METABOLITOS DE NÚCLEO ESTEROIDAL DEL HONGO COMESTIBLE *Laetiporus sulphureus*

Fecha de recepción: 14 de octubre de 2011 • Fecha de aceptación: 19 de noviembre de 2011

STEROID-RELATED METABOLITES FROM EDIBLE MUSHROOM *Laetiporus sulphureus*

Ericsson David Coy Barrera[1,2] • **Ivonne Jeannette** Nieto Ramírez[1]

ABSTRACT

Eighteen steroid-related compounds were found to be chemical constituents for a steroidal fraction obtained from the fruiting body of the edible mushroom *Laetiporus sulphureus* on the basis of GC-MS analyses. Compounds were found to be mostly associated to the ergostane and lanostane moieties. Lanost-8,25-dien-3β-ol **3** (8.8%), lanost-7,9(11),24-trien-3β,15α-diol (polycarpol) **11** (12.7%), 15-norlanost-9(11)-en-3β,14,15-triol **13** (15.3%), and 3β,15α-dihydroxy-24-methylenelanost-8-en-21-oic acid (sulphurenic acid) **18** (15.4%), were found to be the main compounds.

Key words: Polyporaceae, lanostane-type acids, ketosteoids, polyhydroxysteroids.

1 Grupo de Investigación en Química de Hongos Macromicetos, Departamento de Química, Facultad de Ciencias, Universidad Nacional de Colombia Sede Bogotá, Bogotá D.C., Colombia.
2 Facultad de Ciencias Básicas, Universidad Militar Nueva Granada, Campus Nueva Granada, Cajicá, Colombia
Autor para Correspondencia: ericsson.coy@unimilitar.edu.co

RESUMEN

A partir de la fracción esteroidal obtenida del cuerpo fructífero del hongo comestible *Laetiporus sulphureus* fueron encontrados dieciocho compuestos con núcleo esteroidal mediante análisis por CG-EM. Los compuestos estuvieron asociados principalmente a estructuras tipo ergostano y lanostano. Lanost-8,25-dien-3β-ol **3** (8.8%), lanost-7,9(11),24-trien-3β,15α-diol (policarpol) **11** (12.7%), 15-norlanost-9(11)-en-3β,14,15-triol **13** (15.3%), y ácido 3β,15α-dihidroxi-24-metilenlanost-8-en-21-oico (ácido sulfurénico) **18** (15.4%) fueron los componentes mayoritarios encontrados en la mencionada fracción esteroidal.

Palabras clave: Polyporaceae, Ácidos lanostánicos, cetoesteoides, polihidroxiesteroides.

INTRODUCTION

Polyporaceae fungi have been found to contain a wide-range of steroid-related compounds, which had encouraged studies in several applications, e.g. at pharmacological field, since they had exhibited anti-inflammatory (Yasukawa *et al.*, 1998) and anti-microbial (Hashimoto et al. 1987) activities, as well as effects towards rheumatism, metastasis and periodontal diseases (Kawagishi *et al.*, 1997), immunostimulation and modulation (Lung and Huang, 2011), and hypertension (Cheng et al., 1996), among others. *Laetiporus sulphureus* is an ectomycorrhizae, edible fungus which is widely distribuited in Colombia preferently in Andina region, whose common name is "chicken-of-the-woods". *L. sulphureus* is also used in wine production and as a source of gibberellic acid and cytokinin (Leon *et al.*, 2004).

Chemical studies on *L. sulphureus* have led to found compounds mainly related to the ergosta- and lanostane-type (Leon *et al.*, 2004; Kac *et al.*, 1984; Fried *et al.*, 1964). In addition, *L. sulphureus* was found to possess esters, aldehydes and aromatic compounds as main components in its volatile fraction (Rapior *et al.*, 2000), sterols such as ergost-7,22-dien-3β-ol, ergosterol, ergost-7-en-3β-ol and 24-ethylcholestan-3β-ol (Coy and Nieto; 2009; Kac *et al.*, 1984; Yokokawa, 1980), lanostane-type compounds such as sulphurenic and eburicoic acids (Coy and Nieto; 2009; Leon *et al.*, 2004; Fried *et al.*, 1964), fatty acids, benzofurane glycoside, acetylenic acids (Yokokawa, 1980; Yoshikawa *et al.*, 2001), laetiporic acids (which provide typical coloration to fungus) (Davoli *et al.*, 2005) and N-phenethylhexadecanamide (Shiono *et al.*, 2005). As part of our research on Colombian macromicete fungi, a chemical exploration was carried out on steroidal fraction from fruiting body of *L. sulphureus* detecting the presence of a set of eighteen steroid-related compounds (four sterols, four ketosteroids, seven polyhydroxysterols, and three lanostane-type acids) on the basis of Gas Chromatography-Mass Spectrometry (GC-MS) analyses. To the best of our knowledge, there are no reports about keto and polyhydroxy steroid-related on *L. sulphureus*.

