

wines. It is suggested that the hydroxide groups would react with the DMDC and the combination would decrease the chance that the DMDC would react with ammonia to form the methylcarbamate. More data are required before that can be verified. Surprisingly little is known about these reactions with DMDC.

Methylcarbamate has been investigated for carcinogenic activity (Pound, 1967). The report states that it has none. The toxic level is reported at LD₅₀: 500 mg/kg taken intraperitoneally (U.S. Department of Health, Education and Welfare, 1973).

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Geosmin, the Earthy Component of Table Beet Odor

The volatile components of red table beets (*Beta vulgaris* L., C.V. Ruby Queen) were prepared by fractional steam distillation, solvent extraction, and concentration, and then separated into acid, base, and neutral fractions by further solvent extraction. The base fraction had a potato-like odor, the neutral part had a distinct earth-like odor, and the acid fraction was almost odorless. Analysis of the neutral fractions by gas chromatography and mass spectrometry yielded a single major earthy smelling component identified as geosmin, a C₁₂ terpene-like compound known to be produced by soil organisms of the order Actinomycetes.

"Earthy" odors have been described in several vegetables and vegetable products as being due to pyrazine compounds (Deck and Chang, 1965; Parliment and Epstein, 1973; Buttery and Ling, 1973). However, the "earthy" or "musty" odor caused by the contamination of food and water supplies by Actinomycetes (Morris, 1962; Romano and Safferman, 1963; Silvey and Roach, 1964) is due to geosmin (*trans*-1,10-dimethyl-*trans*-9-decalol) identified by Gerber (1968), Marshall and Hochstetler (1968), and Kikuchi et al. (1972). This paper shows that geosmin is also a major component of beet essence and is responsible for this vegetable's characteristic "earthy" odor.

EXPERIMENTAL SECTION

Beet Juice. Red table beets (*Beta vulgaris* L., C.V. Ruby Queen) grown at this station during the 1973 season were graded for size (2.5 to 6.0 cm in diameter), washed, blanched in boiling water for 15 min, and cooled in running water. Beets (500 kg) were then ground in a Fitzpatrick hammermill, mixed with filter aid (KeyCel press aid, 1.5% w/w), and pressed in a hydraulic press, to yield 300 kg of beet juice.

Aqueous Essence. The beet juice was preheated to 100 °C by pumping it through a steam jacketed heat exchanger and then the hot juice was fed continuously to the top of a stainless steel 10 plate distillation column (Moyer and Saravacos, 1968). A clear colorless aqueous essence with a strong beet-like odor was obtained after two cycles through the column representing a 52-fold concentration.

Acid, Base, and Neutral Fractions. Three 300-ml portions of aqueous essence were extracted with equal volumes of Freon 113 (1,1,2-trichlorotrifluoroethane) and the fluorocarbon layer was concentrated at atmospheric pressure by distillation (2 cm o.d. by 90 cm Vigreux) to 30 ml. The concentrated extract was further extracted with 30 ml of 1 N HCl to remove the bases, followed by 30 ml of 1 N NaOH to remove the acids, and then con-

centrated to about 0.3 ml in a 10 plate microdistillation column to yield the neutral fraction. Both the aqueous acid and base layers were neutralized, back extracted with Freon 113, and concentrated to produce the base and acid fractions, respectively.

Gas Chromatography, Odor Evaluations, and Mass Spectrometry. An 80 m × 0.75 mm i.d. stainless steel open tubular gas chromatographic column coated according to the procedure of Mon (1971) with 5% SF 96 and 3% Triton X305 was used in a Varian Model 1440 gas chromatograph. It was operated isothermally at 60 °C for the first 3 min and then programmed at 2 °C/min for 50 min. Helium was used as a carrier gas (12 ml/min) and the output of the column was coupled to a time of flight mass spectrometer (Bendix Model 12 modified with a CVC Mark IV) through a methylsilicone helium separator (Black et al., 1969). Ionization was at 30 °C and 70 eV. The volatile components eluted from the gas chromatograph were detected by monitoring the total ion current of a mass spectrometer and simultaneously evaluating the odor by sniffing the output of the helium separator.

Synthesis of Geosmin. Geosmin was synthesized according to the procedure of Marshall and Hochstetler (1968). The resulting mixture of isomers (50 mg of an almost colorless oil) was separated in a 4 m × 3 mm gas chromatographic column containing Chromosorb W coated with 5% SP 1000 (a Carbowax modified with terephthalic acid, obtained from Sepelco Inc., State College, Pa.). The column was used in the gas chromatograph-mass spectrometer system described above and operated with a carrier gas flow rate of 20 ml/min and the oven temperature was programmed from 60 to 180 °C at 4 °C/min.

