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# Chemical constituents of some higher fungi

## II. Fatty acids Composition of Ascomycetes

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SUMMARY

The nine fungal species from the class Ascomycetes were examined for their FA compositions. Using capillary GC and combined GC/MS, 80 FAs were identified including 43 saturated, 34 monoenoic (the saturated and monoenoic acids were found to possess iso-, anteiso and hydroxy acids), and 3 dienoic and trienoic acids. It was shown that the Ascomycetes species contained very long-chain FAs C24–C28 which occur only rarely in fungi and, likewise, in Ascomycetes.

## Introduction

The Ascomycetes higher fungi are quite widespread in nature and play an important economic role in the human activity.

Previously, their FA composition had been studied by many researchers [4, 8, 14, 17, 23]; it had been shown that the higher fungi characteristically contained palmitic, stearic, linolic and linolenic FAs. Besides these acids, Basidiomycetes and Enthomophthorales were found to contain acids C26–C30 [6, 15], while Ascomycetes and mycelial fungi possessed additional acids C16:3 and C18:4 [2, 9, 10]. An unusual 9,10-cis-epoxyoctadecanoic acid was found in Cronartium immitis at 40% from the total FAs [3]. Arachidonic acid (20:4) was detected in certain Ascomycetes species at 0.4 to 4.2%, e.g. in Penicillium (order Euroliales) [23].

The present work deals with the FA composition from Ascomycetes in some detail.

## Experiment

The fungal species to be studied were sampled in the nature reserve "Samarskaya Luka" near Togliatti in August 1990. Freshly collected species were thoroughly cleansed of extraneous matter and only clean fungal tissues were used for homogenization in a high-speed unit. Lipids were extracted in three stages and the extracts were combined as described previously [5, 6]. The lipid extracts were submitted to triple washing in cooled 0.9% KCl. Total lipids were determined as described elsewhere [5].

Methyl esters of corresponding FAs were prepared by alkaline hydrolysis and reaction of free acids with $BF_3$-methanol [16].

The oxazolines and/or their trimethylsilyl (TMS) ethers were prepared by modification of the reference methods [21, 24]: 5 mg dicyclohexylcarboimide was added to a solution of 5 mg FAs in 1 ml $CH_2Cl_2$. After stirring (10 min) 5 mg 2-amino-2-methylpropanol was added (20



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Table 1. The saturated fatty acids.

