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65. Tsukinaka Yamana, Yūzō Mizukami,*1 and Fujio Ichimura*2 : On the Acid Hydrolysis Mechanism of Ethyl D-Glucuronate.

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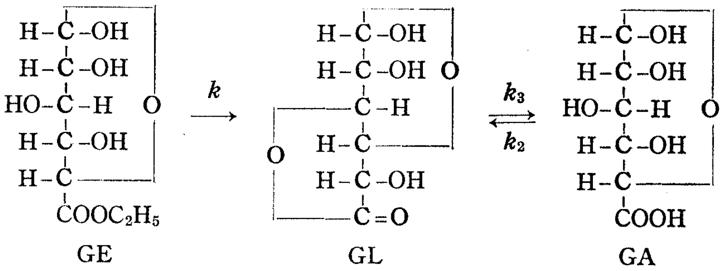
It has previously been reported[1] by the kinetic investigation, that in acid solution (0.1N~0.3N HCl) D-glucuronic acid amide is hydrolyzed to D-glucuronic acid (GA), which further undergoes to D-glucuronic acid lactone (GL) by reversible reaction. Meanwhile, Nitta, *et al.*[2] have suggested, as a result of paper partition chromatographic analysis, that the hydrolysis of ethyl D-glucuronate (GE) in buffer solution (pH 6.86 and 3.85) proceeds through the following steps:



(A)

The previous kinetic investigation was designed to ascertain possible mechanism of the acid hydrolysis of ethyl D-glucuronate.

Experimental

Material——Ethyl D-glucuronate (GE) was prepared from D-glucuronic acid lactone (Chūgai Pharmaceutical Co.) by treatment with Amberlite IR-4B in ethanol following the procedure of Hirasaka and Umemoto,[3] and gave m. p. 133~134°.

Kinetic Studies—— Solution was prepared to contain 5.0×10^{-3} M GE in 0.1 N HCl, and was kept in the thermostatically controlled bath, the temperature of which was regulated at 75°. The temperature variations were less than 0.1°.

Samples for assay were withdrawn at appropriate intervals.

Assay Procedure——Hydroxamic acid method[4] was used for the assay of both GE and GL. Determination of amounts of GE and GL in the samples was based on the different rate of formation of the hydroxamic acid derivative of GA from GE or GL at different concentration of alkali.

The procedure practically used was essentially the same as that of the previous report[5] for the similar assay of D-glucuronic acid amide and GL, except that 1.8N NaOH was used instead of 1.5 N NaOH.

Result and Discussion

Precision of the Assay for Ethyl D-Glucuronate (GE) and D-Glucuronic Acid Lactone (GL)——One milliliter aliquots of solutions containing $1.25 \times 10^{-6}M$, $2.50 \times 10^{-6}M$, $3.75 \times$

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1) T. Yamana, Y. Mizukami, S. Asahi: Yakugaku Zasshi, **84**, 699 (1964).

2) Y. Nitta, J. Ide, N. Ogikubo: *Ibid.*, **82**, 1669 (1962).

3) Y. Hirasaka, K. Umemoto: *Ibid.*, **82**, 1676 (1962).

4) F. Bergmann: Anal. Chem., **24**, 1367 (1952).

5) T. Yamana, S. Asahi, J. Aoki: Yakuzaigaku, **22**, 60 (1962).

$10^{-6}M$, and $5.00\times10^{-6}M$ of GE and GL per milliliter, respectively, were analyzed according to the hydroxamic acid method. The absorption measurements were using HITACHI photoelectric photometer model EPO-B with a No. 47 filter. The values of absorbance are plotted *versus* concentration of GE and GL in Fig. 1.

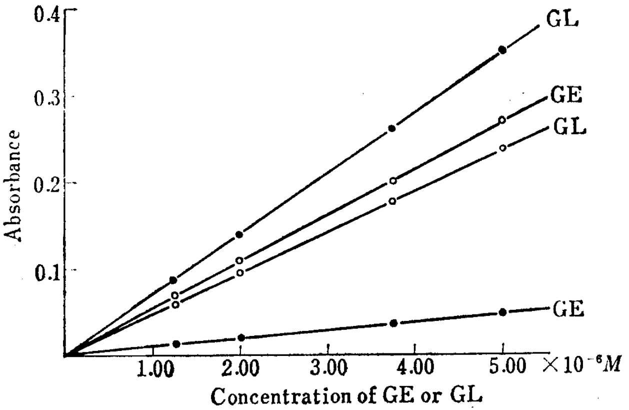

Fig. 1. Relation between Absorbance of Fe $^{+++}$-Hydroxamic Acid Derivative Complex of D-Glucuronic Acid formed from Ethyl D-Glucuronate (GE) or D-Glucuronic Acid Lactone (GL) and Their Concentration

—●— 1.8 N NaOH —○— 3.5 N NaOH

Result from the Rate Study on the Breakdown of Ethyl D-Glucuronate (GE)——The observed breakdown of GE in 0.1 N HCl at 75° is given in Table I and Fig. 2, and the result conform to the pseudo first-order reaction. The calculated pseudo first-order rate constant k gave 10.8×10^{-3} (min^{-1}).

Results from the Rate Studies on the Formation of D-Glucuronic Acid Lactone (GL)——The observed formation of GL during the hydrolysis of GE in 0.1 N HCl at 75° are given in Table I and Fig. 2.

