pesticides presents a completely new potential source of these compounds. It is significant indeed that other metabolites, e.g., TCAB and bis-substituted triazene have already been detected in soils that have been treated with anilines or aniline-containing compounds.

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Identification of Offensive Odor Compounds from Potato Processing Plant Waste Effluent Irrigation Fields

The volatile components from an Idaho potato processing waste effluent irrigation field were isolated and analyzed using chemical and adsorption chromatography separation techniques together with combined capillary gas chromatography–mass spectrometry. The components with the most offensive odors were identified as skatole (ca. 0.5 ppm of the soil) and geosmin (ca. 24 ppb of the soil).

The aqueous waste from major potato processing plants in Idaho generally is first neutralized and treated to remove solids. The resulting effluent, which may amount to more than 1000 gal/min, contains some small amount of solids and dissolved matter. This is run out onto fields which are used frequently for growing crops, taking advantage of the soluble nitrogen content of the effluent. Unfortunately, at certain times of the year, particularly during warm weather, the fields may develop odors which are offensive. The present study was undertaken to determine the nature of the compounds responsible for the offensive odors. Such basic information could aid in finding a solution to this problem.

EXPERIMENTAL SECTION

Materials. Authentic skatole was Eastman No. 1357. Other authentic samples were obtained from reliable commercial sources and purified by gas chromatography (GLC).

Several samples of soil were obtained from the waste effluent irrigation fields of a major potato processor in Idaho. They were shipped to Berkeley in polyethylene bags contained in wooden boxes.

Isolation of Volatile Oil. A 4-L volume of soil (4.7 kg) was placed in a 12-L flask together with sufficient water (ca. 4 L) to cover it. The mixture was treated for 3 h using vacuum steam distillation continuous extraction (Likens:Nickerson head) at 100 mmHg pressure with the aqueous soil at about 50 °C. Hexane was used as the extracting solvent. A refrigerated water/ethanol mixture at 0 °C was used to cool the condensor. The hexane extract was dried over sodium sulfate and filtered, and the solvent was removed at atmospheric pressure using low hold-up Vigreux distillation columns.

Separation into Fractions. The volatile oil was taken up in hexane (100 mL) and extracted in a separatory funnel with hydrochloric acid (4 N, 2 × 20 mL) to give (after neutralization with excess $NaHCO_3$) a basic fraction (1). The hexane solution was then extracted with sodium hydroxide solution (10%, 2 × 20 mL) to give (after acidification with dilute HCl) an acidic fraction (2). The remaining hexane solution (the neutral fraction, 3) was dried over sodium sulfate and placed on a column of activated alumina (Woelm neutral activity grade 1, 14 × 1.5 cm). The column was washed through with more hexane (200 mL) to give a hydrocarbon fraction (3a), then with diethyl ether (200 mL) to give an intermediate polarity fraction (3b), and finally with ether/methanol (10:1, 100 mL) to give an "alcohol" fraction (3c).

Capillary GLC–MS. A Pyrex glass capillary column, 150 m long by 0.64 mm i.d. coated with Tween 20 containing 5% Igepal CO-880 was used. Several different temperature programming conditions were used with combined GLC, mass spectrometry (GLC–MS). For the identification of skatole, the column was kept at a constant temperature of 170 °C with a helium carrier gas flow velocity of 50 cm/s. The column was coupled to a modified Consolidated 21-620 mass spectrometer through a silicone rubber membrane molecular separator.

Odor Evaluation. The odor threshold of skatole was obtained using procedures described previously (Guadagni et al., 1963) with Teflon bottles and tubing as containers for the odor solutions. Odor quality evaluation of fractions throughout was carried out by three-four experienced odor judges.

RESULTS AND DISCUSSION

The volatile oil, obtained from the potato processing waste effluent field soil, amounted to 10 parts per million parts (ppm) of the soil. This oil was found to be an extremely complex mixture of components by capillary GLC.