| Class | Ascomycetes | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Orders | Endomycetales | | | | | | | Lycoperdales | |
| Families | Polyporiaceae | | | | Poriaceae | Hymenochaetaceae | | Lycoperdales | |
| Genera | Daedaleopsis | | | Coriolus | Daedalea | Phellinus | | Lycoperdon | |
| Species | confragosa | sp. 1 | sp. 2 | zonatus | quercina | sp. 1 | sp. 2 | perlatum | pussilum |
| iso-12:0 | 0.08 | 0.11 | 0.04 | 0.20 | 0.18 | 0.31 | 0.20 | 0.65 | 0.51 |
| n-12:0 | 0.37 | 0.27 | 0.43 | 0.47 | 0.26 | 0.52 | 0.44 | 1.55 | 2.16 |
| n-13:0 | 0.21 | 0.23 | 0.27 | 0.32 | 0.26 | 0.22 | 0.20 | 0.75 | 0.46 |
| iso-12:0-OH | – | – | – | 0.04 | 0.04 | 0.04 | 0.05 | 0.10 | 0.15 |
| iso-14:0 | 0.50 | 0.49 | 0.39 | 0.36 | 0.35 | 0.65 | 0.59 | 1.10 | 1.08 |
| n-14:0 | 1.04 | 1.29 | 0.85 | 1.23 | 1.28 | 0.61 | 1.17 | 4.47 | 3.35 |
| anteiso-13:0-OH | – | 0.04 | 0.08 | 0.08 | – | 0.04 | – | 0.10 | 0.05 |
| iso-15:0 | 0.12 | 0.08 | 0.04 | 0.20 | 0.18 | 0.17 | 0.15 | 1.05 | 1.18 |
| anteiso-15:0 | 0.25 | 0.27 | 0.12 | 0.24 | 0.35 | 0.31 | 0.29 | 1.45 | 1.60 |
| n-15:0 | 0.67 | 0.57 | 0.66 | 0.67 | 0.92 | 0.57 | 0.54 | 2.20 | 2.32 |
| iso-14:0-OH | 0.04 | 0.08 | 0.04 | – | – | 0.04 | 0.10 | 0.10 | 0.21 |
| n-14:0-OH | 0.17 | 0.11 | 0.16 | 0.04 | 0.09 | 0.17 | 0.15 | 0.05 | 0.21 |
| iso-16:0 | 0.87 | 1.21 | 1.59 | 1.15 | 1.63 | 3.53 | 2.73 | 2.59 | 2.63 |
| n-16:0 | 19.03 | 17.60 | 20.82 | 18.03 | 18.53 | 17.30 | 14.70 | 9.96 | 10.38 |
| anteiso-15:0-OH | – | 0.08 | 0.12 | 0.16 | 0.04 | 0.04 | 0.20 | 0.05 | 0.10 |
| n-15:0-OH | 0.08 | 0.11 | 0.19 | 0.28 | 0.22 | 0.17 | 0.24 | 0.20 | 0.26 |
| iso-17:0 | 0.46 | 0.46 | 0.58 | 0.32 | 0.31 | 0.44 | 0.44 | 0.75 | 0.67 |
| anteiso-17:0 | 0.79 | 0.68 | 0.81 | 0.47 | 0.26 | 0.74 | 0.73 | 1.45 | 1.44 |
| n-17:0 | 0.62 | 0.72 | 0.66 | 1.35 | 0.53 | 0.57 | 1.12 | 1.75 | 1.44 |
| iso-16:0-OH | 0.04 | 0.11 | 0.08 | 0.12 | 0.04 | 0.13 | 0.05 | 0.15 | 0.10 |
| n-16:0-OH | 0.25 | 0.30 | 0.35 | 0.51 | 0.53 | 0.44 | 0.73 | 0.85 | 0.77 |
| n-18:0 | 6.04 | 8.15 | 7.33 | 6.89 | 8.85 | 10.50 | 4.83 | 2.10 | 1.96 |
| anteiso-17:0-OH | 0.08 | 0.19 | 0.27 | 0.12 | 0.26 | 0.09 | 0.05 | – | – |
