Phytochemistry, 1976, Vol. 15, pp. 1717–1721. Pergamon Press. Printed in England.

DIPEPTIDE PRECURSOR OF GARLIC ODOUR IN *MARASMIUS* SPECIES

ROLF GMELIN*, HANS-HEINO LUXA*, KLAUS ROTH† and GERHARD HÖFLE‡

*Institute for Pharmacognosy and Phytochemistry; †Institute for Organic Chemistry, Freie Universität Berlin-Dahlem; ‡Institute for Organic Chemistry, Technische Universität Berlin-Charlottenburg, W. Germany

(*Revised received* 20 *May* 1976)

Key Word Index—*Marasmius alliaceus*; *M. scorodonius*; *M. prasiosmus*; Basidiomycetae; Tricholomataceae; odour formation; γ-glutamyl-*S*-alkyl-L-cysteine sulphoxide type substrates; γ-glutamyl transpeptidase; C-S lyase.

Abstract—γ-Glutamyl-marasmine, a new natural dipeptide containing an unusual cysteine sulphoxide moiety has been isolated from the Basidiomyceteous mushrooms *Marasmius alliaceus*, *M. scorodonius* and *M. prasiosmus*, which are known for their garlic like odour. It is shown that this compound is the common natural precursor and that its two step enzymatic cleavage leads to the odorous substances. In the first step γ-glutamyl-marasmine is cleaved by a γ-glutamyl transpeptidase. The formed marasmine is split in a second enzymatic reaction by a C-S lyase into pyruvic acid, ammonia and an unstable sulfur compound, which decomposes to form the odorous secondary products.

INTRODUCTION

The odours and flavours of mushrooms occur in great variety. As described in a recent review [1] a number of these odorous compounds could be characterized as alcohols, aldehydes, ketones, esters, lactones etc. Typical odours sometimes may be useful in systematic classification and in distinguishing closely related mushroom species by organoleptic criteria [2]. Some mushrooms are particularly appreciated as food or spices for their pleasant odours and flavours.

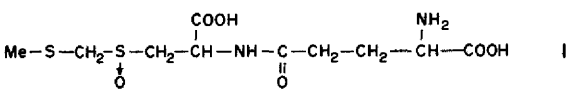
As expressed by their Latin and German names the mycelium and carpophores of *Marasmius alliaceus* Jacq., *M. scorodonius* (Fr.) Quel. and *M. prasiosmus* Fr. ("Knoblauch-Schwindlinge"), develop a strong, garlic like odour—especially when wet or when crushed with water. In this paper we describe our studies on the nature and origin of this odour.

RESULTS AND DISCUSSION

We observed in the extracts of mushrooms a *S* containing substance giving a brick red colouration with ninhydrin, which decolourized K_2PtI_6 reagent on TLC. By TL electrophoresis this substance migrated anodically indicating its acidic character. Upon incubation of the dried and powdered mushrooms with H_2O this substance gradually disappeared with concomitant formation of pyruvic acid, ammonia and a sharp tasting compound which soon decomposed into volatile products with a characteristic odour. This indicated that the original compound in the mushrooms represents a substrate in an enzymatic process leading to the odorous substance. Extracts from mushrooms were obtained by boiling powdered dried fruit bodies with 70% MeOH. After repeated ion exchange chromatography on Amberlite IR 120 (H form), Lewatit MP 5080 (acetate form) and DEAE A 25 Sephadex (acetate form) the precursor was obtained as colourless crystals in *ca* 1% yield per dry wt. Elemental analysis of the precursor gave values for $C_{10}H_{18}N_2O_6S_2 \cdot 1/2\ H_2O$. A strong peak at *m/e* 327 for the $(M+1)^+$ ion in the field desorption MS confirmed the molecular formula $C_{10}H_{18}N_2O_6S_2$ (MW 326.4).

