

An Analysis of the Flavor of the Dried Mushroom, *Boletus edulis*

Alan F. Thomas

Coupled gas chromatography-mass spectrometry of an extract of the dried mushroom, *Boletus edulis*, has led to the identification of ca. 70 constituents, including nine pyrazines and seven 2-

formylpyrroles. Two of the latter have not been described in the literature, and one of them, 1-(2-phenylethyl)-2-formylpyrrole, was synthesized.

There is, surprisingly, little work published about the volatile part of the flavor of mushrooms. Much of the voluminous literature on mushroom flavor is of doubtful value since many workers, not aware of the enormous strength of the compounds actually responsible for the flavor, have been quick to ascribe it to nonvolatile bases (Craske and Reuter, 1965) and other nonvolatile compounds, without demonstrating conclusively any real association. Using dried *Cortinellus berkeleyanus* mushrooms, Take and Otsuka (1965) pointed out that the participation of organic acids and amino acids in the flavor was slight, although guanylic acid contributed. The following compounds have been associated with the flavor of mushrooms: from *Lentinus edodes* (the Japanese "shiitake" mushroom), guanosine 5'-monophosphate (Nagajima *et al.*, 1961) and lenthionine (1,2,3,5,6-pentathiepin) (Monta and Kobayashi, 1966; Wada *et al.*, 1967); from *Phallus impudicus* ("stinkhorn"), methyl mercaptan, hydrogen sulfide, and a number of aldehydes and acids (List and Freund, 1968), particularly 2-phenylcrotonaldehyde (List and Freund, 1967); from *Armillaria matsutake*, 1-octen-3-ol (Murahashi, 1936); from *Trichoderma viride*, 6-pentyl- α -pyrone (Collins and Halim, 1972). Preceding the present study on *Boletus edulis*, Cronin and Ward (1971), using a gas chromatograph coupled to a mass spectrometer, identified 14 substances, of which only two (1-octen-3-one and 1-octen-3-ol) had mushroom odors and, indeed, were already known to have (Stark and Forss, 1964).

A full literature survey of mushroom volatiles has been published by Herout (1968).

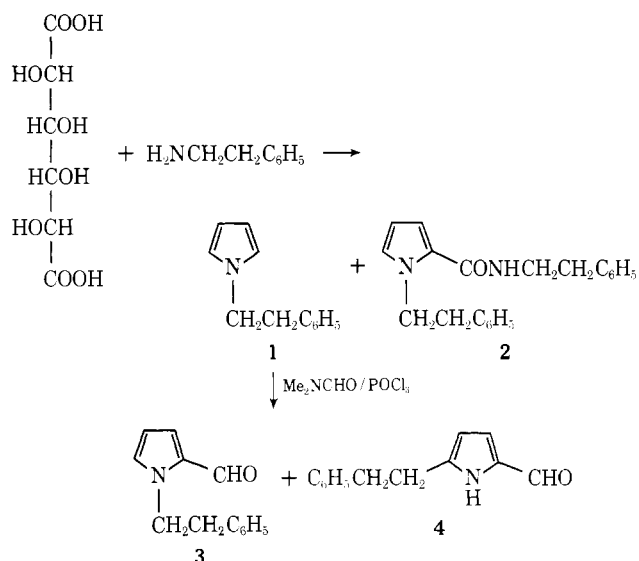
MATERIALS AND METHODS

The study was carried out with the dried *B. edulis* mushrooms commercially available in Poland.

If dried mushrooms are extracted, there is little or no characteristic flavor isolable from organic solvents. It is first necessary to heat in water before extraction (*cf.* Take and Otsuka, 1965), when pentane extraction yields a residue possessing a powerful smell of mushroom and humus. Microdistillation in high vacuum was possible without excessive loss of flavor, and the resulting distillate was examined by injection onto a glass capillary column coupled with a mass spectrometer. The mass spectra thus obtained were compared with spectra in the collection of our laboratories, and all identifications mentioned below were possible in this way, with two exceptions. These were 1-(2-phenylethyl)-2-formylpyrrole (peak 124 in Figure 1) and its 5-methyl homolog (peak 125) and, for direct comparison, the former was synthesized.