MATERIALS AND METHODS

General experimental procedures

GC-MS analyses were performed on a HP 6890 Gas Chromatograph (Capillary Column HP5-MS 30m, 0.25mm D.I. and 25µm; Carry Gas He 4.5 to 1 mL/min; split 1:10 mode; temperature from 90°C to 300 °C a 5°C/min) coupled to HP 5973 Mass Spectrometer (70eV scan mode). Column chromatography (CC) was performed using silica gel (Merck®, particle size 0.063-0.200 mm), in isocratic or gradient elution, and sephadex LH-20 in isocratic elution (Sigma®); preparative TLC was carried out on silica gel-coated chromatoplates (Merck®, $PF_{254+366}$, particle size 0.040-0.060 mm).

Fungus material

Fungus material of *L. Sulphureus* was collected in Tequendama region, Cundinamarca Department, Colombia, in March 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 (COL411969).

Extraction and analysis

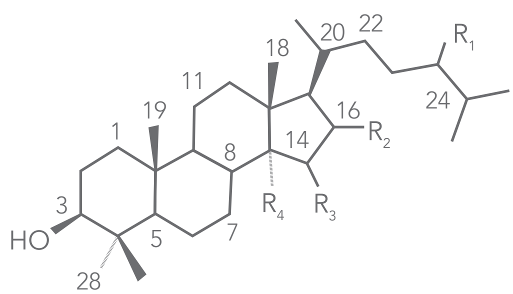
Dried fruiting body of *L. sulphureus* (335 g) was ground and steeped in ethanol (96%) by six days at room temperature. Ethanol extract (35.2 g) was concentrated and partitioned with chloroform:water 1:1 to obtain chloroform subextract (9.1 g) (steroidal fraction), which was further fractionated by column chromatography (CC) on silica gel eluted with $CHCl_3$ to AcOEt:MeOH (95:5). This fractionation yielded six fractions: A (122 mg), B (194 mg), C (597 mg), D (112 mg), E (1312 mg) and F (1674 mg). Composition of fractions A and B was reported in a previous work (Coy and Nieto, 2009), which afforded fraction A to be a multi-component mixture of fatty acid-related compounds, and fraction B as a mixture of fungi common ergostane-type sterols. Fraction C was fractionated by CC on silica gel ($CHCl_3$ to $CHCl_3$:AcOEt 50:50) yielding compounds **1-4**. Similar procedure was used for depurating fractions D and F, which yielded compounds **5-8** and **9-15**, respectively. Fraction E was fractionated by CC on sephadex LH-20 using Hexane:CH_2Cl_2:MeOH (1:1:1) as eluting mixture, affording compounds **16-18**. Compounds **1-18** were analyzed by GC-MS, and their identification was based on mass spectral interpretation on comparing mass spectra and retention time data with reported literature. Composition was determined by relative area on gas chromatogram (Table 1).

