

Occurrence of N-methyl-N-formylhydrazones in Mycelia of *Gyromitra esculenta*

Marjatta Raudaskoski and Heikki Pyysalo

Department of Botany, University of Turku, Turku, and Food Research Laboratory, Technical Research Centre of Finland, Espoo, Finland

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N-methyl-N-formylhydrazones, the toxic compounds identified in the fruitbodies of the false morel *Gyromitra esculenta*, were also found in the mycelia grown from isolated ascospores. The amounts of acetaldehyde N-methyl-N-formylhydrazone, the main hydrazone compound of the false morel, varied significantly among the strains studied. Especially low levels occurred in some of the strains isolated from one fruitbody.

Introduction

The fruitbodies of false morel, *Gyromitra esculenta* Pers. Fr., contain several toxic N-methyl-N-formylhydrazones (MFH) (Fig. 1) the best known of which is acetaldehyde N-methyl-N-formylhydrazone, called gyromitrin or ethylidene gyromitrin [1, 2]. The average amount of ethylidene gyromitrin in the fresh Finnish mushroom is 50 mg per kg, whereas the average combined value of higher homologues of ethylidene gyromitrin is 7 mg per kg fruitbodies.

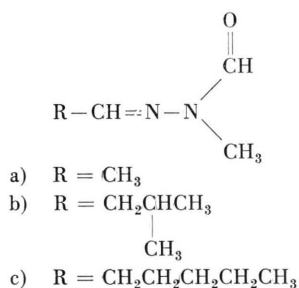


Fig. 1. The structure of acetaldehyde MFH (ethylidene gyromitrin) (a), 3-methyl butanal MFH (b), and hexanal MFH (c).

Unlike most other macrofungi containing toxic compounds, the mycelia of *G. esculenta* can be grown in culture. A mycelial culture is started by isolating one germinated ascospore [3]. The cultures make it possible to study the occurrence of ethylidene gyromitrin and the other toxic MFH-compounds in mycelia and the effect of different environmental conditions on their production. The

mycelial cultures can be preserved on synthetic media in the cold, and experiments with the same strain can be repeated. Further, the use of monosporic cultures provides a mean of observing genetic variation in the production of MFH-compounds among different monosporic strains.

Experimental

Spores were isolated from dried fruitbodies collected from different parts of Finland in June 1973 and preserved at room temperature in paper bags. Three samples of fruitbodies originated from northern Finland: 3b from Sevetijärvi (lat. 69°29' N and long. 28°39' E), A from Inari (lat. 68°57' N and long. 27°8' E), and B from Sodankylä (lat. 67°28' N and long. 26°40' E). Two samples were from eastern Finland: I from Ilomantsi (lat. 62°40' N and long. 30°55' E) and G4 from Juva (lat. 61°50' N and long. 27°53' E). P fruitbodies were collected in Littoinen, southern Finland (lat. 60°25' N and long. 22°27' E). Only one fruitbody was used from each sample, but several monosporic strains were isolated from each fruitbody by a process reported previously [3].

In each experiment pieces of agar with hyphae were cut with a micropipette from the edge of well-growing monosporous cultures and transferred with a needle to a Petri dish. The agar medium was completely covered with a cellophane membrane [3] to prevent the penetration of the mycelium into the medium, while at the same time allowing the nutrients to reach the mycelium. Each monosporic strain tested was inoculated to 10 replica plates, on which it was grown for 12–14 days. The mycelium was harvested from membranes with a razor

Requests for reprints should be sent to Ph. D. Heikki Pyysalo, Technical Research Centre of Finland, Food Research Laboratory, Biologinkuja 1, SF-02150 Espoo 15, Finland.



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blade for the estimation of ethylidene gyromitrin and the other toxic compounds.

The MFH-compounds were analysed by a high resolution glass capillary gas chromatography (GLC) technique [2]. MFH-compounds were extracted from the deep frozen mycelium into 50 ml of water-saturated diethyl ether in a Soxhlet apparatus over a period of 10 hours. The dried ether extract was evaporated in vacuum into 0.5 ml volume. A 50-m glass capillary column and FID were used in the GLC analysis.

Synthetic MFH compounds whose structures had been confirmed by ^1H NMR and mass spectra [2, 4] were used as reference compounds in quantitative GLC analysis.

Results

In the first experiment the mycelia of two strains, one originating from northern and the other from eastern Finland, were grown at 20 °C in both continuous light and darkness and tested for ethylidene gyromitrin. The mycelia of both strains contained approximately the same amount of ethylidene gyromitrin independent of illumination. The media on which the mycelia had grown were also analysed, but no secretion of ethylidene gyromitrin or other MFH-compounds had occurred into media.

Twelve monosporic strains were further analysed for their contents of ethylidene gyromitrin and of the next most abundant hydrazones, hexanal N-methyl-N-formylhydrazone and 3-methylbutanal N-methyl-N-formylhydrazone. Six of the strains studied originated from different parts of Finland (Table I) and six were isolated from the same *3b* fruitbody (Table II). All the strains were grown simultaneously at 20 °C, in rhythmic light, for 14 days. During this time the mycelia of some strains spread over

Table I. Amounts of N-methyl-N-formylhydrazones (mg/kg) in mycelia of six strains originating from different parts of Finland.

