UDC 547.854.72

142. **Masashi Tomoda** : Colorimetric Determination of Pentoses. IV.[1] Determination with Orcinol Reagent.

(*Kyoritsu College of Pharmacy*[*1])

Hydrochloric acid solution containing the iron salt of orcinol is known as the qualitative and quantitative reagent for pentoses.[2~4] Numerous methods using this reagent have been reported but substances other than pentose undergo coloration with this reagent, such as hexoses, hexuronic acids, sialic acids, and deoxypentoses, and there is still the question of specificity of this reagent to be solved.[5,6]

It had been shown in Part II of this series[7] that the orcinol reagent with a mixture of hydrochloric and acetic acids as the solvent showed a good specificity, although the degree of coloration was low. It was later found that the presence of an iron salt in this reagent increased the degree of this coloration and that this also eliminated the coloration of D-glucose, which interferes in nutrition analyses, and of deoxyribose, which interferes in the analyses of nucleic acids. This new method is considered to be better than the existing method both in the degree of coloration and specificity.

The coloration of pentoses and ribonucleic acid by the orcinol-hydrochloric acid-acetic acid reagent containing iron salt reaches the maximum by heating in a boiling water bath for 30 minutes. The effect of iron salts is similar with ferric ammonium sulfate and ferric chloride, although the coloration is slightly lower with the former salt. Absorption maximum of the color by the reagent not containing iron salt is at 605 mμ, and the addition of iron salt gives another maximum at 665 mμ, which increases in intensity as the concentration of the iron salt increases, as shown in Fig. 1. Absorbance at both absorption maxima reaches the maximum in the presence of 0.01 mole of ferric chloride and there is no change of absorbance by further increase of the iron concentration.

Absorbance of five samples containing 20 μg./ml. of D-xylose was measured at 665 mμ using the new orcinol reagent and the values obtained were 0.328 for average value

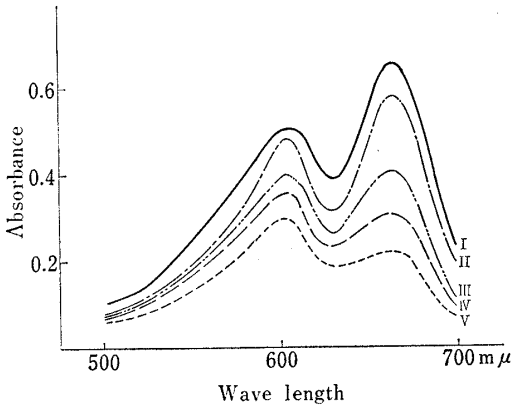


Fig. 1. Absorption Curves for the Colours Produced by Orcinol-Fe^{3+} Reagent

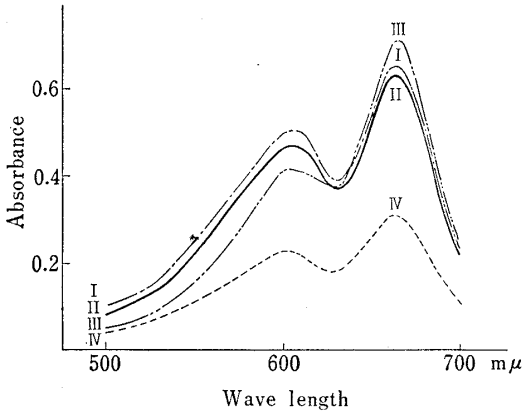
I : 10 $\times 10^{-3}M$ $FeCl_3$ in Reag.
II : 4 $\times 10^{-3}M$ $FeCl_3$ in Reag.
III : 1 $\times 10^{-3}M$ $FeCl_3$ in Reag.
IV : $0.5 \times 10^{-3}M$ $FeCl_3$ in Reag.
V : $0.1 \times 10^{-3}M$ $FeCl_3$ in Reag.
sample : xylose 40 μg./ml.

*1 Shiba-Kōen, Minato-ku, Tokyo (友田正司).

1) Part III : This Bulletin, **11**, 806 (1963).
2) G.L. Miller, R.H. Golder, E.E. Miller : Anal. Chem., **23**, 903 (1951).
3) W.R. Fernell, H.K. King : Analyst. **78**. 80 (1953).
4) M. Tomoda : Bunseki Kagaku. **5**. 249 (1956).
5) Z. Dische : Methods of Biochem. Anal., **2**, 349 (1955).
6) M. Tomoda, S. Kamiya, M. Kimura : Yakugaku Zasshi, **82**, 126 (1962).
7) M. Tomoda, S. Kamiya : *Ibid.*, **82**, 1447 (1962).

