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STEROL COMPOSITION OF THE MACROMYCETE FUNGUS *Laetiporus sulphureus*

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The sterol composition of macromycete fungus Laetiporus sulphureus *was analyzed by GC-MS. The fungus contained mainly C16 to C20 fatty acids, C16 to C24 fatty acid ethyl esters, and C28, C29, and C30, Δ^5, Δ^7, and $\Delta^{5,7}$ conventional sterols, and in minor amounts Δ^0 analogues with saturated and unsaturated side chains. Moreover, ergosterol peroxide and cerevisterol were identified. This sterol pattern was compared with those of other members of the family Polyporaceae reported in the literature.*

Key words: *Laetiporus sulphureus*, Polyporaceae, macromycetes, sterols, GC-MS, fatty acids and derivatives.

Laetiporus sulphureus is a saprophyte and edible fungus. It is widely distribuited in Colombia, especially in Andina region, and it is commonly known as "chicken of the woods". *L. sulphureus* is used in wine production and as a source of gibberellic acid and cytokinin [1]. Previously reported were volatile fraction components such as esters, aldehydes, and aromatic compounds, mainly [2] lanostane-type compounds such as sulfurenic and eburicoic acids [3], sterols such as ergost-7,22-dien-3β-ol, ergosterol, ergost-7-en-3β-ol, and 24-ethylcholestan-3β-ol [4], benzofuran glycoside, acetylenic acids [5–7], and laetiporic acids; these last provide the characteristic coloration to fungus [8]. Biological studies have revealed that secondary metabolites isolated from *Laetiporus sulphureus* exhibit antithrombin [9], and antioxidant activities [10], cytotoxicity on HL-60 cells [1], and antimicrobial, cytotoxic, and dopamine D2 receptor agonistic activities [11]. The present work corresponds to the sterol fraction analysis obtained from the chloroformic extract from *L. sulphureus* by GC-MS. This fraction contains seven fatty acid ethyl esters, five fatty acids, ten sterols, and one unsaturated ergostane-type hydrocarbon. Moreover, ergosterol peroxide and cerevisterol were isolated. Those were identified by NMR, ^{13}C NMR, 2D NMR, and comparison with bibliographic data.

Constituent fraction A2 shows in the mass spectra the ions at *m/z* (frag., %): 239 (M^+–45, M^+–CH_3CH_2O, 5.2), 73 (CH_3CH_2O–C≡O^+, 14.8), and 88(CH_3-CH_2-O-CO^+H=CH_2, 100). This last fragment corresponds to McLafferty rearrangement, and it is the major peak. Moreover, this spectra shows methylene group consecutive losses as the pattern fragmentation for hydrocabonated chain: 45, 59, 73, 87, 101, 115, 129, 143…M–15. Previous ions are characteristic for fatty acid ethyl esters and are shown in Table 1. The major compound is ethyl 9,12-octadecadienoate determined by the GC area.

Constituent fraction B shows peaks in the mass spectra at *m/z* (%) 60 (48.6) caused by acetic acid loss and ion $(CH_2)_2CO_2H^+$ at *m/z* (int.) 73 (100) produced by McLafferty rearrangement, and it is the spectrum base peak. Mass spectrometric analyses permitted identifing them as fatty acids as indicated in Table 2. The major compound is palmitic acid determined by the fraction GC profile.

Fraction C3 shows sterol typical profile on the MS. It shows methyl loss (M^+–15), water loss (M^+–18), methyl and water loss (M^+–33), side chain loss with H-transpositions sometimes, C and D ring cleavage, and *m/z* 69 if C_{25} unsaturation exists [12].

The characteristic fragments of each sterol are shown in Table 3 as ergost-7-en-3-ol (**1**), ergost-7,22-dien-3-ol (**2**) (majority), ergost-5,7,22-trien-3-ol (ergosterol) (**3**), ergost-5,7,9(11),22-tetraen-3-ol (dehydroergosterol) (**4**), 24-methylenelanost-7,9-dien-3-ol (**6**), 24-methylenelanost-8-en-3-ol (obtusifoldienol) (**7**), 4,4-dimethylergost-24-en-3-ol (**8**), 4-methylergost-5,7,25-trien-3-ol (**9**), and two isomers (probably epimers) from 4-methyllergost-7,14,25-trien-3-ol (**10**).

