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Hydrocarbon Constituents from White Strains of the Mushroom *Agaricus bisporus* (Lange) Singer

The study of the chemical composition of the cultivated mushroom *Agaricus bisporus* (Lange) Singer has been limited. Two white strains of the *A. bisporus* mushroom were analyzed for their hydrocarbon content. Hexane extractable lipids were 1.81% and 1.37% (dry weight) for The Pennsylvania State University strains 310 and 342, respectively. Hydrocarbons were isolated and identified by TLC, GC, and GC–MS. *n*-Alkanes from pentadecane to hentriacontane were identified and quantitated in each mushroom strain. Strains 310 and 342 contained 6.1 and 5.1 ppm of total *n*-alkanes/dry weight of mushrooms, respectively. Several possible functions of alkanes present in the fruiting body are discussed.

Agaricus bisporus (Lange) Singer is a cultivated mushroom that is commonly grown by the mushroom industry in the United States. Almost 400 million pounds of mushrooms are produced each year with Pennsylvania contributing the largest amount (~60%).

The literature concerning the chemical composition ($M_r \leq 450$) of the *A. bisporus* mushroom has been limited mainly to the isolation and identification of L-glutamyl derivatives (Daniels et al., 1961; Levenberg, 1961; Vogel et al., 1977), volatile components (Cronin and Ward, 1971), and free fatty acids (Holtz and Schisler, 1971).

Supplementation of compost with specific polar lipids (e.g., fatty acid esters) or various plant oils (e.g., corn or soybean oils) has been shown to produce increased mycelium growth and mushroom yields (Wardle and Schisler, 1969; Schisler, 1976). Since fatty acids and/or their derivatives have been found in *A. bisporus*, the possible presence and function of nonpolar lipids, specifically hydrocarbons, have been of interest to us. Therefore, this report examines the hydrocarbon components of the sporophore of two white strains of the cultivated mushroom *A. bisporus*.

MATERIALS AND METHODS

Growing Procedure. *A. bisporus* mushrooms (strains 310 and 342) were grown from mushroom spawn, obtained from the Pennsylvania State University spawn bank, on standard compost under temperature- and humidity-controlled conditions (Schisler and Patton, 1972). Mushrooms were harvested at first fruiting, and extraction procedures were initiated within 1 h. The growing procedures were performed in duplicate.

Sample Preparation. Hydrocarbons were isolated from each strain by homogenizing mushrooms with redistilled pesticide-grade hexane. The resulting suspension was filtered under vacuum, and the remaining solid was rinsed with spectrograde methanol and hexane. The hexane layer was separated and dried over anhydrous $MgSO_4$. After filtration, hexane was removed by rotary evaporation, resulting in a yellow oil. The yellow oil was subjected to analytical separation and identification techniques. In addition, a solvent blank was prepared by using the above extraction procedure.

Analytical Techniques. The mushroom extracts were subjected to thin-layer chromatography (TLC) as described by Kostelc et al. (1980) in order to isolate the hydrocarbon fraction. Quantitative gas chromatography (GC) of the hydrocarbon fraction was carried out on a Hewlett-Packard 7610A instrument equipped with a flame ionization detector. Glass columns (1.8 m × 2 mm i.d.) packed with 3% OV-1 on 100–120-mesh Gas-Chrom Q (Applied Science Division, State College, PA 16801) were temperature programmed from 100 to 280 °C at 6 °C/min with a 2-min delay at 100 °C.

Gas chromatography–mass spectrometry (GC–MS) was performed on a Finnigan 3200 gas chromatograph–mass spectrometer with an interactive 6000 data system. Glass columns similar to those described above were used for GC–MS analyses. A temperature program from 140 to 260 °C at 15 °C/min with a 1-min delay at 140 °C was employed. Electron impact spectra were recorded from 35 to 450 amu at 70 eV. Identification of unknowns resulted from a comparison of chromatographic retention times and mass spectra with that of authentic *n*-alkanes. Quanti-

