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Odor and Volatile Compounds in Liquid Swine Manure. II. Steam-distillable Substances

Akio Yasuhara* and Keiichiro Fuwa**

Division of Chemistry and Physics, National Institute For Environmental Studies, Yatabe, Tsukuba, Ibaraki 300-21

***Faculty of Science, University of Tokyo, Hongo, Bunkyo, Tokyo 113*

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Many aliphatic hydrocarbons, nitrogen-containing compounds, and other substances were isolated from neutral or alkaline liquid swine manure by steam distillation and column chromatography, and identified by means of gas chromatography-mass spectrometry. Aliphatic hydrocarbons have an oily odor, and nitrogen-containing substances, particularly indole, skatole, and *o*-aminoacetophenone, have a strong malodor. Sulfur-containing substances which have a strong malodor have been detected in a trace quantity.

Malodor has been the most frequent nuisance except noise in Japan. Swine buildings are among the most important sources of this malodor. Liquid swine manure evolves a very strong and offensive odor, mainly by means of an anaerobically microbial decomposition. Only a little knowledge has been obtained about the chemical nature of the odor of liquid swine manure. Seventeen carboxylic acids and four phenols in acidic liquid swine manure have been reported in a previous paper.[1] The odor of neutral or slightly alkaline swine manure is different from that of acidic swine manure. Anaerobically decomposed liquid swine manure shows a neutral or slightly alkaline nature. It has been thought that amines and sulfur-containing compounds play an important role with regard to malodor.[2,3] Steam distillation is a useful method to isolate volatile compounds containing odorous substances, although it is apt to lose very volatile compounds such as lower amines, or to decompose unstable substances such as sulfur-containing material.

This paper will deal with the separation by steam distillation and column chromatography of odorous components in neutral or slightly alkaline liquid swine manure, and with the results of the application of gas chromatography-mass spectrometry and the organoleptic technique to the determination of the compounds most responsible for the malodor.

Experimental

Gas Chromatography. A Shimadzu Model GC-5A gas chromatograph was equipped with dual-flame ionization detectors, a matrix temperature programmer, a dual-pen recorder, and a Takeda Riken -*tr*-2215 A digital integrator. The flame detectors were operated at a hydrogen-flow rate of 50 ml/min and an air-flow rate of 0.5 l/min. Some apparatus was also used for the organoleptic tests, as will be described below. The gas-chromatographic conditions were as follows: injector and detector temperatures, 300 °C; column temperatures for Fraction **A**, 80 °C for a minute, followed by an increase to 280 °C at a rate of 2 °C/min, and then held at 280 °C for 30 min; column temperatures for the other fractions, 70 °C for 3 min, followed by an increase to 270 °C at a rate of 8 °C/min, and then held at 270 °C until the completion of the analysis; carrier-gas (nitrogen) flow rate, 43 ml/min at 5 kg/cm^2 for Fraction **A** or 20 ml/min at 5 kg/cm^2 for the other fractions. A 3 m × 3 mm i.d. glass column packed with 5% Silicone SE-30 on 60- to 80-mesh, acid-washed, DMCS-treated Chromosorb W was used.

Mass Spectrometry. A JEOL Model JMS-D 100 mass spectrometer was connected with a JEOL JGC-20 K gas chromatograph and a JEOL JMA-2000 data-treatment system. The gas-chromatographic conditions were the same as above except for the size of the column. A 3 m × 2 mm i.d. glass column packed with 5% Silicone SE-30 on 60- to 80-mesh, acid-washed, DMCS-treated Chromosorb W was used. The mass-spectrometric conditions were as follows: ion-source temperature, 135 °C; ionizing current, 3×10^{-4} A; ionizing voltage, 25 V; accelerating voltage, 3 kV; scan range, *m/e* 35 to 400; scan time, 2.1 s; scan interval, 5 s. The data-treatment procedures were the addition of the mass spectra on several scans, the subtraction of the integrated background-mass spectra from the added mass spectra, and normalization by means of the most intense peak.

Organoleptic Test. This test was carried out by smelling the odor of compounds progressively eluted from the outlet of the gas chromatograph, which was modified as follows in order to study the quality of odors: the gas eluted from the end of the column was divided into two streams in a 10 : 1 ratio, a eleventh of the gas was led into the flame-ionization detector, and ten elevenths of the gas was emitted from the outlet of gas chromatograph for smelling. A 3 m × 3 mm i.d. stainless column was used for this test.