After acidic hydrolysis of the precursor under mild conditions (2N HCl; 100° for 2 h) L-glutamic acid could be detected by TLC indicating the presence of a labile γ-glutamyl linkage. Reductive treatment with Raney nickel in water afforded γ-L-glutamyl-L-alanine. According to its occurrence and peptidic character the precursor is named γ-glutamyl-marasmine (**1**). It is readily soluble in water giving slightly acidic solutions which gradually decompose with the formation of the typical odour of the parent mushrooms. This odour can be ascribed to products derived from the sulphenic acid Me-S-CH_2-SOH which is formed from **1** by an α,β-elimination reaction [3]. The other fragment, γ-glutamyl amidoacrylic acid can be detected on Si gel TLC (R_f 0.2) and by the yellow coloration with K_2PtI_6 reagent, which is typical of acyl amidoacrylic acids.

The complete structure of **1** as γ-L-glutamyl-3(methylthiomethylsulphinyl)-L-alanine could be unequivocally derived from the data obtained by 270 MHz PMR and 20 MHz ^{13}C-NMR and is in agreement with the product of Raney nickel degradation.



1

In D_2O solution the PMR spectrum exhibits a complex five spin system, an AB, an ABX system and a three proton singlet. The five spin system consists of a multiplet centred at δ 2.20 (2H) which is coupled to a distorted triplet at δ 2.55 (J = 7.5 Hz, 2H) and a triplet at δ 3.93 (J = 6.5 Hz, 1H). It can be assigned to a glutamic acid derivative and as observed with this type of compound, addition of acid caused a downfield and addition of base

PHYTO 15/11—J

an upfield shift in these signals [4]. Since only the α proton and not the γ protons are shifted significantly ($\Delta\delta_\alpha = -0.59$ ppm) in basic solution the γ carboxyl group must be substituted. The ABX system with signals at δ 3.39; 3.48 and 4.79 ($J_{AB} = 14$ Hz, $J_{AX} = 11$ Hz, $J_{BX} = 3.5$ Hz) suggests the presence of a cysteine fragment which is presumably bound to the γ carboxyl group of the glutamic acid. As above its free carboxyl group causes an upfield shift ($\Delta\delta = -0.57$ ppm) of the α proton in basic solution. The Me singlet with a chemical shift of δ 2.30 must be a MeS which is linked via a methylene group to the S of the cysteine. The chemical shift difference between the methylene proton of *ca* 0.2 ppm clearly demonstrates the vicinity of the asymmetric SO group. This structural moiety has already been found in the antibiotic sparsomycine [5], which is a cysteinol analogue of marasmine.

The results of the ^{13}C-NMR investigation of **1** are summarized in Fig. 1. The assignment of the various signals is based on ^{13}C {^{1}H}-off resonance decoupling experiments and by comparison with the corresponding amino acids. The peptide bond has to be in the γ-position of the glutamic acid since there is no signal for a γ carboxyl group. The signal of the α carbon in the cysteine part of **1** at 48.6 ppm is shifted to higher field compared with that of cysteine (58.4 ppm) [6], cystine (55.8 ppm) [7] or glutathione (Glu(α-OH)-Cys-Gly-OH 55.8 ppm [8].

Both mushrooms, *M. alliaceus* and *M. scorodonius*, contain a γ-glutamyl transpeptidase (EC 2.3.2.2) as demonstrated by the cleavage of glutathione and γ-L-glutamyl-4-nitroanilide and by the transfer of the γ-glutamyl residue to an acceptor e.g. L-methionine or hydroxylamine [9]. 4-Nitroaniline formed in this reaction was determined photometrically at 410 nm [10], γ-L-glutamyl-L-methionine and γ-L-glutamyl-hydroxylamine were detected by PC and TLC. Comparable results were obtained with crude enzyme preparations from both mushrooms at pH 8–9. Furthermore a C-S lyase (EC 4.4.1.1.-n) could be extracted from the mycelium and carpophores of *M. scorodonius* and M. alliaceus by 66mM Pi buffer pH 8 which was very similar in its substrate specificity to that of a C-S lyase from the endosperm of seeds of *Albizzia lophantha* (Mimosaceae) [11]. It is also activated by pyridoxal phosphate (PALP) and shows the same broad activity spectrum against different substrates (L-cysteine, *S*-alkyl-or aralkyl- and aryl-L-cysteines, L-djenkolic acid and the corresponding sulphoxides) affording H_2S,R-SH,$CH_2(SH)_2$ or R-S-SO-R respectively, pyruvic acid and ammonia. In the absence of γ-glutamyl transpeptidase **1** was not attacked by C-S lyases as demonstrated with a crude enzyme preparation from *A. lophantha* seeds according to Schwimmer [12].