RESULTS AND DISCUSSION

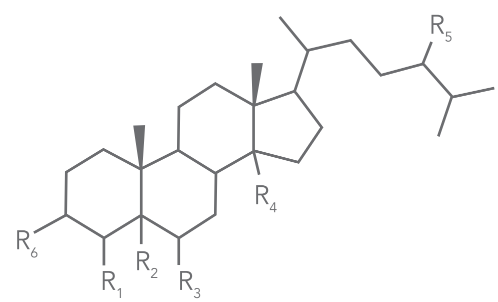
Analyses of MS data (see Table 1) for compounds **1-18** led us to establish their structures as steroid-related compounds, which were identified as lanost-22-en-3β-ol **1**, 4,4-dimethylergost-22-en-3β-ol **2**, lanost-8,25-dien-3β-ol **3**, 24-ethylcholest-5,7,22-trien-3β-ol **4,** ergost-5,7,9(11)-trien-3-ona **5**, ergost-4,6,8(14),15-tetraen-3-one **6**, ergost-4,6,8(14),22-tetraen-3-one **7**, ergost-4,6,15,22-tetraen-3-one **8**, 4,4,14,20-tetramethylpregn-8-en-3β,22-diol **9**, 28-norlanost-8,22-dien-3β,24-diol **10**, lanost-7,9(11),24-triene-3β,15α-diol (polycarpol) **11**, 4,4,14,20-tetramethylpregn-8-en-3β,7,15,22-tetraol **12**, 30-norlanost-9(11)-en-3β,14,15-triol **13**, ergost-7,9(11),22-trien-3β,5,14-triol **14**, ergosta-7,22-dien-3β,5,6-triol **15**, 3β,16α-dihydroxy-24-methylenelanost-7,9(11)-dien-21-oic acid **16,** 3β-hydroxy-24-methylenelanost-8-en-21-oic acid (eburicoic acid) **17,** 3β,15α-dihydroxy-24-methylenelanost-8-en-21-oic acid (sulphurenic acid) **18**, whose structures are shown in Figure 1.

According to their mass spectra, all compounds showed a typical sterol profile (Table 1), consisting of methyl loss (M^+–15), water loss (M^+–18), combination of methyl and water loss (M^+–33), side chain loss with eventual H-transposition, as well as C and D ring cleavage and m/z 69 ocurrence whether Δ^{25} is kept (Diekman and Djerassi, 1967). Model MS fragmentations for compound **3** are exhibited in Figure 2.

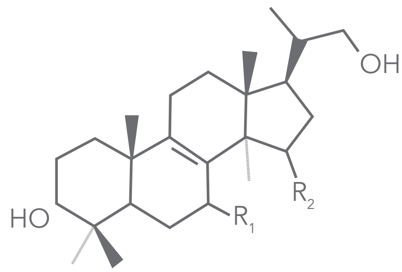
Fungi steroids are known to have a wide-variety of structures; however in *L. sulphureus* is kept the steroid skeleton related to the ergostane- and lanostane-type (Coy and Nieto, 2009; Leon et al., 2004; Yokokawa, 1980; Kac et al., 1984; Fried et al., 1964), exhibited in sterols **1-4**, ketosteroids **5-8**, polyhydroxysterols **9-15** and lanostane-type acids **16-18**. Positions of the functional groups make the difference such as hydroxy, carbonyl, methoxy, ethoxy on side chain at C-17, as well as insaturations Δ^5, Δ^8, $\Delta^{9,11}$, Δ^7 and Δ^4 at the steroid moiety and Δ^{22}, Δ^{24} and Δ^{25} on side-chain, being Δ^4-insaturation a very common feature for keto-steroids. Interestingly, the lanostane-type sterol **11** (polycarpol) could be considered the biosynthetic precursor for the lanostane-type acid **18** (sulphurenic acid), a biologically important, characteristic constituent of *L. sulphureus* (Fried et al., 1964). Lanost-8,25-dien-3β-ol **3** (8.8%), lanost-7,9(11),24-trien-3β,15α-diol (polycarpol) **11** (12.7%), 15-norlanost-9(11)-en-3β,14,15-triol **13** (15.3%), 3β,15α-dihydroxy-24-methylenelanost-8-en-21-oic acid (sulphurenic acid) **18** (15.4%), were found to be the main compounds.

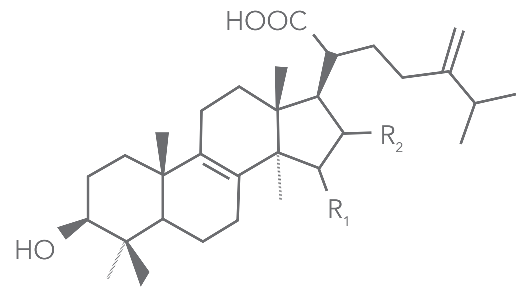


	R_1	R_2	R_3	R_4	Δ
1	H	H	H	CH_3	22
2	CH_3	H	H	H	22
3	H	H	H	CH_3	8,25
4	CH_2CH_3	H	H	CH_3	5,7,22
11	H	H	OH	H	7,9(11)24
13	H	OH	OH	H	9(11)



