

23. Goro Chihara : Measurement of Infrared Absorption Spectra by Absorption on Japanese Hand-made Paper and Its Application to Paper Chromatography.

(Division of Biochemistry, Central Clinical Laboratory, Tokyo University Hospital*)

Studies on the combination of paper chromatography and infrared spectral measurement are desirable but past studies were chiefly on the spectral measurement of the extract obtained from chromatogram already developed. Some attempts have been made to measure the infrared spectrum directly from the developed filter paper^{1,2)} but none of these seems to have made any headway.

Examination of various filter papers and Japanese hand-made papers was made and it was found that a good quality hand-made paper from Kochi Prefecture ("Tenguchō," a very thin paper made from pure fibers of *Broussonetia Kazinoki* SIEB. and of *Edgeworthia papyrifera* SIEB. et ZUCC.) is suited both for infrared absorption spectral measurement and paper chromatography.

In this method, a sample is absorbed by this paper, impregnated with liquid paraffin to avoid running, and this is submitted to infrared spectral measurement. This method also offers one means of minimizing the amount of sample used, 0.1 mg. of a sample being sufficient to determine the absorption of a carbonyl group.

In general, absorption energy in the infrared region is only a fraction of that in the visible or ultraviolet region. Therefore, it is not possible to carry out sensitive measurement with a sample of around 20 γ , which is the original purpose of paper chromatography, but the measurement can be made with a minimum of 100 γ of any sample. It may be possible to apply this method in case the measurement in visible and ultraviolet region is meaningless.

Method and Results

1) **Apparatus**—Measurement was made on the Hilger H-800 type, double-beam, recording infrared spectrophotometer with NaCl or KBr prism.

2) **Japanese Hand-made Paper**—The paper has been made from olden times by hand, using the fiber of *Broussonetia Kazinoki* SIEB. and of *Edgeworthia papyrifera* SIEB. et ZUCC., and consists almost entirely of pure α -cellulose-I, free from glue and other impurities. The choice of this paper is very important and the highest quality paper made in Kochi Prefecture was used throughout the present work.

a) The paper was soaked in 18% NaOH at 20° for 30 mins. and 82% was recovered.

b) X-Ray diffraction was measured by the use of Cu-K α line, at 35 kV, 12 mA, and $2\theta = 5\sim 35^\circ$, and the curve shown in Fig. 1 was obtained. This result indicates that this paper consists of pure α -cellulose-I and the degree of crystallization may be around 70%.

c) The thickness of the paper used was 0.028~0.034 mm.

3) **Comparison of Infrared Absorption Spectra of Japanese Paper and Filter Papers :**

a) Liquid paraffin was applied to the Japanese paper to avoid dispersion and its infrared absorption spectrum was measured. The results are presented in Fig. 2(a), measured with NaCl prism in the region of 4000~650 cm^{-1} , and in Fig. 3(a), measured with KBr prism in the region of 1000~350 cm^{-1} . These results also indicate that this paper is made of cellulose-I with a high degree of crystallization.

The absorption of this Japanese paper shows almost 90% transmittance in the region of 2700~1500 cm^{-1} and absorption in the region of 900~350 cm^{-1} is very weak. This indicates that the paper is available for the measurement of absorptions for carbonyl compounds, out-of-plane vibration of

* Hongo, Tokyo (千原吳郎).

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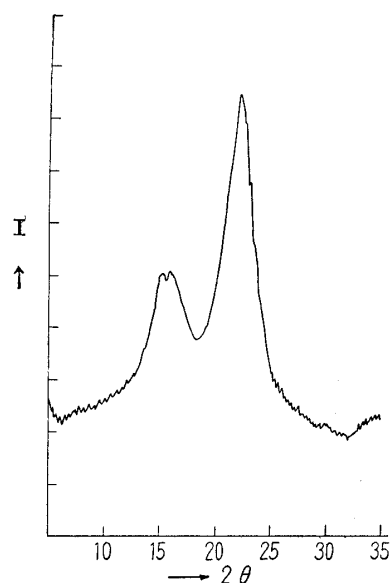


Fig. 1.
X-Ray Diffraction Pattern of
Japanese Hand-made Paper

benzene ring, and the region of KBr.

b) Cellulose absorption can be compensated almost wholly by the use of a reference by double-beam spectrometer. The curves (b) in Figs. 2 and 3 are absorptions of the blank so obtained.

c) Absorption of various filter papers, measured for comparison, impregnated with liquid paraffin to avoid dispersion, is shown in Fig. 2. No significant differences were found with Whatman No. 1, Schleicher Schull No. 2043a and b, and Toyo Roshi Nos. 50, 51, and 52.