On disc electrophoresis on 7.5% polycrylamide gels this new fungal C-S lyase was separated into 6 distinct zones of isoenzymes which are very similar to the isoenzyme patterns of other C-S lyases from different sources (e.g. seeds or roots of *Albizzia lophantha*). The gels were

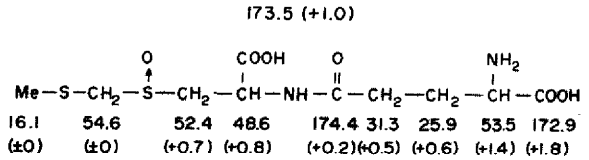


Fig. 1.

incubated in 0.25 M Tris–citrate buffer pH 8 containing L-cysteine (10mM) and Pb acetate (5mM) and traces of PALP. After a short time dark brown precipitates appeared at the individual locations of C-S lyase isoenzymes. Extracts from *M. alliaceus* and *M. prasiosmus* were far less active than that from *M. scorodonius*. This buffered L-cysteine–Pb acetate–PALP reagent was also very effective and sensitive in histochemical experiments.

From the above observations it can be concluded that in both species a two step enzymatic process is responsible for the odour formation from **1**. 1) The precursor, γ-glutamyl-marasmine, is cleaved first by a γ-glutamyl transpeptidase into marasmine, Me-S-CH_2-SO-CH_2-$CH(NH_2)$COOH, and a γ-glutamyl residue transferred on endogenous acceptors e.g. amino acids. 2) The formed marasmine is split by C-S lyase into pyruvic acid, ammonia and presumably CH_3-S-CH_2-SOH, which forms the sulphinic ester Me-S-CH_2-S-SO-CH_2-S-CH_3 (**3**) in close analogy to the formation of allicine (allyl-S-SO-allyl) from alliine (S-allyl-L-cysteine sulphoxide) [13]. The sulphinic ester **3** can be recognized by the transient sharp taste, characteristic of R-S-SO-R type compounds when fresh fruit bodies of *M. scorodonius* or *M. prasiosmus* are chewed. **3** is very unstable and decomposes rapidly into not yet defined polysulphides with the smell of the odorous products derived from methylenedithiol. Methylenedithiol is the primary product of C-S lyase attack on the endogenous substrate L-djenkolic acid in seeds of *Albizzia lophantha* [11].

The mycelia of *M. scorodonius* grown on the surface of nutrient agar also develop the typical odour of the fruit bodies. Extracts obtained by grinding the mycelium in Pi buffer contained γ-glutamyl transpeptidase and C-S lyase. In MeOH extracts **1** could be detected by TLC and PC. *M. prasiosmus*, which is described [2] as having an outstandingly strong odour and sharp taste, also contains according to our TLC and PC results γ-glutamyl-marasmine, accompanied by a C-S lyase and a γ-glutamyl transpeptidase. Since most of the rare fungal material was used for the isolation of **1**, detailed enzymatic studies could not be carried out. As referred to in the mycological literature [2] and expressed by their names many species in the genera *Marasmius*, *Micromphale*, *Collybia* develop similar, partly unpleasant odours. As will be described in a forthcoming paper they also contain closely related γ-glutamyl peptides of L-cysteine derivatives and enzymes as the *Marasmius* species.

Our results on the odour formation in three mushrooms appear to represent a new basic model for similar proeesses of odour formation, evidently widely distributed in the plant kingdom. *Allium* species, for example, are known for their active excretion of volatile sulphur compounds and moreover for their content of numerous γ-glutamyl derivatives of *S*-alkyl-L-cysteines as described in the important contributions by Virtanen and Matikkala on the constituents of *Allium cepa*, *A. sativum*, *A. porrum* and *A. schoenoprasum* [14]. The nature and role of such excretion processes, whose initial step may be an attack of γ-glutamyl transpeptidase followed by a C-S lyase cleavage, as well as the exact histochemical location of the participating enzymes at the sites of excretion deserves further attention. The role of γ-glutamyl transpeptidases in plants may be related to a proposed γ-glutamyl cycle recently described by Meister *et al.* [15]. which seems to be involved in the active transport of amino acids in animal kidney.