Initially the oil was separated into three main fractions, an acid fraction (1), a basic fraction (2), and a neutral fraction (3). Odor evaluation of these fractions indicated that the main offensive odor was in the neutral fraction (3). Further separation of fraction 3 using alumina chromatography gave three subfractions, i.e., a hydrocarbon fraction (3a), an intermediate polarity fraction (3b), and an "alcohol" fraction (3c). Odor evaluation of the subfractions indicated that the main offensive odor was in 3c. GLC separation of subfraction 3c with odor evaluation of the effluent from the GLC column indicated that the main offensive odor corresponded to the retention time of the main GLC peak. The compound corresponding to this GLC peak was found to have both mass spectrum (major ions *131*, 130, 77, 103, 65, 51) and GLC retention time (Kovat's Index 2329 Tween 20 capillary GLC column) consistent with that of skatole (3-methylindole).

Another component with a strong odor, which also occurred in the alcohol fraction, was geosmin (*trans*-1,10-dimethyl-*trans*-9-decalol) whose mass spectrum (parent ion 182, major ions 112, 69, 55, 43, 41) and GLC retention time (Kovat's Index 1716 on Tween 20 capillary) were also consistent with that of an authentic sample. Measurement of GLC peak areas and calculations based on the amount of volatile oil showed that the concentrations in the original soil were of the order of 0.5 ppm for skatole and 25 parts per 10^9 parts (ppb) for geosmin.

Other major components identified in the volatile oil, which however did not seem to contribute much to the total odor, were 5-methylfurfural, benzaldehyde, styrene, acetophenone, and indole. All had mass spectra and GLC retention data consistent with that of authenic samples.

Skatole has long been known to be an odorous component of feces (Noller, 1957; Brieger, 1877), and it can then be seen that this would be an objectionable odor. Its odor threshold in water solution was found in the present work to be 0.2 ppb of water (with 95% confidence limits of 0.16–0.25 ppb). There would seem to be an adequate amount (0.5 ppm) in the soil to provide a strong odor.

The concentration of geosmin at 25 ppb of the odorous Idaho soil is about 25 times the concentration found by some of the authors previously (Buttery and Garibaldi, 1976) in "normal" garden soil. The odor threshold of geosmin had been found (Buttery et al., 1976) to be 0.02 ppb of water and it would seem that geosmin must also contribute to the odor of the Idaho soil, blending with the skatole. Geosmin apparently can be a desirable odor, for example, as a flavor of red beets (Murray et al., 1975). But geosmin can also be a "musty, moldy, earthy" off flavor of dry beans (Buttery et al., 1976), and drinking water supplies (Rosen et al., 1970). It seems likely that the blending of geosmin with skatole would add to the offensive odor.

It might be noted that, although skatole is a secondary amine and secondary amines are usually reasonably basic, skatole was found in the neutral fraction in the present work. It was not extracted with 4 N hydrochloric acid. However, it is known that indole, like pyrrole, has "basic and acidic properties...about the same as those of water" (Noller, 1957). The nitrogen in skatole does not show the normal basic properties of secondary amines.

It is known that geosmin is produced by *Actinomycetes* (Gerber, 1968). The formation of skatole seems less specific and it is probably produced by a number of different species of bacteria. It has been reported to be formed by *Clostridium skatol* (Fellers and Clough, 1925) and *Escherichia coli* (Hopkins and Cole, 1903). It has been known for many years that skatole is formed by the putrification (which must involve bacteria) of protein material (Noller, 1957). Skatole is apparently a metabolic product formed from the breakdown of the amino acid tryptophan which occurs in reasonable amounts in potatoes (Synge, 1977).

The detection of skatole was repeated (using GLC–MS) with several soil samples received from Idaho. Most of the odor judges described the odor of the soil samples as being "pigsty"-like. It is interesting that skatole has been found to be a major volatile component of material in pigsties (Spoelstra, 1977) along with a number of fatty acids and phenolic compounds. Solutions of skatole in water at concentrations of the order of 1 ppm were also described by most judges as being pigsty like and similar to the odor of the samples of soil.

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