Chemistry. A general method for preparing N-substituted pyrroles consists in treating pyrrolythallium with an alkyl halide (Candy and Jones, 1971). With 2-phenylethyl bromide, the only isolable materials by this procedure were pyrrole and styrene. Pyrolysis of mucic acid in the presence of a primary amine yields pyrroles (Pictet and Steinmann, 1902; Reichstein, 1927) and using phenylethyl-

amine in this reaction, 17% of 1-(2-phenylethyl)pyrrole (1) was obtained, together with the 2-phenylethylamide (2) of the corresponding pyrrole 2-carboxylic acid. Formylation was effected with dimethylformamide and phosphorus oxychloride, and the product (3) was compared with the substance identified in mushrooms. The formylation reaction resulted in some migration of the phenylethyl group from nitrogen to carbon, so that 3 had to be purified from the contaminating C-alkylated formylpyrrole (4) by chromatography on silica gel.



EXPERIMENTAL SECTION

Gas chromatography was carried out on a Carlo Erba Fractovap model GT, fitted with a glass capillary column, details of which are in the legend to Figure 1. The injection port was at 200°, and the carrier gas (helium) was at an inlet pressure of 0.7 Atm. A split led to the flame ionization detector, the main exit leading, *via* a Becker-Ryhage-type helium separator, to the inlet of an Atlas CH4 mass spectrometer.

Nmr spectra were measured in CCl₄ on a Hitachi Perkin-Elmer R-20B instrument, and chemical shifts are given in ppm with tetramethylsilane as 0.00 ppm. Mass spectra independently of the coupling experiment were measured on an Atlas CH4 mass spectrometer with an inlet temperature of about 150° and electrons of 70 eV energy. Figures for mass spectra are given as *m/e* (relative abundance).

Extraction of Dried *Boletus edulis*. In all operations connected with the extraction, no direct contact with plastic tubing was allowed, a careful purification of the pentane used was effected by distillation first, over sodium, then alone, when the accompanying isopentane was removed, and all distillations and extractions were carried out in systems closed with traps cooled in liquid nitrogen.

The dried mushrooms (338 g) were macerated in a blender with 2 l. of distilled water, and then the mixture

Firmenich SA, Research Laboratory, 1211 Geneva 8, Switzerland.