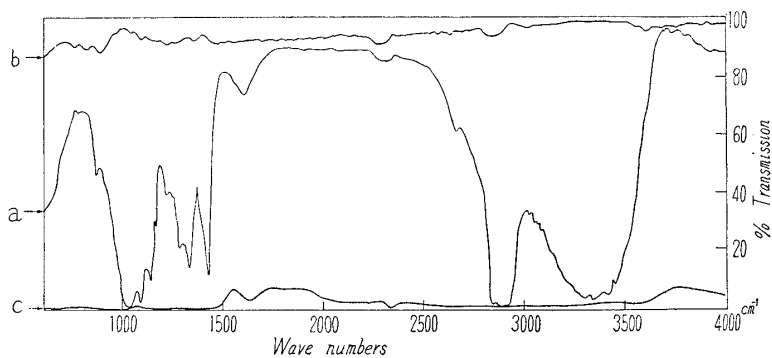


Fig. 2. Comparison of Infrared Spectra of Japanese Hand-made Paper and Various Filter Papers

a: Japanese paper. b: Absorption compensated by the use of the same Japanese paper for reference. c: Whatman No. 1, Schleicher Schull No. 2043a and b, Toyo Roshi Nos. 50, 51, and 52.

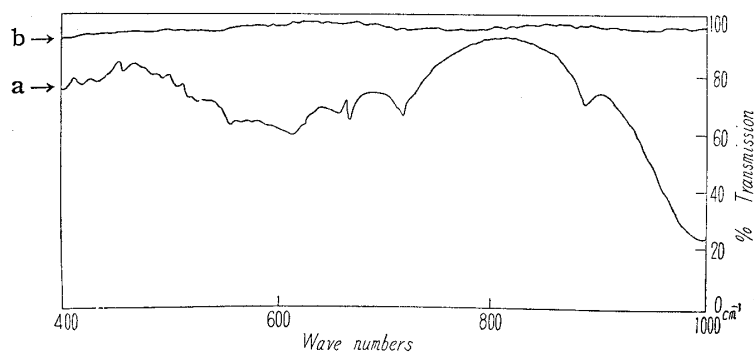


Fig. 3. Infrared Spectra of Japanese Hand-made Paper (KBr region)

a: Japanese paper. b: Absorption compensated by the use of the same Japanese paper as reference.

4) Measurement of Infrared Absorption Spectrum by Adsorption on Japanese Hand-made Paper

—The sample is absorbed by the Japanese hand-made paper, painted with liquid paraffin, and submitted to spectral measurement. When the sample is a liquid, it is used as such and when solid, it is dissolved in a suitable solvent, preferably highly volatile, and the solution is soaked by the paper uniformly. The size of the paper should be the size of the focal point of light, the size being less than 2×10 mm. in the case of Hilger Model H 800. It is, therefore, possible to minimize the size and the use of a micro-illuminator would offer further scope of interesting applications.

Fig. 4(a) gives the absorption of acetophenone by this method, using 0.1 mg. of a sample and a blank paper as reference. For the sake of comparison, absorption of acetophenone measured in 0.1-mm. thickness is given in the same Fig. 4(b). These curves indicate that the absorptions of carbonyl and out-of-plane vibration of a benzene ring have been taken with a very small amount of sample.

Fig. 5. shows the absorption of *m*-cresol measured under the same conditions with 0.1 mg. of a sample and out-of-plane vibration of benzene ring is apparent.

