Sterols and fatty acids in *Peziza muralis*

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Abstract: Composition of sterols and fatty acids in fruiting bodies of fungus *Peziza muralis* were determined by gas chromatography – mass spectometry. Sterol distribution (e.g., brassicasterol occurrence) confirms phylogenetic relations to the order Tuberales. Nontypical lipid composition reflects extreme conditions (e.g., sunny, dry, and mineral-rich niches) where this fungus occurs.

Key words: *Peziza muralis*, lipids, sterols, brassicasterol, fatty acids.

Résumé : La composition des stérols et celle des acides gras des structures de fructification du champignon *Peziza muralis* ont été déterminées par chromatographie gazeuse et spectrographie de masse. Le distribution des stérols (p. ex. l'occurrence de brassicastérol) a confirmé les relations phylogénétiques avec l'ordre des Tubérales. La composition des lipides nonspécifiques reflète des conditions extrêmes, comme les niches riches en minéraux, ensoleillées et sèches, où le champignon se développe.

Mots clés : *Peziza muralis*, lipides, stérols, brassicastérol, acides gras.
[Traduit par la rédaction]

Fungi seldom occur in sunny and dry places. They typically prefer wet and shadowy niches. There are, however, some exceptions such as some plant parasites belonging to Ustilaginales (e.g., *Ustilago*), various rusts, and some Clavicipitales (e.g., *Claviceps purpurea* - Ergot). These sun-loving fungi had to develop special mechanisms to protect themselves against intensive UV rays. They normally developed pigments (e.g., melanins) to absorb light in broad spectral bands (cf. the dark colour of the above mentioned fungi) (Zhdanova and Vasilevskaya 1988; Zhdanova et al. 1990). Despite of the protection by the pigmented cell wall and quite common light-induced DNA-repair mechanisms, there are probably some other protection mechanisms developed by these fungi in the course of phylogenesis.

It was found that many of these fungi contain as one principal cellular sterol, brassicasterol, and the contents of ergosterol is strongly reduced (Weete and Laseter 1974; Ragsdale 1975; Křen et al. 1986). Ergosterol is normally the typical dominant sterol component in cell membranes of fungi. This sterol, however, could become a "Trojan-horse" upon exposition to sun. UV light is known to activate conjugated double bonds ($\Delta^{5,7}$) in ergosterol, thus creating a reactive radical. These radicals are consequently responsible for cell membrane disruption. This would be a rationale for (probably phylogenetical) reduction of ergosterol content and its replacement by brassicasterol (lacking this conjugated double bond system and therefore UV stable).

There is, however, an exception to this theory. Species of the order Tuber (Tuberales) (truffles), ascomycetous fungi with hypogean fruiting bodies, contain a high proportion of brassicasterol and their ergosterol content is strongly reduced (Weete et al. 1985). The question is, why did these fungi develop this assumed protective mechanism? Tuberales are phylogenetically younger members of the order Pezizales.

Species belonging to order Tuberales typically occur in dry, sun-exposed places, rich in minerals. They commonly occur at older scenes of fire (rich in potassium) or dumping places with rubbish (rich in calcium). Therefore, if species belonging to Pezizales would contain a reduced content of ergosterol and an elevated content of brassicasterol or another sterol lacking the conjugated double bond system, it could be expected that high brassicasterol in Tuberales would be a simple phylogenetical artefact or rudiment.

To confirm this hypothesis we have determined sterol and fatty acid content in species *Peziza muralis* that occurs quite often in the Czech Republic. This is, according to our knowledge, the first lipid analysis in this genus.

Fruiting bodies (17 pieces of size ranging from 24 to 64 g, mostly mature) of *P. muralis* were collected in a barn where lime was spilt in the Letovice region (South Moravia, Czech Republic). The same fungus was previously found in places rich in calcium (a dumping place with rubbish and a destroyed wall partly covered with soil), however in smaller amounts not sufficient for thorough analysis. The fungi were taxonomically determined by (the late) Dr. Marta Semerdžieva, Institute of Microbiology, Prague. The fungal material was processed immediately upon collection.