	R_1	R_2	R_3	R_4	R_5	R_6	Δ
5	H	H	H	H	CH_3	C=O	5.7.9(11)
6	H	H	H	H	CH_3	C=O	4,6,8(14),15
7	H	H	H	H	CH_3	C=O	4,6,8(14),22
8	H	H	H	H	CH_3	C=O	4,6,15,22
10	CH_3	H	H	CH_3	OH	βOH	8,22
14	H	OH	H	OH	CH_3	βOH	7,9(11),22
15	H	OH	OH	H	CH_3	βOH	7,22





	R_1	R_2	Δ
9	H	H	8
12	OH	OH	8

	R_1	R_2	Δ
16	H	OH	7,9(11)
17	H	H	8
18	OH	H	8

Figure 1. Structures of steroid-related constituents from *L. sulphureus*

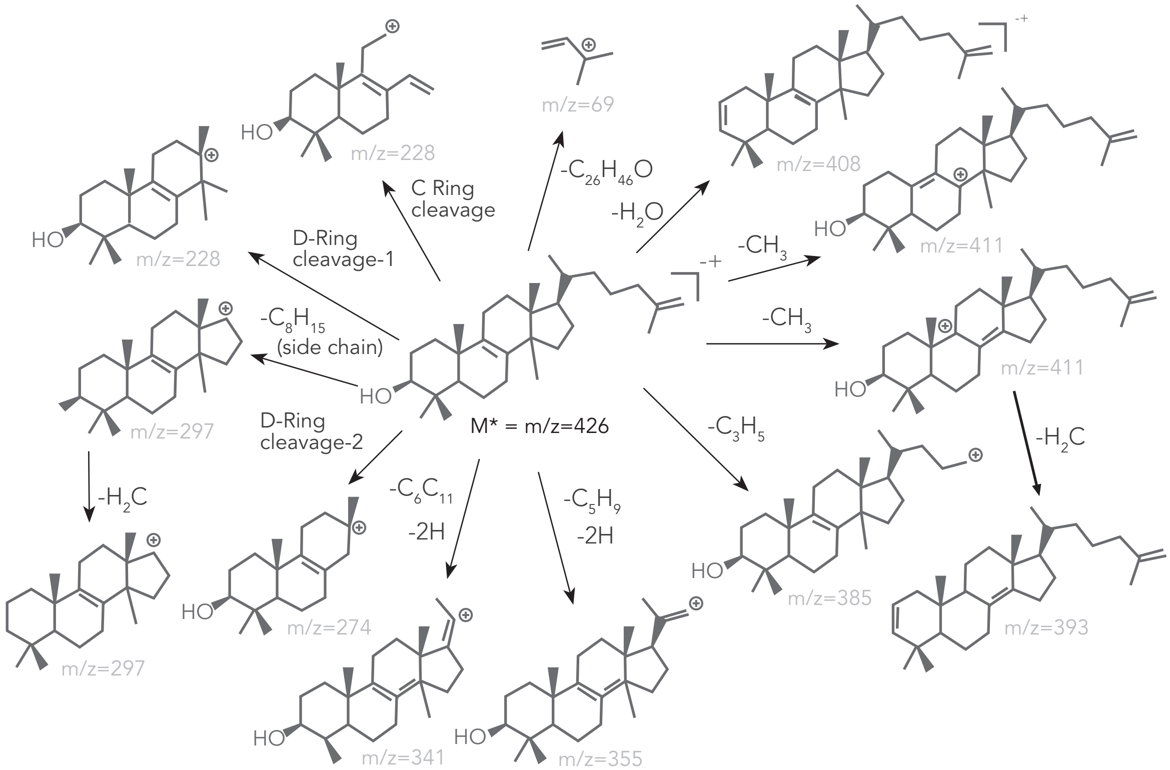


Figure 2. Main MS profile-derived fragmentations for compound 3.

These type of compounds are mostly common in marine organisms like sponge *Spongionella gracillis* (Piccialli and Sica, 1987), as well as several fungi from basidiomycete and ascomycete classes such as *Lactarium volemus* y *Agaricus blazei* (Yue et al., 2001; Kawagishi et al., 1988), and *Tuber indicum* (Jinming et al., 2001), respectively.