Sampling Procedure and Fractionation. The experiments reported here were performed using accumulated liquid swine manure collected from pits under confinement swine buildings near Takezono, Tsukuba New Town, Ibaraki, Japan The liquid manure (52 l) was steam-distilled. The distillate was saturated with sodium chloride and extracted two times with

Table 1. Fractionation of the extract by liquid chromatography

First chromatography[a)]		Second chromatography[b)]		Fraction
Solvent	V/ml	Solvent	V/ml	
Hexane	150	Hexane	150	**A**
		Hexane	300	**B**
Benzene	350	Benzene	100	**C**
		Benzene	100	**D**
Ether	650	Benzene	100	**E**
		Benzene	50	**F**
		Benzene	100	**G**

a) Silica gel was used as an adsorbent. b) Alumina was used as an adsorbent.

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* Author to whom correspondence should be addressed.

dichloromethane. The extract was evaporated by means of a Kvrderna-Danish concentrator under atmospheric pressure. The concentrated residue was dissolved in ether (50 ml), and the solution was washed with an aqueous solution of sodium hydroxide in order to eliminate the phenol compounds. The solution was then concentrated and chromatographed over silica gel (60 g) (Wako Gel obtained from Wako Pure Chemicals Industries). As elution solvents, hexane, benzene, and ether were used. Each fraction was chromatographed again over neutral alumina (90 g), obtained from the Merck Co., Ltd. The details of the first and second chromatographies are shown in Table 1.

Results and Discussion

The steam-distillate had a malodor, but it was relatively different from the odor of neutral or slightly alkaline liquid swine manure. It is considered that lower aliphatic amines might be lost and some sulfur-containing compounds might decompose during steam distillation. Fractions **A**, **C**, **D**, **F**, and **G** have a malodor. Figures 1 to 7 show gas chromatograms of Fractions **A**, **B**, **C**, **D**, **E**, **F**, and **G** respectively. A large quantity of extracted matter was contained in Fractions **A** and **C**.

The components included in each fraction were identified by gas chromatography-mass spectrometry. No other identification method such as IR, NMR, and UV, has been practical because of the small quantity of extracted matter and the difficulty of separating components of a similar chemical nature. Fraction **A** had a roasted rubber-like or oily odor, and it consisted mainly of alkanes whose origins were unknown. Table 2 shows the results of the identification of Fraction **A** and the content ratio of each component, which was calculated from the areas of the peaks on the gas chromatogram, corrected by the relative sensitivity of each normal alkane against the gas-chromatographic detector. 2,6-Di-*t*-butyl-*p*-cresol, only a little of which was present in Fraction **A**, was the main component in Fraction **B**; its presence in liquid swine manure has already been reported in a previous paper.[1] From the mass spec-

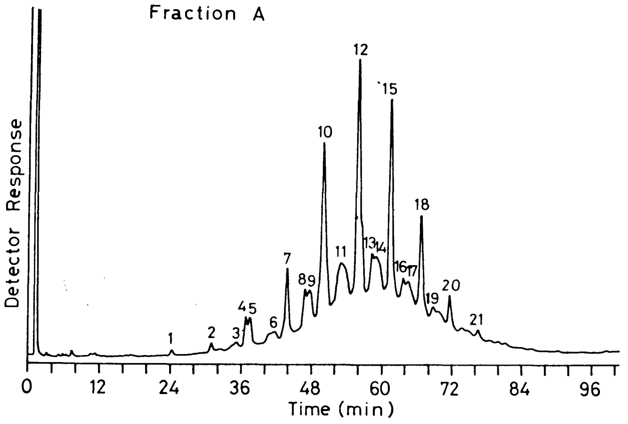

Fig. 1. Gas chromatogram of fraction **A**.

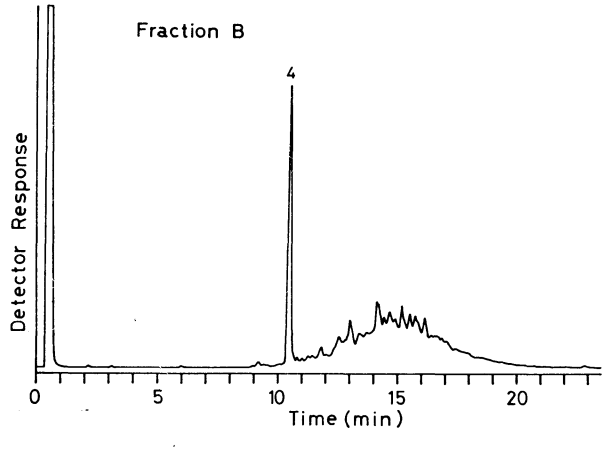

Fig. 2. Gas chromatogram of fraction **B**.