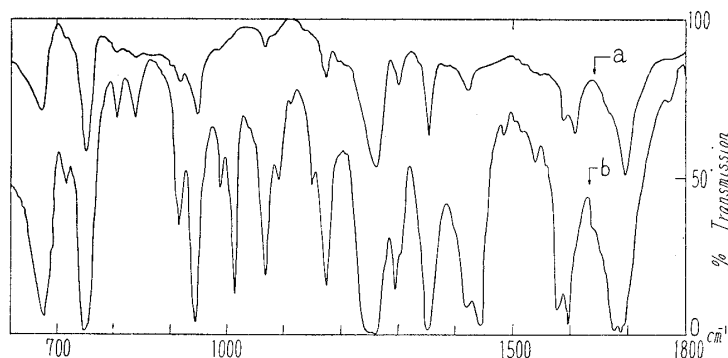


Fig. 4. Infrared Absorption Spectra of Acetophenone
 a : 0.1 mg. of sample by absorption on Japanese hand-made paper. Blank paper used as reference.
 b : In 0.1-mm. thickness, by the capillary method.

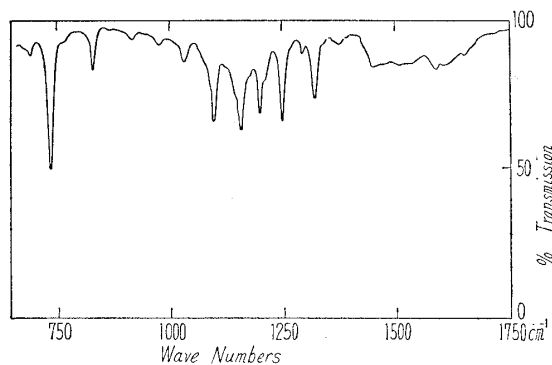


Fig. 5
 Infrared Absorption Spectra of *m*-Cresol
 (Japanese Hand-made paper)

5) **Application to Paper Chromatography**—The use of the same Japanese hand-made paper to paper chromatography results in faster rate of solvent ascending and a good partitioning. The filter paper used in paper chromatography serves merely as a carrier and its thickness is not an important factor. Some examples will be given below for actual use of the Japanese paper, which was cut perpendicular to the direction of fibers into strips of 1.5 cm. in width and about 30 cm. long.

a) About 100 γ of the ether extract from fungal component of *Penicillium islandicum* Sopp. was developed in the usual manner, using a mixed solvent of Me_2CO :petr. benzene: H_2O (5:5:3.5). Similar to the use of filter papers, the band of erythroskyrin³⁾ separated out. This portion was cut out, painted with liquid paraffin, the beam of infrared ray was adjusted to this band, and its spectrum was measured, using a blank paper for reference. The curve obtained is given in Fig. 6, together with that of pure erythroskyrin taken by the usual Nujol method. As will be seen from this figure, the positions of absorption in both curves agree and this indicates that identification is possible and it will offer a means of making some assumptions about the structure of unknown substances.

b) The same method was applied to paper chromatography of inorganic substances. About 200 γ of Na_2SO_4 was used as an aqueous solution and developed with a mixed solvent system of MeOH :

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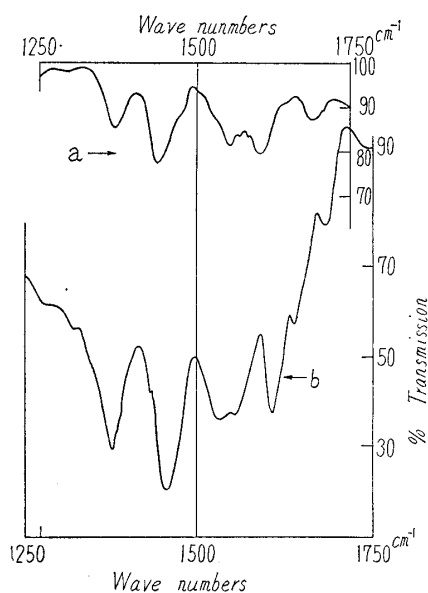


Fig. 6.

Infrared Spectra of Erythrokyrin

- a : Erythrokyrin from paper chromatogram on Japanese paper.
b : Pure erythrokyrin in Nujol.

H₂O:BuOH(1:1:3). The portion of the paper corresponding to the position of SO₄²⁻, separately detected under the same conditions with BaCl₂ and rhodizonic acid,⁴⁾ was cut out and submitted to infrared spectral measurement as in the foregoing case. The absorption curve thereby obtained is shown in Fig. 7. For this measurement, KBr prism was used and the absorption of SO₄²⁻ can be observed at 620 cm⁻¹.