Similar compounds have been isolated from species from other taxonomic kingdom (ergostane- and lanostane-type is highly distributed on fungi and marine organisms). This fact lets to state fungi could have a polyphylogenetic origin, indicating a short connection with a common producer such as a symbiotic microorganism. Hence, from Basidiomycete fungi *Lactarium volemus* and *Agaricus blazei* have been isolated (22E,24R)-ergost-7,22-dien-3β,5α,6β,9α-tetraol (Yue et al., 2001; Kawagishi et al., 1988); from Ascomycetes class such as *Tuber indicum* was isolated cerevisterol (and its glycoside) and ketosteroid (22E, 24R)-ergost-4,6,8(14),22-tetraen-3-one (Jinming et al., 2001), and from sponge *Spongionella gracillis* were isolated eight polyhydroxysterols which have three OH groups at C-3, C-5 and C-6 differentiate by the side-chain shape (Piccialli and Sica, 1987). Similarly, cerevisterol [(22E, 24R)-ergost-dien-7,22-3β,5α,6β-triol] has been isolated from *L. volemus*, *T. indicum* and *S. gracillis*. Thus, keto- and polyhydroysteroid-type are metabolites commonly found in species of both macromycete fungi and marine sponges. These considerations suggest a close relationship between land and marine species, far away by taxonomy, but their differences could be due to evolutive processes, and metabolic shift leading may be the result of intensification of steroid biosynthesis and/or oxidative sterol transformations in this fungus due to its ectomycorrhizae character.

Fungi steroids are known to have a wide-variety of structures; however in *L. sulphureus* is kept the steroid skeleton related to the ergostane- and lanostane-type