RESULTS AND DISCUSSION

The concentrated extract of beet essence described above has the characteristic odor of beets. However, the neutral fraction retained the earthy character of the whole

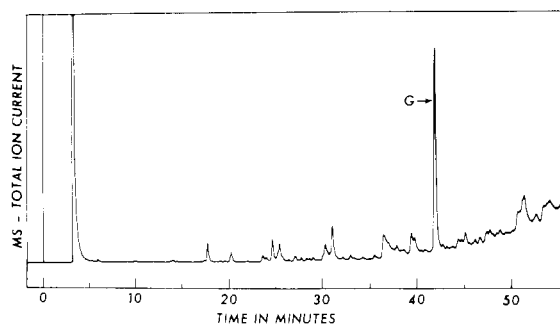


Figure 1.

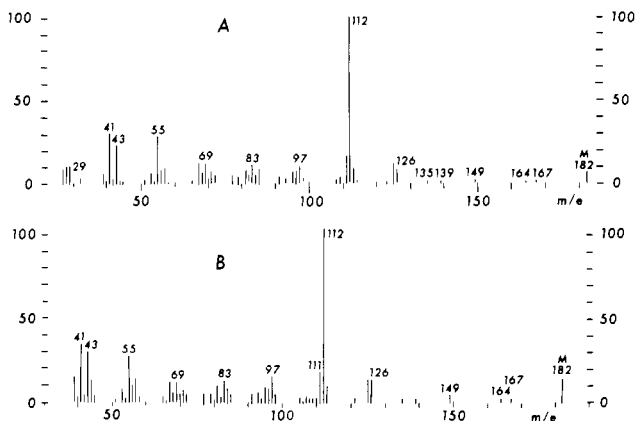


Figure 2.

essence and the basic fraction had a potato-like odor. The acid fraction was almost odorless. Figure 1 shows the gas chromatogram obtained from the concentrated neutral fraction of the beet essence. The strongest odor detected in this chromatogram was an earthy odor associated with peak G. The mass spectrum of peak G shown in Figure 2A is virtually identical with the spectrum of authentic geosmin, Figure 2B (Buttery, 1974; Kikuchi et al., 1972).

The retention time of synthetic geosmin prepared according to Marshall and Hochstetler (1968) and chromatographed in a packed column was 28.7 min and its mass spectrum and odor were virtually identical with that of peak G which had a retention time of 28.6 min on the same column. The *cis*-9-decalol isomer of geosmin had a

very similar spectrum to that of geosmin but a retention time of 29.5 min and an odor similar to camphor or cedar. Thus, the character of beet odor can be described as a combination of a potato-like odor, from the basic compounds, and the earthy odor of geosmin.

The fact that geosmin is known to be produced by soil organisms raises the question of its origin in beets. In the experiments reported here the washing and blanching procedures eliminate the possibility of contamination by soil. However, beets may absorb geosmin from soil during growth or storage. If so then why do not other root crops such as carrots and turnips also have a strong earthy odor due to the absorption of geosmin? The possibility that geosmin is absorbed by beets from the soil rather than produced metabolically by the beet is presently being investigated.

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Uptake of Benomyl by the Cultivated Mushroom, *Agaricus bisporus*

Fruiting bodies of *Agaricus bisporus*, grown under commercial conditions in substrates treated with 40 $\mu\text{g/g}$ of benomyl at different stages of cultivation, were found to contain from 0.67 to 3.7 $\mu\text{g/g}$ of benomyl residues. Mushrooms from substrates treated by drenching contained the highest quantities of residues, whereas those treated by mixing benomyl with the spawning material contained the least. Although translocation from vegetative mycelium into fruiting bodies occurred, accumulation of residues in the mushrooms was not indicated.

Although the systemic fungicide benomyl (methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate) has been shown to be effective for control of the major fungal diseases of the cultivated mushroom, *Agaricus bisporus* (Gandy, 1971; Holmes et al., 1971; Peake, 1972; Snel and Fletcher, 1971), and is widely used by growers in Europe and England, little published information is available

concerning the presence or absence of residues in fruiting bodies harvested from treated cultures. Snel and Fletcher (1971), in a study which dealt mainly with disease control by benomyl, mentioned that residues of the fungicide were not present in mushrooms harvested from pots treated with 5 or 10 $\mu\text{g/g}$ of a wettable powder formulation. In contrast to this, preliminary studies in our laboratory