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Table I. *n*-Alkanes in White Strains of *A. bisporus*

n-alkanes	percentages: strain 310 ± SEM	percentages: strain 342 ± SEM
pentadecane	0.8 ± 0.2	1.3 ± 0.6
hexadecane	2.5 ± 0.4	3.7 ± 1.1
heptadecane	8.7 ± 2.2	11.7 ± 4.3
octadecane	13.8 ± 1.7	14.4 ± 1.2
nonadecane	16.0 ± 1.5	17.5 ± 1.9
eicosane	11.1 ± 1.2	13.9 ± 0.5
heneicosane	6.2 ± 0.6	5.9 ± 0.7
docosane	5.6 ± 0.4	4.7 ± 0.3
tricosane	3.8 ± 0.1	3.7 ± 0.4
tetracosane	2.6 ± 0.2	3.0 ± 0.5
pentacosane	7.5 ± 0.5	4.4 ± 0.4
hexacosane	2.1 ± 0.6	2.1 ± 0.7
heptacosane	2.0 ± 0.4	1.7 ± 1.0
octacosane	6.9 ± 0.5	5.6 ± 1.5
nonacosane	2.7 ± 1.2	1.9 ± 0.9
triacontane	1.9 ± 0.4	1.4 ± 0.6
hentriacontane	5.8 ± 2.7	3.1 ± 1.1

tation was performed using a hydrocarbon external standard (*n*-heptadecane), and peak areas were determined by either triangulation or computerized digital integration.

Statistical Analysis. The differential effects of the group profiles were examined by a mixed model analysis of variance and covariance where profiles are contrasted in a partial repeated measures design.

RESULTS AND DISCUSSION

A. bisporus mushrooms (strains 310 and 342) yielded 1.81% and 1.37% (dry weight) hexane-extractable lipids, respectively. These values resemble the lipid values (1.31%–1.76%) of another cultivated mushroom *Agaricus campestris* (Hughes, 1962).

Hydrocarbons were isolated from each mushroom extract by thin-layer chromatography and then by gas chromatography. The gas chromatographic trace resembled a homologous series of *n*-alkanes with 15–31 carbons. Trace amounts of compounds which have retention times similar to that of methyl-branched alkanes also appeared to be present. Gas chromatography–mass spectrometry analyses confirmed the presence of the *n*-alkanes since the fragmentation pattern of each compound was identical with mass spectra of the corresponding standard *n*-alkanes. In addition, fragmentation pattern analysis indicated the presence of some monoolefins albeit in trace amounts.

Table I lists the *n*-alkanes isolated from each strain of *A. bisporus* and their relative percentages. The raw data were corrected for any hydrocarbon impurities found in the solvent blank, and the percentages reflect this correction. Strains 310 and 342 have been found to contain a mean value of 6051 and 5105 ppb of *n*-alkanes/dry weight of mushrooms (i.e., 1 ng of compound/g of dry mushroom equals 1 ppb). These results illustrate a similar alkane pattern for both white strains of mushrooms with nonadecane constituting the largest amount in each strain followed by octadecane, eicosane, heptadecane, etc. Statistical analysis in which profiles from each strain were contrasted showed no significant difference at the $P < 0.10$ level for both the transformed data (percentages) as well as the raw data (not presented here).

The hydrocarbon profiles from these mushroom strains show a odd/even carbon ratio of ~1 unlike the odd-carbon dominance of waxes from other higher plant forms (Eglinton and Hamilton, 1963). In addition, an extensive profile of *n*-alkanes (15–31 carbons), with nonadecane predominating, was observed in these mushrooms whereas various plant waxes usually show predominant odd-carbon *n*-alkanes of either 25, 27, 29, or 31 carbons. These differences of odd/even carbon ratio and carbon length dominance hypothetically may be important in determining plant evolution.

The isolation and identification of *n*-alkanes with 15–31 carbons in two white strains of the cultivated mushroom *A. bisporus* has been presented in this report. The function of alkanes and/or hydrocarbons, in general, in the chemical matrix of the sporophore is still unknown. Hydrocarbons may function as deterrents to moisture evaporation in the sporophore since water constitutes ~90% of its weight. On the other hand, the presence of alkanes in the fruiting body suggests the possible existence of a mechanism for the utilization of compost nutrients, since mycelium growth and mushroom yields are increased due to compost supplementation with long-chained fatty acid esters or various plant oils of high hydrocarbon content.

Furthermore, the presence of alkanes in *A. bisporus* may allow for facile differentiation of mushroom strains by utilization of the alkane profiles as demonstrated by Eglinton and Hamilton (1963). As reported here, the two white strains of *A. bisporus* do not have statistically different alkane profiles; however, white strains of *A. bisporus*, in general, may have similar alkane profiles. Consequently, alkane profile differences may exist between types of strains (e.g., white vs. brown) or more importantly among varieties. Further research is necessary to determine if such a profile is useful in other discriminations of mushroom types.

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