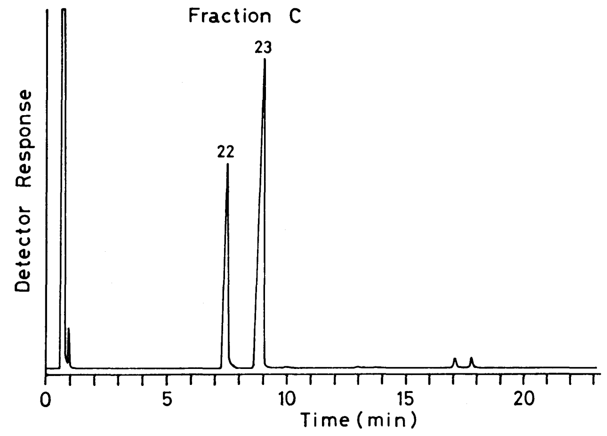

Fig. 3. Gas chromatogram of fraction **C**.

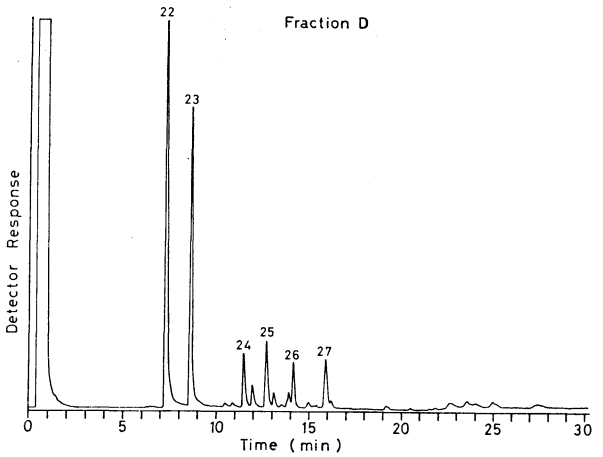

Fig. 4. Gas chromatogram of fraction **D**.

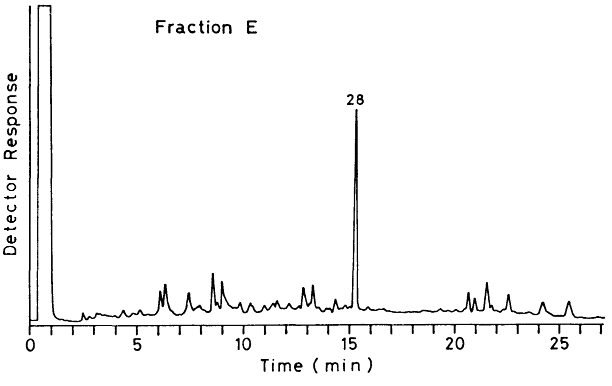

Fig. 5. Gas chromatogram of fraction **E**.

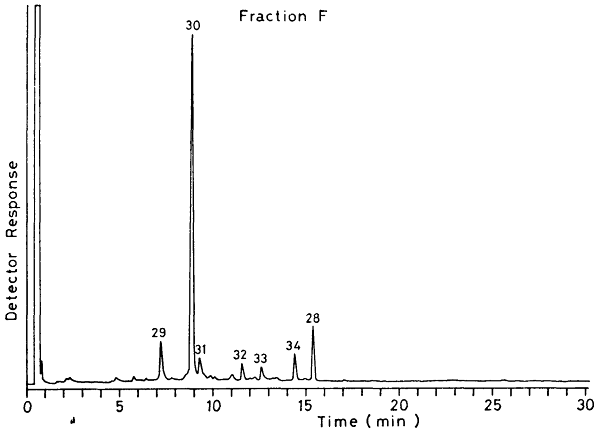

Fig. 6. Gas chromatogram of fraction **F**.