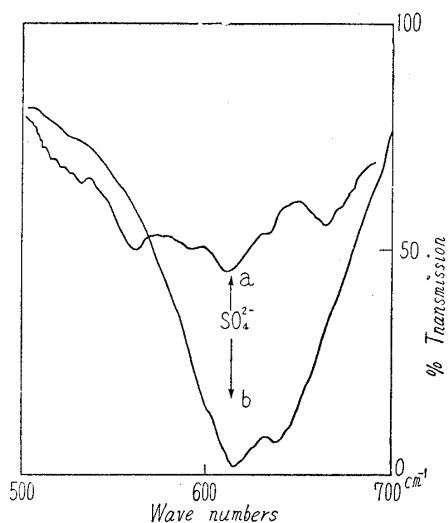


Fig. 7.

Infrared Spectra of Sodium Sulfate
Paper Chromatography on
Japanese Paper

- a : From paper chromatogram of SO₄²⁻
b : Na₂SO₄ (in Nujol)

Conclusion

This method of using Japanese hand-made paper for infrared spectral measurement is not applicable to volatile substances and good result cannot be obtained with substances which are washed by liquid paraffin. At times, a shift of absorption is observed due to the effect of OH of cellulose.

In the application of this method to paper partition chromatography, a fairly larger amount than usual, at least around 100 γ , of the substance must be used. In such a case, development may be impeded. There is a certain limit to the use of this method and it is not as general compared to the densitometer of ultraviolet and visible regions. It is an effective method, however, when some structural conjecture is required or for examination of reactions on a filter paper.

The author expresses his deep gratitude to Prof. S. Shibata of the Pharmaceutical Institute of this University for his kind and unflinching guidance and encouragement during the course of this work,

to Prof. H. Sofue of Department of Applied Chemistry, Faculty of Technology, of this University, and to Dr. S. Fukuhara for the donation of the hand-made paper used in this work and for many valuable informations on this paper, and to Messrs. I. Kitagawa, M. Miyazaki, and O. Tanaka for technical help. A part of expenses for the present work was defrayed by a Grant in Aid for Scientific Research from the Ministry of Education.

Summary

Minimization of samples for infrared spectral measurement was attempted by the use of a Japanese folkcraft, hand-made paper, impregnated with liquid paraffin. By this method, it is possible to measure 0.1 mg. of a sample.

This hand-made paper was also used for paper chromatography and the developed paper was submitted directly to infrared spectral measurement. Component of *Penicillium islandicum* and inorganic sulfate ion were determined by spectral measurement of their chromatogram.

(Received November 7, 1957)

UDC 615.782 : 547.554

24. Yutaka Kasuya : Chemicopharmacological Studies on Antispasmodic Action. XII. Structure-Activity Relationship of Aralkylamines.

(Pharmaceutical Institute, Medical Faculty, University of Tokyo*)

It is a matter of common knowledge that synthetic antispasmodics have atropine-like effect as well as papaverine-like effect. Many investigators have described the relationship between chemical structure and spasmolytic activity, but they were mainly concerned with atropine-like activity.

How the papaverine-like effect is elicited, and how that effect is related to the atropine-like effect, are problems of great interest. The author already reported that some diphenylbutanolamines have considerable atropine- and papaverine-like activities.¹⁾ A series of compounds related to the diphenylbutanolamines have been synthesised in this laboratory.

This report presents various screening data and discusses some of the relationships between structural modifications (changes of radicals attached to nitrogen, of distance between OH and N, existence of OH and optical isomer, etc.) and above two principal activities.

Method

1. *Estimation of Atropine-like Activity* : Tests were carried out with intestinal segments (20~30 mm.) isolated from white mice (male), weighing between 15 and 20 g., fasted for 12 hrs. before sacrifice.

After suspending the segment in Tyrode solution maintained at 26°(adventure of this temperature was described previously¹⁾), acetylcholine (ACh) was added to the bath. ACh concentration in the bath was 1.2×10^{-7} g./cc.²⁾

The spasmolytic drug was added to the bath solution two mins. after ACh and the resultant reduction in contracture occurring within 5 mins. after addition of spasmolytic drug was expressed in

* Hongo, Tokyo (粕谷 豊).

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2) When assaying the atropine-like activity of compounds having both atropine-like and papaverine-like effect, it is desirable to limit the concentration of ACh as low as possible. There is a risk that high dose of preparation required for antagonism against high ACh dose will display the papaverine-like effect simultaneously with the atropine-like effect.