

mentary contact with borosilicate glass such as would occur in sample collection, reagent addition, etc. Very serious errors can be introduced by any prolonged contact of aqueous reagents or unknowns with borosilicate glass. The amounts extracted by bases are sufficiently large so that the contamination would be noted in examination of analytical results. With acid solutions, however, many samples analyzed would contain unknown concentrations of boron of the same order of magnitude as that extracted from glass and the error might go unnoticed.

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Geosmin and Methylisoborneol in Garden Soil

Geosmin (*trans*-1,10-dimethyl-*trans*-9-decalol) and methylisoborneol (1,2,7,7-tetramethyl-2-norbornanol), known volatiles from actinomycetes, have been isolated and characterized in ordinary garden soil using mass spectrometry and capillary gas chromatography.

As vegetables are, of course, normally grown in soil, one possible source of vegetable volatile flavor components is the soil itself. This is probably particularly true with root vegetables such as carrots, potatoes, and beets but may also be true with other vegetables (e.g., beans and peas) by transferral of the compounds from the roots to other parts of the plant with possible concentration. Some of these volatile flavor compounds may be desirable, but abnormal conditions in the soil may bring about the transferral of off-flavors.

The most likely source of soil volatiles are the microorganisms common to soil such as *Streptomyces* and *Pseudomonas*. Volatiles produced by *Streptomyces* have been rather thoroughly studied particularly by Gerber (1968, 1969, 1971, 1972) and have been shown by Rosen et al. (1970) to be responsible for earthy-musty off-odors in public water supplies. The compounds geosmin and methylisoborneol were thought to be the most responsible. The odor of earth itself has been attributed (Gerber and Lechevalier, 1965) largely to the presence of geosmin. However, as far as we can determine no one has actually previously characterized geosmin or methylisoborneol in earth.

We undertook to study the volatile components of ordinary garden soil to characterize the important aroma components and to learn something about their role in vegetable flavor.

EXPERIMENTAL SECTION

Materials. Methylisoborneol was synthesized by the reaction of camphor with methylmagnesium iodide as outlined by Medsker et al. (1969). It was purified from unreacted camphor by gas-liquid chromatography (GLC) separation using a 3 m × 0.635 cm o.d. aluminum column packed with 80-100 mesh Chromosorb P coated with 10% Silicone SF96(100) and 0.5% Igepal CO-880.

An authentic sample of geosmin was kindly supplied by Dr. Nancy Gerber. Additional geosmin was obtained from the cell-free media of *Streptomyces phaeochromogenes* NRRL B-3559 which showed 0.06 ppm of geosmin, 1 ppm of methylisoborneol, and 0.2 ppm of a sesquiterpene alcohol which gave a mass spectrum with a molecular ion of 222 and a base peak at *m/e* 59. This last compound is

apparently different from the sesquiterpenols already reported by Gerber (1971, 1972) from streptomycetes.

Isolation of Volatile Constituents from Garden Earth. A 5-l. volume of earth (4.5 kg) from the garden of the grounds of the authors' laboratory was placed in a 12-l. flask, covered with odor-free water, and treated for 3 h using vacuum steam distillation continuous extraction (Likens-Nickerson head) at 100 mm pressure with the liquid at about 50 °C. Hexane was used as the extracting solvent, with ice water cooling of the condenser. The hexane extract was dried over anhydrous sodium sulfate, filtered, and concentrated using low hold up distillation columns in the usual way.

A blank run made with the odor-free water showed no detectable amounts of geosmin or methylisoborneol.

Mass Spectrometry. The mass spectrometer was a modified Consolidated 21-620 cycloidal type mass spectrometer using 70 V ionization voltage. For the volatiles from earth a capillary GLC column (150 m × 0.75 mm i.d., stainless steel, coated with Tween 20 containing 5% Igepal CO-880) was coupled to the mass spectrometer using a silicone membrane molecular separator. The column was programmed from 70 to 170 °C at 0.5 °C/min and held at the upper limit.

RESULTS AND DISCUSSION

Vacuum steam distillation continuous extraction of garden soil gave an oil (of the order of 1 part per 10⁶ parts of soil) which was analyzed by capillary GLC. By smelling the end of the capillary GLC column as the compounds were eluted, several earthy odor components were detected. The column was then coupled to the mass spectrometer and the mass spectra of the compounds measured. The peak having the most intense earthy odor was geosmin. Its mass spectrum (parent ion 182, major ions 112, 43, 41, 55, and 69) and GLC retention data (Kovat's index 1716) were consistent with those of an authentic sample of geosmin. The concentration of geosmin was of the order of 0.1% of the volatile oil and therefore of the order of 1 part per 10⁹ parts of the soil.

