Phytochemistry, Vol. 22, No. 3, pp. 705–709, 1983.
Printed in Great Britain.

0031-9422/83/030705-05$03.00/0

VOLATILE AROMA COMPONENTS, PARTICULARLY GLUCOSINOLATE PRODUCTS, OF COOKED EDIBLE MUSHROOM (*AGARICUS BISPORUS*) AND COOKED DRIED MUSHROOM

ALEXANDER J. MACLEOD and SHNEHLATA D. PANCHASARA

Department of Chemistry, Queen Elizabeth College, University of London, Campden Hill Road, London, W8 7AH, U.K.

(*Revised received* 4 *August* 1982)

Key Word Index—*Agaricus bisporus*; Agaricaceae; mushroom; aroma volatiles; glucosinolates.

Abstract—The aroma volatiles of cooked edible mushroom (*Agaricus bisporus*) were extracted and concentrated to a valid essence using well-established techniques. Analysis by conventional GC and GC/MS showed at least 40 components (total *ca* 150 μg/g), including trace amounts of benzyl isothiocyanate which indicated the presence of benzylglucosinolate in *A. bisporus*. Previously identified C_8 components, which are important to the characteristic flavour of mushroom, were also obtained, with oct-1-en-3-ol contributing *ca* 59% (*ca* 88 μg/g) to the sample. A new mushroom volatile was detected, cyclo-octanol (*ca* 24%, 36 μg/g), presumably formed by cyclization of oct-1-en-3-ol during cooking. The C_8 compounds comprised *ca* 98% of the sample. The volatiles of cooked dried mushrooms (total *ca* 500 μg/g) were very different and only one of the seven C_8 compounds, oct-1-en-3-one (*ca* 1.5%), survived processing. A number of pyrazines and other thermally-produced artefacts of processing were recognized. Benzyl cyanide as well as benzyl isothiocyanate was identified in dried mushroom samples, providing further evidence of the presence of benzylglucosinolate in edible mushroom. No 2-phenethylglucosinolate products could be detected in any mushroom samples, even using highly sensitive SIM searches during GC/MS.

INTRODUCTION

Natural loci of glucosinolates are dicotyledons and they are ubiquitously present within the order Capparales. For many years it was considered that glucosinolates were limited to this order, but their sporadic occurrence outside the Capparales is now well recognized, although still rather rare. Nevertheless, the occurrence of glucosinolates in species of fungi would be rather unexpected, but in 1935 Friese reported the detection of isothiocyanates (benzyl or 2-phenethyl) in extracts of a large number of mushroom species [1]. Isothiocyanates are one of the main enzymic degradation products of glucosinolates, and although it is possible that the compounds detected by Friese [1] could have been of non-glucosinolate origin, this is less likely, and the author himself was of the opinion that they were produced from specific glycosides. Although the species studied by Friese do not constitute today's more common edible mushrooms, the widespread detection of isothiocyanate in all species examined [1] encourages consideration that such compounds might also be produced by the more common edible mushrooms.

Whilst suspicion might be directed at such old work in the field of aroma volatiles, it is perhaps surprising that this original study [1] has been ignored in relevant modern reviews [2, 3]. In other reviews the work has been slightly misquoted and 2-phenethyl thiocyanate has been listed as an aroma volatile [4]. This would be particularly unexpected since, of the *ca* 80 known glucosinolates, only three appear capable of degradation to yield thiocyanate (not thiocyanate ion), namely, allyl-, benzyl- and 4-(methylthio)butylglucosinolates.

To evaluate the possible presence of glucosinolate in the common edible mushroom, we analysed the aroma volatiles of *Agaricus bisporus* by conventional procedures, but with specific attention to the detection of glucosinolate products (i.e. isothiocyanates, thiocyanates and nitriles). Furthermore, since so few studies have been carried out on the flavour chemistry of mushroom [3], it was considered appropriate to conduct, in addition, a more broad-based examination of the mushroom volatiles. Mushrooms can be consumed either raw or cooked, but in general relatively little attention has been devoted specifically to the aroma volatiles of the cooked product [5, 6]. Thus, the volatiles of cooked (boiled) mushrooms were studied in this project. As well as fresh mushrooms, the commercially dried product was also analysed, since this too has not been well studied [7–9].