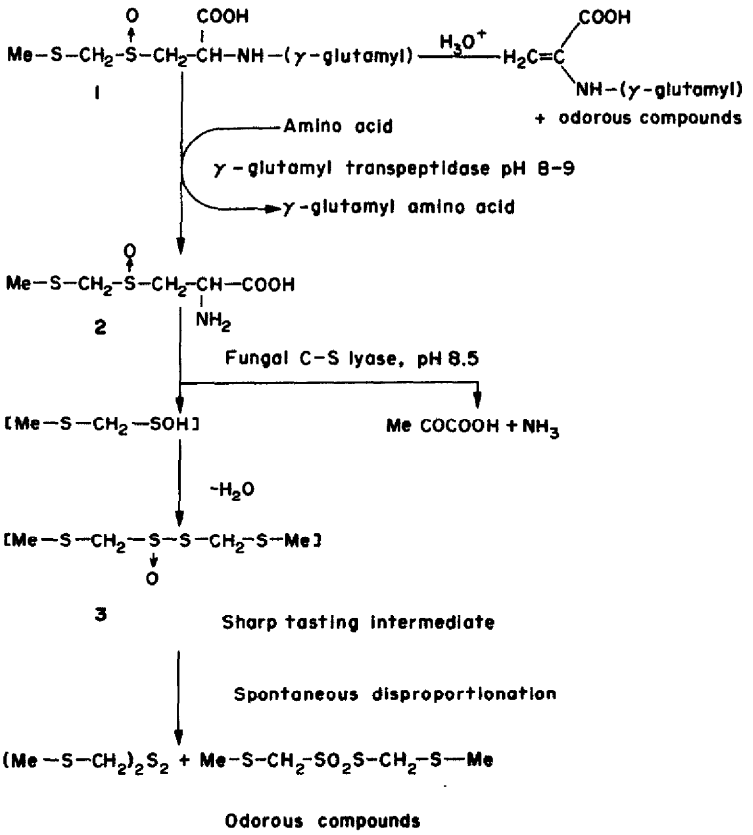


Fig. 2.

When this work was finished, Prof. Dr. Sh. I. Hatanaka, Tokyo, kindly drew our attention to a report, which was not accessible to us. Yasumoto *et al.* [16] have recently come to similar conclusions with *Lentinus edodes*, shii-take, the popular Japanese edible mushroom. Its flavour substances of the lenthionine type, which have been isolated and structurally elucidated a few years ago by Morita and Kobayashi [17], are formed by an analogous enzymatic process. The precursor, a structurally unknown derivative of γ-L-glytamyl-L-cysteine [18] is evidently closely related to γ-glutamyl-marasmine.

EXPERIMENTAL

Fruit bodies of *M. alliaceus* were collected near Bonn and Tuebingen (BRD)(leg. W. St. and B. Oe.), of *M. scorodonius* in the Berlin area and near Bozen (Italy)(leg. R. Gm., H.H.L. and A. Sch.), of *M. prasiosmus* near Eschwege (BRD)(leg. E. Ge.) during summer 1975. The fresh fruit bodies were carefully dried in a current of warm air and kept in a desiccator. They were ground immediately prior to extraction. Mycelia of *M. scorodonius* were obtained from Centralbureau voor Schimmelcultures, Baarn, Holland. They were grown on Merckobakt Sabouraud-Glucose 2% agar in petri dishes. The harvested mycelia were freeze dried and kept in the desiccator. All derivatives of L-cysteine were prepared according to original procedures as cited in ref. [11]. TLC and PC were developed with *n*-BuOH–HOAc–H_2O–*iso*PrOH 8:2:5:3.

