Studies on Cycasin, a New Toxic Glycoside, of *Cycas revoluta* Thunb.

Part VIII. Polarographically Determined Velocity Constants of the Alkali- or Acid-Decomposition of Cycasin and Macrozamin

Kotaro NISHIDA, Akira KOBAYASHI, Tomonori NAGAHAMA, and Junko NAWATA

(*Laboratory of Biochemistry*)

Introduction

It has been suggested in this series repeatedly that cycasin or macrozamin, viz. its aliphatic azoxy-group, is exceedingly labile in an alkaline solution, though is stable in an acidic one. Here is presented the polarographically determined decomposition velocity of these glycosides with sodium hydroxide or hydrochloric acid. In the kinetic research of the chemical reactions, polarography was used first by Heyrovský and thereafter has been unfolded its wide application for various reaction-systems.[1] This method is assumed to be the most appropriate and convenient one for the present purpose, too, since it is free from the troubles by the co-existing sugars which are produced as a result of the decomposition of the glycosides.[2][3]

Experimental Methods

Unless otherwise noted, the materials, the apparatus, and the procedures were those of the same as reported in the just preceding paper.[3]

The alkali-decomposition of the glycosides was examined at low temperature in the following way. A definite volume of the solution of glycoside, containing potassium chloride and gelatin, and that of sodium hydroxide were incubated separately at definite temperature. After the temperature-equilibrium was attained, the both solutions were mixed together (zero-time). Immediately, the reaction-mixture was transferred into an electrolytic cell, through which hydrogen gas was bubbled somewhat vigorously for about 1.5 minutes, and then the recording was started. Two methods of measurements[1] were applied; one was the recording of the whole polarographic waves at intervals of 2 to 5 minutes, and the other that of the current-time curve at a definite potential. The concentration of potassium chloride in the electrolyte and that of the depolarizer at zero-time were made as 0.2 N and 2.5×10^{-4} M, respectively. Mercury pool was used as the anode.

In the case of hydrochloric acid, scarcely any hydrolysis of glycosides was recognized at room temperature. For the investigation of the reactions at high temperature, another procedure should be employed. Test tubes of about 6 cm

length with slender neck was used as the reaction vessel, in which 1 ml each of glycoside solution, 5×10^{-3} M, and hydrochloric acid, 0.2, 0.4, 0.6, 0.8, or 1.0 N, were poured. Sealed tubes were immersed in a water bath of constant temperature for a definite period, and then cooled rapidly with running water. One ml of the contents was filled up to 10 ml with 0.1 N hydrochloric acid containing 0.2 N potassium chloride and gelatin, and polarographied as usual.

The velocity constants were calculated in an ordinary way. On the assumption that the reaction is first order, the wave height at each period, h_1* and h_2 mm, were directly inserted in the equation (I), where k_1* and t* were the velocity constants and the reaction time in minutes.

$$k_1 = \frac{2.303}{t} \cdot \log \frac{h_1}{h_2} \qquad \text{(I)}$$

The amounts of remained glycoside were found, if necessary, by the use of calibration curves, which were drawn up, in the case of alkali-decomposition, on the unbuffered solution of the glycosides containing only potassium chloride and gelatin.