| anteiso-19:0 | 0.21 | 0.23 | 0.19 | 0.12 | 0.31 | – | 0.20 | 0.45 | 0.36 |
| n-19:0 | 0.04 | 0.53 | 0.47 | 0.36 | 0.62 | 0.09 | 0.24 | 0.25 | 0.05 |
| n-20:0 | 0.96 | 0.72 | 1.20 | 0.99 | 1.85 | 0.48 | 0.88 | 1.55 | 1.91 |
| anteiso-19:0-OH | 0.08 | 0.04 | 0.19 | 0.08 | 0.13 | 0.13 | – | 0.05 | 0.10 |
| n-19:0-OH | 0.79 | 0.57 | 0.66 | 0.44 | 0.35 | 0.70 | 0.59 | 0.20 | 0.15 |
| n-21:0 | 0.50 | 0.38 | 0.35 | 0.20 | 0.31 | 0.39 | 0.68 | 0.60 | 0.31 |
| iso-20:0-OH | 0.25 | 0.30 | 0.54 | 0.83 | 0.40 | 0.61 | 0.44 | – | 0.05 |
| n-20:0-OH | 0.67 | 0.87 | 0.70 | 0.95 | 1.37 | 0.74 | 0.59 | 0.90 | 1.18 |
| n-22:0 | 1.00 | 1.06 | 1.20 | 0.87 | 0.70 | 1.09 | 0.93 | 2.40 | 1.91 |
| iso-21:0-OH | 0.21 | 0.08 | 0.12 | 0.08 | 0.18 | 0.31 | 0.24 | 0.40 | 0.36 |
| n-21:0-OH | 0.25 | 0.30 | 0.19 | 0.28 | 0.40 | 0.35 | 0.49 | 0.20 | 0.15 |
| n-23:0 | 0.50 | 0.57 | 0.54 | 0.75 | 0.75 | 0.57 | 0.59 | 0.55 | 0.46 |
| iso-22:0-OH | 0.29 | 0.19 | 0.35 | 0.16 | 0.26 | 0.04 | 0.24 | 0.24 | 0.15 |
| n-22:0-OH | 1.00 | 0.99 | 1.24 | 0.71 | 1.88 | 1.44 | 0.88 | 0.80 | 0.72 |
| n-24:0 | 0.58 | 0.68 | 0.66 | 0.95 | 1.37 | 1.92 | 1.81 | 2.59 | 3.14 |
| n-23:0-OH | 0.37 | 0.27 | 0.19 | 0.32 | 0.40 | 0.09 | 0.24 | 0.30 | 0.36 |
| n-25:0 | 0.50 | 0.34 | 0.19 | 0.28 | 0.40 | 0.17 | 0.15 | 0.80 | 0.62 |
| n-24:0-OH | 1.75 | 2.05 | 1.47 | 1.23 | 1.28 | 1.35 | 3.56 | 2.25 | 1.91 |
| n-26:0 | 0.08 | 0.11 | 0.19 | 0.08 | 0.18 | 0.17 | – | 0.40 | 0.36 |
| n-28:0 | 0.21 | 0.27 | 0.12 | 0.16 | 0.09 | 0.26 | 0.24 | 0.40 | 0.36 |
| normal-acids | 32.35 | 33.49 | 35.94 | 33.60 | 36.90 | 35.43 | 28.52 | 32.32 | 31.19 |
| iso-acids | 2.03 | 2.35 | 2.64 | 2.23 | 2.65 | 5.10 | 4.11 | 6.14 | 6.07 |
| anteiso-acids | 1.25 | 1.18 | 1.12 | 0.83 | 0.92 | 1.05 | 1.22 | 3.35 | 3.40 |
| iso-acids-OH | 0.83 | 0.76 | 1.13 | 1.23 | 0.92 | 1.17 | 1.12 | 0.99 | 1.02 |
| anteiso-acids-OH | 0.16 | 0.35 | 0.66 | 0.44 | 0.43 | 0.30 | 0.25 | 0.20 | 0.25 |
| acids-OH | 5.33 | 5.57 | 5.15 | 4.76 | 6.45 | 5.45 | 7.47 | 5.75 | 5.71 |
| the sum HO-acids | 6.32 | 6.68 | 6.94 | 6.43 | 7.80 | 6.92 | 8.84 | 6.94 | 6.98 |
| Saturated | 41.95 | 43.70 | 46.64 | 43.09 | 48.27 | 48.50 | 42.69 | 48.75 | 47.64 |