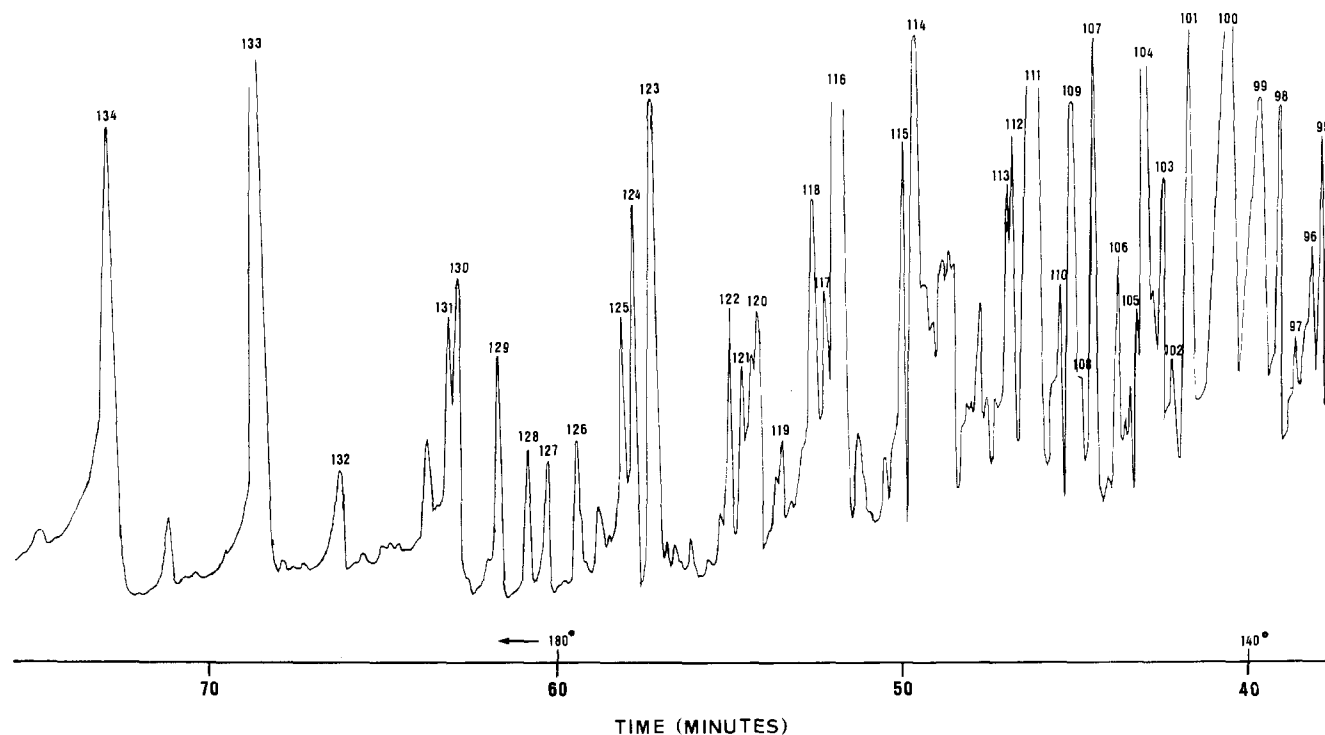


Figure 1. Gas chromatogram of *B. edulis* concentrate: 0.30 mm i.d. \times 33 m glass column coated with UCON HB 5100 and preconditioned according to Grob and Voellmin (1970)*. Starting temperature, 60°, programmed 2°/min to 180°. *Prepared by H. & J. Jaeggi,

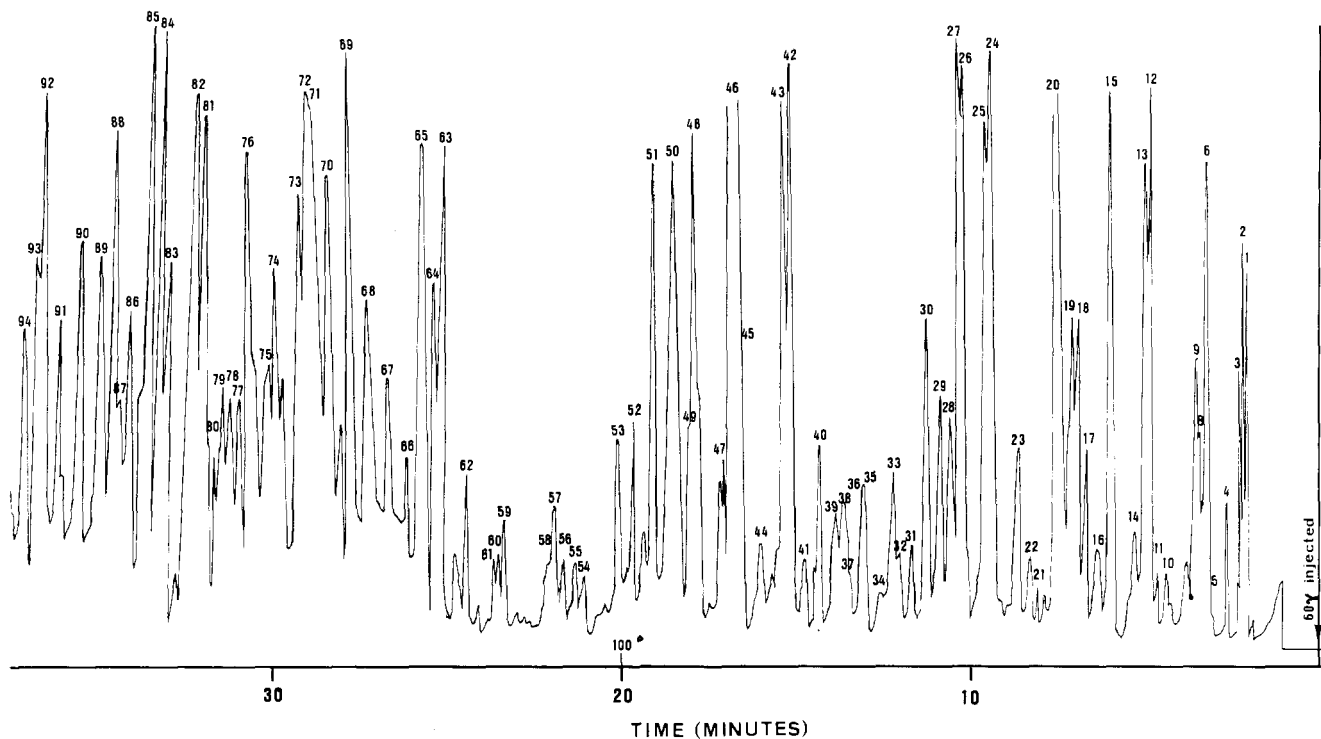
Table I. Compounds Identified by Ms-Glc Coupling of Extract from Dried *Boletus edulis*. Numbers Refer to the Peaks Illustrated in Figure 1