Results and Discussion

1. Alkali-Decomposition An example of the features of alkali-decomposition

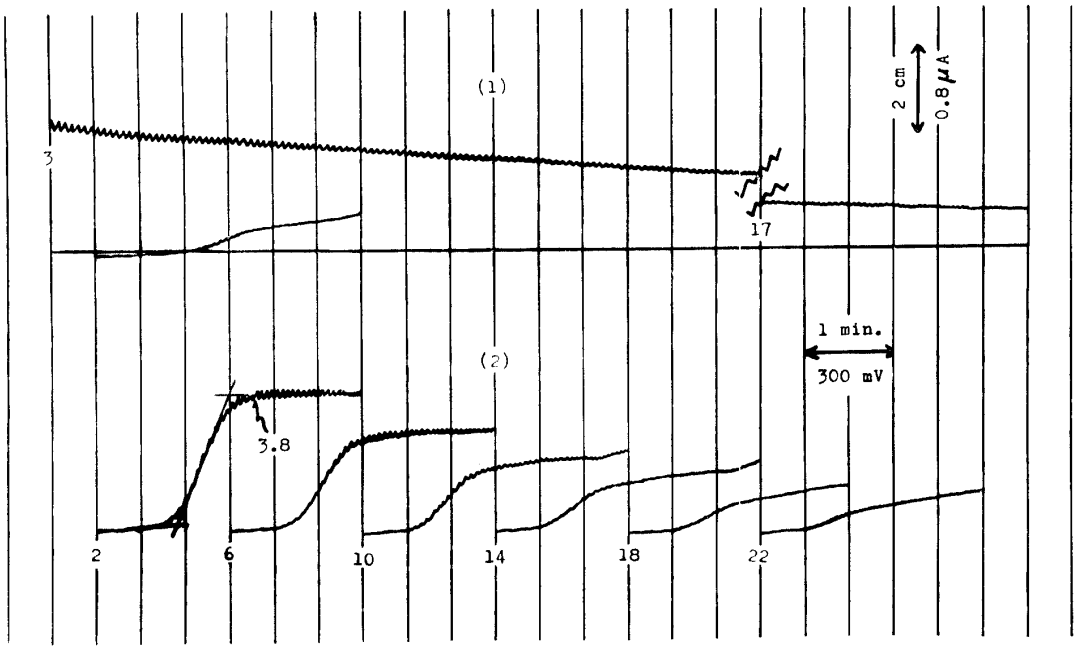

Fig. 1. Decomposition of cycasin with sodium hydroxide
Cycasin: 2.5×10^{-1} M at zero-time, NaOH: 0.3 N, KCl: 0.2 N, Gelatin: 0.004%. Temp: 25° C. (1): Current-time curve at −1.0 V (2): Each polarogram starts from −0.6 V. Correction for the cathode potential was omitted. Figures show the time of reaction.

* The following symbols are used without notice in this paper; k_1: velocity constant calculated for first order reaction. k_2: that for second order reaction. h: polarographic wave height, mm. t: reaction time, min.

was shown in Fig. 1 as a current-time curve and as polarograms. These two methods of measurements, together with the calculated k_1, were comparingly examined under various conditions of alkali-concentration and of temperature. When the reaction proceeded extremely fast, the former method proved to be far more convenient than the latter by which only a few polarograms could be recorded. By the former, however, satisfactory results were not obtained excepting a few case, because it was difficult to decide an appropriate potential which shows the corresponding position on the limiting current of the polarograms throughout the reaction.

Table 1. Measurement-series in alkali-decomposition at 25° C

Initial concentration of glycosides : 2.5×10^{-4} M

Concn. of NaOH	Cycasin			Macrozamin		
	t,* min.	h, mm	$k_1 \times 10^2$	t, min.	h, mm	$k_1 \times 10^2$
0.1 N	4.2	44 8	—	4.2	33.2	—
	9.1	37.3	3.69	9.1	26.7	4.45
	14.1	31.4	3.59	13.7	21.8	4.42
	18.8	26.1	3.70	18.7	17.8	4.30
	23.7	22.0	3.65	23.4	14.3	4.39
	28.5	18.4	3.63	28.5	11.4	4.40
	33.5	15.3	3.67	33.6	9.6	4.22
	38.4	12.5	3.73	38.5	7.6	4 30
	43.4	10.3	3.75	43.5	6.4	4.20
			av. 3 68 ± 0.05**			av. 4.34 ± 0.09
0.2	4.3	35.9	—	4.1	27.5	—
	8 8	26.0	7.17	8.8	18.8	8.09
	13.5	18.6	7.15	13.6	12.8	8.04
	18.4	12.9	7.26	18.6	8.8	7.86
	23.3	9.1	7.23	23.6	6.2	7.64
	28.2	6.7	7.07	28.6	4.6	7.61
			av. 7.17 ± 0.08			av. 7.85 ± 0.20
0.3	3.8	30.2	—	4.1	23.5	—
	7.7	20.2	10.31	7.9	15.4	11.13
	11.5	13.7	10.27	11.7	9.6	11.78
	15.3	9.2	10.34	15.6	6.4	11.31
	19.3	6.0	10.43	19.5	4.1	11.34
	23.2	4.1	10.29	23.5	3.1	10.44
			av. 10.33 ± 0.06			av. 11.20 ± 0.44
0.4	3.8	26.3	—	4.0	21.3	—
	7.5	15.7	13.95	6.8	14.3	14.36
	11.3	9.8	13.17	9.7	9.6	13.98
	15.3	5.7	13.29	12.6	6.9	13.33
	19.3	3.2	13.59	15.6	4.6	13.21
			av. 13.50 ± 0.30	18.5	3.3	12.89
						av. 13.55 ± 0.54
0.5	3.7	23.2	—	4.1	17.0	—
	6.6	14.2	16.62	6.9	10.6	17.02
	9.4	8.4	17.82	9.8	6.5	16.87
	12.3	5.5	16.74	12.7	4.3	16.00
	15.2	3.5	16.45	15.6	2.9	15.38
			av. 16.91 ± 0.54	18.7	2.0	14.68
						av. 15.99 ± 0.89

