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18. Keiichiro Hayashi and Yohei Hashimoto : Studies on the Microanalysis of Essential Oil Components. III.[1] The Detection of Aldehydes by Paper Chromatography.

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The authors already reported[1,2] studies on chromatographic separation of essential oil components including alcohols or some volatile oil compounds which are important for perfume preparation.

Several chromatographic methods of aldehyde separations have appeared hitherto, including the work reported by Newcombe *et al.*[3] by which separation of vanillin from its mixture with methylvanillin was performed by using a paper impregnate with sodium hydrogen sulfite.

In the present series of experiments the authors successfully carried out a method by which aldehyde perfumes were well separated on a filter paper. As it was mentioned in the previous paper,[2] almost all kinds of essential oil components, or compounds which are difficultly soluble in aqueous solvent could be separated on silicone-treated paper to form well separated spots. By the same procedure, numerous aldehydes were treated in this laboratory with excellent results.

The other method was tried to convert aldehyde and lactone into water-soluble hydrogen sulfite compounds, which would make it possible to treat them by the usual partition chromatography with aqueous stationary phase on paper.

The aldehydes of 8~12 carbon atoms are distributed widely in lemongrass, sweet orange, lemon, nelori oils, and vanillin and coumarin are also found in vanilla and tonka beans. It was ascertained that vanilla flavors containing several homologs, such as methylvanillin, ethylvanillin, heliotropin, or coumarin are readily chromatographed on paper.

The colored paper chromatograms were also examined by a double-beam, automatic recording densitometer and a quantitative curve with linear relation between the amount of sample and reading of curves was obtained. In this way a new procedure of chromatographic separation of aldehydes for perfume analysis or estimation of flavors was established.

I] Chromatography with Silicone-treated Paper

The method using a silicone-treated filter paper was applied to the separation of carbonyl compounds to form clearly outlined spots on papergram after spraying. Other procedure can be performed in much the same way which was mentioned in the previous reports.[1,2]

A filter paper strip (Toyo Roshi No. 52, 1.5×18 cm.) was immersed in 2% benzene solution of Dow Corning Silicone 1107. After evaporation of benzene, the strip was heated to 150° during 0.5 hr. While an orange red or yellow color appeared in the presence of aromatic aldehydes, aliphatic aldehydes, such as citronellal, usually appeared yellow by reagent (A). The exposure of paper chromatogram in vapor of ammonia was often effective in revealing yellow colored spots, and therefore, more intense coloration was obtained by treatment with reagent (C).

The following solvents and color reagents were used.

Color Reagents :

(A) 0.1% solution of 2,4-dinitrophenylhydrazine.

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1) Part II. K. Hayashi, Y. Hashimoto : This Bulletin, **4**, 496(1956).
2) T. Kariyone, K. Hayashi : *Ibid.*, **4**, 494(1956).
3) A. G. Newcombe, S. G. Reid : Nature, **455**, 172(1953).

(B) 2% solution of *p*-phenylenediamine, 3% H_2O_2, and 2*N* AcOH
(C) 1% solution of *p*-nitroaniline in conc. HCl

The yellow condensation product of *p*-nitroaniline with aliphatic and aromatic aldehydes is a kind of a Schiff base.

Developing Solvents :

(1) 5% Ether in ligroine (70~80°)
(2) Ligroine (70~80°)
(3) Methanol
(4) Benzene-ethanol (1:1)

The sample solution (usually dissolved in 10~20 volumes of hexane or EtOH) was spotted at the start line drawn 3 cm. from the end of paper strip and then developed.

Table I. Rf Value of Aliphatic Aldehydes on Silicone-treated Paper

Solvent	(1)	(2)	(3)	(4)
Caproaldehyde	.52	.62	.56	.55
Enanthaldehyde	.59	.66	.63	.64
Caprylaldehyde	.68	.70	.68	.69
Pelargonaldehyde	.74	.73	.70	.72
Decanal	.80	.82	.76	.81
Undecanal	.84	.84	.83	.82
Lauraldehyde	.86	.88	.92	.90

Table II. Rf Value of Aromatic and Terpenic Aldehydes

Solvent	(1)	(2)
Benzaldehyde	.62	.74
p-Tolualdehyde	.60	.68
p-Anisaldehyde	.64	.66
Cinnamaldehyde	.72	.69
Citronellal	.72	.76
Citral	.75	.72

The excellent separation was observed in the case of aldehydes of 6~12 carbons, which belong to aromatic terpenic aldehyde, after trying several kinds of developing solvents.

