Studies on Cow's Urine

Part III. Examination of an Oil Obtained from Cow's Urine by Gas Chromatography

By Rikisaku SUEMITSU, Shin-ichi FUJITA and Hiroshi MATSUBARA

*Department of Technical Chemistry, Faculty of Technology,
Doshisha University, Kyoto.*

Received May 17, 1965

An oil obtained from cow's urine was examined by means of gas chromatography. Ethylbenzene, phenol, *m*-cresol, *p*-cresol, and *p*-ethylphenol were identified as the major components of the oil, while there were at least four components still remaining unknown.

A hypothesis concerning the degradation of equol,¹⁾ 7-hydroxy-3-(4'-hydroxy) chroman, to *p*-cresol and *p*-ethylphenol in the urine was proposed.

INTRODUCTION

As reported previously,^{2,3)} equol and an oil were isolated from cow's urine, when active carbon was used as the adsorbent. This oil was apparently the cause of the peculiar odour of cow's urine, and *p*-cresol and phenol were identified as the chief components.

Though *p*-cresol and phenol are chiefly responsible for the peculiar odour of the oil, judging from its odour, there seemed some other components present except for them. Thus the oil was analysed by means of gas chromatography.

Apiezon grease L (AG-L), Polyethylene glycol-6000 (PEG-6000), and 2,4-Xylenylphosphate were employed as the stationary phases for the separation and identification of components of the oil. The components were identified by comparing these retention times with those of the known samples.

EXPERIMENTAL

Apparatus The gas chromatographic equipment used here was a Yanagimoto type GCG-3.

1) E. Anderson and F. Marrian, *J. Biol. Chem.*, **127**, 649 (1939).

2) R. Suemitsu and M. Hiura, *J. Agr. Chem. Soc. Japan*, **27**, 825 (1953).

3) R. Suemitsu, M. Hiura, and M. Nakajima, *ibid*, **29**, 591 (1955).

Material The method to isolate the oil was previously reported.²⁾ The physical properties of the oil which was separated in 1953 are as follows: b. p. 35~60°C/10 mmHg, d_4^{20} 1.014, n_D^{20} 1.509.

Procedure It was found that the combination columns of AG-L and PEG-6000 were the most effective after examination of a few other stationary phases. The oil was submitted to gas chromatography under the following conditions.

Column: AG-L (ϕ 0.5×200 cm) and PEG-6000 (ϕ 0.5×100 cm) Temperature: 170°C, Carrier gas: H₂, Flow rate: 100 ml/min.

The mixture of ethylbenzene, phenol, *o*-, *m*- and *p*-cresol was also examined as the standard samples

FIG. 1. Chromatogram of the Oil Obtained from Cow's Urine.

FIG. 2. Chromatogram of a Mixture of *o*, *m*, *p*-Cresols, Phenol, and Ethylbenzene.

Peak No.	Compound	Peak No.	Compound
1	Ethylbenzene	3	<i>o</i> -Cresol
2	Phenol	4	<i>m</i> -Cresol & <i>p</i> -Cresol

AG-L and PEG-6000, 170°C, 100 ml/min. H₂

under the same conditions. The chromatograms were presented in Fig. 1 and 2 respectively. As shown in Fig. 1 and 2, the retention time of the peak 4 coincided with that of phenol. Considering the peak 6, the authors supposed that the main peak of the chromatogram was ascribable to *p*-cresol or *m*-cresol. It is difficult to separate the isomers by gas chromatography employing the ordinary stationary phases as shown in Fig. 2, for their boiling points are quite close each other. It was reported⁴⁾ that they were successfully separated each other by gas chromatography employing 2,4-xylenylphosphate as a stationary phase. Then it was employed in our experiment. Experimental conditions were as follows:

Column: 2,4-xylenylphosphate (ϕ 0.5 $\times$ 800 cm); Temperature: 120°C, Carrier gas: H₂; Flow: 150ml/min.

The mixture of ethylbenzene, phenol, *o*-, *m*-, and

FIG. 3. Chromatogram of the Oil Obtained from Cow's Urine.

2,4-Xylenylphosphate, 120°C, 150 ml/min. H₂.

FIG. 4. Chromatogram of a Mixture of *o*, *m*, *p*-Cresols, Phenol, and Ethylbenzene.

Peak No.	Compound
1	Ethylbenzene
2	Phenol
3	<i>o</i> -Cresol
4	<i>p</i> -Cresol
5	<i>m</i> -Cresol

2,4-Xylenylphosphate, 120°C, 150 ml/min. H₂.