If the mechanism (A) can be valid for the hydrolysis, according to Rakowski,[6] the concentration of GL ([GL]) at time t is as follows:

Table I. Breakdown of Ethyl D-Glucuronate (GE) and Formation of D-Glucuronic Acid Lactone (GL) in 0.1 N HCl at 75°

Time (min.)	GE Found (%)	GL Found (%)	$k_1\times10^3$	GL Calcd. Mech. (A) (%)	GL Calcd. Mech. (B) (%)	GL Calcd. Mech. (C) (%)
30	71.4	13.9	5.3	23.3	5.0	14.0
45	61.2	19.4	5.2	30.2	9.3	19.7
60	53.2	24.4	5.3	35.3	14.1	24.6
75	44.1	28.3	5.1	39.0	18.6	28.7
90	36.5	32.5	5.5	41.8	23.1	32.3
120	26.5	38.5	5.7	45.6	30.5	38.0
150	19.0	42.6	5.8	48.0	35.0	42.1
average			5.4			

$$[\mathrm{GL}]=\frac{k_2[\mathrm{GE}]_0}{k_2+k_3}[1-e^{-(k_2+k_3)t}]+\frac{(k-k_2)[\mathrm{GE}]_0}{k_2+k_3-k}[e^{-kt}-e^{-(k_2+k_3)t}] \qquad (1)$$

where $[\mathrm{GE}]_0$ is the initial concentration of GE.

The numerical values for k was given in this report, and for k_2 and k_3 were also given in the previous report,[7] as 14.8×10^{-3} (min^{-1}) and 13.1×10^{-3} (min^{-1}), respectively.

On substitution of these numerical values into equation (1), the increasing concentration of GL during the hydrolysis may be given as are shown in Table I and Fig. 2.

However, there is observed a large difference between the values observed and calculated. And this might be ascribed to the wrong application of mechanism (A) for the analysis of the hydrolysis.

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[6] R. A. Alberty, W. G. Miller: J. Chem. Phys., **26**, 1231 (1957). (A. Rakowski: Z. physik. Chem., **57**, 321 (1907)).

[7] T. Yamana, Y. Mizukami, S. Asahi: Yakugaku Zasshi, **84**, 696 (1964).

Next, if the following mechanism (B) be assumed:

$$\mathrm{GE} \xrightarrow{k} \mathrm{GA} \underset{k_3}{\overset{k_2}{\rightleftarrows}} \mathrm{GL} \tag{B}$$

the concentration of GL at time t is shown[6] as follows:

$$[\mathrm{GL}]=\frac{k_2[\mathrm{GE}]_0}{k_2+k_3}[1-e^{-(k_2+k_3)t}]-\frac{k_2[\mathrm{GE}]_0}{k_2+k_3-k}[e^{-kt}-e^{-(k_2+k_3)t}] \tag{2}$$

The concentration at various times of GL, thus calculated, using equation (2), which are shown in Table I and Fig. 2, do also differ from those of observed. This indicates that the mechanism (B) would also be excluded

Now it is suggested that hydrolysis reaction of GE may proceed a mechanism:

$$\begin{tikzcd} & \mathrm{GL} \arrow[d, shift right, "k_3"'] \\ \mathrm{GE} \arrow[ur, "k_1"] \arrow[dr, "k_4"'] & \\ & \mathrm{GA} \arrow[u, shift right, "k_2"'] \end{tikzcd} \tag{C}$$

In this case, according to Alberty and Miller,[6] the concentration of GL at time t is calculated by the following equation:

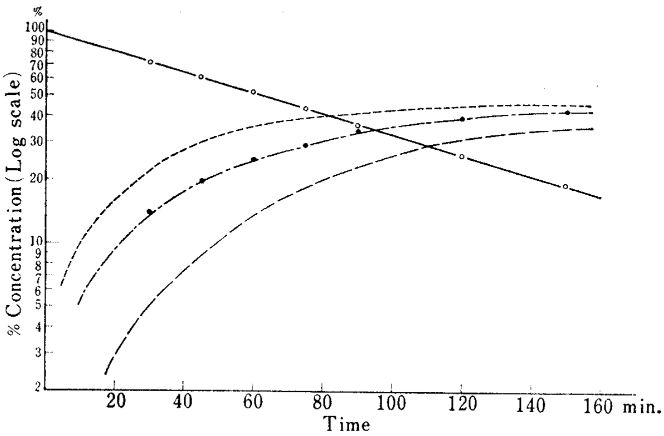

Fig. 2. Disappearance of Ethyl D-Glucuronate and Formation of D-Glucuronic Acid Lactone at 75°

Found:	Ethyl D-glucuronate	○
	D-Glucuronic acid lactone	●
Calculated D-glucuronic acid lactone used Mechanism (A)		---------
〃 Mechanism (B)		— — — —
〃 Mechanism (C)		—-—-—

$$[\mathrm{GL}]=\frac{k_2[\mathrm{GE}]_0}{k_2+k_3}[1-e^{-(k_2+k_3)t}]+\frac{(k_1-k_2)[\mathrm{GE}]_0}{(k_2+k_3)-(k_1+k_4)}[e^{-(k_1+k_4)t}-e^{-(k_2+k_3)t}] \tag{3}$$

Since the sum of k_1 and k_4 is the degradation rate constant k of GE, and k_2 and k_3 are given already, we can solve this equation with respect to k_1. The results of calculation are given in Table I.

As can be seen in Table I, at every time, the calculated k_1 values show reasonable agreement with each another.

The k_4 value is also obtained from the difference of k and k_1 values.

The calculated concentration of GL at various times during the hydrolysis thus obtained by use of these rate constants according to the mechanism (C), are tabulated and plotted in Table I and Fig 2, and are in good agreement with those observed. This fact strongly validates the proposed mechanism (C) for the acid hydrolysis of GE.

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Summary

The kinetics of hydrolysis of ethyl D-glucuronate in acid solution have been studied. It has been shown that the ester at first hydrolyzed to both D-glucuronic acid and its lactone, then they reached to equilibrium.

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