II) Chromatography with Sodium Hydrogen Sulfite Compounds

The method of $NaHSO_3$-treated paper was also applied to the chromatography of hydrogen sulfite adduct of aldehydes. As it will be described in the method of purification, coumarin also combines with hydrogen sulfite to form a complex, though it has no aldehyde group in its molecule.[4] The coumarin is fixed, thereby forming a stable, non-volatile, soluble compound. This method can also be applied to other coumarin derivatives.

Color Reagents for Vanillin, Ethylvanillin, Methylvanillin, and Heliotropin

(A) 0.5% solution of 2,4-dinitropheylhydrazine in 2*N* H_2SO_4
(B) 2% solution of *p*-phenylenediamine, 3% H_2O_2, and 2*N* AcOH[5]
(C) 1% solution of *p*-nitroaniline in conc. HCl
(D) Dark violet fluorescence under ultraviolet light by immersing paper into 5% KOH solution.

Detection for coumarin was made by the application of (D) and a green fluorescence appeared under ultraviolet light.

Table III. Rf Value of Aldehyde-Sodium Hydrogen Sulfite Adduct

Solvent	(1)	(2)
Vanillin	.13	.12
Ethylvaillin	.32	.38
Methylvanillin	.54	.58
Heliotropin	.72	.70
Coumarin	.50	.66

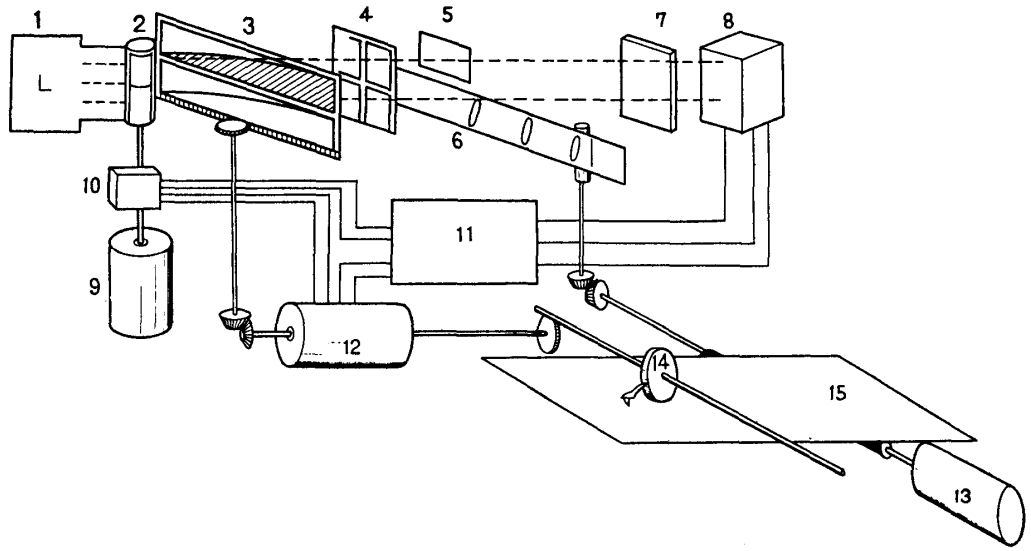
Developing Solvents

(1) Ligroine (70~80°)
(2) Hexane

4) U. S. Pat. 1,945,184; 1,945,182; French Pat. 722, 406(1931).
5) F. Feigl : "Spot Tests," Elsevier Inc., Amsterdam, **11**, 153(1953).

The hydorogen sulfite adduct of aldehydes could usually be dissolved in 10~20 volumes of water. The sample solution was spotted on the start line drawn 7 cm. from the end of paper strip and then developed by the solvent. After 5~6 hrs.' development, the paper strip was taken out and dried.

