Communications to the Editor

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ODOROUS METABOLITES OF AN ACELLULAR SLIME MOLD, PHYSARUM POLYCEPHALUM SCHW., AND A BASIDIOMYCETE, PHALLUS IMPUDICUS PERS.

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Geosmin, having an earthy-musty smell, 2-methylquinoline, linalool, and other volatile compounds were detected from the culture of an acellular slime mold *Physarum polycephalum* SCHW.. Perillene, phenylacetaldehyde, β-phenethyl alcohol, veratrole, guaiacol, phenol, and related compounds were detected as volatile metabolites of a basidiomycete *Phallus impudicus* PERS.

KEYWORDS —— geosmin; 2-methylquinoline; phenylacetaldehyde; slime mold; *Physarum polycephalum;* basidiomycete; *Phallus impudicus;* earthy-musty odor; odorous metabolite; water supply

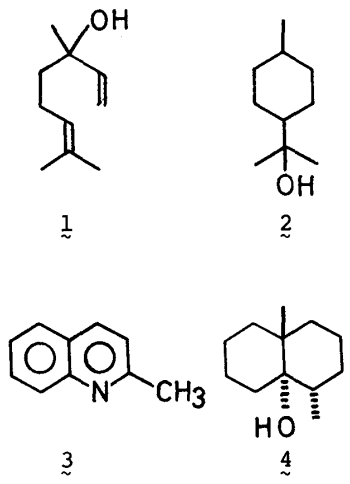
Geosmin (4)[1] and 2-methylisoborneol,[2] produced by several actinomycetes and other microorganisms,[3] have received a great deal of attention because they are responsible for the unpleasant earthy-musty odor and taste sometimes occurring in public water supplies. In connection with our study on odorous compounds in water supplies, we recently examined the volatile metabolites of a strongly earthy-musty smelling slime mold, *Physarum polycephalum* SCHW., and a putrid smelling basidiomycete, *Phallus impudicus* PERS.

Microplasmodia of *Physarum polycephalum* were grown on a semi-defined medium containing yeast extract and tryptone[4] at 26°C for 10-14 days and the whole culture (200 ml) was subjected repeatedly to steam distillation in order to concentrate the volatile substances. Extraction of the final distillate with ether and careful concentration of the extract gave a small amount of oily substance with an earthy-musty odor. Gas chromatographic (GC) analysis of this substance indicated the presence of a compound which has the same retention time as authentic dl-geosmin (4) (Fig. 1, peak D). The substance was further purified by silica gel chromatography (0.8 x 1.5 cm column) and eluting with ether-pentane (3:7) to give a colorless oil (7 mg) which was then examined by combined gas chromatography-mass spectrometry (GC-MS).[5]

The mass spectrum of peak D showed the molecular ion peak at m/z 182 (Calcd for $C_{12}H_{22}O$: 182.1665, Found: 182.1710) together with characteristic peaks at m/z 167, 164, 149, and 112, as shown in Fig. 2. This pattern was quite identical with that of authentic dl-geosmin (4) taken under the same condition and thus the peak D was identified as 4. On the other hand, the GC-mass spectrum of peak C gave the molecular ion peak at m/z 143 (Calcd for $C_{10}H_9N$: 143.0734, Found: 143.0739) and fragment

peaks at m/z 128 (Calcd for C_9H_6N: 128.0499, Found: 128.0486), 115, 101, 89, 75, and 63.[6] This was finally identified as 2-methylquinoline (3) by GC and GC-MS comparisons with an authentic sample of 3. The biological activity of this compound is now under investigation.

Other significant peaks A, B, and E were assigned to linalool (1), α-terpineol (2), and hexadecanal, respectively, based on the GC-MS analyses.



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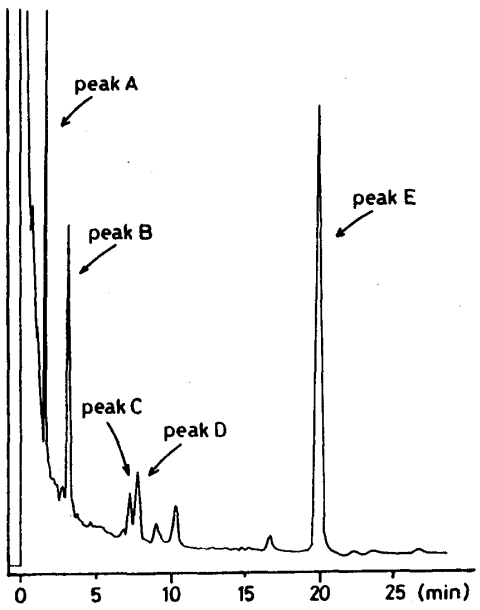