* The time adopted as t is not when a polarogram begins, but when the limiting current part indicates the wave height which was determined by the usual drawing, as shown in Fig. 1.

** mean error

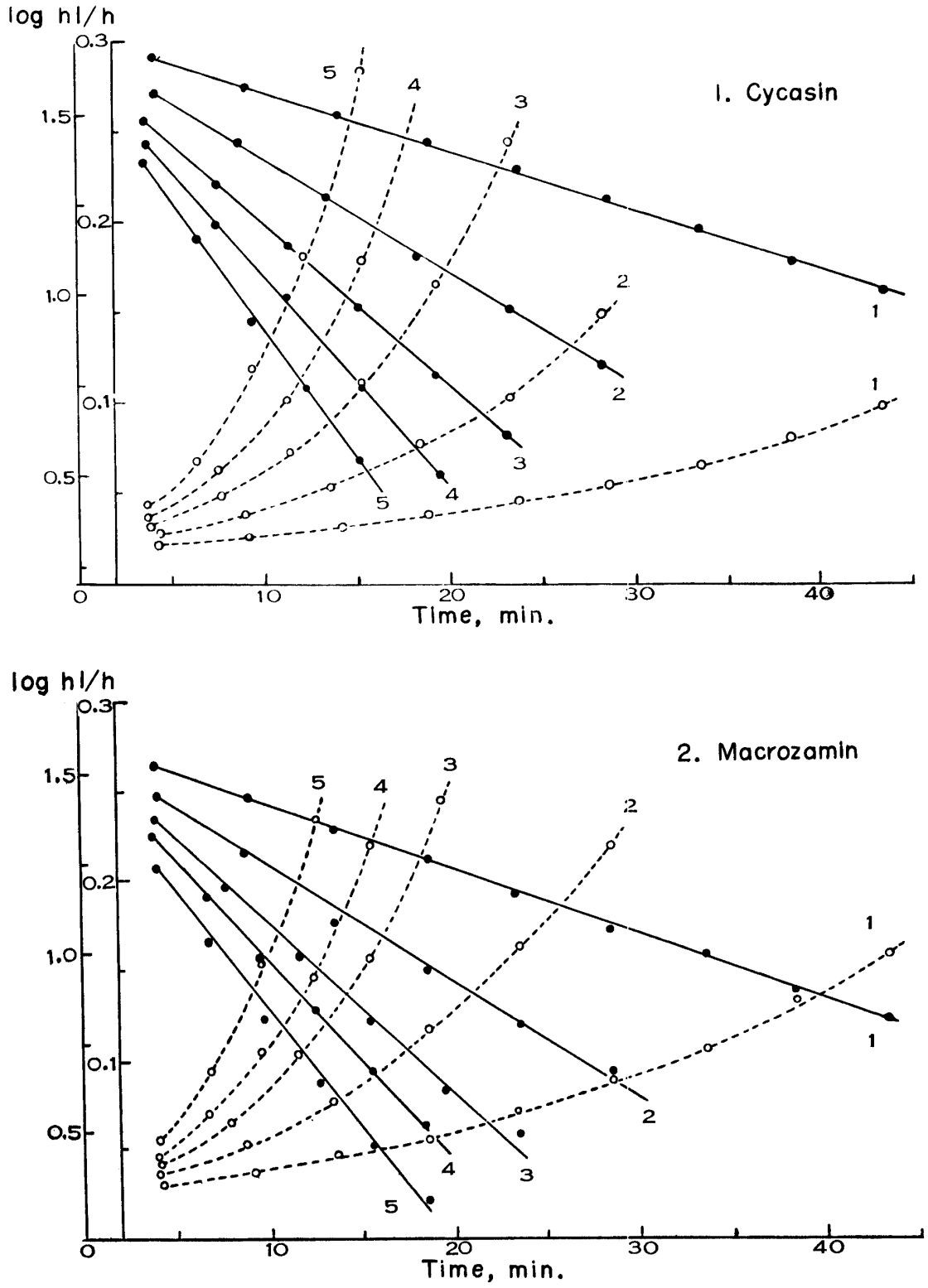

Fig. 2. Relationships between time and logarithms or reciprocals of the wave height in alkali-decomposition at 25° C

—●—, log*h*. --○--, 1/*h*. Figures show the normality of NaOH ×10.

Being more acceptable in its constancy upon the whole, the velocity constants determined by the latter method are discussed in the following part.

(a) Decomposition with Alkali of Various Concentration The changes of h during the reaction at 25° C and the calculated k_1 were shown in Table 1. Although slight changes were observed in the case of macrozamin, it would be said that a good constancy of k_1 was obtained. Calculation of the velocity constants were also carried out according to the bimolecular reaction velocity equation of integral form, in which not the wave height but the amounts of glycoside and of alkali were inserted. The data were, however, absurd for example the value obtained on cycasin was approximately 0.4 which scarcely varied irrespectively with various alkali-concentration. It is probably because of the extreme