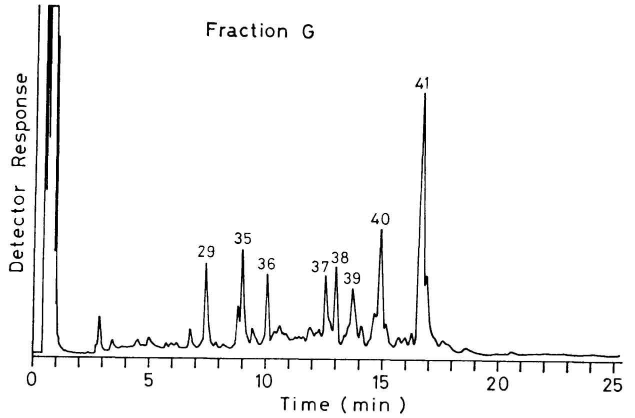

Fig. 7. Gas chromatogram of fraction **G**.

TABLE 2. COMPONENTS OF FRACTION **A**

Peak number	Name	Content ratio (%)
1	Tridecane	0.1
2	Tetradecane	0.2
3	Pentadecane isomer	0.4
4	2,6-Di-*t*-butyl-*p*-cresol	2.0
5	Pentadecane	
6	$C_{16}H_{30}$	0.7
7	Hexadecane	2.4
8	Octadecane isomer	6.2
9	Heptadecane isomer	
10	Heptadecane	8.7
11	Nonadecane isomer	12.4
12	Octadecane	14.7
13	Nonadecane isomer	11.5
14	Nonadecane isomer	
15	Nonadecane	12.3
16	Eicosane isomer	8.2
17	Eicosane isomer	
18	Eicosane	7.6
19	Heneicosane isomer	1.8
20	Heneicosane	6.9
21	Docosane	0.2

TABLE 3. RESULTS OF IDENTIFICATION OF FRACTIONS, **C**, **D**, **E**, **F**, AND **G**

Peak number	Name
22	Indole
23	Skatole
24	Tetradecanal
25	Pentadecanal
26	Hexadecanal
27	$C_{18}H_{34}O$
28	Dibutyl phthalate
29	*o*-Aminoacetophenone
30	Unknown
31	Unknown
32	Unknown
33	Unknown
34	Unknown
35	2-(1-Propenyl)-6-methoxyphenol
36	3,4-Dimethoxyacetophenone
37	2-(Butylamino)benzoxazole
38	2-Tridecanol
39	Unknown
40	Unknown
41	Unknown

trum normal alkanes can be distinguished from isoalkanes, but structure determinations of the isoalkanes are almost impossible using only the mass spectra.

Table 3 shows the results of the identification of Fractions **C** to **G**. Fraction **C** consisted almost entirely of indole and skatole, which are well-known to have a fecal odor and which had already been detected in liquid animal manure.[4] However, the odors of indole and skatole are quite different from that of the swine manure, although they are thought to contribute to the total odor of the swine manure. Fraction **D** contained some aldehydes besides indole and skatole. However, the aldehydes did not play any role in the odor of swine manure. Fraction **E** consisted exclusively of dibutyl phthalate (DBP), which was also confirmed, with a peak enhancement, by the co-injection of an authentic substance into the gas chromatograph. DBP is an odorless compound and a well-known pollutant; thus, its origin in liquid swine manure may not be a metabolic process, but, rather, it may be a pollution from the synthetic polymers.

Fractions **F** and **G** had a characteristic and tenacious malodor. The organoleptic test showed that a compound with a peak number of 29 had the odor. It was concluded from its mass spectrum to be aminoacetophenone. Further, it was confirmed to be *o*-aminoacetophenone by means of a comparison with an authentic substance using gas chromatography. As the odor of this compound has been recognized for the first time by column chromatography, it must fuse with odors of other odoriferous substances to become a new malodor. Many components in Fractions **F** and **G** could not be identified by mass spectrometry alone.

Trace components which did not appear as peaks in the gas chromatograms with the detector's gain, shown in Figs, 1, 3, 5, and 6, were also identified by gas chromatography-mass spectrometry; most of them were odoriferous. They are trithiapentane and terathiahexane in Fraction **A**; ethylindole, 2-pentadecanone, and 2-hexadecanone in Fraction **C**; 3,3-dimethyl-2-thiapentane and benzothiazole in Fraction **E**, and quinazoline in Fraction **F**.

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References

1) A. Yasuhara and K. Fuwa, *Bull. Chem. Soc. Jpn.*, **50**, 731 (1977).

2) J. R. Miner and T. E. Hazen, *Trans. Am. Soc. Agr. Eng.*, **12**, 772 (1969).

3) J. Ronald Miner, "Odors from Confined Livestock Production," Office of Research and Development, U. S. Environmental Protection Agency, Washington, D.C., 1974.

4) W. E. Burnett, *Environ. Sci. Technol.*, **3**, 744 (1969).