1. Hexanal	25. 3-Ethyl-2,5-dimethylpyrazine	50. 3-Methylbutyric acid	100. Octanoic acid
2. 3-Hexanol	26. Isomer of 25	51. 3-Decen-2-one	101. Eugenol
3. 2-Hexanol	27. 1-Octen-3-ol	52. 2-Undecanone	104. 2-Formyl-1-heptylpyrrole
6. 2-Heptanone	28. Tetramethylpyrazine	56. α -Terpineol	105. Nerolidol (?)
7. 2-Methyltetrahydrofuran-3-one	30. 5-Methylfurfural	57. Pentanoic acid	111. Nonanoic acid
8. Methylpyrazine	31. Butyrolactone	59. A 2-formyldimethylthiophene	113. Dimethyl phthalate
9. 1-Hexanol	32. 4-Hydroxypentanoic acid lactone	65. 2-Formyl-1-(3-methylbutyl)pyrrole	115. p -Vinylphenol
12. 2,5-Dimethylpyrazine	33. 2-Methylbutyrolactone	70. Heptanoic acid	116. Decanoic acid
13. 2,6-Dimethylpyrazine	34. 2-Ethyl-3,5,6-trimethylpyrazine	72. Isomer of 70	118. Diethyl phthalate
14. 2,3-Dimethylpyrazine	35. Thiobutyrolactone	73. A methylbutyl acetamide	121. A chlorocresol
15. Dimethyl trisulfide	37. 1-Ethyl-2-formylpyrrole	75. 2-Acetylpyrrole	122. Vanillin
16. Furfural	38. 2-Decanone	79. 2-Formylpyrrole	123. Phenylacetic acid
17. 2-Ethyl-5-methylpyrazine	39. Trithiolan (2,5-dimethyl-1,3,4-trithiacyclopentane) + 2-acetyl-5-methylfuran	80. 2(?) -Methylquinazoline	124. 2-Formyl-1-(2-phenylethyl)pyrrole
18. Isomer of 17	42. Furfurylmethanol	82. Phenol	125. 2-Formyl-5-methyl-1-(2-phenylethyl)pyrrole
19. 2,3,6-Trimethylpyrazine + ? 3-methylcyclohex-5-enone	43. 2-Formylthiophene	84. o (?) -Cresol	127. Hydrocinnamic acid
22. 3-Octen-2-one		85. Biphenyl	130. Anthracene or phenanthrene
23. 2-Acetylfuran		86. 2-Propionylpyrrole	131. Dibutyl phthalate
24. Benzaldehyde + isomer of 25		87. 1-Undecanol (?)	132. Cinnamic acid

was heated for 2 hr on a water bath (95°) and filtered. A further 2 l. of distilled water was added to the residue, and the mixture was again heated for 2 hr and filtered. The aqueous extract possessed the typical mushroom smell, and was extracted with pentane for 1 week. The pentane extract was concentrated at 30° to leave 0.3 g of material, which was sealed in a tube at 0.001 mm pressure while being cooled in liquid nitrogen and then distilled by cooling the other end of the tube while gently warming the part containing the extract. About 0.05 g of distillate was thus obtained, and although there was a slight deterioration of the odor during this process, the smell was still strongly mushroom-like, and the material obtained in this

way was injected into the capillary column. Conditions of the chromatogram are given in Figure 1.

1-(2-Phenylethyl)pyrrole. A mixture of 30 g of mucic acid and 20 g of 2-phenylethylamine was heated slowly (1 hr) to 180° at a pressure of ca. 70 mm. Water distilled at first, and when no more was left to be distilled, the pressure was lowered to 10 mm and then finally to 0.001 mm, simultaneously raising the temperature of the bath to 200°. Two fractions of distillate were obtained, bp 65–105° and 105–160° (0.001 mm). The lower boiling fraction was taken up in pentane and washed with sodium hydroxide (10%), hydrochloric acid (10%), again with sodium hydroxide, and then water. After removal of the pentane,



Labor für Gaschromatographie, 9043 Trogen, Switzerland.