highness of the concentration of alkali compared with that of the glycoside. The decrease of the former during the reaction can. therefore, be neglected in this case, and the equation (II) of second order is applied. The marks a and x represent respectively the amount of the initial and decreased glycoside.

$$k_2 = \frac{1}{t} \cdot \left(\frac{1}{a-x} - \frac{1}{a} \right) \qquad \text{(II)}$$

The equation shows that if the reaction is second order, the relationship between time and reciprocals of the remained amount of glycoside, accordingly reciprocals of the wave height, is linear. On the other hand logarithms of the wave height is proportional with time in a first order reaction. These relationships were shown in Fig. 2.

From these results it is clearly deduced that the alkali-decomposition of cycasin is a reaction of first order, in which sodium hydroxide, or hydroxy ion, has only a catalytic action. Satisfying the relation of equation (III), where k_c and [ ] denote the catalytic coefficient and concentration, the reaction velocity constants varies proportionally with varying alkali-concentration as shown in Fig. 3.

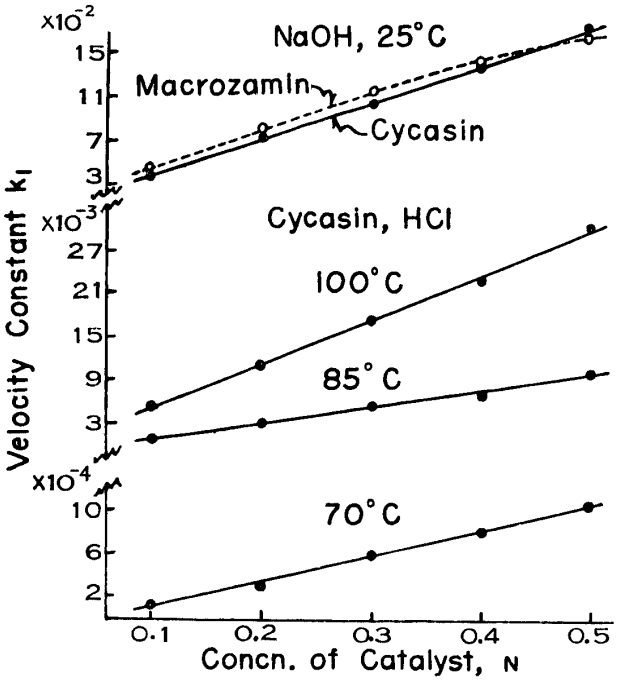

Fig. 3. Relationships between the velocity constants and concentration of the catalyst