Isolation of **1**. Portions of powdered carpophores of *M. alliaceus* or *M. scorodonius* (30g) were placed in 300 ml boiling H_2O and boiling continued with stirring for 15 min. Filtrates were mixed with an equal vol of MeOH and the flocculent ppt. filtered off. After evaporation of MeOH, H_2O was added to 1 l. The solns were run through a column of 200 ml Amberlite IR 120 (H form) and after washing with 500 ml H_2O the amino acids and peptides were eluted with 2% NH_4OH soln. The ninhydrin positive fraction was evaporated and the residue taken up in 500 ml H_2O. The resulting soln was percolated through a column containing 100 ml Lewatit MP 5080 (acetate form). On elution with an HOAc gradient (1 to 10%) γ-glutamyl-marasmine appeared sharply separated after the glutamic acid fraction. In a final step the slightly coloured peptide was rechromatographed on a column of 50 ml DEAE A25 Sephadex (acetate form) with an HOAc gradient as above. The fraction containing the precursor was concentrated to a small vol and after addition of EtOH to the warm soln γ-glutamyl-marasmine crystallized as fine white needles. Yield about 1.3% with *M. alliaceus*, 1% with *M. scorodonius* mp 174° (decomp.); $[\alpha]_D^{20} = -5.5°$ (H_2O; *c* 0.1) Found: C, 36.00; H, 5.51; N, 8.10; S, 18.27; $C_{10}H_{18}N_2O_6S_2$. 1/2 H_2O (MW 335.41) requires: C, 35.80; H, 5.70; N, 8.35; S, 19.13%.

Acid hydrolysis. **1** (100mg) was dissolved in 10 ml 2N HCl and the soln heated for 2 hr at 100° and afterwards evaporated *in vacuo*. The residue was dissolved in H_2O and the soln passed through a small column of Amberlite IR 120 (H^+ form). Elution with 5% NH_4OH soln yielded, after evaporation and crystallization from hot H_2O made slightly acidic with a few drops of HCO_2H, glutamic acid (identified by PC and TLC).

Reductive cleavage. **1** (100mg) was dissolved in 10 ml H_2O and after the addition of 1g freshly prepared Raney Ni the mixture was stirred for 4 hr. The filtrate was treated with H_2S, and the sulphide filtered off. Ion exchange column chromatography on Amberlite IR 120 (H form) as for glutamic acid yielded about 40 mg crystals (H_2O–EtOH), mp 199°, $[\alpha]_D^{20} + 7.0°$ (0.5 N-HCl; *c* 0.1). The IR spectra of the product and an authentic sample of γ-glutamyl-L-alanine [19] in KBr were identical.

TLC and PC. Powdered mushrooms or dried mycelium (1g) were extracted by boiling in 7 ml 60% MeOH. 5 μl portions of the filtrates were spotted on TLC (Si gel; cellulose) or 20 μl portions on PC sheets MN 214. R_f values for **1**: Si gel TLC 0.125, cellulose TLC 0.24, MN 214 PC 0.35. (brick red color with ninhydrin; slow decolouration of K_2PtI_6 reagent; grey–brown spots with 0.1 $AgNO_3$ soln–conc NH_4OH soln 5:1; 120°; 15 min).

TL electrophoresis. Portions of the extracts (20–50 μl) were spotted on TL plates cellulose MN 300 or Si gel plates. 1% solns of α-alanine, glutamic acid, aspartic acid, S-β-carboxyethyl-L-cysteine, glutathione and picric acid (5 μl) were applied for comparison.The layers were slightly and evenly sprayed with 33mM Pi buffer pH 6.5 or with pyr–HOAc–H_2O buffer pH 4.9. Electrophoresis was run at 600 V and 30 mA for 30 min. The dried plates were sprayed with ninhydrin or K_2PtI_6 soln. Distance of anodic migration relative to α-alanine:

	Pi buffer pH 6.5 cellulose (cm)	pyr–HOAc–buffer pH 4.9 Si gel (cm)	cellulose (cm)
1	3.0	5.2	3.0
glu	4.7	6.5	4.0
asp	5.5	7.5	5.1
S-β-cecys	4.6	5.2	3.0
glutathione	3.0	5.2	3.0
picric acid	3.8	7.8	3.5

Preliminary enzyme screening test. Crushed mycelium or powdered fruit bodies (10g) of *M. scorodonius* and *M. alliaceus* were stirred in 50 ml 0.66 M Pi buffer pH 8 for 30 min. Mixtures were filtered under pressure through a layer of cellulose powder. Gel filtration through Sephadex G25 resulted in odourless protein fractions containing the enzyme complex. 5 fold concentration was achieved by addition of dry Sephadex G25 and filtration after 30 min.