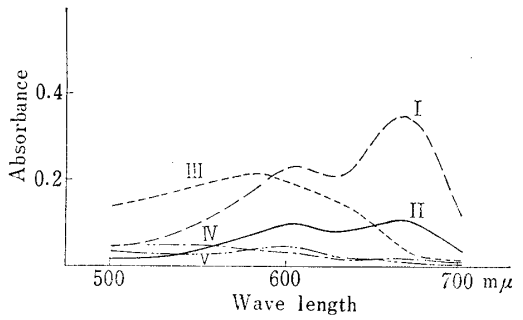
of absorbance, 0.0037 for standard deviation, and 1.1% for coefficient of variation. Absorption curves for pentoses and sodium ribonucleate in aqueous solution are shown in Fig. 2 and those for D-fructose, L-rhamnose, D-glucuronic acid, D-galacturonic acid, and N-acetylneuraminic acid in Fig. 3. Absorption curves for D-xylose, D-ribose, and ribonucleic acid are of the same shape. Absorbance of L-arabinose at 665 mμ is greater than that of other pentoses but that at 605 mμ is lower. Absorption curve for uronic acids is similar to that of pentoses. While the coloration of D-glucuronic acid is weaker, that of D-galacturonic is fairly strong. N-Acetylneuraminic acid shows coloration with absorption maximum at 580 mμ. Determination of sialic acids with orcinol reagent has been reported to date[8,9] and it seems possible to use the present method, which gives a high degree of coloration, for the determination of sialic acid. However, its absorbance at 665 mμ is small and has little effect on the determination of pentoses. Absorbance of D-fructose at 665 mμ is less than 3% of that of pentoses. L-Rhamnose shows some absorption in a shorter wave length region below 620 mμ but gives almost no effect at 665 mμ. D-Glucose, D-glucosamine, and deoxyribonucleic acid do not undergo coloration under these conditions. It is considered possible, from these experimental results, to carry out colorimetric determination of pentoses by measurement of absorption at 665 mμ.

Fig. 4 shows the result of absorption measurement at 665 mμ with L-arabinose, D-xylose, D-ribose, and sodium ribonucleate, varying their concentration. It is seen



I : xylose 40 μg./ml.
II : ribose 40 μg./ml.
III : arabinose 40 μg./ml.
IV : RNA-Na 100 μg./ml.

Fig. 2. Absorption Curves for the Colours Produced by Orcinol-Fe^{3+} Reagent



I : galacturonic acid V : rhamnose
II : glucurone sample : 40 μg./ml.
III : N-acetyl neuraminic acid
IV : fructose

Fig. 3. Absorption Curves for the Colours Produced by Orcinol-Fe^{3+} Reagent

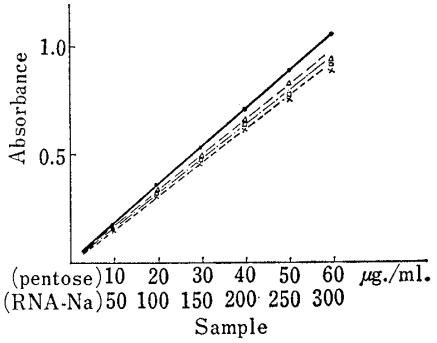


Fig. 4. Optical Density at 665 mμ of Various Amounts of Pentoses and RNA

•—— : •arabinose
△— — : △xylose
□—·— : □ribose
×------ : ×RNA-Na

8) I. Werner : Acta Soc. Med. Upsalien., **57**, 230 (1952).
9) L. Svennerholm : Arkiv Kemi, **10**, 577 (1957).

that the coloration is linearly proportional to the concentration and that determination can be made with 50 μg./ml. of pentoses and within 250 μg./ml. of ribonucleic acid. It is clear from the experimental results reported in Part I of this series[6] that pentosans can be determined under the present conditions.

The degree of coloration of D-xylose is 92% and that of D-ribose is 89% of that of L-arabinose at 665 mμ. Presence of serum albumin or sodium chloride with pentose had no effect on its coloration.