The crushed sample was extracted overnight (three nights, every time with fresh solution) by $CHCl_3$–CH_3OH (2:1, v/v). The total lipids were saponified and extracted according to Parks et al. (1985). Briefly, a 1-g portion of crude lipid was suspended in 1.5 mL of 60% KOH in water and 2 mL methanol. The mixture was refluxed 2 h at 70°C. After cooling, the unsaponifiable material was removed from the mixture by three 5-mL washes with hexane. Then the mixture was diluted to 50 mL with water and acidified by HCl to pH 1. Fatty acids were extracted by 3 × 10 mL of diethyl ether.

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Sterol acetates prepared according to Parks et al. (1985) were identified and quantified by total ion current by gas chromatography – mass spectrometry (GC–MS) (Shimadzu QP 1000, Shimadzu. Kyoto, Japan), equipped with a fused silica capillary column (30 m × 0.32 mm i.d. × 0.25 μm SPB-1, Supelco) and using splitless injection with He as a carrier gas. The oven temperature was programmed from 100 to 320°C at 4°/min. The ionization energy was 70 eV with an electron-multiplier voltage of 2.5 kV.

Major sterols (acetates) at relative retention times of 1.12, 1.26, 1.34, and 1.46 (Table 1) were analyzed in greater detail. The gas–liquid chromatographic (GLC) and MS data (Table 2) for these sterols were in agreement with previously published data (Itoh et al. 1982; Knights 1967). Retention times in GLC are given for sterol acetates (excluding squalene) relative to cholesterol acetate. Mass spectra were obtained also on sterol acetates.

Fatty acid methyl esters were prepared by reaction with BF_3–CH_3OH (Dembitsky et al. 1993). They were identified by GC–MS under the following conditions: column used, 30 m × 0.32 mm i.d. × 0.25 μm Supelcowax 10, Supelco; temperature gradient, 5°/min from 140 to 240°C. Other conditions were same as above.

The distribution of fatty acids (FAs) in *P. muralis* is given in Table 3. Similar to other fungal species, palmitic and oleic acids were the major components found. Using the capillary column, we succeded to separate some topoisomers, e.g., 18:1*n*11, 18:1*n*9, and 18:1*n*7. Occurrence of the topoisomers in fungi is not common; however, this could be ascribed less to biosynthetic capabilities of the organism than to advanced analytical methods used. Much more interesting is a finding of FAs with chains exceeding 18 carbons, as these FAs cause lowering of cell membrane permeability. Branched FAs that occur in fungi quite seldom have opposite effects. We suppose that *P. muralis* exposed to extreme conditions may control cell membrane permeability by these two mechanisms (i.e., elongation of FAs to over 18 carbon atoms and (or) methyl branching of the FA straight chains). Both groups of FAs mentioned above are considerably less sensitive to the environmental conditions than, for example, the polyene FAs 18:3*n*6 and 18:3*n*3.

This also supports the finding of two very rare FAs, i-17:1*n*9 and ai-17:1*n*9. In general, branched FAs having only a single double bond are less prone to oxidation, so that they can often be found in thermophilic bacteria (from hot springs) and yeast (Russell 1989).

Hydroxy-FAs make less than 1% of total FAs of which 2-hydroxypalmitic and 2-hydroxystearic acids form 65 and 35%, respectively. This observation is in agreement with previously published data, e.g., *Daedaleopsis confragosa*, *Coriolus zonalus*, or *Daedalea quercina* (belonging to Acomycetes, Endomycetales) (Dembitsky et al. 1993), as well as with the data published for Agaricales in the review of Solberg (1989).

Sterol distribution in *P. muralis* was identified by GC–MS (Table 1). Sterols with a conjugated system $\Delta^{5,7}$ make up only 45% of total sterols. Ergosterol accounts for half of all the $\Delta^{5,7}$ sterols. The rest is formed by its biosynthetic precursors (e.g., 5,7-ergostadienol) and by its metabolic product (ergostatetraenol). Thus, more than 55% of total sterols consist of intermediates of the pathway leading from squalene to ergosterol or the products of side metabolic pathways, e.g, 24-desmethylsterols (cholestan derivatives). This pattern demonstrates the unbalanced (not stabilized) biosynthesis of sterols. Brassicasterol (5,22-ergostadienol) forms up to 13.5% of total sterols.