RESULTS AND DISCUSSION

Aroma volatiles of cooked mushrooms (*Agaricus bisporus*) were extracted in a Likens and Nickerson [10] apparatus, as modified by MacLeod and Cave [11], and extracts were concentrated by low temperature–high vacuum distillation [11]. This procedure has been shown to be especially sensitive and to produce concentrated, valid essences. It has been successfully employed for glucosinolate-containing systems [12–16]. Essences obtained from fresh mushrooms in this manner possessed genuine, characteristic mushroom aroma on re-dilution. They were examined by routine temperature-programmed gas chromatography and constituents were identified as far as possible by GC/MS, using both electron impact ionization (EI) and chemical ionization (CI) techniques. Specific searches for glucosinolate products were made by selected ion monitoring (SIM), using a multi-peak monitoring (MPM) unit.

Table 1 lists the components identified in the fresh

Table 1. Volatile aroma components of cooked fresh mushrooms

Peak No.	Component*	RR_t (min)	Relative abundance (%)	μg/g	Odour quality
1	Hexane	2.9	0.09	0.13	—
2	Dichloromethane	4.1	0.13	0.22	yeast
3	Chloroform	5.7	0.03	0.05	solvent
4	Toluene	6.3	0.11	0.16	pungent
5	Hexanal	7.2	tr	tr	green, grass
6	Xylene (*m* or *p*)	8.4	0.57	0.84	—
7	3-Methylbutan-1-ol	8.7	tr	tr	mushroom soup
8	Pyridine plus *o*-xylene	9.3	tr	tr	—
9	? Cyclopentadiene	10.3	0.22	0.32	—
10	Octan-3-one	10.4	4.11	6.12	sweet, ester, pear drops
11	Oct-1-en-3-one	11.2	0.36	0.53	raw mushroom
12	Hex-1-en-3-ol	11.9	0.26	0.39	green, grass
13	Dimethylformamide	12.1	0.08	0.12	—
14	Octan-3-ol	12.3	9.29	13.80	sweet, floral, earthy, aniseed
15	Oct-1-en-3-ol	13.2	59.02	87.71	mushroom, earthy, buttery, new potatoes
16	Furfural	13.5	0.09	0.14	roast meat
17	A dichlorobenzene	13.9	0.05	0.10	—
18	Benzaldehyde	14.0	0.32	0.47	almond
19	Octan-1-ol	14.7	0.55	0.81	scented, soap
20	A dichlorobenzene	14.8	tr	tr	—
21	Oct-2-en-1-ol	14.9	tr	tr	—
22	3,5,5-Trimethylcyclohex-2-enone	15.0	tr	tr	—
23	Cyclo-octanol	15.1	24.35	36.19	fresh cut cucumber, fresh cut grass, floral, fragrant
24	Phenylacetaldehyde	15.5	0.15	0.22	almond, roses
25	? Amino compound	16.6	tr	tr	—
26	Sesquiterpene (? α-humulene)	16.8	tr	tr	—
27	Unsaturated alcohol	17.0	tr	tr	—
28	Benzyl alcohol	18.9	0.10	0.15	fried mushroom
29	? Saturated hydrocarbon	19.0	tr	tr	—
30	*N*-Phenylpyrrole	19.2	tr	tr	—
31	? Saturated hydrocarbon	19.8	tr	tr	—
32	Benzyl isothiocyanate	23.3	tr	tr	—
33	? Saturated hydrocarbon	26.1	tr	tr	—
34	? Saturated hydrocarbon	45.3	tr	tr	—

tr, Trace.

*Five unidentified components were also detected, all present in only trace amounts.

mushroom essence, together with retention data, quantitative data and odour qualities of the GC peaks, as determined by odour assessment at a splitter at the exit of the GC column. As many as 40 constituents were detected, of which 27 (comprising over 99.5%) were positively identified from a comparison of their mass spectra with the literature [17, 18]. A further eight components were partially or tentatively characterized. In total nearly 150 μg of aroma volatiles were obtained per gram of fresh mushrooms, which represents quite a high concentration.

Benzyl isothiocyanate (peak 32) was positively identified in the mushroom sample, although it was produced in only trace amounts (i.e. less than 0.1 μg/g). However, the mass spectrum of benzyl isothiocyanate is not highly diagnostic and basically consists of only two main peaks, at *m/z* 91 (100%) and at *m/z* 149 (M^+, 11%) [19]. Together with its low concentration, this might partly explain why previous workers have missed this component in mushroom samples. The appropriate GC peak in our mushroom essence gave a good mass spectrum, which matched perfectly that of the genuine compound, and the RR_t (reference standard, benzyl alcohol) of the GC peak also agreed exactly with that of the pure benzyl isothiocyanate. This identification of benzyl isothiocyanate can be taken as strong evidence of the presence of the glucosinolate precursor in mushrooms, but no other benzylglucosinolate degradation products could be detected, despite careful SIM searches (on *m/z* 90 and 117 for the nitrile and on *m/z* 91 and 149 for the thiocyanate). Although SIM does not in itself provide a positive criterion of identity, it is a most valuable technique in showing the absence of a component.