Fig.1.Gas Chromatogram of the Odorous Metabolites of *Physarum polycephalum* (peak A:linalool, peak B: *a*-terpineol, peak C: 2-methylquinoline, peak D: geosmin, peak E: hexadecanal)

Conditions: column, 2% OV-17 (2m x 3mm i.d.); column temp., 100-200°C (3°C/min); inj. temp., 150°C; carrier gas, N_2 (35 ml/min)

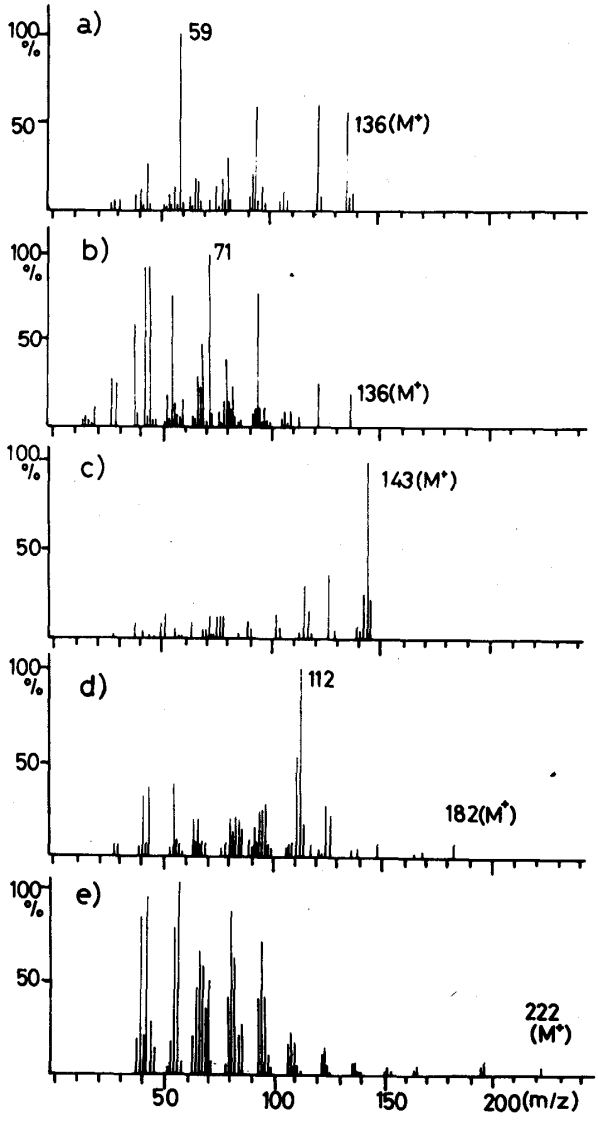


Fig.2. Mass Spectra of the Odorous Metabolites of *Physarum polycephalum*
a) peak A (linalool)
b) peak B (*a*-terpineol)
c) peak C (2-methylquinoline)
d) peak D (geosmin)
e) peak E (hexadecanal)

Conditions: GC column, 2% OV-17 (2m x 2mm i.d.); column temp., 100-200°C (3°C/min); carrier gas, He (1kg/cm^2); MS ionization voltage, 70 eV; accelerating voltage, 3 kV

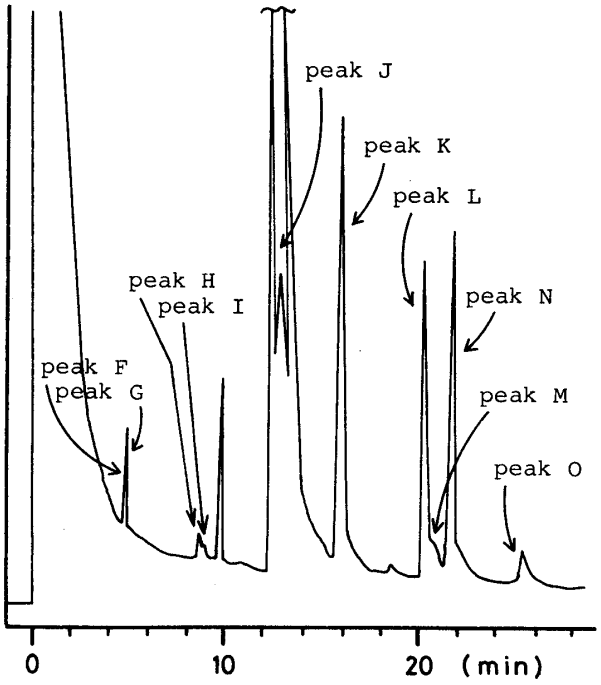
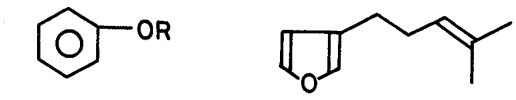


