Geosmin, a Musty Off-Flavor of Dry Beans

A musty odor fraction of off-flavored dry white navy beans was found by the combination of capillary gas chromatography and mass spectrometry to contain the compound geosmin (*trans*-1,10-dimethyl-*trans*-9-decalol) in sufficient amounts for it to be responsible for the musty off-flavor. Geosmin is a known musty off-flavor contaminant of some water supplies.

Dried beans (*Phaseolus vulgaris*) are, in themselves, a fairly bland food. For this reason, the presence of only very low concentrations of foreign volatile compounds can result in objectionable off-flavors. Such off-flavors can lead to the rejection of large quantities of beans for food purposes. One of these off-flavors has been described by bean processors as musty, moldy, earthy. The present work was aimed at characterizing the compound mainly responsible for the odor of a musty fraction isolated from samples of such off-flavored dry beans.

The authors had previously made a study of the volatiles of normal dry beans (Buttery et al., 1975).

EXPERIMENTAL SECTION

Materials. Off-flavored, dry white navy beans were obtained from a Michigan bean dealer. Authentic geosmin was obtained from the cell-free medium of a pure Actinomycetes culture (cf. Gerber and Lechevalier, 1965) using vacuum steam distillation continuous extraction with hexane, drying the hexane over sodium sulfate and concentrating in the usual way. This was followed by gas–liquid chromatography (GLC) separation on a 1 m × 0.64 cm o.d. aluminum column packed with Chromosorb P coated with 20% Carbowax 20M at 120°C, 10 psi of He inlet pressure. The geosmin was well separated from other components at 12 min retention time.

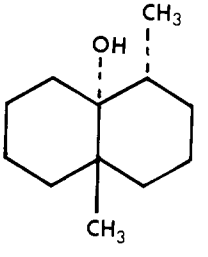
Isolation of the Volatile Oil. Dry white navy beans (20 kg) were placed in a 90-l. glass-lined container, covered with water (odor-free), and treated (4 hr) using vacuum steam distillation continuous extraction (Likens head) at 100 mm pressure and with the beans at about 45°C. Hexane was the extracting solvent and the condensor was cooled with ice water. The hexane extract was dried over sodium sulfate and placed on a short column (1.2 × 8 cm) of activated silica gel. The hydrocarbon components were eluted with 500 ml of pentane and discarded. The oxygenated components were then eluted with 500 ml of diethyl ether (freshly distilled). Concentration of the ether solution in the usual way gave the oxygenated fraction (10 μl).

Capillary GLC Mass Spectral Analysis. The GLC column was a 150 m × 0.75 mm i.d. stainless steel capillary coated with Tween 20 containing 5% Igepal CO-880. The column was programmed from 100 to 170°C at 0.5°C per minute and held at the upper limit. Helium at an inlet pressure of 8 psi was the carrier gas. The musty odor component was first located by odor evaluation of the effluent from the column by three experienced odor judges. The column was coupled to a modified Consolidated 21-620 mass spectrometer using a silicone membrane molecular separator and the mass spectrum of the musty odor component recorded.

Odor Threshold of Geosmin. The threshold of geosmin was determined by a modification of the procedure described by Guadagni et al. (1963). The original method was modified by substituting Teflon bottles and tubing for polyethylene, and analysis of the sensory data was modified to allow calculation of the 95% confidence limits (Guadagni et al., 1973).

RESULTS AND DISCUSSION

Vacuum steam distillation continuous extraction of a large quantity of off-flavored beans was used to give the volatile oil. This oil was then treated on a short column of silica gel to remove interfering aromatic hydrocarbons. The hydrocarbons were eluted from the silica with pentane and the oxygenated material then eluted with diethyl ether. This oxygenated fraction, which possessed the main musty odor of the bean, after concentration, was resolved by GLC on a capillary column coated with Tween-20. The musty odor component was located by odor evaluation of the effluent from the column by three experienced odor judges familiar with the musty odor of off-flavored beans. Mass spectral analysis of this component showed that it had a mass spectrum consistent with that of geosmin (*trans*-1,10-dimethyl-*trans*-9-decalol) (I), i.e. (two most



I

intense ions every 14 mass units above m/e 34, intensities in parentheses, molecular ion in boldface type) 41 (70), 43 (76); 55 (57), 57 (19); 69 (27), 71 (15); 83 (13), 85 (13); 95 (9), 97 (13); 111 (27), 112 (100); 125 (7), 126 (9); 149 (2); 164 (2), 167 (2); **182** (2). The capillary GLC retention properties (Kovats Index 1716, Tween-20) were also consistent with that of an authentic sample of geosmin.

Authentic geosmin was obtained using GLC from the volatiles of Actinomycetes media following Gerber and Lechevalier (1965). A sample of geosmin was also kindly supplied by Dr. Gerber.