This result confirms the previous work of Freise [1], except that no 2-phenethylglucosinolate degradation products could be detected (again by means of SIM). However, Freise did not claim to detect both isothiocyanates in any of the mushroom species examined [1]. In experiments designed to increase the sensitivity of our

analysis for glucosinolate products, an active thioglucosidase preparation (from mustard) was added to chopped mushroom and the mixture was incubated in distilled water for 1 hr before extraction of volatiles as before. It was hoped that by supplementing endogeneous enzyme activity greater amounts of glucosinolate products would be obtained and that perhaps this might reveal the presence of 2-phenylglucosinolate, but overall the results were the same as in the absence of added enzyme.

Considering the other mushroom volatiles listed in Table 1, it can be seen that the major components were oct-1-en-3-ol (*ca* 59%), cyclo-octanol (*ca* 24%) and octan-3-ol (*ca* 9%). Without doubt, oct-1-en-3-ol is the most important mushroom volatile and previous workers have found it to contribute between *ca* 50% and *ca* 90% of the total volatiles, depending upon the particular species [3]. Dijkstra measured the absolute concentration of oct-1-en-3-ol in 14 mushroom species and found it to vary from less than 0.02 μl/l. to 190 μl/l. [8]. Our figure of *ca* 88 μg/g is nearly the mid-point of this range. However, it is probable that less oct-1-en-3-ol was obtained than might otherwise have been the case, due to the formation of cyclo-octanol (*ca* 24%, 36 μg/g). Surprisingly, this compound has never before been detected in mushroom volatiles, but it is a reasonable deduction that it was formed by cyclization of the oct-1-en-3-ol. Presumably the cyclization occurred as a result of the heating (cooking) process, although previous workers have not detected the product in cooked mushrooms [5, 6]. Thus cooking effectively decreases the proportion of the important oct-1-en-3-ol in the resultant mushrooms.

There is no doubt concerning the identification of the cyclo-octanol in that its mass spectrum agreed well with the literature [17] and with the mass spectrum of an authentic sample recorded on the same instrument. In addition, the RR_t (reference standard, phenylacetaldehyde) of the authentic compound also agreed with that of the suspected cyclo-octanol peak in the mushroom sample. Significant features of the EI mass spectrum of cyclo-octanol were: *m/z* (% rel. int.) 57(100), 55(48), 68(39), 41(38), 67(34), 82(34), 81(32), 69(23), 43(19), 110 $[M-H_2O]^+$ (18), 128 $[M]^+$ (1). The CI mass spectrum was also of diagnostic value, showing an intense peak at *m/z* 111 due to loss of water from the protonated molecular ion (a characteristic feature of the CI spectra of aliphatic alcohols) and a very small peak (2%) at *m/z* 129 due to the protonated molecular ion itself.

As well as being a new volatile components from mushrooms, cyclo-octanol has not apparently been reported as an aroma volatile from any other food system [2], although cyclopentanol has been found in passion fruit [20] and cyclohexanol in roasted peanuts [21]. Other compounds newly detected in mushroom volatiles in this work include hex-1-en-3-ol and *N*-phenylpyrrole. Both gave extremely good and characteristic mass spectra which agreed well with the literature [17, 18]. The hexenol is a lower homologue of oct-1-en-3-ol and some other pyrroles have been identified in dried mushrooms [7], so neither occurrence is particularly surprising.

Current opinion is that a series of compounds containing eight carbon atoms constitutes the primary volatiles contributing to mushroom flavour, namely octan-1-ol, octan-3-ol, octan-3-one, oct-1-en-3-ol, oct-2-en-1-ol and oct-1-en-3-one [3]. All these compounds were detected in our sample and, together with the newly identified cyclo-octanol, they comprised 98% of the essence, a very high percentage for a common group of aroma volatiles. With regard to the odour qualities of these C_8 compounds, most researchers have observed that it is only oct-1-en-3-ol and oct-1-en-3-one which have characteristic mushroom aroma [22, 23]. Our odour assessments (Table 1) were in accord with these previous findings, but in addition a slight mushroom soup aroma was detected at the point at which 3-methylbutan-1-ol eluted from the GC column. Cronin and Ward also detected this compound, but reported no odour quality for its GC peak [22]. Odour descriptions of the other previously identified C_8 compounds were also in good general agreement with earlier work [22–24]. The GC peak due to cyclo-octanol provided a fresh, green aroma with a fragrant undertone. No characteristic aroma was detected at the point when benzyl isothiocyanate eluted from the GC column. Its contribution, if any, to characteristic mushroom flavour is uncertain since it was produced in such relatively small quantities, although its concentration was almost certainly in excess of its odour threshold [25].

