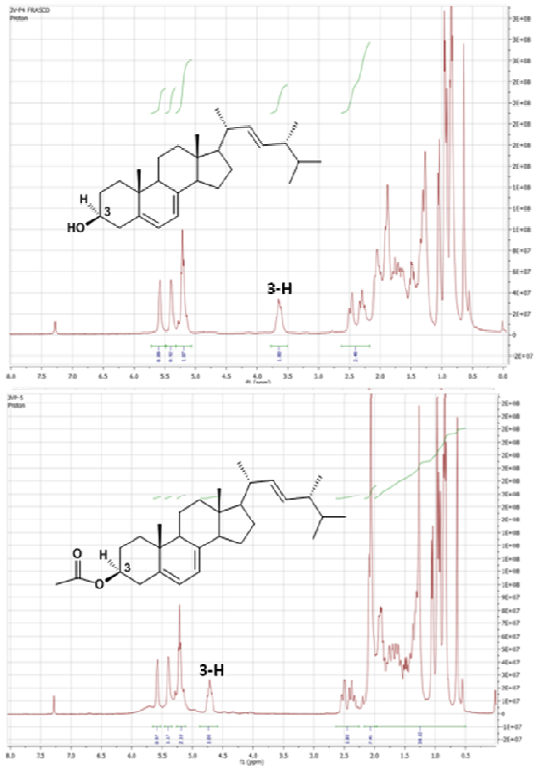

Fig. 2. *^{1}H NMR spectra, compounds **1** and **2**, 300 MHz, $CDCl_3$*

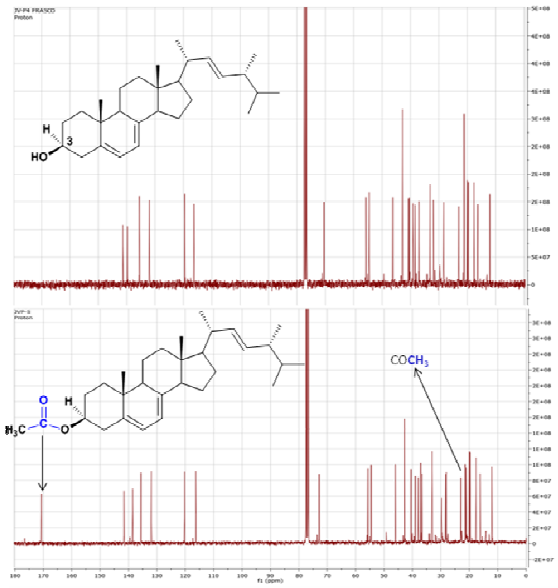

Fig. 3. *^{13}C NMR spectra, compounds **1** and **2**, 75 MHz, $CDCl_3$*

7 kg of *Laetiporus* sp. were submitted to mechanical pressure inside a cylindrical recipient after adding ethanol 95% until disintegration of the sample using a wood tool. The first extraction by maceration lasted 30 days, under agitation and applying mechanical pressure every two days. In addition 5 extractions were made, one every 8 days under agitation. The extracts were concentrated at reduced pressure. The crude extract obtained was yellow and viscous. The crude extract was rinsed with acetone at 15 ^{o}C many times to afford a white precipitate, which was re-crystallized with methanol. The yield was 0.006 % . 30 mg of the white crystals were submitted to NMR analyses.

Acetylation

Crystals of **1** (50.1 mg) were dissolved in 2 mL of acetic anhydride. Pyridine was added (three drops). Reaction time of this mixture was 24 hs. After this period, 50 mL of distilled water was added to provoke the apparition of a white precipitate corresponding to compound **2**. The precipitate was filtered and dried. 30 mg of **2** were dissolved in $CDCl_3$ and submitted to NMR analyses.

*Compound **1***
Ergosterol: ^{13}C NMR (75 MHz, $CDCl_3$) δ 38.4, 32.0,70.4, 40.8, 139.8, 119.6, 116.3, 141.3, 46.2, 37.0, 21.1, 39.1, 42.8, 54,6, 23.0, 28.3, 55.7, 12.0, 17.6, 40.4, 21.1, 135,6, 132.0, 42.8, 33.1, 19.6, 19.9, 16.2

*Compound **2***
3-acetylergosterol: ^{13}C NMR (75 MHz, $CDCl_3$) δ 37.90, 28.10, 72.83, 37.08, 138.53, 120.20, 116.29, 141.53, 46.02, 36.64, 21.11, 39.01, 42.80, 54.51, 22.99, 28.30, 55.68, 12.05, 17.6, 40.5, 21.01, 135.57, 131.96, 40.46, 33.08, 19.65, 19.96, 16.15, 170.6, 21.4

CONCLUSION

Ergosterol (ergosta-5,7,22-trien-3β-ol) and steroids of relative structures of ergosterol have been previously reported: ergosterol peroxide (5,8-epidioxy-5α,8α-ergosta-6,22-dien-3β-ol) from *Inonotus hispidus (Bull.: Fr.) P. Karst* and several other mushrooms [1,9,10]; *Inonotus obliquus* [1,11,12]; *Paecylomices tenuipes* [1,13]; *Tricholoma populinum* [1,14,15]; *Ganoderma applanatum* [1,16]; *Polyporus umbellatus* [17]; ergosterol itself from *Grifola frondosa* [1,18]; 5α-ergosta-7,22-dien-3β-ol from *Ganoderma applanatum* [1,16]. The mushroom *Laetiporus* sp. has been investigated, ergosterol (ergosta-5,7,22-trien-3β-ol) was extracted and crystallized. This is the first time that ergosterol is reported in this genus.

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