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TABLE 1. Identified Esters in *L. sulphureus*

Ester	I_{rel}, %	tr, min	Ester	I_{rel}, %	tr, min
Ethyl hexadecanoate	82	3.616	Ethyl tetracosanoate	15	14.445
Ethyl heptadecanoate	63	4.513	Ethyl pentacosanoate	32	16.017
Ethyl 9,12-octadecadienoate	100	5.373	Ethyl 9,12-tetracosadienoate	20	22.110
Ethyl octadecanoate	70	5.674			

TABLE 2. Identified Fatty Acids in *L. sulphureus*

Acid	I_{rel}, %	tr, min	Acid	I_{rel}, %	tr, min
Palmitic	100	3.408	Stearic	82	5.318
Margaric	30	4.255	Arachidonic	35	5.932
Oleic	95	5.108			

TABLE 3. Sterol Fraction Constituents from *L. sulphureus*

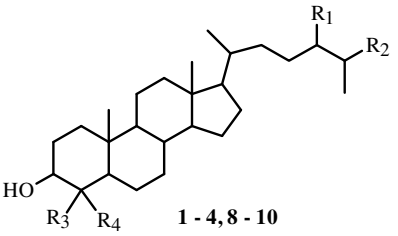
Compound	Mass spectrum (El, 70 eV), *m/z* (I_{rel}, %)	Compound	Mass spectrum (El, 70 eV), *m/z* (I_{rel}, %)
1	400 (M^+, 100), 385 (35), 367 (5), 273 (33), 255 (969), 246 (9), 231 (26), 213 (33), 145 (23)	**7**	440 (M^+, 36), 425 (100), 407 (4), 354 (7), 315 (21), 300 (4), 271 (50), 253 (54), 259 (23), 241 (21)
2	398 (M^+, 23), 383 (13), 380 (1), 365 (4), 337 (8), 273 (29), 271 (100), 255 (37), 246 (21), 213 (17), 145 (23)	**8**	428 (M^+, 40), 410 (6), 303 (100), 300 (4), 261 (5), 243 (5), 208 (5), 175 (17)
3	396 (M^+, 67), 381 (2), 378 (2), 363 (100), 337 (38), 271 (16), 253 (35), 211 (29), 143 (40)	**9**	410 (M^+, 20), 392 (6), 377 (3), 285 (14), 267 (12), 251 (22), 211 (13), 203 (50), 185 (10), 69 (100)
4	394 (M^+, 13), 379 (2), 376 (11), 361 (5), 269 (2), 251 (100), 209 (20), 141 (8)	**10**	410 (M^+, 30), 395 (13), 377 (58), 367 (8), 285 (25), 267 (50), 207 (65), 189 (15), 69 (100)
6	438 (M^+, 70), 423 (20), 405 (14), 354 (7), 311 (100), 271 (50), 253 (54)		

The compound **5** shows ions in its mass spectrum EIMS (70 eV) at *m/z* (I_{rel}, %): 376 (M^+, 50), 361 (4), 251 (100), 235 (20), 225 (4), 209 (10), 156 (17), and it does not show water loss. Moreover, acetylating in acetic anhydride/pyridine overnight of this compound gave no reaction. Thus, it was identify as ergost-3,5,7,9(11),22-pentaen (**5**).

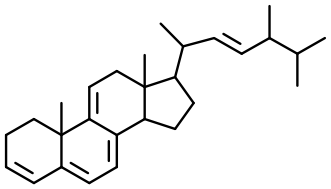
The compound **11** has the following spectroscopic data: mass spectrum EIMS (70 eV), at *m/z* (I_{rel}, %): 428 (M^+, 24), 413 (12), 396 (3), 363 (4), 301 (100); ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.83 (6H, t, J = 3.5, H-26 and H-27), 0.84 (3H, s, H-18), 0.90(3H, s, H-19), 0.92 (3H, d, J = 6, H-25), 1.01 (3H, d, J = 6, H-21), 3.99 (1H, m, H-3), 5.23 (2H, m, H-22 and H-23), 6.26 (1H, d, J = 8, H-7), 6.53 (1H, d, J = 8, H-6); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 66.5 (C-3), 79.4 (C-8), 82.2 (C-5); DEPT: 6 methyls, 4 quaternary C, 7 methylenes and 11 methynes. It was identified by comparison with reported spectroscopic data [13] as ergosta-5α,8α-epidioxy-6,22-diene-3β-ol (ergosterol peroxide, **11**).