Since the fresh mushroom samples employed in this work yielded only a small amount of one isothiocyanate, samples of commercially dried mushrooms were also analysed in the same manner in the hope that they would provide more concentrated aroma essences. Clearly, it was necessary to employ added thioglucosidase enzyme preparation, as already described, since it would be anticipated that the drying process would have inactivated the endogeneous mushroom enzymes [26–28]. Table 2 gives the results obtained, which should also be compared with those in Table 1. The essence obtained from dried mushrooms was more concentrated than that from fresh mushrooms. Thus, *ca* 500 μg of total volatiles were obtained per gram of dried mushroom compared with *ca* 150 μg/g for fresh mushrooms. This facilitated analysis in two important respects. First, in addition to benzyl isothiocyanate it was then possible to positively identify traces of benzyl cyanide. Again, both mass spectrum and RR_t agreed perfectly with those of the reference compound. The identification of both nitrile and isothiocyanate provides virtually positive proof of the existence of the precursor benzylglucosinolate in mushrooms. Secondly, the increased quantity of benzyl isothiocyanate produced enabled it to be detected during GC odour evaluation runs by virtue of its slight lachrymatory quality, even though it co-eluted with another component.

However, it was still not possible to detect any 2-phenethylglucosinolate degradation products in these mushroom samples, despite very careful high sensitivity SIM searches, so the previous claim [1] remains unsubstantiated. It may be that this glucosinolate does occur only in certain species [1], but it can be argued that its occurrence in mushrooms would be very much less likely than benzylglucosinolate for the following reason. Glucosinolates are biosynthesized from α-amino acids such that the side chain is derived from the amino acid side chain. During biosynthesis this side chain may become modified in various limited ways including, in particular, chain elongations. Thus, both benzyl- and 2-phenethyl glucosinolates are biosynthesized from phenylalanine, but for 2-phenethylglucosinolate, chain elongation is necessary. To date all glucosinolates which have been identified in species outside the Capparales are those biosynthesized directly without any chain elongation and, thus, such occurrence of 2-phenethylglucosinolate can be considered, on present evidence, to be somewhat unlikely.

Table 2. Volatile aroma components of cooked dried mushrooms

Peak No.	Component*	RR_t (min)	Relative abundance (%)	μg/g	Odour quality
1	Hexane	3.0	18.95	96.12	solvent, green, metallic, stale
2	3-Methylbutanal	4.6	0.16	0.81	diacetyl-like, pungent
3	Chloroform	5.7	tr	tr	—
4	Toluene	6.3	2.83	14.36	sweet, buttery, sickly
5	Hexanal	7.3	2.90	14.70	green, grass
6	Ethylbenzene	8.2	0.34	1.70	onions
7	Xylene (*m* or *p*)	8.5	0.31	1.58	onions
8	Pyridine plus *o*-xylene	9.2	2.13	10.79	green, solvent
9	2-Pentylfuran	9.9	0.90	4.58	sweet, estery
10	Methylpyrazine	10.5	0.35	1.75	unpleasant
11	Oct-1-en-3-one	11.0	1.45	7.33	mushroom
12	Ethylpyrazine	11.5	1.06	1.45	sweaty, unpleasant
13	2-Ethyl-6-methylpyrazine	12.1	0.76	3.85	—
14	2-Ethyl-5-methylpyrazine	12.5	0.39	1.98	—
15	Furfural plus a dimethylethylpyrazine	13.1	2.43	12.32	musty, mouldy, oily, fatty
16	2-Ethyl-3,5,6-trimethylpyrazine plus 2-acetylfuran	13.4	0.39	1.96	cereal
17	Benzaldehyde	13.9	48.16	244.27	almond-like
18	Undecan-2-one	14.7	0.97	4.92	musty, mouldy
19	Sesquiterpene (? β-farnesene)	15.9	0.15	0.75	nutty, earthy
20	Phenylacetonitrile (benzyl cyanide)	17.7	tr	tr	stale, hay
21	Benzyl alcohol	18.8	9.80	49.69	cereal-like, sweet
22	? 2-Phenylbut-2-enal	20.5	0.16	0.83	—
23	A cresol	21.7	0.22	1.10	—
24	Benzyl isothiocyanate plus a sesquiterpene	23.2	1.70	8.61	lachrymatory, sweet, floral, fragrant
25	Indole	55.0	tr	tr	—

tr, Trace.