γ-Glutamyl transpeptidase tests and assay. Enzyme soln (1 ml) was mixed with 10 ml 0.25 M Tris–citrate buffer containing 50 μmol γ-glutamyl-4-nitroanilide or glutathione and 500 μmol L-methionine or hydroxylamine (pH 8.5). At intervals aliquots were taken. Liberated 4-nitroaniline was measured by A404 or 410 nm ([10]. γ-Glutamyl-L-methionine and γ-glutamyl-hydroxylamine formed were detected by PC or TLC with ninhydrin, K_2PtI_6 and $Fe(NO_3)_3$ reagent, respectively. R_f values for γ-glutamyl-L-methionine: PC 0.39; TLC (Si gel) 0.22; for γ-glutamyl-hydroxylamine: PC 0.13; TLC (Si gel) 0.09.

Test for C-S lyase activity and substrate specificity. The different substrates (50 μmol) (L-cysteine, L-djenkolic acid, *S*-alkyl-L-cysteines and *S*-alkyl-L-cysteine sulphoxides; alkyl = Me, C_2H_5, C_3H_7, allyl-C_6H_5-CH_2) were dissolved in 10 ml 0.1 M Tris buffer pH 8.5 and 1 ml enzyme soln was added. In a parallel series 1 ml of 1 mM aq. soln of PALP was added. After incubation of the mixtures at 37° organoleptic and chemical tests were performed at intervals. a) H_2S was detected by addition of a few drops of 10% Pb acetate soln (positive reaction with L-cysteine, weak reaction with L-djenkolic acid). b) R-SH was detected by sodium nitroprusside and iodine azide tests and by preparation of the corresponding mercuric compounds [11] and organoleptically by the typical mercaptan odours (positive reactions with all tested *S*-alkyl-L-cysteines; identical Hg derivatives as in ref [11]. c) R-S-SO-R type products formed from *S*-alkyl-L-cysteine sulphoxides were detected organoleptically by the sharp and stinging taste followed by the typical odour of the corresponding R-S-S-R compound. For R = benzyl, crystals of C_6H_5-CH_2-S-SO-$CH_2C_6H_5$ (mp 81–83°) separated from the reaction mixture. After 4 hr the formed R-S-SO-R compounds were extracted with $CHCl_3$. The residues after evaporation were dissolved in EtOH and the soln added to an aq. soln of 50 μmol thiamine hydrochloride. 10% TEMED in EtOH was added to adjust to pH 9. After 2 hr the formed alkylthiamine disulphides were extracted with $CHCl_3$ and compared by TLC (Si gel; $CHCl_3$(MeOH–diethylamine 14:5:1)(K_2PtI_6 and $KBiI_4$ reagent: blue or orange spots) with authentic samples as described [19]. d) NH_3 was assayed after microdiffusion photometrically by standard Nesslerization [21]. e) Pyruvic acid was characterized by reaction of the incubation mixtures with a soln of 2,4-dinitrophenylhydrazine in HCl and recrystallization of the precipitated 2,4-dinitrophenylhydrazone (mp) 215–216°). The results by d) and e) showed increased activity in the series with added PALP. The activity of the crude enzyme from *M. scorodonius* was much higher than that from *M. alliaceus*.

Polyacrylamide gel electrophoresis of fungal C-S lyases. The enzyme complex soln (1 ml), 1 ml Tris buffer pH 6.7 and 5 ml satd saccharose soln and a few drops of bromphenol blue soln were mixed [22]. The mixture (50 μl) was applied on the top of tubes with 7.5% polyacrylamide separating gel in pH 8.9 Tris buffer. After electrophoresis for 2 1/2 hr at 2.5 mA per tube the gels were washed twice in 0.1 M Tris buffer pH 8.5 and placed in small test tubes containing a soln of L-cysteine (10 mM) and Pb acetate (5 mM) in 0.25 M Tris citrate buffer pH 8.5 and 2 drops of 1% PALP aq. soln. The time of incubation was *ca* 30 to 60 min for *M. scorodonius* and 120 to 180 min for *M. alliaceus* and *M. prasiosmus*. After the appearance of the brown coloured bands of PbS for the individual C-S lyase isoenzymes the gels were repeatedly washed in 7% HOAc soln and stored in this medium for documentation.