Fig.3. Gas Chromatogram of the Volatile Metabolites of *Phallus impudicus*

(peak F: anisole, peak G: perillene, peak H: acetic acid, peak I: benzaldehyde, peak J: phenylacetaldehyde, peak K: veratrole, peak L: guaiacol, peak M: benzyl alcohol, peak N: β-phenethyl alcohol, peak O: phenol)

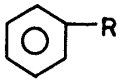
Conditions: column, 2% Thermon-3000 (2m x 3mm i.d.); column temp., 60-200°C (3°C/min); inj. temp., 150°C; carrier gas, N_2 (35 ml/min)



5 : R = CH_3

6 : R = H

7

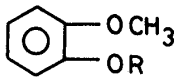


8 : R = CHO

9 : R = CH_2OH

10 : R = CH_2CHO

11 : R = CH_2CH_2OH



12 : R = CH_3

13 : R = H

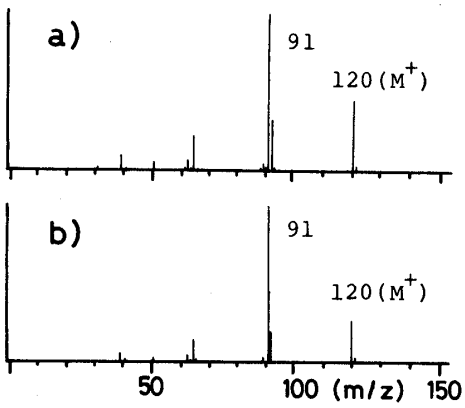


Fig.4. Mass Spectrum of a) the Metabolite (Peak J) of *Phallus impudicus*, and b) an Authentic Sample of Phenylacetaldehyde

Next, we examined the metabolites of *Phallus impudicus*. Caps (130 g) of fruit bodies of *P. impudicus*, freshly collected in October, 1983 at Daisenji Park, Toyama Prefecture, were extracted with ether at room temperature and the solvent was removed by careful evaporation to give an oily residue (1.5 g) having an objectionable smell. Purification of this substance by chromatography on silica gel (3.0 g, 0.8 x 5.5 cm column) using ether-pentane (3:7) gave a colorless oil (about 600 mg), which showed a complex pattern on GC as illustrated in Fig. 3.

Among the GC peaks observed, peaks F,[7] H, I, K, L, M, N, and O were identified as anisole (5), acetic acid,[8] benzaldehyde (8), veratrole (12), guaiacol (13), benzyl alcohol (9), β-phenethyl alcohol (11), and phenol (6), respectively, on the basis of direct GC and GC-MS comparisons with authentic samples.

On the other hand, peak G[7] was determined to be perillene (7) MS m/z: 150 (M^+, Calcd for $C_{10}H_{14}O$: 150.1044, Found: 150.1089) by comparing the MS data with those reported in the literature,[6] although direct comparison was not performed.

The most prominent peak (peak J) could be assigned to phenylacetaldehyde (10). The GC-mass spectrum (Fig. 4) corresponding to the peak J was identical with that of authentic phenylacetaldehyde (10), measured under the same conditions.

Our present results provide the first example of detection of geosmin (4) from the metabolites of the acellular slime mold and suggest that, besides actinomycetes, blue green algae and fungi, slime molds may also be responsible for the unpleasant earthy-musty odor and taste in public water supplies. It is of particular interest that geosmin (4) is a metabolite widely distributed among microorganisms. From *Phallus impudicus* ten volatile compounds were isolated, among which phenylacetaldehyde (10) may be mainly responsible for the unpleasant smell of this mushroom. It should be mentioned that 10 is one of the metabolites of fungi isolated from bottom deposits of a water reservoir.[3d] It is also of interest that phenol (6) was detected as a metabolite of the mushroom.

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4) J. Daniel and H. H. Baldwin, "Cell Physiology," Vol. 1, ed. by D. M. Prescott, Academic Press Inc., New York, 1964, pp. 9-41.
5) GC-MS and GC-high resolution MS measurements were made with a JEOL JMS-D300 instrument.
6) E. Stenhagen, S. Abrahamson, and F. W. McLafferty (eds.), "Registry of Mass Spectral Data," Vol. 1, John Wiley & Sons, Inc., New York, 1974, pp. 331 and 388.
7) Peak F and peak G were not separated by a 2% Thermon-3000 column (Fig. 3), but distinctly separated when using a 2% OV-17 column.
8) Acetic acid and phenylacetaldehyde were already reported as the metabolites of this mushroom, see P.H. List and B. Freund, *Planta Medica*, Supplement 123 (1968).

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