$$k_1 = k_c[\text{catalyst}] \qquad \text{(III)}$$

The decomposition of macrozamin, too, can be deduced to be first order, especially from the curves in Fig. 2-2, nevertheless some doubts are left. Though a fairly well constancy was obtained, the k_1 for macrozamin has yet a decreasing tendency with time. The greater value of k_1 than that for cycasin under the same condition, or the convex curve in Fig. 3 is also incomprehensible. These facts would suggest a necessity of con-

sidering another reaction mechanism for macrozamin, or the limits of this method of determination, and are further discussed in the latter part.

(b) Decomposition at Various Temperature Influences of temperature upon the velocity constants were inspected as for the reaction with 0.1 N sodium hydroxide. The results were shown in Table 2. Between logarithms of k_1 and reciprocals of absolute temperature, a linear relation was obtained as shown in Fig. 4, from which the activation energy, E_A, was calculated according to the equation (IV). It amounted on cycasin to 18,800 cal. The linearity in macrozamin was not so satisfactory for the correct value of E_A, which was yet roughly 13,600 cal.

$$E_A = 4.57 \cdot \tan\alpha' \quad \text{(IV)}$$

Table 2. Velocity constants of alkali-decomposition at various temperature

Initial concentration of glycosides: 2.5×10^{-1} M
Concentration of NaOH: 0.1 N

Temp.	Cycasin $k_1 \times 10^2$	Macrozamin $k_1 \times 10^2$
30° C	6.34 ± 0.19	6.90 ± 0.23
40°	13.15 ± 0.69	12.89 ± 0.67
50°	30.80 ± 1.27	27.24 ± 1.96

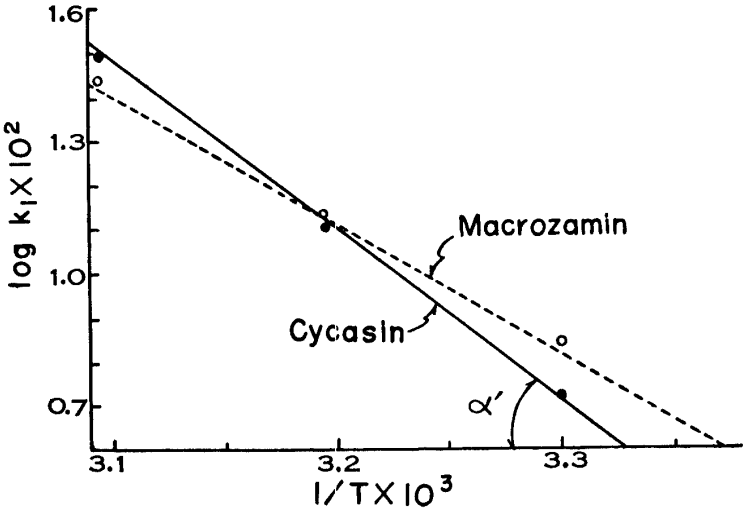

Fig. 4. Determination of the activation energy in alkali-decomposition

2. Acid-Hydrolysis of Cycasin The reaction proceeded scarcely at 70° C, that is, the decrease of cycasin after 6 hours was only 5.0, 10.1, 17.9, 25.7, or 33.7 % of the initial amount with hydrochloric acid of 0.1, 0.2, 0.3, 0.4, or 0.5 N, respectively. Because of the characters of the experimental methods, it would be inevitable that sufficient results were not obtained. The values of k_1