*Also detected were 13 unidentified compounds, comprising *ca* 2.65% (*ca* 13.39 μg/g) of the samples.

With regard to the other compounds identified in the aroma essence from dried mushrooms, it can be seen from Table 2 that 42 compounds were detected of which 26 (comprising nearly 95%) were positively identified. Three others (*ca* 2%) were partially or tentatively characterized. The major components were benzaldehyde (*ca* 4.8%), hexane (*ca* 19%) and benzyl alcohol (*ca* 10%). Comparing Tables 1 and 2 it is obvious that there is very little similarity between the two sets of data. Clearly, these particular dried mushrooms are a poor substitute and with one exception all the important C_8 aroma components of fresh mushrooms were lost. The only survivor was oct-1-en-3-one (*ca* 1.5%) which is, however, one of the more important mushroom aroma compounds, which provided a determined mushroom aroma to the sample (Table 2). Many new components were detected in the dried mushroom sample, but in general these contributed undesirable flavour characteristics (e.g. the range of pyrazines). Previous workers [7–9] have also studied processed mushrooms, although in general the results obtained were not quite as extreme as those reported here, in that usually some oct-1-en-3-ol was retained in extracts of the dried product.

EXPERIMENTAL

Fresh mushrooms were purchased from a local retailer and were authenticated as *Agaricus bisporus* by basic microscopy. Commercially dried mushrooms were also purchased locally.

Sample preparation. Chopped fresh mushrooms (*ca* 1400 g, accurately weighed) were cooked in boiling distilled H_2O (500 ml) for 30 min and the aroma volatiles were extracted for 2 hr in a Likens and Nickerson [10] apparatus as modified by MacLeod and Cave [11] using triple-distilled 2-methylbutane (30 ml) as solvent. The extract was concd to 1.0 ml by low temp.–high vacuum distillation [11]. Chopped, dried mushrooms (*ca* 60 g, accurately weighed) were rehydrated, incubated in distilled H_2O (400 ml) at room temp. with a thioglucosidase enzyme preparation (1.5 g) for 1 hr, cooked and analysed as before. Fresh mushrooms were also incubated with enzyme, in the same manner, before cooking and extraction of volatiles.

Enzyme preparation. A stable thioglucoside glucohydrolase extract was prepared from commercial mustard powder by well-established procedures [29]. Its activity was confirmed by its degradation of synthesized benzyl- and 2-phenethylglucosinolates [30].

GC. Essences were examined by GC with heated FID. Most routine analyses were performed using a 1.5 m × 4 mm i.d. glass column packed with 10% PEG 20M coated on 100–120 BSS mesh acid-washed Diatomite C. N_2 carrier gas (30 ml/min) was used and the temp. programme was 60° for 5 min followed by an increase of 12°/min to 180° for the remainder of the run. Isothermal runs (at 110° or 150°) were carried out for accurate quantification of components and for accurate measurements of

RR_Is of glucosinolate products. Instrument detector and injector temp. was 250° and typically 1 μl of essence was injected. An 18 ft column was used for GC/MS and for GC-odour evaluations.

GC/MS. A Kratos MS 25 instrument was used, linked on-line to a Kratos DS 50S data processing system. The same GC conditions as noted above were employed, but using He as carrier gas. A single-stage, all-glass jet separator was operated at 250°. Both EI- and CI-MS were performed, and the MPM unit (for SIM) and the retrospective single ion monitoring facility of the data system were extensively employed in specific searches for glucosinolate degradation products. Significant operating parameters of the mass spectrometer during EI work were: ionization potential, 70 eV; ionization current, 100 μA; source temp., 230°; accelerating voltage, 1.5 kV; resolution, 600; scan speed, 1 sec/decade (repetitive throughout run). Identical conditions were employed during CI-MS except for the following: reagent gas, methane or isobutane; ionization potential, 100–110 eV; emission current, 5 mA.