Table 2. The monoenes fatty acids.

| Species | D. confragosa | D. sp. 1 | D. sp. 2 | C. zonatus | D. quercina | Ph. sp. 1 | Ph. sp. 2 | L. perlatum | L. pussilum |
|---|---|---|---|---|---|---|---|---|---|
| 12:1 (n-7) | 0.12 | 0.04 | 0.27 | 0.24 | 0.22 | 0.04 | 0.39 | 0.45 | 0.57 |
| anteiso-13:1 (n-8) | – | – | – | – | 0.04 | – | – | – | 0.10 |
| 13:1 (n-8) | 0.12 | 0.08 | 0.08 | – | – | 0.09 | 0.05 | 0.05 | 0.15 |
| 14:1 (n-9) | 0.72 | 0.61 | 0.78 | 0.75 | 0.92 | 0.52 | 1.12 | 1.25 | 1.18 |
| 15:1 (n-8) | 0.50 | 0.64 | 0.54 | 0.44 | 0.57 | 0.44 | 0.44 | 1.85 | 1.65 |
| 16:1 (n-9) | 6.82 | 6.09 | 9.93 | 15.73 | 9.73 | 14.81 | 15.22 | 6.04 | 6.13 |
| 15:1-OH | 0.12 | 0.15 | 0.08 | 0.08 | 0.13 | 0.13 | 0.05 | 0.10 | 0.00 |
| iso-17:1 (n-8) | 0.25 | 0.19 | 0.16 | 0.08 | – | 0.13 | 0.24 | 0.40 | 0.57 |
| anteiso-17:1 (n-8) | 0.33 | 0.34 | 0.43 | 0.20 | 0.09 | 0.26 | 0.44 | 0.50 | 0.41 |
| 17:1 (n-8) | 0.50 | 0.19 | 0.35 | 0.28 | 0.18 | 0.22 | 0.20 | 1.69 | 1.57 |
| 17:1 (n-10) | 1.29 | 1.02 | 0.97 | 0.71 | 0.26 | 0.35 | 0.44 | 1.05 | 1.29 |
| iso-16:1 (n-9)-OH | – | – | – | 0.08 | 0.13 | – | – | 0.10 | 0.21 |
| 16:1 (n-9)-OH | 0.08 | 0.11 | 0.04 | 0.12 | 0.09 | 0.13 | 0.10 | 0.05 | 0.05 |
| 18:1 (n-9) | 15.99 | 18.51 | 12.68 | 11.23 | 10.56 | 12.81 | 9.26 | 11.40 | 12.09 |
| 18:1 (n-11) | 2.25 | 1.18 | 1.01 | 0.59 | 1.89 | 1.70 | 4.10 | 1.05 | 0.88 |
| anteiso-17:1 (n-8)-OH | 0.12 | 0.08 | 0.04 | – | – | 0.04 | 0.10 | 0.05 | 0.10 |
| 17:1 (n-8)-OH | 0.25 | 0.15 | 0.12 | 0.04 | 0.09 | 0.09 | 0.05 | 0.05 | 0.05 |
| 19:1 (n-10) | 0.29 | 0.38 | 0.35 | 0.24 | 0.53 | – | 0.34 | 0.45 | 0.51 |
| 18:1 (n-9)-OH | – | – | – | 0.12 | 0.09 | 0.13 | 0.20 | 0.25 | 0.36 |
| 20:1 (n-9) | 0.71 | 0.68 | 0.78 | 0.24 | 0.18 | 0.22 | 0.34 | 0.60 | 1.75 |
| 20:1 (n-11) | 1.91 | 1.40 | 1.90 | 0.24 | 2.25 | 2.44 | 2.34 | 0.15 | 0.05 |
| 22:1 (n-13) | 2.25 | 1.82 | 2.44 | 1.45 | 4.01 | 2.09 | 2.69 | 2.10 | 0.57 |
| anteiso-21:1 (n-12)-OH | – | – | – | 0.04 | – | – | – | 0.25 | 0.36 |
| iso-22:1 (n-13)-OH | – | – | – | 0.08 | 0.04 | – | – | 0.40 | 0.36 |
| 22:1 (n-13)-OH | 0.50 | 0.42 | 0.50 | 0.47 | 0.62 | – | 0.34 | 0.96 | 0.93 |
| 24:1 (n-13) | 0.25 | 0.30 | 0.47 | 1.07 | 0.44 | 0.48 | 1.22 | 0.95 | 1.80 |
| 24:1 (n-15) | 1.62 | 2.35 | 1.59 | 1.35 | 1.23 | 1.35 | 2.05 | 0.75 | 0.46 |
| iso-24:1 (n-15)-OH | – | – | – | – | 0.18 | – | – | 0.25 | 0.31 |
| 24:1 (n-15)-OH | 0.50 | 0.30 | 0.27 | 0.47 | 0.26 | 0.13 | – | 0.40 | 0.36 |
| 26:1 (n-17) | 0.50 | 0.57 | 0.66 | 0.12 | 0.22 | 0.31 | 0.29 | 0.90 | 0.82 |
| 25:1 (n-16)-OH | – | – | – | – | – | – | – | 0.20 | 0.15 |
| iso-26:1 (n-17)-OH | – | – | – | – | – | – | – | 0.10 | 0.10 |
| 26:1-OH | 0.04 | 0.08 | 0.04 | 0.04 | – | 0.04 | – | 0.25 | 0.15 |
| 28:1-OH | – | – | – | – | – | – | – | 0.10 | 0.10 |
| iso-monoenes | 0.25 | 0.19 | 0.16 | 0.08 | – | 0.13 | 0.24 | 0.40 | 0.57 |
| anteiso-monoenes | 0.33 | 0.34 | 0.43 | 0.20 | 0.13 | 0.26 | 0.44 | 0.50 | 0.51 |
| iso-monoenes-OH | – | – | – | 0.16 | 0.35 | – | – | 0.85 | 0.98 |
| anteiso-monoenes-OH | 0.12 | 0.08 | 0.04 | 0.04 | – | 0.04 | 0.10 | 0.30 | 0.46 |
| monoenes-OH | 1.49 | 1.21 | 1.05 | 1.34 | 1.28 | 0.65 | 0.74 | 2.36 | 2.10 |
| the sum HO-acids | 1.61 | 1.29 | 1.09 | 1.54 | 1.63 | 0.69 | 0.84 | 3.41 | 3.54 |
| Monoenes | 35.84 | 35.86 | 34.80 | 34.68 | 33.19 | 37.87 | 40.85 | 30.73 | 31.47 |

Table 3. The dienes and trienes fatty acids.