This supports the hypothesis of modification of cellular lipids by environmental factors: the organism replaces common components of cell membranes by others that are more stable and more suitable for survival in extreme conditions. This

Table 1. Distribution of sterols in fruiting bodies of *P. muralis*.

Sterol	% of total sterols	RRT[a]
Squalene	0.95	0.64
7,24-Cholestadienol	1.46	1.07
5,22-Ergostadienol (brassicasterol)	13.44	1.12
8,24-Cholestadienol	6.36	1.16
4-Methyl-8-cholestenol	0.29	1.18
4-Methyl-7-cholestenol	0.66	1.21
5,7,22-Ergostatrienol	20.16	1.26
5,24(28)-Ergostadienol	5.26	1.27
5-Ergostenol	2.70	1.29
7,22-Ergostadienol	5.54	1.30
4,14-Dimethyl-8,24-cholestadienol	1.24	1.31
5,7,22,24(28)-Ergostatetraenol	14.76	1.34
8,24(28)-Ergostadienol	3.80	1.40
5,7-Ergostadienol	10.37	1.46
7,24(28)-Ergostadienol	4.53	1.49
7-Ergostenol	6.14	1.51
4-Methyl-7,24-ergostadienol	1.39	1.57
24-Methyl-8,24(28)-lanostadienol	0.58	1.75
24-Methyl-8-lanostenol	0.37	1.78

[a] RRT, relative retention time to cholesterol acetate.

Table 2. Chromatographic and mass-spectral properties of major sterols.

Sterol	RRT[a]	*m/z* (intensity) M⁺	M-AcOH	M-AcOH-Me	M-AcOH-C_1 to C_3	M-SC-AcOH	M-SC-AcOH-2H	M-SC-AcOH-26
Brassicasterol	1.12	440(4)	380(58)	365(47)	339(6)	255(32)	253(9)	—
Ergosterol	1.26	438(18)	378(100)	363(71)	337(11)	253(97)	251(22)	227(17)
Ergostatetraenol	1.34	436(9)	376(42)	361(28)	335(8)	253(59)	251(9)	227(8)
Ergostadienol	1.46	440(16)	380(100)	365(87)	339(18)	253(66)	251(18)	227(26)

[a] RRT, relative retention time to cholesterol acetate

Note: SC, side chain; AcOH, acetic acid; Me, CH_3 group.

Table 3. Distribution of fatty acids in fruiting bodies of *P. muralis*.

Fatty acid	% of total fatty acids
14:0	1.09
i-15:0	0.52
ai-15:0	0.85
15:0	1.24
15:1*n*7	0.67
i-16:0	1.42
16:0	13.18
16:1*n*9	5.08
16:1*n*7	3.28
i-17:0	0.70
ai-17:0	1.07
i-17:1*n*9	0.38
ai-17:1*n*9	0.48
17:0	1.32
17:1*n*9	0.64
17:1*n*7	0.78
18:0	4.05
18:1*n*11	3.56
18:1*n*9	27.14
18:1*n*7	1.71
18:2*n*6	8.04
18:3*n*6	4.57
ai-19:0	0.09
18:3*n*3	6.96
20:0	1.69
20:1*n*11	0.70
20:1*n*9	1.25
22:0	0.40
22:1*n*11	0.69
24:0	0.55
24:1*n*11	0.24
24:1*n*9	0.32

Note: i,isoacid; ai, anteisoacid.

pattern obviously survived as an artifact in evolutionary younger members of Tuberales (Weete et al. 1985) that developed from Pezizales.

It is clear, however, that not only environmental conditions account for the occurrence of brassicasterol but also physiological requirements of the organism, as demonstrated in *Gibberella fujikuroi* (Nes and Heupel 1986; Nes and Le 1990). Ergosterol and brassicasterol are synthesized by two independent pathways and they are not interconvertible (Nes and Le 1990). This endorses more the evolutionary reinforcement of the brassicasterol biosynthesis pathway in *P. muralis*. Both these sterols, together with 5-ergostenol (22-dihydrobrassicasterol), have an unsubstitutable architectural role (bulk and regulatory) that was demonstrated by reverting of the inhibitory effect of 2,3-iminosqualene on growth of *G. fujikuroi* (Nes and Heupel 1986).

The analytical data of lipids are probably the only ones within this genus and they should contribute to the chemotaxonomy of Ascomycetina.

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