Phytochemistry, Vol. 20, No. 8, pp. 2021–2022, 1981.
Printed in Great Britain.

0031–9422/81/082021–02 $02.00/0
© 1981 Pergamon Press Ltd.

VOLATILE COMPOUNDS FROM THE MYCELIUM OF THE MUSHROOM *AGARICUS BISPORUS*

JOHN FREDERICK GROVE

Agricultural Research Council, Unit of Invertebrate Chemistry and Physiology, University of Sussex, Falmer, Brighton, BN1 9RQ, U.K.

(*Received* 21 *November* 1980)

Key Word Index—*Agaricus bisporus*; mushroom; volatile secondary metabolites; tetrachloro-1,4-dimethoxybenzene; 2,4-nonadienal; 2,4-decadienal; C_5–C_8 alcohols, aldehydes and ketones.

Abstract—The pattern of volatiles from the mycelium of two commercial strains of *Agaricus bisporus*, grown in axenic culture on a semi-synthetic medium, was found to be broadly similar to that of the volatiles identified from sporophores. Tetrachloro-1,4-dimethoxybenzene, a known secondary metabolite of several Basidiomycetes, was found in the mycelium though not in the sporophores. [^{36}Cl]Tetrachloro-1,4-dimethoxybenzene was obtained when sodium [^{36}Cl]chloride was added to the medium.

INTRODUCTION

As part of an investigation into the source of the volatiles present in mushroom houses during the spawn-running phase (vegetative growth) of commercial mushroom production, we have studied, by coupled gas chromatography/mass spectrometry (GC/MS), the neutral volatiles obtained by steam distillation of the mycelium of *Agaricus bisporus* grown in axenic culture on a semi-synthetic medium.

Volatiles, particularly flavour components, obtainable from *A. bisporus* sporophores, usually grown on compost, have been well documented [1–9]. Although the C_8-alcohols and ketones octan-3-ol, oct-1-en-3-ol, oct-2-en-1-ol, octan-3-one and oct-1-en-3-one, together with benzyl alcohol, benzaldehyde, hexanal and 3-methylbutanol predominate among these volatiles and account for 96% of the total [5], no neutral compounds containing more than five carbon atoms have hitherto been reported amongst volatiles from the mycelium [10–13]. Ethylene, acetaldehyde, acetone, ethanol, ethyl acetate and 3-methylbutanol have been identified by previous workers [10, 12]. In the present work all the above-mentioned volatiles, previously reported from the sporophores have also been identified, together with 2,4-nonadienal, 2,4-decadienal and tetrachloro-1,4-dimethoxybenzene.

RESULTS AND DISCUSSION

Of the standard techniques available for the isolation of volatiles, distillation in steam, rather than low-temperature vacuum distillation, was thought likely to come closer to the microclimate operating in mushroom house spawn-running conditions. We have recorded the volatiles obtainable by this technique, though the formation of volatiles from non-volatile components at temperatures higher than ambient is known [4] and cannot be excluded here.

Gas chromatograms were obtained isothermally: this mode of operation gives more stable conditions in the ion source in coupled GC/MS, an important factor in high-resolution work. Compounds were identified by their mass spectra and GC retention times which were checked against those obtained with authentic specimens: they are listed in Table 1. The pattern of volatiles was the same from both *A. bisporus* strains. With the exception of 2,4-nonadienal and 2,4-decadienal, presumably autoxidation products of linoleate [14, 15], and the unidentified sesquiterpenoid giving a parent ion of composition $C_{15}H_{24}$, they are known volatiles of *A. bisporus* sporophores.

Table 1. Volatile compounds identified from mycelium of *A. bisporus* strains D.621 and S.22

	Chromatographic data	
	Temp. (°)	Retention time (min)
Ethyl acetate	50	1.5
3-Methylbutanol	80	1.1
n-Hexanal		1.4
Octan-3-one		3.1
Octan-3-ol		3.4
Oct-1-en-3-ol		3.5
Oct-1-en-3-one		3.8
Benzaldehyde	110	2.2
Oct-2-en-1-ol		2.9
Benzyl alcohol		3.0
2,4-Nonadienal	160	1.4
2,4-Decadienal		2.5
Unidentified $C_{15}H_{24}$		3.5
Tetrachloro-1,4-dimethoxybenzene		10.0

Furfural, a dimethylpyrazine and phenylacetaldehyde were also identified, but were present in the malt extract medium.

Tetrachloro-1,4-dimethoxybenzene (drosophilin A methyl ether) is a known metabolic product of several Basidiomycetes. Although it was absent from sporophores of D.621 strain grown on composted straw it has previously been detected in commercial mushrooms [16]. The isolation of [^{36}Cl]tetrachloro-1,4-dimethoxybenzene from S.22 strain mycelium grown on a medium to which sodium [^{36}Cl]chloride had been added establishes tetrachloro-1,4-dimethoxybenzene as a genuine metabolic product of *A. bisporus*. This proof was necessary as the compound could arise by microbial degradation of the environmental contaminant pentachlorophenol [17].

