Note

[Chem. Pharm. Bull. 14(8) 915~916 (1966)] UDC 547.857.8.08

Katsuichi Shuto, Kimiko Matsuo, and Masamichi Tsuboi: A Rapid Analysis of Inosinic Acid and Guanylic Acid in Their Mixture by Means of a Bromination Reaction.

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In this note, we present a simple method for determining inosine residue content in a system containing guanosine-, cytidine-, and/or uridine-residues. The method is based upon the difference in reactivity between hypoxanthine and some other bases toward bromine reagents. It has been found by several authors[1~6] that, on mixing a certain amount of bromination reagents, thymidylic acid, cytidylic acid, guanylic acid and uridylic acid react quantitatively, whereas adenylic acid (or adenine or adenosine residue) remains unchanged. We have recently found that inosinic acid (or inosine residue) also remains unchanged under similar conditions.

5'-Inosinic acid (IMP) and 5'-guanylic acid (GMP) samples used in the present experiment were both supplied by Central Research Laboratory, Ajinomoto Company, Ltd. Each of these samples gives only one spot in paper chromatography. One mg. of each was dissolved into 1 ml. of distilled water, and to this solution 19 ml. of 1 *N* sulfuric acid was added. After 15 hours, the ultraviolet absorption spectrum of the sulfuric acid solution was recorded (full line in Fig. 1). Then, 0.04 ml. of N-bromoacetamide solution (30 mmol./L. in H_2O) was added to 3 ml. of the sulfuric acid solution. After 2

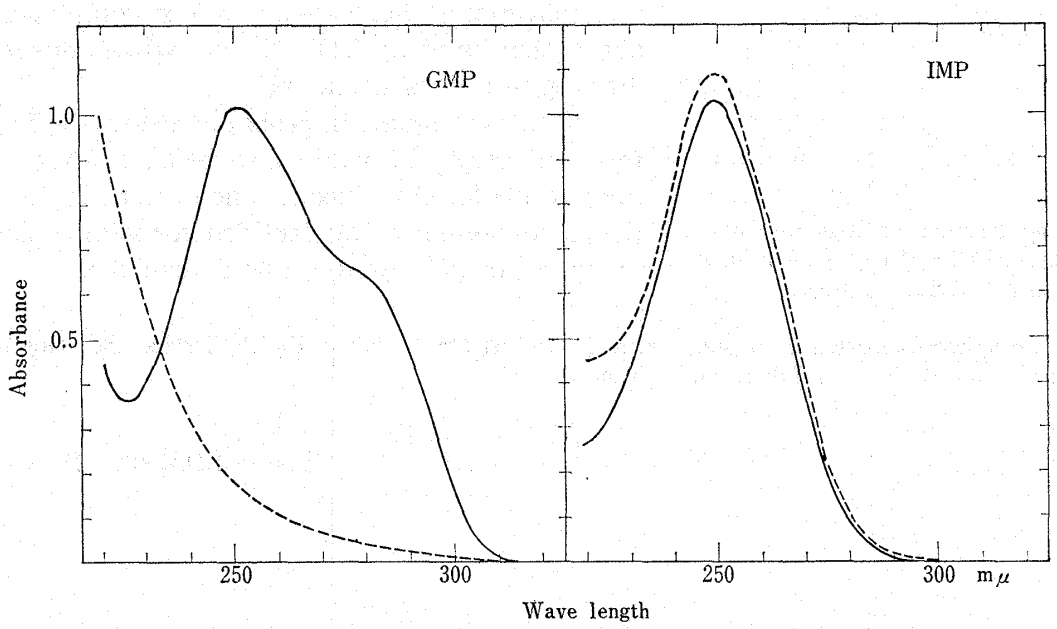

Fig. 1. Ultraviolet Absorption Spectra of Guanylic Acid (GMP) and Inosinic Acid (IMP) in 1*N* Sulfuric Acid before (——) and after (- - -) Treatment with N-Bromoacetamide

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hours, the ultraviolet absorption spectrum of the solution was again recorded (broken line in Fig. 1). As is seen in Fig. 1, a bromination causes a disappearance of the 230~280 mμ absorption band of GMP, while this does not take place with IMP at all. A few mixtures of IMP and GMP were prepared, and the same experiment was made with each of these mixtures. The mol fraction of each mixture was determined on the basis of a phosphorus analysis[7,8] The absorbance at 250 mμ (where GMP and IMP give nearly equal molar extinction coefficient before the bromination) was compared before and after the bromination. The ratio of the absorbance of the solution after the bromination *vs.* that before the bromination was plotted against the mol fraction of inosinic acid content in the mixture (Fig. 2). A straight line resulted indicates that an accurate quantitative analysis of a GMP + IMP mixture is possible by the above stated procedure.

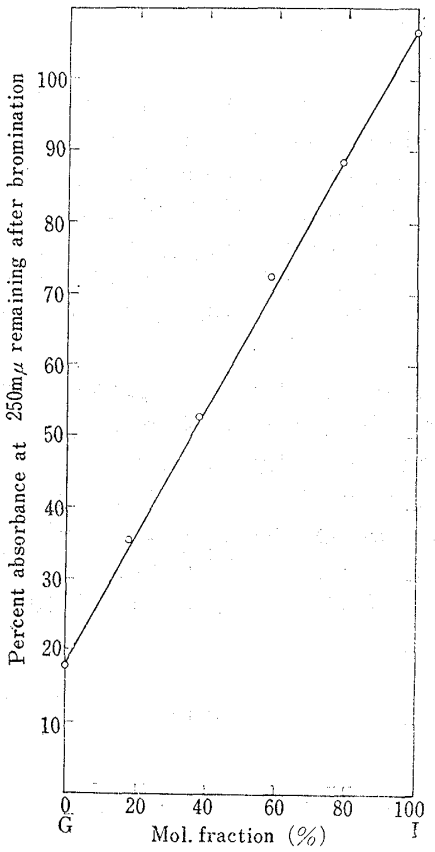

Fig. 2. An Example of Standard Curve for a Quantitative Analysis of Inosinic and Guanylic Acids mixtures

Similar experiments have been made with different amounts (0.015~0.085 ml.) of N-bromoacetamide. It was found that, if the amount is less than 0.03 ml., the bromination of the guanine ring is not completed within two hours. With the amount stated above (0.04 ml.), on the other hand, this bromination is completed by one hour. With any of the amounts examined, no decrease in the absorbance of inosine residue is detectable. In the conditions similar to that stated above, guanosine, guanosine-5′-diphosphate, uridine-5′-monophosphate, and cytidine-5′-monophosphate were found to lose completely their absorption band at 230~280 mμ, while adenosine-5′-diphosphate does not at all.

What we found is probably useful in a field of food industry. It would also be useful in determining the content of inosinic acid residue in a synthetic copolymer of nucleotides containing guanylic, uridylic, and/or cytidylic acid residues.

This work was supported by a grant from the United States Public Health Service (No. GM 10024-02) and a grant from Ministry of Education of Japan.

(Received December 13, 1965)

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