Notes

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Spectrophotometric Determination of Oxalacetic Acid in the Presence of Aluminum Ion

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A simple and sensitive spectrophotometric method for the determination of oxalacetic acid in the presence of aluminum ion has been developed by the measurement of the difference in absorbance ΔA of the reaction mixture as a function of time.

Keywords——oxalacetic acid; acetone dicarboxylic acid; aluminum; aluminum ion-catalized decomposition; complex; spectrophotometric determination

For the determination of oxalacetic acid in biological materials, chemical methods[2] based on the formation of hydrazones have been used but these methods cannot discriminate oxalacetic acid, α-keto acid, and β-keto acid. On the other hand, there have been many reports[3] published on the decarboxylation of oxalacetic acid in the presence of multivalent cations. Metal ions such as Al^{3+}, Fe^{3+}, Cu^{2+}, and Zn^{2+} can catalize only decarboxylation of β-keto-dicarboxylic acids, but not that of β-keto-monocarboxylic acids. Krebs and Eggleston[4] determined oxalacetic acid in a buffered solution of pH 5 in the presence of Al^{3+}. This method is based on the measurement of CO_2 evolved by the use of a Warburg manometer. We attempted to establish a spectrophotometric determination method of oxalacetic acid based on the decarboxylation catalized by aluminum ion.

Effect of pH

Oxalacetic acid is stable in strongly acidic (pH 1) and in strongly alkaline (pH 13) solutions. The solution of oxalacetic acid was adjusted to various pH in the acidic side by the addition of 0.1 M acetate buffer in order to avoid the hydrolysis of aluminum ion. When aluminum nitrate solution was added to an oxalacetic acid solution, the absorption peak at various pH appeared at 265 nm. These absorbances reached maximum values within 3 min after the addition of aluminum ion and then decreased gradually. Time-absorbance curves of these solutions are shown in Fig. 1. At pH 4, the value of absorbance of aluminum complex increases, and its decomposition is fast. From these results, the optimum pH value for the decomposition was found to be 4.

In order to study the effect of temperature, a mixture of 1.4×10^{-4} M oxalacetic acid and 2.5×10^{-3} M aluminum nitrate was allowed to react at various temperatures, and time-absorbance curves were recorded at 265 nm. As shown in Fig. 2, at a low temperature, the absorbance of aluminum complex increases and decomposition of the complex is accelerated at a high temperature. On the other hand, maintenance of the temperature of the cell compart-

1) Location: *Gofuku Toyama.*

2) K. Soda, *Agr. Biol. Chem.* (Tokyo), **31**, 1054 (1967); F.A. Isherwood and D.H. Gruickshank, *Nature* (London), **173**, 121 (1954).

3) H.A. Krebs, *Biochem. J.*, **36**, 303 (1942); A. Kornberg, S. Ochoa, and A.H. Mehler, *J. Biol. Chem.*, **174**, 159 (1948); J.F. Speck, *ibid.*, **178**, 315 (1949).

4) H.A. Krebs and L.V. Eggleston, *Biochem. J.*, **39**, 408 (1945).

ment at low or high temperatures is difficult. Taking these facts into consideration, the optimal reaction temperature was chosen as 20° or 30°

The same reaction using aluminum chloride or aluminum sulfate in place of aluminum nitrate showed no difference.

Calibration curves were prepared from the plots of oxalacetic acid concentration *vs.* the difference in absorbance, ΔA, of the complex, as shown in Fig. 1. When the time at the point

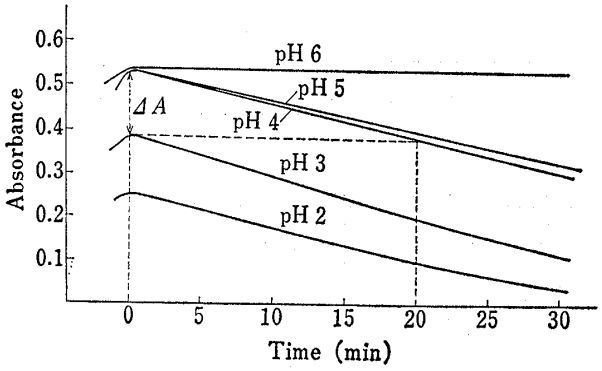

Fig. 1. Effect of pH on Time-Absorbance Curve at 20°

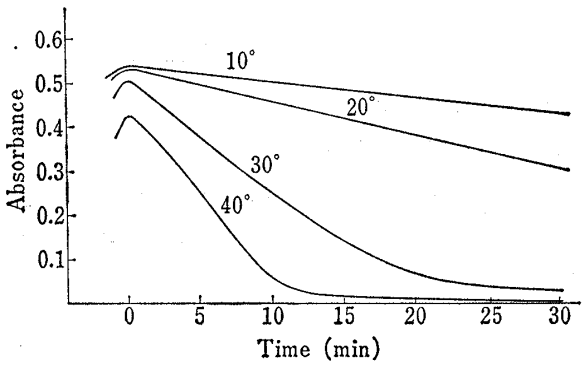

Fig. 2. Effect of Temperature on Time-Absorbance Curve at pH 4

of maximum absorbance of the solution is defined as zero time, the differences in absorbance ΔA were measured after 10, 20, and 60 min. As shown in Fig. 3, calibration curves were obtained as a straight line, and the optimal reaction time was chosen to be 20 min. It was found referring to the calibration curve that oxalacetic acid can be determined in the concentration range of 1.75×10^{-5} to 1.4×10^{-4} M. Compared with the manometric method, which requires 7.0×10^{-3} M of oxalacetic acid and the reaction time of 60 min, this spectrophotometric method has a considerable sensitivity for oxalacetic acid and it is possible to carry out the determination in a short time.