Quantitative assessment. Sample preparation and concn were conducted with quantitative accuracy so that known aliquots of the mushroom samples were analysed. Quantitative data were then derived both from the trace obtained from the TIC monitor during GC/MS and from the FID trace during routine GC. Known amounts of a selection of identified components (hex-1-en-3-ol, benzaldehyde, benzyl alcohol, methylpyrazine, benzyl isothiocyanate) were injected under the same analytical conditions to assess a range of detector response factors.

Odour assessment. Aromas of the separated components of the essences were assessed at an odour port following GC. An outlet splitter set at 10:1 diverted the major fraction of the eluent through a heated line to the outside of the GC oven for aroma assessment by a total of three subjects, two of whom were experienced in aroma analysis using this technique. An injection vol. of 10 μl was necessary.

Acknowledgements—Thanks are due to Mr. W. G. Gunn and Mr. A. E. Cakebread for operation of the GC/MS and data processing system. We much appreciate the assistance of Dr. G. MacLeod and Mrs. J. Ames in odour assessments.

REFERENCES

1. Freise, F. W. (1935) *Perfum. Essent. Oil Rec.* **26**, 91.
2. Van Straten, S., de Vrijer, F. and de Beauveser, J. C. (1977) *Volatile Compounds in Foods* 4th edn. Central Institute for Nutrition and Food Research TNO, Zeist.
3. Maga, J. A. (1981) *J. Agric. Food Chem.* **29**, 1.
4. Johnson, A. E., Nursten, H. E. and Williams, A. A. (1971) *Chem. Ind. (London)* 1212.
5. Picardi, S. M. and Issenberg, P. (1973) *J. Agric. Food Chem.* **21**, 959.
6. Card, A. and Avisse, C. (1977) *Ann. Technol. Agric.* **27**, 287.
7. Thomas, A. F. (1973) *J. Agric. Food Chem.* **21**, 955.
8. Dijkstra, F. Y. (1976) *Z. Lebensm. Unters. Forsch.* **160**, 401.
9. Sulkowska, J. and Kaminski, E. (1977) *Acta Aliment. Pol.* **3**, 409.
10. Likens, S. T. and Nickerson, G. B. (1964) *Proc. Am. Soc. Brew. Chem.* 5.
11. MacLeod, A. J. and Cave, S. J. (1975) *J. Sci. Food Agric.* **26**, 351.
12. MacLeod, A. J. and Islam, R. (1975) *J. Sci. Food Agric.* **26**, 1545.
13. MacLeod, A. J. and Islam, R. (1976) *J. Sci. Food Agric.* **27**, 909.
14. Gil, V. and MacLeod, A. J. (1980) *Phytochemistry* **19**, 227.
15. Gil, V. and MacLeod, A. J. (1980) *Phytochemistry* **19**, 1369.
16. Gil, V. and MacLeod, A. J. (1980) *Phytochemistry* **19**, 1657.
17. (1974) *Eight Peak Index of Mass Spectra* 2nd edn. Mass Spectrometry Data Centre, AWRE, Aldermaston.
18. Jennings, W. and Shibamoto, T. (1980) *Qualitative Analysis of Flavour and Fragrance Volatiles by Glass Capillary Gas Chromatography.* Academic Press, New York.
19. Gil, V. and MacLeod, A. J. (1980) *Phytochemistry* **19**, 1365.
20. Winter, M. and Kloti, R. (1972) *Helv. Chim. Acta* **55**, 1916.
21. Walradt, J. P., Pittet, A. O., Kinlin, T. E., Muralidhara, R. and Sanderson, A. (1971) *J. Agric. Food Chem.* **19**, 972.
22. Cronin, D. A. and Ward, M. K. (1971) *J. Sci. Food Agric.* **22**, 477.
23. Pyysalo, H. and Suihko, M. (1976) *Lebensm. Wiss. Technol.* **9**, 371.
24. Wasowicz, E. (1974) *Bull. Acad. Pol. Sci., Ser. Sci. Biol.* **28**, 269.
25. Buttery, R. G., Guadagni, D. G., Ling, L. C., Seifert, R. M. and Lipton, W. (1976) *J. Agric. Food Chem.* **24**, 829.
26. MacLeod, A. J. and MacLeod, G. (1970) *J. Food Sci.* **35**, 734.
27. MacLeod, A. J. and MacLeod, G. (1970) *J. Food Sci.* **35**, 739.
28. MacLeod, A. J. and Pikk, H. E. (1979) *J. Food Sci.* **44**, 1183.
29. Schwimmer, S. (1961) *Acta Chem. Scand.* **15**, 535.
30. Gil, V. and MacLeod, A. J. (1980) *Tetrahedron* **36**, 779.