FIG. 5. Chromatogram of the Oil Corresponding to the Peak of No. 8.

2,4-Xylenylphosphate, 135°C, 120 ml/min. H₂.

4) V. T. Brooks, *Chem. and Ind.*, **17**, 1317, (1959).

p-cresol was also examined as the standard samples by the same method as in Fig. 2. The chromatograms obtained were presented in Fig. 3 and 4. Though the peaks of *m*- and *p*-cresols were not sufficiently separated, their presence were clearly recognized. Then, ethylbenzene, phenol, *m*-cresol, and *p*-cresol were identified as components of the oil.

As the peak 8 occupied the considerable large part of the chromatograms, an attempt to separate the

component from the others by gas chromatographic method was undertaken. A glass-traps,⁵⁾ cooled with the freezing mixture, was equipped to condense the gas at the end of the column. In order to prevent the contamination of other components and obtain the substance purely, ether was injected to wash out the component before and after cutting the component of the peak 8. The experiment was carried out under the following conditions.

Column: 2,4-xyleneiphosphate (ϕ 0.5 $\times$ 300 cm); Temperature: 135°C, Carrier gas: H₂; Flow rate 120 ml/min.

In these procedures, a kind of colorless oil, having a peculiar odour, was obtained. As this oil was supposed to be *p*-ethylphenol, it was submitted to gas chromatography again and its retention time was compared with that of the authentic sample. As shown in Fig. 5 and 6, the retention time of the oil coincided with that of *p*-ethylphenol. It was impossible to separate the component of *p*-ethylphenol from the others completely by gas chromatographic method because of the small quantity of the sample. Therefore, small parts of *m*- and *p*-cresols appeared to remain as shown in Fig. 5. The infrared spectra of two substances, however, were essentially identical as shown in Fig. 7. Consequently, *p*-ethylphenol was proved to be a component of the oil.

FIG. 6. Chromatogram of *p*-Ethylphenol.
2,4-Xylenylphosphate, 135°C, 120 ml/min. H₂.

FIG. 7. Infrared Spectra of *p*-Ethylphenol (A) and the Oil Corresponding to the Peak of No. 8.

5) H. Sugisawa, *Kagaku to Kogyo*, **18**, 417 (1965).

Quantitative Determination of the Compound in the Oil The determination was carried out by measuring areas of the peaks of Fig. 1. As shown in Fig. 3, peaks of *m*- and *p*-cresols were not sufficiently separated for measurement of each area. Then, a smaller quantity of the oil was injected to accomplish the separation and to obtain a ratio of *p*-cresol to *m*-cresol. The chromatogram obtained was shown in Fig. 8 and the components of the oil obtained from cow's urine were shown in Table I.

FIG. 8. Chromatogram of the Oil Corresponding to the Peaks of No. 6 and No. 7.

2,4-Xylenylphosphate, 120°C, 100 ml/min. H₂.

TABLE I. COMPONENTS OF THE OIL OBTAINED FROM COW'S URINE

Peak. No.	Rt.	Component	%
1	1.5	Unknown	2.1
2	2.3	Ethylbenzene	0.8
3	7.9	Unknown	0.2
4	10.2	Unknown	0.3
5	11.3	Phenol	0.4
6	15.2	<i>p</i> -Cresol	47.0
7	15.3	<i>m</i> -Cresol	16.7
8	22.6	<i>p</i> -Ethylphenol	31.3
9	32.8	Unknown	1.2

DISCUSSION

As previously reported, phenol and *p*-cresol

were identified as chief components of the oil obtained from cow's urine. In this paper, *m*-cresol, *p*-ethylphenol, and ethylbenzene were furthermore identified. Consequently, the peculiar odour of cow's urine is ascribable to phenol, *m*- and *p*-cresols, *p*-ethylphenol, and ethylbenzene.

The structure of equol is shown in [I]. If equol is decomposed in the dotted line I as shown in the chart, *p*-cresol should be the product whereas in the dotted line II, *p*-ethylphenol should be obtained. As equol was adsorbed from cow's flesh urine with active carbon used as a adsorbent, and could not be adsorbed from the urine after three days'. Therefore equol would be easily degraded in the urine. On the occurrence of cresols in cow's urine, some other mechanisms may be taken into consideration. In fact, equol has no relation with the occurrence of *m*-cresol. However, it might be said that equol is degraded to *p*-cresol and *p*-ethylphenol by some enzyme system in the urine.

Acknowledgement. The authors wish to express their thanks to Dr. M. Hiura of Rakuno College, Nopporo, for valuable advice and encouragement.