(III) **Estimation of Aldehydes by Scanning Paper Chromatograms with Automatic Recording Densitometer**



1 Lamp house
2 Rotary Sector
3 Adjustable Slit
4 Slit
5 Standard Filter paper
6 Sample Filter paper
7 Colored glass filter
8 Photomultiplier
9 Synchronous Motor
10 Phase Shifter
11 Amplifier (low cycle triple amplification)
12 Contary Motor
13 Warren Motor
14 Recording Pen
15 Recording Paper

Fig. 1. Densitometer

Among the numerous results obtained by densitometry, a typical procedure is described in the present paper by which a rapid estimation of vanillin was carried out. 1% standard solution of vanillin was spotted on the start line of a filter paper (Toyo Roshi No. 52, 2×40 cm.) with a micropipet to deposit 50 γ of a sample. In a second paper strip of experimental series 100 γ was spotted. These strips were developed with ligroine (70~80°) or hexane system solvents by the usual ascending method, dried, and sprayed with color reagent (A). The densitometry should preferably be completed during 1 hr., starting 2 min. after spraying. Since the background of paper strip is colored yellow by reagent (A), orange red spots of aldehyde can be scanned well by cobalt glass filter which is inserted in the position 7 (Fig. 1). The quantitative analysis of each compound was determined by automatic recording densitometer for paper chromatogram. The ratio of concentration for various densities is shown in Table IV. The scanning of vanillin quality is shown in Fig. 2, and the area occupied by each peak on the curves, caused by vanillin spot, was measured. A linear relation was proved to exist between the amount of sample and area measured (Table IV) by repeating at least several experiments simultaneously.

Table IV. Densitometric Data of Vanillin

Amount (γ)	Area (1)	Area (2)
50	17.5	30.5
100	34.5	63.5
150	55.0	90.5
200	—	118.0
250	—	148.5

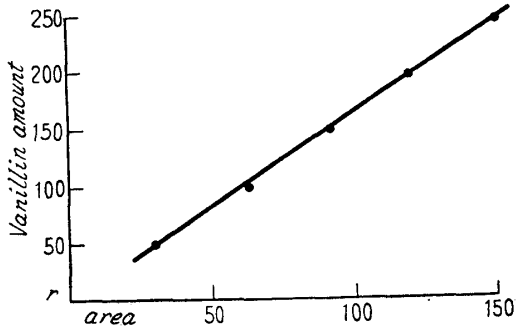


Fig. 2. Relation between Area and Amount

It is noteworthy that paper chromatogram sprayed with tinted reagent was measured only by using a densitometer of double beam system, inserting filter paper of the same color as the background of papergams as contrast 5 in Fig. 1, though it was often necessary in these experiments to examine chromatograms with orange spots on yellow background.

The densitometer used in this work was constructed in the authors' laboratory by the support of Toyo Filter Paper Co. and also by the suggestion of Mr. K. Amaya, Scientific Faculty, Kobe University.

Experimental

Preparation of $NaHSO_3$-treated Paper—The filter paper strip (Toyo Roshi No. 52, 2×40 cm.) was immersed in 2% solution of $NaHSO_3$, excess solution was removed by pressing it between two sheets of dry filter paper, and dried at room temperature.

Chromatography of Aldehydes by Hydrogen Sulfite Method—The sample containing aldehydes or lactones was mixed with several vols. of 30~35% $NaHSO_3$ solution and heated on a water bath until the crystallization occured, by which the formation of $NaHSO_3$ adduct was completed. It is preferable to heat the solution above the melting point of the sample. This treatment should be done especially in the case of coumarins. The crystals of a bisulfite adduct are filtered and washed with ether or EtOH.

Summary

The analysis of aldehydes which are available for perfume preparation was successfully carried out by paper chromatography. A method of converting aldehydes into hydrogen sulfite compounds, which makes it possible to treat them by the usual paritition chromatography with aqueous stationary phase, was studied. Studies were also made on developing aldehydes by a reversed phase chromatography on paper previously treated with an organic silicone resin. These two methods are considered to be helpful in the microanalysis of perfumes containig several aldehyde components. Quantitative results were also described by double-beam, automatic recording densitometer.[6]

(Recieved December 3, 1956)

6) Y. Hashimoto: "The Recent Progress in Paper Chromatography." Symposium at the Kinki Local Meeting of the Pharmaceutical Society of Japan, October 20, 1956.