| Species | D. confragosa | D. sp. 1 | D. sp. 2 | C. zonatus | D. quercina | Ph. sp. 1 | Ph. sp. 2 | L. perlatum | L. pussilum |
|---|---|---|---|---|---|---|---|---|---|
| 18:2 (n-9) | 11.28 | 12.40 | 9.54 | 14.07 | 9.82 | 5.53 | 6.49 | 9.43 | 9.47 |
| 18:3 (n-6) | 2.33 | 1.40 | 1.09 | 0.36 | 1.37 | 0.22 | 0.44 | 3.09 | 2.94 |
| 18:3 (n-9) | 6.41 | 4.82 | 6.25 | 5.98 | 5.59 | 6.80 | 8.01 | 3.59 | 3.86 |
| The sum of dienes and trienes | 20.02 | 18.62 | 16.88 | 20.41 | 16.78 | 12.55 | 14.94 | 16.11 | 16.27 |

deg. C, 4 hr). The evaporated mixture was dissolved in 1 ml diethylether and treated with 0.5 ml $SOCl_2$ (20 deg. C, 1 hr), washed in ice cold water and eluted through a column with anhydrous sodium sulfate and silica gel. The eluate was evaporated, dissolved in pyridine, and heated with trimethylsilylchloride (50 deg. C, 4 hr). Methyl esters were identified in a Shimadzu QP-1000 apparatus with a fused silica capillary column (60 m × 0.32 mm ID/SPB-1; Supelco, USA), using splitless injection and helium as the carrier gas. Oven temperature was programmed from 100 to 320 deg. C, at the rate 4 deg. C/min. Ionization energy was 70 eV, and electron multiplier voltage was 2.5 kV. TMS-ethers of oxazolines were identified under conditions similar to those of the methyl ester identification, the only difference being the column temperature programmed from 150 to 300 deg. C at the rate 5 deg. C/min.

Abbreviations: GC, gas chromatography; GC/MS, combined gas chromatography/mass spectrometry; FA, fatty acid.

## Results and Discussion

Our FA analysis showed that the main FAs in the studied species were the saturated FAs having chain lengths from C12 to C28 (Table 1) and varing from 41,95% in D. confragosa to 48.75% in L. perlatum. Iso-acids, including those with a hydroxy group, varied in the range 2.86–7.13%. Anteiso-acids, also with a hydroxy group, constituted lower amounts, 1.27 to 3.65%. The content of hydroxy acids among saturated FAs varied from 6.32–8.84%. Hydroxy acids often occur within the different orders of fungi [17, 22, 23]. They were identified in many species of sclerotial fungi [11–13] with the maximum amount observed in the order Hypocreales at 64% [13]. Our data indicated 2 to 3 times higher levels of iso- and anteiso-acids in Basidiomycetes in comparison with Ascomycetes [7]. Hydroxy acid totals in Basidiomycetes were similar to those in Ascomycetes, as were their respective levels of saturated FAs. Hydroxy acid n-18:O–OH had been previously identified amoung saturated FAs from the fungus Claviceps purpurea at the level of 22 to 33% [23]. Long-chain dihydroxy and hydroxy FAs with C22–C26 have been found in ceramides, sphingolipids and glycolipids of various fungi and yeasts [1, 4, 18, 22].

Total monoenoic FAs, similarly to saturated, have been found to contain iso-, anteiso-, and hydroxy acids with chain lengths C12–C26 (Table 2) varying from 30.73–31.47% for the family Lycoperdales, to 34.68–35.86% for the family Polyporiaceae, and at the higher level to 37.87–40.85% for the family Hymenochaetaceae. The level of iso- and anteiso-moenes did not exceed 1% in all studied species. Total hydroxy acids among monoenes constituted less amounts than among saturated acids and varied from 0.69 to 3.54%. However, certain Ascomycetes are known to possess OH–18:1 which was found, according to different authors who studied Claviceps purpurea, to vary from 5.9 to 41.8% [19, 23]. Among monoenoic acids from Basidiomycetes, the level of total hydroxy acids was also higher than in Ascomycetes [7] and lower than in saturated FAs and varied from 0.69 to 3.54% (Table 2). Monoenoic compounds included very long-chain FAs C24–C28. Acid 24:1 has been found to possess 4 isomers with a hydroxy group present in 2 isomers. Acids 26:1 is represented by 3 isomers, and, again, a hydroxy group is present in 2 isomers. The longest acid, both among saturated and monoenoic compounds, is acid C28. The hydroxy group is in position 2 in all the acids.

Only 3 acids were identified among dienes and trienes: 18:2(n–9), 18:3(n–6) and 18:3(n–9) (Table 3). The total dienes/trienes were lower than saturated acids and monoenes and varied from 16.11 to 20.02%. The amount of acid 18:2 in many studied Ascomycetes species is known to be much higher than that detected in our study; in the case of Claviceps purpurea this amount is as high as 65.4% [23].

Thus, our examination of the FAs from Ascomycetes showed that the higher fungal species mentioned contained a considerably broad set of 80 FAs which included iso-, anteiso- and hydroxy acids as well as very long-chain FAs C24–C28.

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*Key words:* Ascomycetes, fatty acids; hydroxy acids

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