Comp.	%[a]	EIMS m/z(Fragment, %)[b]
1	2.0	428(M^+, 5.8), 413(M^+-CH_3, 2.3), 410(M^+-H_2O, 6.8), 395(M^+-H_2O-CH_3, 5.2), 385(M^+-C_3H_7, 10.3), 371(M^+-C_4H_9, 9.7), 358(M^+-C_4H_9-CH_3, 3.4), 343(M^+-C_6H_{11}-2H, 6.1), 317(M^+-side chain, 100), 299(M^+-side chain-CH_3, 48.5), 276(D ring cleavage, 10.5), 259(D ring cleavage-H2O, 7.4), 207(C ring cleavage-H, 28.8), 179(C ring cleavage-CH_3, 32.4), 161(C ring cleavage-2H-H_2O, 31.3).
2	2.5	428(M^+, 23.5), 413(M^+-CH_3, 24.0), 410(M^+-H_2O, 16.2), 395(M^+-H_2O-CH_3, 17.0), 385(M^+-C_3H_7, 13.3), 371(M^+-C_4H_9, 7.6), 357(M^+-C_5H_{11}, 5.2), 329(M^+-C_7H_{13}-2H, 17.3), 303(M^+-side chain, 100), 285(M^+-side chain-H_2O, 42.1), 270(M^+-side chain-H_2O-CH_3, 17.3), 262(D ring cleavage, 10.5), 247(D ring cleavage-CH_3, 13.8), 233(C ring cleavage-2H, 5.3), 207(C ring cleavage-H, 20.0), 152 (B ring cleavage-2H, 15.3)
3	8.8	426(M^+, 7.1), 411(M^+-CH_3, 91.8), 408(M^+-H_2O, 12.9), 393(M^+-H_2O-CH_3, 10.0), 385(M^+-C_3H_5, 23.5), 355(M^+-C_5H_9-2H, 6.7), 341(M^+-C_6H_{11}-2H, 6.0), 315(M^+-side chain, 36.5), 300(M^+-side chain-CH_3, 7.1), 297(M^+-side chain-H_2O, 6.5), 288(D ring cleavage 1, 20.0), 274(D ring cleavage 2, 22.5), 259(D ring cleavage-CH_3, 20.3), 247(C ring cleavage-H, 16.5), 233(C ring cleavage-CH_2, 18.8), 207(20.0), 154(20.5), 145(20.0), 55(C_3H_5, 100).
4	3.2	410(M^+, 8.2), 395(M^+-CH_3, 35.3), 381(M^+-C_2H_5, 24.7), 377(M^+-CH_3-H_2O, 7.9), 367(M^+-C_3H_7, 20.0), 325(M^+-C_6H_{13}, 8.7), 281(M^+-C_8H_{15}-H_2O, 43.5), 271(M^+- side chain, 61.2), 253(M^+-side chain-H_2O, 100), 241(M^+-side chain-2CH_3, 11.2), 229(D ring cleavage-CH_3, 20.0), 203(C ring cleavage, 46.5), 191(C ring cleavage-CH_3, 32.9), 173(C ring cleavage-CH_3-H_2O, 25.3).
5	0.6	394(M^+, 5.9), 379(M^+-CH_3, 50.6), 364(M^+-2CH_3, 10.2), 295(M^+-C_7H_{15}, 10.0), 281(M^+-C_7H_{15}-CH_2, 11.0), 267(M^+-side chain, 100), 252(M^+-side chain-CH_3, 35.3), 207(M^+-$C_{15}H_{27}$,18.8).
6	1.1	392(M^+, 10.6), 377(M^+-CH_3, 4.2), 293(M^+-C_7H_{15}, 5.1), 281(M^+-$C_{22}H_{32}$-CH_3, 8.5), 266 (M^+-side chain+H, 45.2), 249(M^+-side chain-CH_3-H, 100.0), 207(M^+-$C_{15}H_{27}$,18.8).
7	0.4	392(M^+, 43.5), 377(M^+-CH_3, 14.1), 349(M^+-C_3H_7, 8.2), 293(M^+-C_7H_{15}-2H, 11.8), 281(M^+-$C_{22}H_{32}$-CH_3, 22.9), 267(M^+-side chain, 100), 252(M^+-side chain-CH_3, 49.4), 240(D ring cleavage, 70.6), 207(M^+-$C_{15}H_{27}$, 76.5), 193(M^+-$C_{14}H_{25}$, 25.9), 179(M^+-$C_{13}H_{23}$, 21.2), 165(M^+-$C_{12}H_{21}$, 20.0).
8	0.3	392(M^+, 15.3), 377(M^+-CH_3, 10.6), 349(M^+-C_3H_7, 7.1), 281(M^+- $C_{22}H_{32}$-CH_3, 10.6), 268(M^+-side chain (C_9H_{17})+H, 100), 253(M^+- side chain +H-CH_3, 24.7), 240(D ring cleavage, 7.1), 228(D ring cleavage-CH_2, 3.5), 214(C ring cleavage-H, 20.0) 207(M^+-$C_{15}H_{26}$+H, 22.3), 173(C ring cleavage, 20.0).
9	0.2	374(M^+, 23.5), 359(M^+-CH_3, 100), 356(M^+-H_2O, 21.2), 344(M^+-2CH^3, 17.6), 338(M^+-2H_2O, 27.1), 315(M^+-side chain, 23.5), 300(M^+-side chain-CH_3, 41.1), 282(M^+-side chain-CH_3-H_2O, 17.6), 274(D ring cleavage, 14.1), 267(M^+-side chain-2CH_3-H O, 20.0), 249(C ring cleavage 1-2H, 24.7), 233(C ring cleavage 2, 17.6).