Compound **12**. Spectroscopic data: mass spectrum (EI, 70eV) *m/z* (I_{rel}, %): 412 (M^+-H_2O, 65), 397 (22), 394 (37), 382 (60), 379 (100), 376 (5), 287 (11), 269 (57), 251 (62), 227 (27); ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 5.28 (1H, br.d, J = 5, H-7), 5.21 (1H, dd, J = 15 and 7, H-23), 5.14 (1H, dd, J = 15 and 7, H-1), 3.95 (1H, m, H-3), 3.54 (1H, br.s, H-6), 2.14 (1H, q, J = 13, Hax-4), 1.78 (1H, dd, J = 13 and 5, Heq-4), 1.04 (3H, s, H-19), 1.01 (3H, d, J = 6.9, H-21), 0.91 (3H, d, J = 6.9, H-28), 0.84 (3H, d, J = 6.9, H-26 or H-27), 0.82 (3H, d, J = 6.9 , H-26 or H-27), 0.59 (3H, s, H-18); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 143.2 (C-8), 135.5 (C-22), 132.0 (C-23), 117.5 (C-7), 67.2 (C-3), 73.0 (C-5), 75.8 (C-6). These data are in total concordance with reported data for ergosta-7,22-dien-3,5,6-triol (cerevisterol) [14].

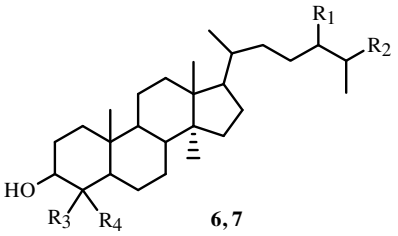
The compounds found in the fruiting body of *L. sulphureus* are evidence that ergostane-type sterols are common in the fungus kingdom. However, lanostane and 24- and 25-methylenelanostane-type sterols have significance in chemotaxonomy because they are found specially in the Polyporaceae family. Moreover, sterols of ergostane and lanostane type have unsaturations in C_5, C_7, C_8, C_9, C_{11}, and C_{22}. The major sterol is ergost-7,22-dien-3-ol; it has been found in several Polyporaceae fungi.



1 - 4, 8 - 10



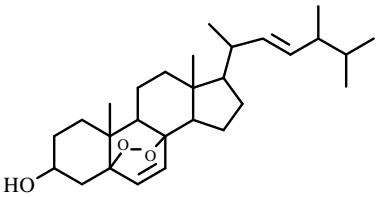
5



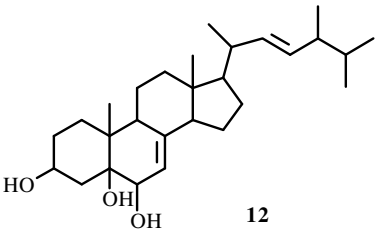
6, 7

1 - 4: $R_1 = R_2 = CH_3$, $R_3 = R_4 = H$
1: Δ^7; **2:** $\Delta^{5,22}$; **3:** $\Delta^{5,7,22}$; **4:** $\Delta^{5,7,9(11),22}$
8: $R_1 = CH_2$, $R_2 = R_3 = R_4 = CH_3$, Δ^{24}
9, 10: $R_1 = CH_3$, $R_2 = CH_2$, $R_3 = H$, $R_4 = CH_3$
9: $\Delta^{5,7,25}$; **10:** $\Delta^{7,14,25}$

6, 7: $R_1 = CH_2$, $R_2 = R_3 = R_4 = CH_3$
6: $\Delta^{7,9,24}$; **7:** $\Delta^{8,24}$



11



12

The esters and fatty acids reported in this work have been reported for the fungus kingdom, except margaric and arachidonic acids, wich are reported for the first time. For sterols, only compounds **1**, **2**, and **3** have been reported in this fungus. The others are novel.

EXPERIMENTAL

General Experimental Procedures. CC using Merck® silica gel (particle size between 0.063 and 0.200 mm), with isocratic or gradient elution, and isocratic elution using Sigma® Sephadex LH-20, and/or preparative TLC on Merck ® Silica gel $PF_{254+366}$ (particle size between 0.040 and 0.060 mm). GC-MS on HP 6890 gas chromatograph (capillary column HP5-MS 30 m, 0.25 mm D.I. and 25 μm; carrier gas He 4.5 to 1 mL/min; split 1:10 mode; temperature from 90°C to 300°C at 5°C/min) coupled to HP 5973 mass spectrometer (70 eV scan mode). NMR spectra were taken with a Bruker Avance 400 spectrometer with standard pulse sequences operating at 400 MHz in ^1H NMR and 100 MHz in ^{13}C NMR, using $CDCl_3$ as solvent and TMS as internal standard.