Table 3. Velocity constants of acid-hydrolysis of cycasin

Initial concentration of cycasin: 2.5×10^{-3} M

Temp.	70° C	85°	100°
Concn. of HCl	$k_1 \times 10^4$	$k_1 \times 10^3$	$k_1 \times 10^2$
0.1 N	1.21 ± 0.20	1.59 ± 0.03	5.62 ± 0.25
0.2	2.88 ± 0.15	3.12 ± 0.07	11.25 ± 0.44
0.3	5.58 ± 0.26	4.90 ± 0.26	17.50 ± 0.80
0.4	8.06 ± 0.32	6.95 ± 0.12	22.67 ± 1.72
0.5	10.59 ± 0.30	9.40 ± 0.09	29.66 ± 2.63

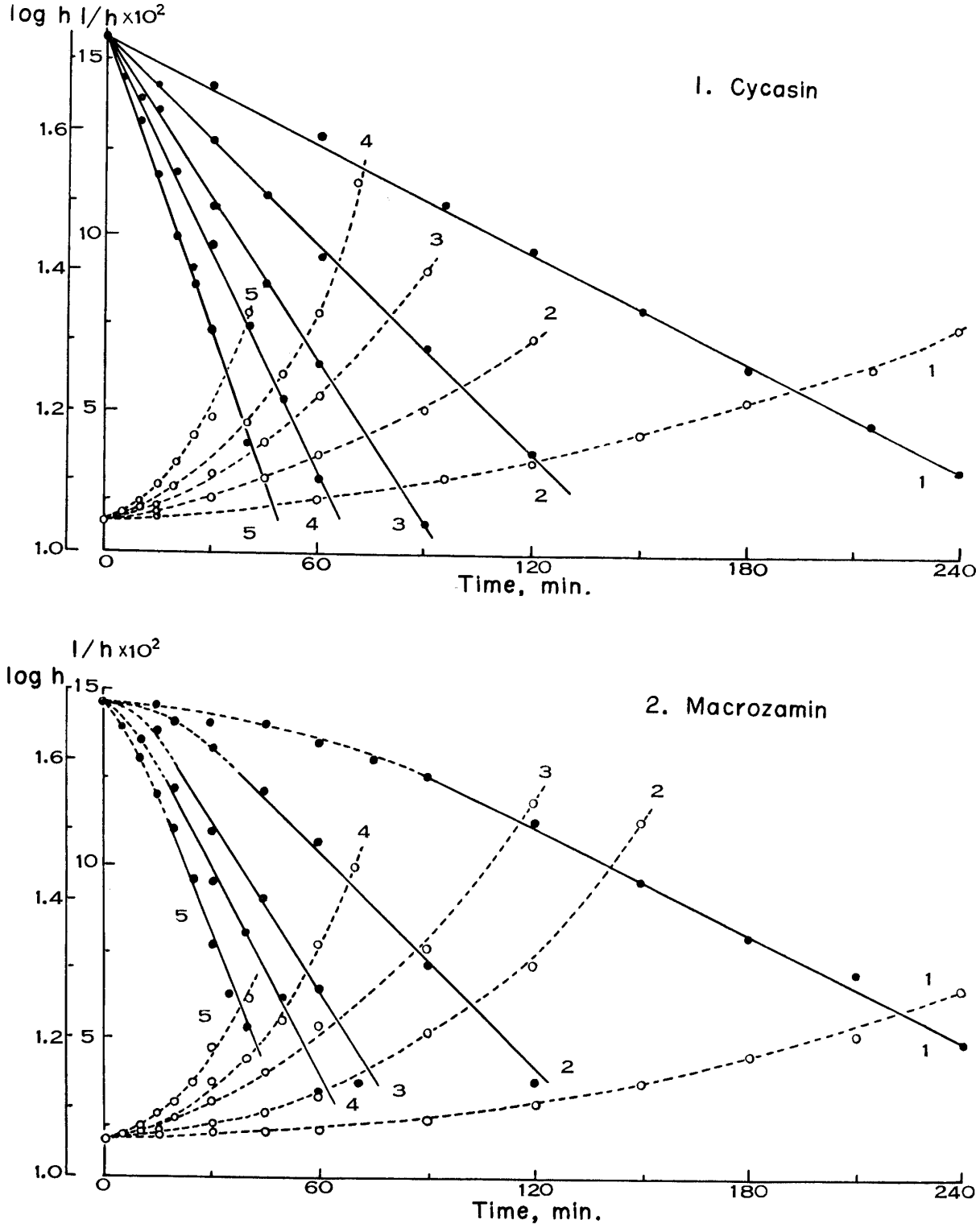

Fig. 5. Relationships between time and logarithms or reciprocals of the wave height in acid-hydrolysis at 100° C