10	1.1	428(M^+, 14.1), 413(M^+-CH_3, 45.9), 410(M^+-H_2O, 7.1), 395(M^+-H_2O-CH_3, 9.4), 385(M^+-C_3H_7, 20.0), 377(M^+-CH_3-2H_2O, 11.8), 355(M^+-C_4H_9O, 12.9), 327(M^+-$C_6H_{11}O$-2H, 25.3), 301(M^+- side chain ($C_8H_{15}O$), 100), 286(M^+- side chain ($C_8H_{15}O$)-CH_3, 100), 267(M^+-side chain -CH_3-H_2O-H, 29.4), 241(D ring cleavage-CH_3-H_2O), 207(C ring cleavage- H, 20.0), 191(C ring cleavage-CH_3, 14.1).
11	12.7	440(M^+, 14.1), 425(M^+-CH_3, 8.23), 407((M^+-H_2O-CH_3, 25.9), 404(M^+-2H_2O, 10.6), 379(M^+-C_3H_7-H_2O, 20.0), 374(M^+-2CH_3-2H_2O, 10.6), 355(M^+-$C_6H_{11}O$-2H, 10.0), 329(M^+-side chain, 7.1), 311(M^+-side chain-H_2O, 100), 302(D ring cleavage, 9.4), 293(M^+-side chain-2H_2O-H, 23.5), 281(M^+-side chain-2CH_3-H_2O-H, 31.8), 271(M^+-side chain-C_3H_6O(A ring cleavage), 21.8), 257(D ring cleavage-CH_3, 10.0), 191(C ring cleavage, 10.0)
12	3.7	406(M^+, 72.9), 391(M^+-CH_3, 100), 388(M^+-H_2O, 22.3), 373(M^+-CH_3-H_2O, 16.5), 370(M^+-2H_2O, 21.2), 347(M^+-side chain, 12.4), 334(M^+-4H_2O, 14.7), 329(M^+-side chain-H_2O, 64.7), 311(M^+-side chain-2H_2O, 10.0), 296(M^+-side chain-2H_2O-CH_3, 23.5), 290(D ring cleavage, 8.2), 222(C ring cleavage, 23.5).
13	15.3	446(M^+, 8.2), 431(M^+-CH_3, 36.4), 428(M^+-H_2O, 9.5), 416(M^+-2CH_3, 7.3), 413(M^+-CH_3-H_2O, 6.5), 410(M^+-2H_2O, 10.2), 398(M^+-2CH_3-H_2O, 7.0), 375((M^+-C_5H_{11}, 14.2), 361(M^+-C_6H_{13}, 8.3), 333(M^+-side chain, 13.6), 315(M^+-side chain-H_2O, 100), 276(D ring cleavage, 14.0), 258(D ring cleavage-H_2O, 15.2), 231(C ring cleavage-H_2O, 34.1)
14	3.1	428(M^+, 7.2), 410(M^+-H_2O, 25.8), 395(M^+-H_2O-CH_3, 19.7), 385(M^+-C_3H_7, 11.0), 377(M^+-CH_3-2H_2O, 45.3), 303(M^+-side chain, 21.2), 285(M^+-side chain-H_2O, 68.8), 267(M^+-side chain-2H_2O, 100), 262(D ring cleavage, 12.5), 252(M^+-side chain-2H_2O-CH_3, 25.6), 246(D ring cleavage-CH_3-H, 28.4), 231(28.4).
15	2.7	412(M^+-H_2O, 65.2), 397(M^+-H_2O-CH_3, 22.3), 394(M^+-2H_2O, 37.3), 382(M^+-H_2O-2CH_3, 60.4), 379(M^+-2H_2O-CH_3, 100), 376(M^+-3H_2O, 4.5), 287(11.3), 269(57.3), 251(61.7), y 227(27.3).
16	1.6	484(M^+, 33.2), 469(M^+-CH_3, 15.2), 466(M^+-H_2O, 18.3), 451(M^+-CH_3-H_2O, 15.4), 438(M^+-HCOOH, 21.0), 401(M^+-C_6H_{11}, 11.8), 372((M^+-C_7H_{13}, 14.2), 329(M^+-side chain, 40.2), 311(M^+-side chain-H_2O, 100), 293(M^+-side chain-2H_2O, 24.7), 204(C ring cleavage, 24.6), 45($HOCO^+$, 14.3)
17	1.9	470(M^+, 35.7), 455(M^+-CH_3, 100), 452(M^+-H_2O, 14.3), 437(M^+-CH_3-H_2O, 39.8), 424(M^+-HCOOH, 44.7), 387(M^+-C_6H_{11}, 36.3), 315(M^+-side chain, 38.5), 274(D ring cleavage, 29.5), 205(C ring cleavage-H, 17.7).
18	15.4	486(M^+, 51.3), 471(M^+-CH_3, 23.5), 468(M^+-H_2O, 31.9), 453(M^+-CH_3-H_2O, 25.4), 444(M^+-HCOOH, 28.2), 420(M^+-2H_2O, 17.9), 403(M^+-C_6H_{11}, 11.8), 331(M^+-side chain, 23.2), 313(M^+-side chain-H_2O, 100), 295(M^+-side chain-2H_2O, 24.7), 206(C ring cleavage, 24.6), 45($HOCO^+$, 18.5).

Table 1. Composition and MS data of steroid-related constituents from *L. sulphureus*
a. Composition by relative area on gas chromatogram | b. MS data obtained by GC-MS analyses.