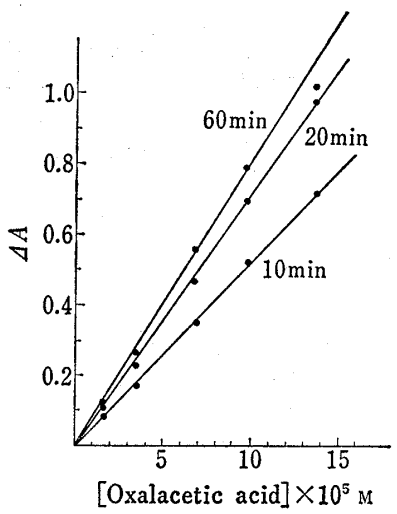

Fig. 3. Calibration Curve for Oxalacetic Acid at 20°

In addition, some other ketonic acid was determined by this method. The stability of acetoacetic acid, α-ketoglutaric acid, and pyruvic acid was not influenced by the addition of aluminum ion. On the other hand, acetone-dicarboxylic acid was rapidly decomposed into acetoacetic acid and CO_2. Thus acetone-dicarboxylic acid could be determined by the above-mentioned method. The calibration curve was prepared, and a liner relationship was found in the concentration range of 1.77×10^{-5} to 1.40×10^{-4} M of acetone-dicarboxylic acid.

Experimental

Chemicals——Oxalacetic acid was obtained by hydrolysis of diethyl oxalacetate according to the method of Krampitz and Werkman.[5] Acetone-dicarboxylic acid and inorganic substances were reagent grade commercial chemicals and used without further purification.

Spectrophotometry——Absorption spectra were taken with the Hitachi Model 101 and 124 recording spectrophotometer (Hitachi Ltd., Tokyo) with a 10 mm cell-path.

Assay Procedure——To 4 ml of a sample solution buffered at pH 4, 4 ml of $Al(NO_3)_3$ solution (5×10^{-3} M) buffered at pH 4 with 0.1 M acetate buffer was added with stirring. An aliquot was rapidly transferred into

5) L.O. Kramppitz and C.H. Werkmann, *Biochem. J.*, 35, 595 (1941).

a glass-stoppered quartz cell, and submitted to absorption measurement. The temperature of the cell compartment was kept at 20° throughout the measurements. As shown in Fig. 1, when the time at the point of maximum absorbance of the solution is defined as zero time, the rate of change in the absorbance of the solution with time was automatically recorded at 265 nm with $Al(NO_3)_3$ solution as blank. After the addition of Al ion, the absorbance reached maximum value within 3 min and the difference in absorbance ΔA was measured after 20 min. From the calibration plots of ΔA *vs.* concentration, the amount of oxalacetic acid was calculated.

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Quantum Statistical Calculation for the Correlation of Biological Activity and Chemical Structure. IV.[1] Subtrates for a Rabbit Kidney Reductase

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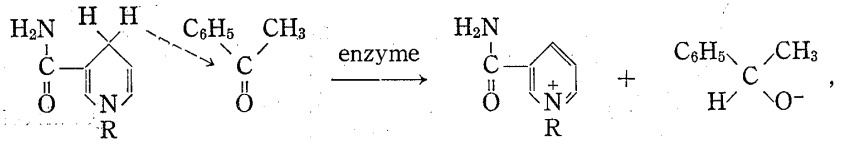
A structure-activity relationship based on a quantum statistical model was pursued in this study. The results of the calculations show that the model is good in relating the maximum velocity of the different acetophenone substrates for rabbit kidney reductase to the stretching vibrational frequency of the carbonyl group.

Keywords——structure-activity relationship; quantum statistical model; mathematical model; substrates; acetophenone derivative; infrared spectroscopic data; enzyme

In order to investigate the structure-activity relationship for compounds of substituted acetophenones, which are used as substrates for rabbit kidney reductase,[3] we have computed an equation based on the quantum statistical model.[1,4] The results of these calculations are shown in Table I and II.



According to the following reaction model of Hermann, *et al.*[5] the relationship between the maximum velocity of the different acetophenone substrates for rabbit kidney reductase was established in terms of quantum chemical parameters. We decided to use Eq. (1) developed in our early publications[4] to correlate their data:

$$-\log(k_0) = c_1 - c_2\theta, \tag{1}$$

$$\ln(1/C) = k_1 - k_2\theta, \tag{2}$$

where $\theta = \boldsymbol{h}c\tilde{\nu}/2\boldsymbol{k}T$, $\tilde{\nu}$ is the wave number of the stretching vibrational mode of the carbonyl group, $\boldsymbol{c}$ is the speed of light, $\boldsymbol{h}$ is the Planck constant, $\boldsymbol{k}$ is the Boltzman constant, and T is

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3) H.W. Culp and R.E. McMahon, *J. Biol. Chem.*, **243**, 848 (1968).
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5) R.B. Hermann, H.W. Culp, R.E. McMahon, and M.M. Marsh, *J. Med. Chem.*, **12**, 749 (1969).