—●—, log*h*. --○--, 1/*h*. Figures show the normality of HCl ×10

are shown in Table 3. It is also shown in Fig. 5 that the reaction is deduced to be first order. Linear relationships, which satisfy the equation (III) as well as in the case of alkali, were obtained between k_1 and acid-concentration with respect to the reaction at each temperature (Fig. 3). Being accordingly indifferent to the acid-concentration, the activation energy was determined by the data with 0.1 N acid. Good proportionality was not obtained between logarithms of k_1 and reciprocals of absolute temperature, probably because of the erroneous value of k_1 at 70° C. The activation energy was, therefore, calculated in this case according to the equation (V) with use of the velocity constants, k_1 and k_1' at 373° and 358° K, T and T', respectively. It amounted to 22,400 cal.

$$E_A = 4.57 \cdot \frac{TT'}{T-T'} \cdot \log \frac{k_1}{k_1'} \qquad \text{(V)}$$

3. Acid-Hydrolysis of Macrozamin The appearances of the reaction are shown in Fig. 5-2. The retardation of the reaction during the early stage presents subjects to be argued. The points, on which hydrolysis with acid is presumed to occur, are two in macrozamin (VI), while one in cycasin (VII), where Xyl, Glu, and Agl denote the residues of xylose, glucose, and aglycone, respectively. Being not able to exist independently, the liberated aglycone decomposes rapidly, probably too fast to be measured, into low molecular compounds.[4] It is, therefore, considered

$$\underset{\uparrow\,(3)}{\text{Xyl-O}}\text{-}\underset{\uparrow\,(2)}{\text{Glu-O}}\text{-Agl} \quad \text{(VI)}, \qquad \underset{\uparrow\,(1)}{\text{Glu-O}}\text{-Agl} \quad \text{(VII)}$$

that in the case of cycasin the rate-determining step in the total reaction (VIII) is the splitting of the glucosidic linkage (1), and the reaction can be pursued directly

$$\text{Glu-O-CH}_2\text{-N}=\underset{\downarrow \atop \text{O}}{\text{N}}\text{-CH}_3 + H_2O = \text{Glucose} + CH_3OH + N_2 + HCHO \qquad \text{(VIII)}$$

according to the decrease of wave height. On the other hand, macrozamin may have possibilities of giving some intermediates, since its two oxygen-bridges are assumed to be resembled to each other in their hydrolysis-velocities. When the splitting on the point (3) precedes that on (2), xylose is liberated together with cycasin, which decomposes further into glucose and aglycone, or low molecular final products. If the reverse is the fact, the intermediate obtained first would be primeverose, which then splits into xylose and glucose. The formation of these intermediates should cause the following changes of the wave height. In the first case, the wave height is unchangeable or rather is enhanced slightly, because cycasin shows a little higher wave than equimolecular macrozamin does. In the latter, however, it will be lessened without delay accompanying with the advance of the reaction. Xylose or primeverose, too, can be ignored here with respect to their polarograms as well as glucose.

By these assumptions it may be made comprehensible in the hydrolysis of macrozamin that lag periods, or even higher waves than the initial ones, are observed at the early stage, or that the curves of Fig. 5-2, when slided to the right side along the lateral axis, conforms approximately with that of Fig. 5-1.

