willardiine,[20] *etc.*[9,11,12] have been described in *Citrullus* and *Fagus* seedlings. These results suggest that enzymes catalyzing the formation of β-substituted alanines from O-acetyl-L-serine have different substrate specificities when prepared from different species.

O-Ureidoserine(**4**) conceivably might be an intermediate in the biosynthesis of cycloserine(**8**) formed from β-aminoxy-alanine(**7**) by decarbamoylation.

A more detailed investigation of the enzymes from higher plants responsible for the formations of O-ureidoserine and other types of β-substituted alanines[21] is in progress in our laboratory.

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Faculty of Pharmaceutical Sciences,
University of Chiba,
Yayoi-cho 1-33, Chiba-shi, 280 Japan

Isamu Murakoshi
Fumio Ikegami
Kaoru Harada
Joju Haginiwa

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Determination of Uric Acid using 4-Amino-3-hydrazino-5-mercapto-1,2,4-triazole and Urate Oxidase-Catalase System

A new method of colorimetric determination of uric acid, using uricase and catalase is reported. This method is based on the combination of enzyme reactions and colorimetric procedure with using 4-amino-3-hydrazino-1,2,4-triazole, a highly sensitive reagent for formaldehyde. The color developed in a straight line passing through the original point at least within the absorbance unit of 1.5 at 550 nm, corresponding to 16 mg/dl of the uric acid concentration of the sample.

Keywords——determination; uric acid; urate oxidase; catalase; 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole

Determination of uric acid in biological fluids is essential to diagnose the case as gout. Until now, for the assay of uric acid the phosphotungstic acid method[1] and the ultraviolet method have been most commonly employed.[2]

Recently, urate oxidase-catalase enzyme system has been introduced into the assay of uric acid by Kageyama.[3] In this assay system final product, lutidine derivative, which is

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3) N. Kageyama, *Clin. Chim. Acta*, **31**, 421 (1971).

determined colorimetrically, has the absorbance maximum at 410 nm and the sensitivity for uric acid was relatively low.

Therefore, the new colorimetric determination method for uric acid, which is more sensitive than the former and one can assay at longer wave length in order to remove the interference by bilirubin, has been desired in clinical chemistry field.

The method described here utilizes 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole(AT), a highly sensitive reagent for formaldehyde.[4] AT was found to react with formaldehyde generated by the action of catalase on methanol at pH 9.5 which at the same time optimul hydrogen ion concentration for urate oxidase.

Reagents

1) Alkaline reagent: 5 N KOH.
2) Color reagent: 0.5% 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole in 0.5 N HCl.
3) Oxidizing reagent: 0.75% KIO_4 in 0.2 N KOH.

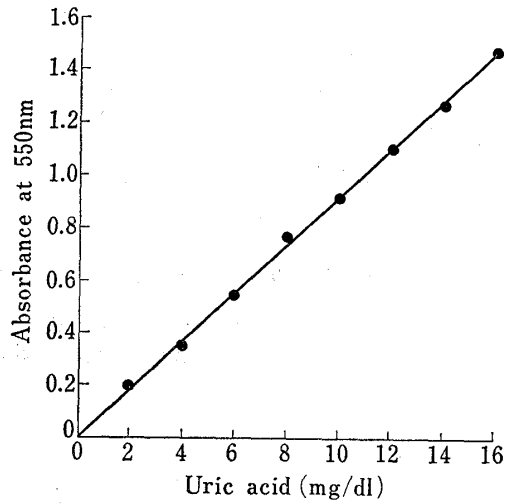

Fig. 1. Calibration Curve of Uric Acid

Procedures

The reaction mixture composed with 0.6 ml of 0.2 M borate buffer (pH 9.5) containing catalase at 1200 IU/ml, 0.1 ml of 70% (v/v) methanol, 0.1 ml of urate oxidase at 0.6 U/ml and 0.2 ml of the sample to be tested was incubated for 30 min at 37°. After the incubation to this reaction mixture was added 1 ml of alkaline reagent and color reagent with shaking and then added 1 ml of oxidizing reagent with vigorous shaking. The absorbance of the mixture was measured against blank at the absorption maximum (550 nm).

Fig. 1 shows the calibration curve of uric acid. The color developed in a straight line passing through the original point at least within the absorbance unit of 1.5 at 550 nm, corresponding 16 mg uric acid/dl in a sample.

On studying the effect of bilirubin (2—10 mg/dl) and ascorbic acid (0.5—2.0 mg/dl) on this colorization process and the value of uric acid determined, no interference was found.

Details of the experiment will be reported in the near future.

Department of Clinical Biochemistry,
Tokyo College of Pharmacy,
Horinouchi 1432-1, Hachioji,
Tokyo 192-03, Japan

TAKAFUMI WATANABE
TETSUYA SUGA

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