Strain	Fresh weights of mycelia [g]	Acetaldehyde MFH	3-Methylbutanal MFH	Hexanal MFH
<i>A</i> ₁	11.107	67.9	4.0	3.1
<i>B</i> ₁	10.109	34.8	11.2	12.1
<i>P</i> ₁	11.642	66.7	5.7	15.2
<i>G</i> ₄ ₁	6.351	54.7	1.4	7.1
<i>I</i> ₁	5.464	66.4	11.0	17.2
<i>3b</i> ₂	0.970	35.2	2.2	1.4

Table II. Amounts of N-methyl-N-formylhydrazones (mg/kg) in mycelia of six strains originating from the same fruitbody.

Strain	Fresh weights of mycelia [g]	Acetaldehyde MFH	3-Methylbutanal MFH	Hexanal MFH
<i>3b</i> ₃	8.316	43.6	8.7	16.3
<i>3b</i> ₄	3.496	49.7	—*	—*
<i>3b</i> ₅	8.992	8.7	1.8	3.2
<i>3b</i> ₆	6.226	9.7	8.7	4.1
<i>3b</i> ₇	6.849	29.4	1.3	9.9
<i>3b</i> ₈	6.672	50.3	4.0	2.0

* Not analysed.

the whole surface of the agar in the Petri dish, while those of other strains grew much less. The differences in fresh weights of mycelia in Tables I and II indicate the different growth rates of the strains.

Strains *B*₁ and *3b*₂, both originating from North Finland, contained relatively low amounts of ethylidene gyromitrin (Table I). The fresh weight of mycelium of strain *3b*₂ was exceptionally low, however, indicating a poor growth at 20 °C that could be significant for the production of ethylidene gyromitrin. The small sample of strains tested revealed no regional distribution of strains with low or high amounts of ethylidene gyromitrin. The fresh weights of mycelia varied less and the amounts of ethylidene gyromitrin more among strains from the same fruitbody (Table II) than among strains from different fruitbodies (Table I). In two pairs of strains, *3b*₃, *3b*₅ and *3b*₈, *3b*₆ (Table II), whose respective fresh weights of mycelia were almost equal the amount of ethylidene gyromitrin in one member of the pair was five times greater than in the other.

3-Methylbutanal MFH and hexanal MFH were detected in all mycelia analysed (Tables I and II). All the other MFH-compounds identified in fruitbodies [2] were also found in mycelia. Since the samples of mycelia analysed were relatively small, only the amounts of the abundant components could be reliably determined (Tables I and II). In all the strains studied the internal ratio of the amounts of MFH-compounds in mycelia was about the same; ethylidene gyromitrin was the main MFH-compound and the next most abundant components were 3-methylbutanal and hexanal MFH, as in fruitbodies [2].

Further experiments showed that 25 °C was a more optimal temperature than 20 °C for the mycelial growth of strain 3b₂. Two samples of mycelia of strain 3b₂ were grown at 25 °C and analysed for MFH-compounds. In both samples the yield of mycelia was much higher and the amounts of MFH-compounds were still lower than at 20 °C (Table III). A similar result was obtained with strain I₁: the amount of MFH-compounds was decreased at a temperature at which the yield of mycelia was increased.

Table III. Two analyses of mycelia of strain 3b₂ grown at 25 °C for 14 days.

Analysis no	Fresh weights of mycelia [g]	Acetaldehyde MFH [mg/kg]	3-Methylbutanal MFH [mg/kg]	Hexanal MFH [mg/kg]
1	7.8	5.0	0.5	0.2
2	5.8	10.0	0.3	0.1

Discussion

The present study shows that N-methyl-N-formylhydrazones are present in the mycelia of *Gyromitra esculenta* in amounts comparable to those in the fruitbodies. The significance of these toxic compounds to the function and development of the fungus is unclear. It has been suggested that hydrazones might play a role in the rapid growth of the fruitbody [5], but the occurrence of equal amounts of hydrazones in mycelia and fruitbodies is not in agreement with this suggestion.

Analysis of different monosporic strains grown under the same conditions indicated different contents of MFH-compounds among the strains. This could result from genetic variation among the strains in the ability to produce MFH-compounds or in the response of the strains to the environment. The latter would indirectly affect the production of MFH-

compounds [6]. The results presented suggest that both aspects must be considered.

The low fresh weights of mycelia indicated that the environment used was suboptimal for the rapid growth of the strains. Most of the strains had relatively high amounts of MFH-compounds in mycelia. Change in the growth temperature increased the growth rate while decreasing the amount of MFH-compounds, which suggests that the production of MFH-compounds in mycelia is linked to growth in such a way that conditions suboptimal for growth lead to increased production.

Of the strains tested 3b₂ appeared to belong to a group with low content of MFH-compounds, since the amount of the compounds in this strain remained low even in suboptimal growth conditions. Strain 3b₂ originated from the fruitbody 3b from which two other strains with low amounts of MFH-compounds were isolated. Whether the low production of MFH-compounds in these strains is a stable characteristic due to genotype remains to be investigated.

It has been found recently that N-methyl-N-formylhydrazones convert in the stomach to hydrazines, which are carcinogenic and mutagenic [7–12]. It is further known that small amounts of ethylidene gyromitrin remain in the boiled fruitbodies [2]. Consumption of the fruitbodies of *G. esculenta* may therefore be considered questionable. The present observation that some strains of *G. esculenta* contain only small amounts of N-methyl-N-formylhydrazones would prove useful if fruitbodies were to be successfully produced in culture [3]. It would then be necessary to clarify whether MFH-compounds have some role in the development of fruitbodies, and to culture only strains with low MFH contents.

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