The present reagent has better specificity than the known orcinol reagents and is even better than the ferric chloride-phloroglucinol reagent reported in Part I of this series,[6] which has been considered most suitable as the reagent for determination of pentoses and pentosans. The present method gives better coloration and requires shorter time, and it can be used generally as a reagent for the determination of pentoses and pentosans, except in the presence of uronic acids.

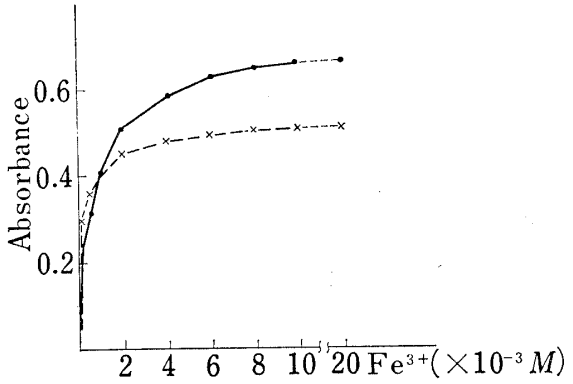
Experimental

The New Orcinol Reagent——A solution was prepared by dissolving orcinol in conc. HCl-AcOH (1:3, v/v) mixture to the concentration of 0.1% and 99 ml. of this solution was mixed with 1 ml. of *M* $FeCl_3$ solution. The reagent should be prepared just before the use.

Measurement of Absorbance and Absorption Curve——The sample solution (1 ml.) was added to each of a series of test tubes (25×200 mm.) with a glass stopper. The reagent (4 ml.) was added and the content of the tube was mixed well. At the same time, a blank (1 ml. of water plus 4 ml. of the reagent) was set up, heated for 30 min. in a boiling water bath, and cooled to room temperature immediately in cold water. The color was stable within 2 hr. The absorbance was measured by Hitachi spectrophotometer type EPU-2 and the absorption curve was measured by Hitachi automatic recording spectrophotometer type EPS-2.

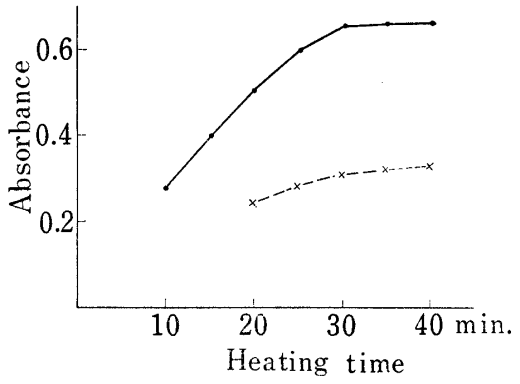
Effect of the Addition of Iron Salt——Relationship between the absorbance at 665 or 605 mμ and the concentration of $FeCl_3$ in the reagent with a sample of D-xylose (40 μg./ml.) is shown in Fig. 5.

Effect of Heating Time——Relationship between the absorbance at 665 mμ and heating time in a boiling water bath with a sample of D-xylose (40 μg./ml.) and sodium ribonucleate (100 μg./ml.) is shown in Fig. 6.



•——•: at 665 mμ sample: xylose 40μg./ml.
×---×: at 605 mμ

Fig. 5. Relationship between the Optical Density and Ferric Ion Concentration



•——•: xylose 40 μg./ml.
×---×: RNA-Na 100 μg./ml.

Fig. 6. Relationship between the Optical Density at 665 mμ and Heating Time

The writer is indebted to Prof. Tamio Yamakawa, Institute for Infectious Diseases, University of Tokyo, for his kindness in providing the sample of N-acetylneuraminic acid. He is grateful to Miss Satoko Kamiya for her technical assistance in the experiment.

Summary

A method using orcinol-ferric chloride-hydrochloric acid-acetic acid reagent was described for the colorimetric determination of pentose, pentosan, or ribonucleic acid.

Coloration by this method was 92% in D-xylose and 89% in D-ribose of that of L-arabinose. Pentoses can be determined up to 10~50 μg./ml. Hexuronic acid showed an absorption curve similar to that of pentoses but its coloration was much lower than that of pentoses. Sialic acid shows a color with an absorption maximum at 580 mμ, but its absorbance at 665 mμ, which is the absorption maximum wave length of pentoses, is low. D-Glucose, D-glucosamine, and deoxyribonucleic acid do not undergo this coloration. Other carbohydrates have very little effect on pentose determination. The presence of serum albumin and sodium chloride does not interfere in the coloration. The present method is better in specificity than other methods which have been used until now.

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