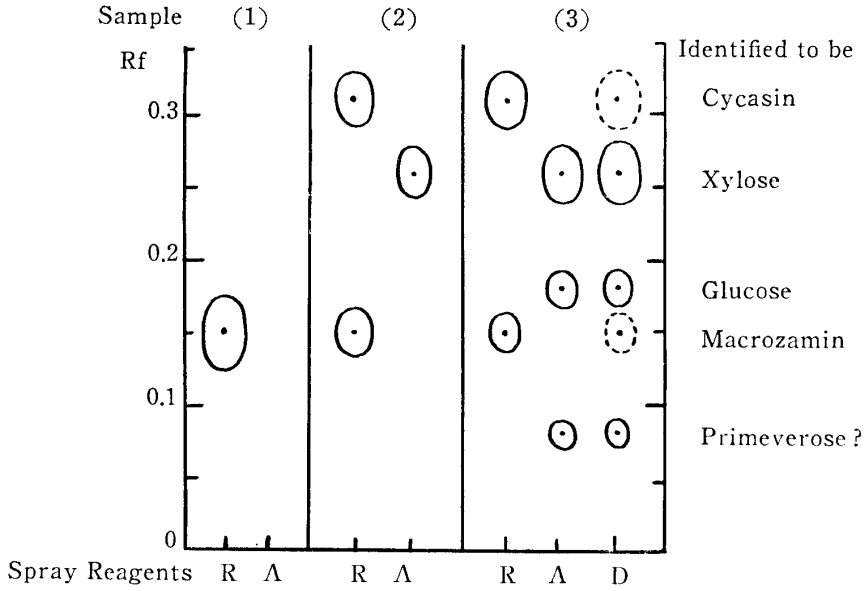

Fig. 6. Intermediates in the acid-hydrolysis of macrozamin, shown in paper chromatogram

Hydrolysis: with 0.1 N HCl, at 100° C. The composition of the reaction mixture was the same as in polarography.
Sample: (1) Macrozamin or zero-time, (2) taken after 10–60 min. at 10 min. intervals. Chromatograms were all resembled to each other, except for somewhat enhanced spots of cycasin and xylose accompanied with the proceeding of the reaction. (3) after 60 min. The plotted amount on the original point was about fifty times of (2) enriched by vacuum condensation of the sample after treatment with anion-resin.
Development: BuOH-AcOH-H_2O (4 : 1 : 1)
Spray reagent: R, resorcin-HCl. A, aniline-hydrogen-phthalate. D, diphenylamine-trichloro acetic acid, as usual.

Further evidences were obtained by paper chromatographical examinations on the hydrolyzate at the lag period under the same conditions as above mentioned. The results were shown in Fig. 6, in which the existence of cycasin and xylose as the main intermediates was clearly exhibited. When the amount of the sample was highly enhanced, spot of glucose and a small spot which was considered to be that of primeverose from its color or Rf value, ill-identified owing to the lack of specimen, were also recognized.

It is thus concluded that the complexity of the reaction must be explained by the two courses, via cycasin and via primeverose, through which the hydrolysis of macrozamin passes to the final products. That the former course is the main reaction, is shown in the above mentioned results. The accumulation of cycasin also proves that being slower in its velocity than the splitting of the bridge (3), that of (1) becomes the rate-determining step in the main reaction. Therefore, the polarographic measurements of the hydrolysis of macrozamin results in that of cycasin.

It is probably because the reaction with alkali seems to occur on the aglycone itself and to be indifferent with the oxygen-bridge, that such a feature is not observed in it. For this reason, the word of decomposition not hydrolysis, is considered to

be suitable in this case. At all events, the order or the mechanisms of the reaction on macrozamin will be fully discussed with further application in parallel of other methods for sugars.

Summary

The alkali- or acid-decomposition of cycasin and macrozamin was polarographically pursued. The reactions were deduced to be first order from the velocity constants or the linear proportionalities of logarithms of wave height with time. The activation energy was determined with the velocity constants at various temperature. Different from the acid-hydrolysis of cycasin, that of macrozamin showed a lag phase, which was explained by the step wise hydrolysis of the two oxygen-bridges in macrozamin. This inference was confirmed also by the fact that as the main intermediate cycasin was caught paper chromatographically together with xylose and a little of primeverose.

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