Calculations from GLC indicated that the concentration of geosmin in the off-flavored beans was of the order of 1 part in 10^9 parts of the bean. It is possible that there could have been some loss of geosmin during the isolation and that the actual figure is higher than this. The quantitative analysis of such low concentrations of volatile material is very difficult. The mean odor threshold of geosmin was 21 parts per 10^{12} parts of water with 95% confidence limits of 17–24 parts per 10^{12} parts of water. Therefore, the concentration of geosmin estimated in the bean was 42–59 times greater than the threshold concentration. The amount of geosmin in the bean, then, would seem sufficient for it to affect the flavor of the bean.

The original work in the literature on the characterization of geosmin (Gerber and Lechevalier, 1965; Gerber, 1968) was carried out in order to identify the objectionable

musty or earthy taint found in some water supplies. It seems possible that the source of the geosmin in the beans then could result from the action of Actinomycetes, either on the beans themselves or in the water supply used during the growing of the beans. In the former case, the beans would have had to be in a moistened condition, i.e., before the beans were dried or by being wet after drying.

A number of musty off-flavored bean samples were studied by the authors. Although geosmin seems to be responsible for the musty off-flavor in the beans used in this study, we do not believe that this is always the case, and feel that other compounds can also be involved in causing musty off-flavor in beans. We have located some of these other compounds by odor evaluation but, up to the present time, have not been able to characterize them.

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A Method for the Determination of Ethylenebis(isothiocyanate) on Food Crops

A method was developed for the determination of ethylenebis(isothiocyanate) on food crops. The method is sensitive to 0.02 ppm and yields mean recoveries of 98% from 0.05 to 10 ppm of added residue. Interferences are not observed with either ethylenebis(dithiocarbamate) or ethylenethiuram monosulfide.

Ethylenebis(isothiocyanate) (EBI) has been postulated as a fungicidal decomposition product of the ethylenebis(dithiocarbamate) fungicides (Thorn and Ludwig, 1962). There is spectroscopic evidence to suggest that EBI can be formed from ethylenethiuram monosulfide, an intermediate in the decomposition of the ethylenebis(dithiocarbamates) (Ludwig et al., 1955). Engst et al. (1968) have presented thin-layer chromatographic evidence for the presence of EBI on tomatoes treated with maneb. The following method was developed to permit the quantitative determination of EBI on food crops treated with ethylenebis(dithiocarbamates).

EXPERIMENTAL SECTION

Materials. Ethylenebis(isothiocyanate) was prepared by thermal decomposition of the bis(ethoxycarbonyl) derivative of disodium ethylenebis(dithiocarbamate) in boiling toluene (Thorn and Huston, 1959). The isothiocyanate was purified by column chromatography on silicic acid (Mallinckrodt, 100 mesh) using hexane–benzene (1:1) as eluent. The infrared spectrum of the purified material in chloroform gave bands characteristic of an isothiocyanate at 2100 and 2125 cm^{-1}, while the 60-MHz NMR spectrum showed a CH_2 singlet at τ 6.13 ppm in deuteriochloroform.

A stock solution of EBI was prepared by dissolving a sufficient amount of the compound in toluene to give a concentration of approximately 200 μg/ml. Standards for gas–liquid chromatography were made by serial dilution of the stock to a concentration of 20 ng/ml. A 5-μl sample of this solution produced a peak with 70% full scale deflection on the gas chromatograph at working attenuation. Solutions of EBI used to fortify crop samples were prepared by dilution of the stock in absolute ethanol. Samples were fortified by the addition of 0.10–0.50 ml immediately before extraction.

Table I. Recovery of EBI from Various Commodities

EBI added, ppm	EBI recovered, %[a]		
	Apple	Tomato	Lettuce
0.047	93.8	85.9	86.8
0.094	108	105	105
0.472	86.5	86.8	90.1
0.944	94.8	99.7	95.9

[a] Values are the means of duplicate determinations.

Procedure. A sample of crop material (5.0 g) was homogenized with absolute ethanol (50 ml) by blending at high speed for 30 sec on a Sorvall Omni-Mixer. The homogenate was filtered through Whatman No. 1 paper and an aliquot (20 ml) of the filtrate placed in a 125-ml separatory funnel. After the addition of 1.0 *M* NaCl (80 ml) the aqueous phase was extracted with toluene (10 ml). The toluene layer was shaken with a fresh portion of 1.0 *M* NaCl (20 ml) and then with 1.0 *M* HCl (10 ml). An aliquot (1–2 ml) of the toluene extract was then passed through a small column of silicic acid prepared by placing a 1-cm bed of silicic acid on a glass wool plug in a pasteur pipet. A sample (5 μl) of effluent was analyzed by gas–liquid chromatography.

Gas–Liquid Chromatography. Samples were analyzed on a Hewlett Packard 5700 A gas chromatograph fitted with a ^{63}Ni electron capture detector and 6 ft × 4 mm i.d. glass column. The column was packed with 5% butanediol succinate on 100–120 mesh Chromosorb W, HP, and was preconditioned at 200°C for 48 hr under a flow of ar-