Plant Material. Fungus material (class Basidiomycetes, order Poriales, family Polyporaceae) was collected in the region of Tequendama, Cundinamarca department (Colombia), in March of 2003. The fungus was identified by mycologist Luis Guillermo Henao of the Instituto de Ciencias Naturales, Universidad Nacional de Colombia, where a voucher specimen is deposited (Col. 411969).

Extraction and Isolation. 335 g of dry fungus (50–60°C, 6 h) was ground and steeped in ethanol (96%) for six days at room temperature. The ethanol extract was concentrated and partitioned with chloroform:water 1:1, to obtain 9.074 g of concentrated chloroform extract (sterol fraction), which was fractionated by CC, yielding four fractions: A (122 mg), B (194 mg), C (597 mg), and D (112 mg), later purified by CC on SiO_2 eluted with $CHCl_3$ to AcOEt–MeOH (95:5). Fraction A was chromatographed on CC eluted with petroleum ether–AcOEt (from 95:5 to 50:50) over SiO_2, yielding three fractions, and the second fraction (named A2) was analyzed by GC-MS. The fraction B was analyzed directly by GC-MS. Fraction C was chromatographed on CC using SiO_2 eluted from $CHCl_3$ to $CHCl_3$–AcOEt (50:50), yielding four fractions, and the third fraction (named C3) was analyzed by GC-MS. Moreover, fraction C4 was submitted to PTLC on SiO_2 and CH_2Cl_2–AcOEt (50:50), yielding compound **11**. Fraction D was chromatographed with CC on Sephadex LH-20 eluted with CH_2Cl_2–Hex–MeOH (1:1:1), yielding five fractions. Fraction D3 was purified using PTLC on SiO_2 eluted from AcOEt to AcOEt–MeOH (85:15), yielding the compound **12**.

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REFERENCES

1. F. Leon, J. Quintana, A. Rivera, F. Estevez, and J. Bermejo, *J. Nat. Prod*., **67,** 2008 (2004).
2. S. Rapior, G. Konska, J. Guillot, C. Andary, and J. Bessiere, *Crypt. Mycol.,* **21**, 67 (2000).
3. J. Fried, P. Grabowich, E. F. Sabo, and A. I. Cohen, *Tetrahedron*, **20**, 2297 (1964).
4. D. Kac, G. Barbieri, M. R. Falco, A. M. Seldes, and E. G. Gros, *Phytochemistry*, **23**, 2686 (1984).
5. H. Yokokawa, *Phytochemistry,* **19**, 2615 (1980).
6. A. Yokoyama, S. Natori, and K. Aoshima, *Phytochemistry*, **14**, 487 (1975).
7. K. Yoshikawa, S. Bando, S. Arihara, E. Matsumura, and S. Katayama, *Chem. Pharm. Bull.*, **49**, 327 (2001).
8. P. Davoli, A. Mucci, L. Schenetti, and R. W. S. Weber, *Phytochemistry,* **66**, 817 (2005).
9. T. Okamura, T. Takeno, M. Dohi, I. Yasumaza, T. Hayashi, M. Toyoda, H. Noda, S. Fukuda, N. Oire, and M. Ohsugi, *J. Biosci. Bioeng*., **89**, 474 (2000).
10. C. Keller, M. Maillard, J. Keller, and K. Hostettmann, *Pharm. Biol*., **40**, 518 (2002).
11. J. K. Zjawiony, *J. Nat. Prod*., **67**, 300 (2004).
12. J. Karliner, H. Budzikiewicz, and C. Djerassi, *J. Org. Chem.,* **31**, 169 (1967).
13. D. S. Kim, N. I. Baek, S. R. Oh, Y. K. Jung, S. I. Lee, H. J. Kim, and K. H. Lee, *Arch. Pharm. Res.,* **20**, 201 (1997)
14. V. Piccialli and D. Sica, *J. Nat. Prod.*, **30**, 915 (1987).