REFERENCES

1. Cheng I-H, Wu D-P, Chiang H-C. 1996. Triterpenoids from *Antrodia cinnamomea*. Phytochemistry, 41:263-267.
2. Coy ED, Nieto IJ. 2009. Sterol composition of the macromycete fungus *Laetiporus sulphureus*. Chemistry of Natural Compounds, 45:193-196.
3. Davoli P, Mucci A, Schenetti L, Weber RWS. 2005. Laetiporic acids, a family of non-carotenoid polyene pigments from fruit-bodies and liquid cultures of *Laetiporus sulphureus* (Polyporales, Fungi). Phytochemistry, 66:817-823.
4. Diekman J, Djerassi C. 1967. Mass spectrometry of some steroid trimethylsilyl esters. The Journal of Organic Chemistry, 32:1005–1012.
5. Fried J, Grabowich P, Sabo EF, Cohen AI. 1964. The structure of sulphurenic acid: a new triterpenoid from *Polyporus sulphureus*. Tetrahedron, 20:2297-2312.
6. Hashimoto T, Tori M, Mizuno Y, Asakawa Y. 1987. Cryptoporic acids A and B, novel bitter drimane sesquiterpenoid ethers of isocitric acid, from the fungus *Cryptoporus volvatus*. Tetrahedron Letters, 28:6303-6304.
7. Jinming G, Lin H, Jikai L. 2001. A novel sterol from Chinese truffles *Tuber indicum*. Steroids, 66:771-775.
8. Kac D, Barbieri G, Falco MR, Seldes AM, Gros EG. 1984. The major sterols from three species of Polyporaceae. Phytochemistry, 23:2686-2687.
9. Kawagishi H, Katsumi R, Sazawa T, Mizuno T, Hagiwara T, Nakamura T. 1988. Cytotoxic steroids from the mushroom *Agaricus blazei*. Phytochemistry, 27:2777-2779.
10. Kawagishi H, Li H, Tanno O, Inoue S, Ikeda S, Ohnishi-Kameyama M, Nagata T. 1997. A lanostane-type triterpene from a mushroom *Daedalea dickinsii*. Phytochemistry, 46, 959-961.
11. Leon F, Quintana J, Rivera A, Estevez F, Bermejo J. 2004. Lanostanoid triterpenes from *Laetiporus sulphureus* and apoptosis induction on HL-60 human myeloid leukemia cells. The Journal of Natural Products, 67:2008-2011.
12. Lung MY, Huang WZ. 2011. Production, purification and tumor necrosis factor-α (TNF- α) release capability of exopolysaccharide from *Laetiporus sulphureus* (Bulliard:Fries) Bondartsey & Singer in submerged cultures. Process Biochemistry. 46:433-439.
13. Piccialli V, Sica D. 1987. Four new trihydroxylated sterols from the sponge *Spongionella gracilis*. The Journal of Natural Products, 50:915-920.

14. Rapior S, Konska G, Guillot J, Andary C, Bessiere J. 2000. Volatile composition of *Laetiporus sulphureus*. Cryptogamie Mycologie, 21:67-72
15. Shiono Y, Tamesada Y, Muravayev YD, Murayama T, Ikeda M. 2005. N-phenethylhexadecanamide from the edible mushroom *Laetiporus sulphureus*. Natural Product Research, 19:363-366.
16. Yasukawa K, Kaminaga T, Kitanaka S, Tai Y, Nunoura Y, Natori S, Takido M. 1998. 3β-p-Hydroxybenzoyldehydrotumulosic acid from *Poria cocos*, and its anti-inflammatory effect. Phytochemistry, 48:1357-1360.
17. Yokokawa H. 1980. Fatty acid and sterol compositions in mushrooms of ten species of Polyporaceae. Phytochemistry, 19:2615-2618.
18. Yoshikawa K, Bando S, Arihara S, Matsumura E, Katayama S. 2001. A benzofuran glycoside and an acetylenic acid from the fungus *Laetiporus sulphureus* var. *miniatus*. Chemical and Pharmaceutical Bulletin, 49:327-329.
19. Yue J-M, Chen S-N, Lin Z-W, Sun H-D. 2001. Sterols from the fungus *Lactarium volemus*. Phytochemistry, 56:801-806.