EXPERIMENTAL

Mass spectra were recorded at 70 eV with a Varian CH5D double-focusing (high-resolution) instrument interfaced with a Varian 620L computer and Statos 21 electrostatic fast printer. The 1.5 m × 0.4 cm i.d. glass GC column was packed with 3% OV17 on 100/120 mesh Gas Chrom Q and was used with a gas flow of 30 ml/min. It was connected through all-glass capillary systems either to a FID (Pye 104 gas chromatograph) or to a Varian two-stage Watson–Biemann separator interfaced with the MS. R_f values are for Merck Si gel H_{254} and $CHCl_3$–MeOH (49:1). With the exception of oct-2-en-1-ol, oct-1-en-3-one and tetrachloro-1,4-dimethoxybenzene, which were prepared by literature methods, compounds were obtained from chemicals suppliers and purified by distillation. Et_2O was freshly purified by passage through a column of activated Al_2O_3 followed by fractional distillation. Water was obtained from an all-glass still. Na_2SO_4 was heated at 140° and stored in a stoppered glass bottle. Glassware was baked out at 200° before use.

A. bisporus strains Somycel (S) 22 and Darlington (D) 621 were used and were maintained on malt agar slopes. The culture medium, containing casein hydrolysate (5 g), malt extract (25 g) and K_2HPO_4 (2 g) in water (1 l.), was autoclaved for 15 min at 1.1 kg/cm^2.

Preparation and steam distillation of the mycelium. An inoculum (1 ml), prepared from a 1-month-old shake culture of the *A. bisporus* strain by maceration of the mycelial pellets under sterile conditions, was added to each of 50 Roux bottles containing the culture medium (100 ml, pH 7.0). After 6 weeks at 23 ± 2° the mycelium was filtered off (suction) and washed with water. The dry weight of an aliquot was determined (D.621, 6.5 g/l; S.22, 6.4 g/l) and the remainder in water (1 l.) was subjected to steam distillation in an all-glass apparatus. The distillate (1 l.) was extracted with Et_2O (2 × ⅓ vol.). The ethereal extract was dried with Na_2SO_4 and concentrated (fractional distillation) to small bulk (0.1–0.2 ml) for GC/MS. Further concn of the extracts from both strains afforded needles of tetrachloro-1,4-dimethoxybenzene, R_f 0.60, identified by mmp (164°) and IR spectrum.

[^{36}Cl]*Tetrachloro-1,4-dimethoxybenzene. A. bisporus* S.22 strain was cultured as described above and after 4 days sodium [^{36}Cl]chloride (50 μCi) in water was divided equally between two bottles. The mycelium, harvested after 6 weeks, was dried, powdered in a container cooled by liquid N_2, and extracted with $CHCl_3$ in a Soxhlet for 12 hr. Prep. TLC of the extract monitored by a Berthold gas-flow plate scanner showed radioactivity concentrated in a spot R_f 0.60. Recovery afforded [^{36}Cl]tetrachloro-1,4-dimethoxybenzene, identified by GC.

Volatiles from A. bisporus *sporophores.* The sporophores (195 g fr. wt, D.621 strain) were cut into small pieces, powdered and steam distilled and the distillate was extracted with Et_2O as described above. The extract was concd to small bulk for GC/MS.

Acknowledgements—I thank N. Claydon and M. Pople for technical assistance, Dr. F. A. Mellon for the mass spectra and Dr. D. Wood for the *A. bisporus* strains and the details of the conditions for their cultivation.

REFERENCES

1. Freytag, W. and Ney, K. H. (1968) *Eur. J. Biochem.* **4**, 315.
2. Cronin, D. A. and Ward, M. K. (1971) *J. Sci. Food Agric.* **22**, 477.
3. Picardi, S. M. and Issenberg, P. (1973) *J. Agric. Food Chem.* **21**, 959.
4. Wasowicz, E. (1974) *Bull. Acad. Pol. Sci.* **22**, 143.
5. Pyysalo, H. (1976) *Acta Chem. Scand.* **30B**, 235.
6. Pyysalo, H. and Suihko, M. (1976) *Lebensm.-Wiss. Technol.* **9**, 371.
7. Dijkstra, F. Y. and Wiken, T. O. (1976) *Z. Lebensm. Unters-Forsch.* **160**, 255.
8. Sulkowska, J. and Kaminski, E. (1977) *Acta Aliment. Pol.* **3**, 409.
9. Card, A. and Arisse, C. (1977) *Ann. Technol. Agric.* **26**, 287.
10. Lockard, J. D. and Kneebone, L. R. (1962) *Mushroom Sci.* **5**, 281.
11. Tschierpe, H. J. and Sinden, J. W. (1965) *Arch. Microbiol.* **52**, 231.
12. Rast, D. and Stauble, E. J. (1970) *New Phytol.* **69**, 557.
13. Stauble, E. J. and Rast, D. (1971) *Experientia* **27**, 886.
14. Patton, S., Barnes, I. J. and Evans, L. E. (1959) *J. Am. Oil Chem. Soc.* **36**, 280.
15. Matsumoto, S. (1971) *Eisei Kagaku* **17**, 347.
16. Buss, H. and Zimmer, L. (1974) *Chemosphere* 123.
17. Suzuki, T. and Nozoe, K. (1972) *Chem. Abstr.* **77**, 4204.