A second component whose odor was evaluated as "very earthy" had a mass spectrum consistent with that of methylisoborneol (parent ion 168, major ions 95, 43, 108,

69, and 55; cf. Medsker et al., 1969). The GLC retention data (Kovats's index 1523) were also consistent with those of an authentic sample of methylisoborneol. The concentration of methylisoborneol in the volatile oil was of the order of 0.4% and therefore of the order of 4 parts per 10^9 parts of the soil.

Other components with earthy odors were detected but could not be characterized in the present work. A number of components with nonearthy odors were also characterized (mass spectrum and GLC retention data consistent with that of an authentic sample). These included benzaldehyde, camphor, hexanol, heptanol, and nonanol.

How much of these compounds is normally transferred to vegetables has yet to be studied although geosmin has recently been characterized in beets (Acree et al., 1976; Murray et al., 1975) and off-flavored dried beans (Buttery et al., 1976).

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Aroma Production by Cultures of *Ceratocystis moniliformis*

Aroma production by the fungus, *Ceratocystis moniliformis*, is described. Sensory evaluation showed the quality and intensity of the aroma to vary with the composition of the culture medium and with the age of the cultures. Combined gas chromatography-mass spectrometry was utilized to identify volatile constituents in cultures characterized as having "fruity-banana", "peach-pear", and "citrus" aromas. The time course of accumulation of volatile constituents in cultures of *C. moniliformis* was followed by headspace gas chromatography. *C. moniliformis* is a potential source of fruit-like essences and/or specific flavor compounds.

There are numerous references in the literature to "fruity" aromas produced by microorganisms. These organisms are potential sources of "natural" fruit essences.

A literature search, plus preliminary studies with several organisms, led to the tentative choice of *Ceratocystis moniliformis*. The present communication describes results which indicate that this organism has potential as a source of flavor essences. *C. moniliformis* could also be useful for biosynthetic studies, since microorganisms generally offer advantages over fruit tissues in terms of introducing potential tracer-labeled precursors and measuring the labeled products (usually excreted into the media).

EXPERIMENTAL SECTION

Cultural Techniques. *Ceratocystis moniliformis* ATCC 12861 was obtained from Dr. Ralph Collins, Department of Botany, University of Connecticut, Storrs. The organism, maintained on 5% potato-dextrose agar slants, was first grown on 5% potato-dextrose agar plates for 4 days at 30 °C. The cells were harvested by flooding each of the plates with 10 ml of sterile, deionized water. The resultant cell suspensions were pooled and a 2-ml aliquot of the cell suspension was used to inoculate 100 ml of liquid media, contained in 250-ml Erlenmeyer flasks. The inoculated flasks were plugged with nonabsorbent cotton wool wrapped in cheesecloth and incubated on a

rotary shaker at 30 °C. Each 100 ml of the basal liquid medium contained 0.1 g of KH_2PO_4 , 0.05 g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.2 mg of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.2 mg of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1 mg of $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 0.1 mg of anhydrous CaCl_2 , 10 mg of thiamin, and 0.5 μg of biotin (Wilson and Lilly, 1958). Various carbohydrate and nitrogen sources were added to the basal medium in quantities which supplied carbon equivalent to 2.5 or 3% dextrose and nitrogen equivalent to 0.1% urea.

Growth. Growth on various sources was assessed by visual comparison, relative to growth on the standard dextrose (2.5 or 3.0%)-urea (0.1%) medium.

Organoleptic Evaluation. A panel consisting of eight members of our Flavor Chemistry Laboratory characterized the aroma of the fungal cultures. The panel members were not restricted to any list of descriptive terms, but they were generally aware that the cultures were being tested for their ability to produce fruitlike aromas. Samples reported to have distinctive or unique flavor notes were reexamined by a highly trained consulting flavorist. He generally confirmed the results of our panel, but pointed out several additional and more subtle notes in the samples.

Separation and Identification of Aroma Compounds. Both the headspace of cultures and a Freon-11 extract of the growth broth of *C. moniliformis* were analyzed for aroma compounds. Headspace gas chroma-