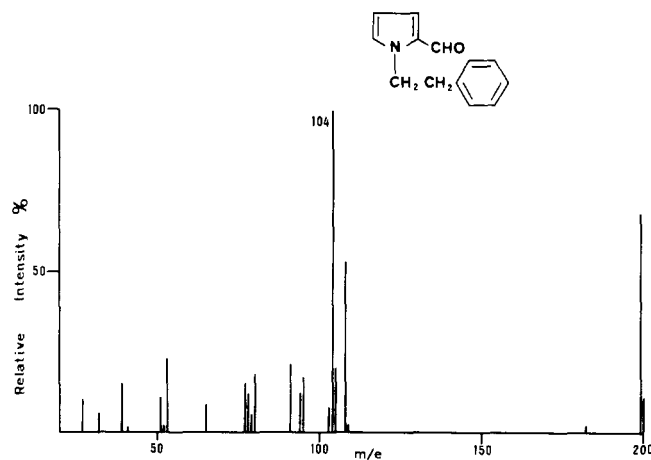


Figure 2. Mass spectrum of 1-(2-phenylethyl)-2-formylpyrrole.

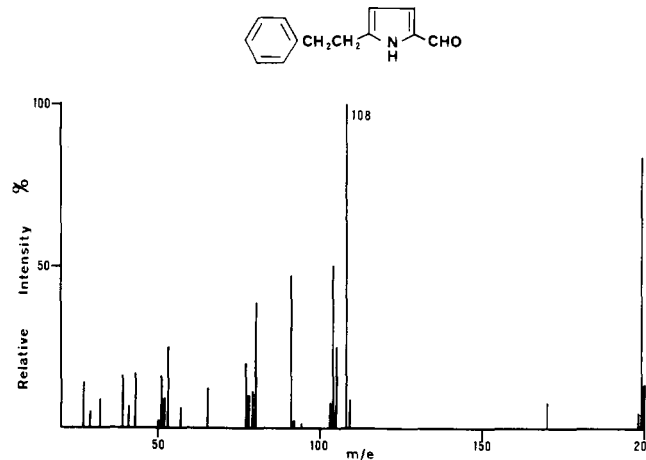


Figure 3. Mass spectrum of 2-formyl-5-(2-phenylethyl)pyrrole.

the residue was distilled at bp 67° (0.001 mm) to give 3 g of the title product, which crystallized on cooling, mp $33\text{--}34.5^\circ$. A further 2 g of this substance was obtained by similar treatment of the higher boiling fraction (see below); nmr spectrum 2.75 (2 H, t, $J = 7.5$ Hz, $\text{C}_6\text{H}_5\text{CH}_2\text{CH}_2$); the coupling of these protons is complex and the figures quoted are those measured from the spectra, *i.e.*, as a triplet rather than the double doublet, 3.78 (2 H, t, $J = 7.5$ Hz, $\text{CH}_2\text{CH}_2\text{N}$), 5.95 and 6.30 (2 H each, t, $J = 2$ Hz, pyrrole protons), 7.00 (5 H, m, phenyl protons); mass spectrum 80 (100), 171 (M^+ , 44), 53 (22), 104 (15).

Anal. Calcd for $\text{C}_{12}\text{H}_{13}\text{N}$: C, 84.17; H, 7.65; N, 8.18. Found: C, 84.32; H, 7.68; N, 8.25.

1-(2-Phenylethyl)pyrrole-2-carboxylic Acid 2-Phenylethylamide (2). The higher boiling fraction from the preceding reaction was washed with pentane; 3 g of material crystallized and was recrystallized from ether-hexane to give the title product 2, mp $75\text{--}76^\circ$. The pentane solution, after the washings described in the previous experiment, yielded 2 g of compound 1: nmr spectrum of 2

2.85 (4 H, m, $\text{C}_6\text{H}_5\text{CH}_2\text{CH}_2$), 3.48 (2 H, q, $J = 7$ Hz, $\text{CH}_2\text{CH}_2\text{N}$), 4.38 (2 H, t, $J = 6$ Hz, $\text{CONHCH}_2\text{CH}_2$), 5.81 (1 H, t, $J = 3$ Hz, pyrrole 4-H), *ca.* 6.3 (2 H, m, pyrrole 3- and 5-H), 7.0-7.15 (10 H, phenyl protons).