Acknowledgements—We thank Prof. Dr. W. Steglich, Bonn, and Prof. Dr. Sh. I. Hatanaka, Tokyo, for discussions and important references, E. Gerhardt, H. Michaelis, B. Oertel, A. Schuster and W. Steglich for valuable samples of rare mushrooms which enabled these investigations. The performance of FD MS spectra by Dr. D. Spitzner and G. Schwinger, Stuttgart-Hohenheim, and financial support by the Deutsche Forschungsgemeinschaft, Bad Godesberg, for the electrophoresis equipment, is gratefully acknowledged.

REFERENCES

1. Pyysalo, H. (1975) Studies on the volatile compounds in mushrooms, Technical Research Centre of Finland, Materials and Processing Technology, Publication 13.
2. Michael, E. and Hennig, B. (1968) *Hanbuch für Pilzfreunde*, 1. Bd.; (1964) Handbuch für Pilzfreunde, 3. Bd.; VEB Gustav Fischer Verlag, Jena.
3. Ostermayer, F. and Tarbell, D. F. (1960) *J. Am. Chem. Soc.* **82**, 3752.
4. Roberts, G. C. K. and Jardetzky, O. –1970) *Adv. Protein Chem.* **24**, 447–545.
5. Wiley, P.-F. and MacKellar, F. A. (1970) *J. Am. Chem. Soc.* **92**, 417.
6. Horsley, W. J., Strenlicht, H. and Cohen, J. S. (1970) *J. Am. Chem. Soc.* **92**, 680.
7. Johnson, L. F. and Jankowski, W. C. (1972) *Carbon-13-NMR-Spectra*, Wiley-Interscience, New York.
8. Breitmaier, E. and Voelter, W. (1974) 13 *CNMR-Spectroscopy*, Verlag Chemie, Weinheim.
9. Thompson, J. F., Turner, D. H. and Gering, R. K. (1964) *Phytochemistry* **3**, 33.
10. Szasz, G. (1970) *Mothoden der enzymatischen Analyse*, Ed. Bergmeyer H. U., 2. Aufl., Verlag Chemie, Weinheim.
11. Gmelin, R., Hasenmaier, G. and Strauss, G. (1957) *Z. Naturforshung* 12*b*, 687.
12. Schwimmer, S. and Kjaer, A. (1960) *Biochim. Biophys. Acta* **42**, 316.
13. Stoll, A. and Seebeck, E. (1948) *Helv. Chim. Acta* **31**, 189.
14. Virtanen, A. I. and Matikkala, E. J. (1964) Report on Primary Plant Substances and Decomposition Reactions in Crushed Plants, Ed. Virtanen, A. I., Biochemical Institute, Helsinki. pp. 114, 116, 172–219.

15. Meister, A. (1973) Glutathione, *Proceedings* 16*th Confer. Germ. Soc. Biol. Chem.* Tübingen pp. 56–88.
16. Yasumoto, K., Iwami, K. and Mitsuda, H. (1974) *Abstracts IXth Internat. Scientif. Congr. of the Cultivation of Edible Fungi.* 5-3. Tokyo, Japan.
17. Morita, K. and Kobayashi, Sh. (1967) *Chem. Pharm. Bull.* **15,** 988; Nishikawa, M., Kamiya, K., Kobayashi, Sh., Morita, K. and Tomiie, Y. (1967) *Chem. Pharm. Bull.* **15,** 756.
18. Yasumoto, K., Iwami, K. and Mitsuda, H. (1971) *Agr. Biol. Chem.* **35,** 2059.
19. Senn Chemicals, CH-8157 Dielsdorf is thanked for a sample of this compound.
20. Matsukawa, T., Kawasaki, H., Iwatsu, T. and Yurugi, S. (1954) *J. Vitaminol.* **1,** 13.
21. Conway, E. J. (1962) *Microdiffusion Analysis and Volumetric Error*, 5th Edit. Crosby Lockwood, London.
22. Davis, B. J. (1964) *Ann. N.Y. Acad. Sci.* **121,** 404.