Anal. Calcd for $\text{C}_{21}\text{H}_{22}\text{N}_2\text{O}$: C, 79.21; H, 6.96; N, 8.80. Found: C, 79.26; H, 6.85; N, 8.76.

1-(2-Phenylethyl)-2-formylpyrrole (*cf.* Silverstein *et al.*, 1963). To 8 g of dimethylformamide at 10° was added dropwise over 15 min 17 g of phosphorus oxychloride. The mixture was stirred for 15 min at room temperature before adding 20 ml of ethylene dichloride. This solution was cooled to 5° and 17 g of 1-(2-phenylethyl)pyrrole in 25 ml of ethylene dichloride was added with stirring and cooling. After the addition was completed, the mixture was heated at reflux for 15 min, then cooled to *ca.* 30° when a solution of 75 g of sodium acetate in 100 ml of water was added, slowly at first and then more rapidly. The mixture was finally heated again at reflux for 15 min with efficient stirring. The organic phase was separated and the aqueous phase extracted with ether. The usual workup yielded crystals, mp $58\text{--}65^\circ$. Thin-layer chromatography demon-

strated the presence of two substances; these were separated by chromatography of 10 g of the mixture on silica gel with hexane-ether (8:2), yielding the following, in order of elution.

1. 7.9 g of 1-(2-phenylethyl)-2-formylpyrrole (3): nmr spectrum 2.92 (2 H, t, $J = 7$ Hz, $C_6H_5CH_2CH_2$), 4.40 (2 H, t, $J = 7$ Hz, $CH_2CH_2N<$), 5.99 (1 H, m, pyrrole 4-H), 6.55 (1 H, br s, pyrrole 5-H), 6.77 (1 H, m, pyrrole 3-H), 7.08 (5 H, s, phenyl protons), 9.46 (1 H, d, $J = 1$ Hz, CHO); mass spectrum, see Figure 2.

2. 1.14 g of 5-(2-phenylethyl)-2-formylpyrrole: nmr spectrum 2.92 (2 H, t, $J = 7$ Hz, $C_6H_5CH_2CH_2$), 4.03 (2 H, t, $J = 7$ Hz, $CH_2CH_2-\alpha$ -pyrrole), 6.40 (2 H, br s, pyrrole 3- and 4-H), 6.8-7.2 (5 H, m, phenyl protons), 9.51 (1 H, s, CHO); mass spectrum, see Figure 3.

RESULTS AND DISCUSSION

Table I lists the peaks identified by mass spectrometry. Only those peaks were numbered (Figure 1) where mass spectra were taken, and it will be noted that there is a relatively high proportion of substances identified. One contributing factor to this success might be that since the mushrooms were dried, the process would encourage formation of substances in a higher state of oxidation: pyrazines, pyrroles, and the like. Indeed, in the large amount of literature dealing with the occurrence of pyrazines in foodstuffs (e.g., Buttery *et al.*, 1971; Goldmann *et al.*, 1967; Stoll *et al.*, 1967; Walradt *et al.*, 1971; Watanabe and Saito, 1971), it is notable that they are particularly associated with roasted or dried materials. Substances of this nature generally give rise to more stable positive ions in the mass spectrometer than, say, the ions from saturated aliphatic compounds. Identification of the sulfur compounds (15, 35, 39, 43) was facilitated by examination of the natural isotope distribution of the parent peak. Peak 39, for example, clearly had the formula $C_4H_8S_3$, and when the trithiolane mass spectrum (Chang *et al.*, 1968; Herz, 1968) was subtracted from the observed mass spectrum for the peak, the remainder fitted exactly with 5-methyl-2-acetylfuran, which also had the same retention time under the conditions used.

Among the compounds listed are some that could be artefacts. Presumably the phthalates (113, 118, 131) came from the plastic bags in which the mushrooms arrived, but there is no proof. The hydrocarbons (85, 130) might be from atmospheric pollution, and the presence of the chlorine-containing compound (121) is unexpected, although substances containing chlorine are known to occur in a Mexican mushroom (Butruille and Dominguez, 1972).

The two 1-phenylethylpyrrole aldehydes (124 and 125) were not available in our collection of spectra, but the mass spectrum of synthetic 1-(2-phenylethyl)-2-formylpyrrole (3) (Figure 2) was identical with that obtained from peak 124 and different from the isomeric substance (Figure 3). Similarity with the 5-methyl homolog (125) left little doubt as to the structure of the latter.

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