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

Part VII. Polarography of Macrozamin, Compared with That of Cycasin

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Introduction

In the preceding report,[1] the polarographic behaviors and determination of cycasin, the toxic principle of the Japanese cycad, were reported. After that, Dr. N. V. Riggs of the University of New England, Australia, who was interested in it, offered to the authors the sample of macrozamin for the sake of polarographic studies. Macrozamin was found in the Australian cycads of genera *Macrozamia* or *Bowenia* etc.,[2] and its structure was proved by Lythgoe *et al.* to be primeverosyloxyazoxymethane.[3] Cycasin is another glycoside, a glucosyl derivative, though it is identical in its aglycone part with macrozamin.[4] Here is described the polarography of macrozamin especially compared with that of cycasin.

Materials and Methods

Macrozamin, isolated from *Bowenia serrata*, m.p. (decomp.) 199°～200° C, and cycasin, isolated in this laboratory from the stem of *Cycas revoluta* Thunb., m.p. (decomp.) 144°～145° C were used. Stock solutions were prepared by dissolving each sample in distilled water in a concentration of 5×10^{-3} M. They were diluted to a definite volume with a buffered solution containing 0.2 N potassium chloride, and were used as the electrolytic solution. For the maintenance of the pH values as 1, 2 to 8, 9 to 12, and 13, N/10 hydrochloric acid, McIlvaine's buffer, Sörensen's buffer, and N/10 sodium hydroxide solutions were used respectively. The capillary for the dropping mercury electrode showed the following characteristic values in 0.2 N potassium chloride solution at −1.5 V vs. N.C.E.; m, rate of mercury flow, was 1.578 mg sec^{-1}, and t, the drop time, was 2.73 sec. The value of $m^{2/3}t^{1/6}$ was accordingly 1.602 $mg^{2/3} sec^{-1/2}$. Prior to the electrolysis, the dissolved oxygen was removed by bubbling of hydrogen gas through the solution. The temperature of the electrolytic solution was, unless otherwise mentioned, kept at 25° C. For this purpose, water of constant temperature was circulated in the outer tube of the electrolytic cell. A normal calomel electrode (N.C.E.) was applied as the reference electrode throughout the experiment. The polarograph employed was a Yanagimoto pen recording type, PA-1.

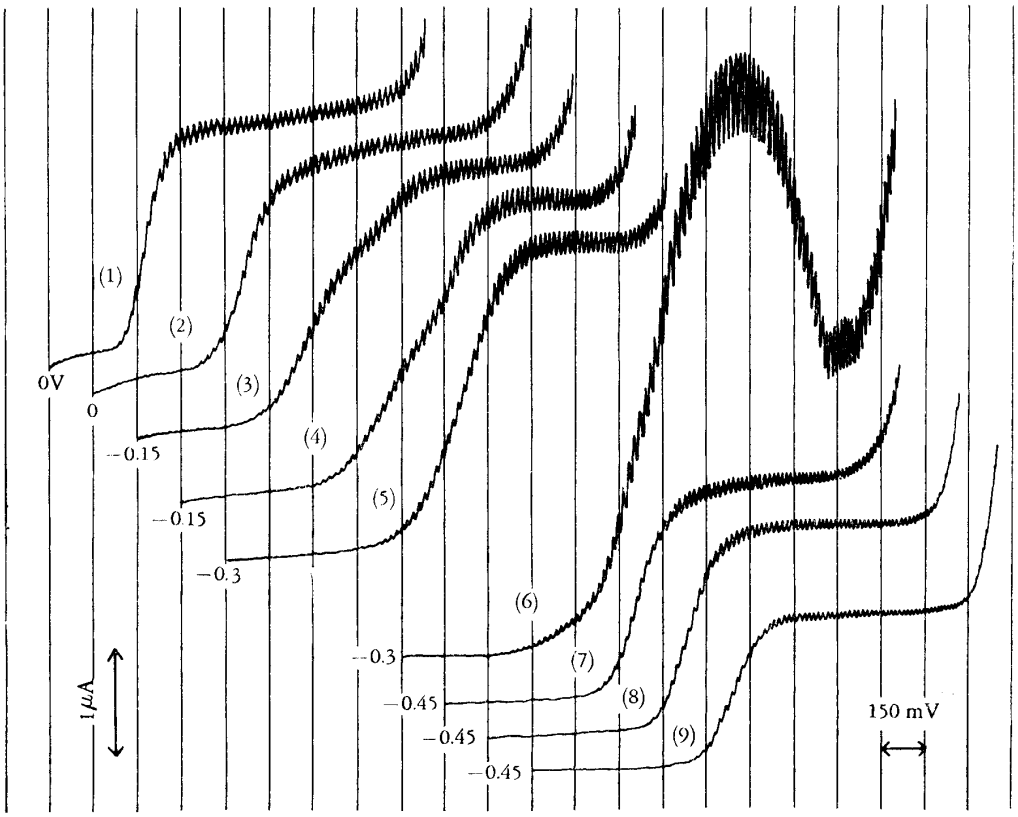

Fig. 1. Polarograms of macrozamin

2.5×10^{-4} M Macrozamin in 0.2 N KCl, at 25° C, at pH (1) 1.0 (2) 3.1, (3) 4.9, (4) 5.8, (5) 7.0, (6) 7.0 (gelatin non-added), (7) 8.9, (8) 10.6, (9) 13.0

Table 1. Relationships between pH, the half-wave potential and the limiting current

2.5×10^{-4} M Macrozamin, gelatin 0.004%, at 25° C.

pH	1st Wave		2nd Wave	
	$E_{1/2}$ vs. N.C.E.	i	$E_{1/2}$ vs. N.C.E.	i
1.0	−0.33 V	1.90 μA		
2.6	−0.45	1.76		
3.1	−0.51	1.70		
4.1	−0.62	1.64		
4.9	−0.71	1.32		
5.5	−0.75	1.18	−1.08 V	0.78 μA
5.8	−0.78	0.88	−1.10	1.00
6.0			−1.10	1.32
6.3			−1.11	1.48
7.0			−1.09	2.75
7.5			−1.10	2.40
8.2			−1.10	2.12
8.9			−1.10	1.97
10.0			−1.09	1.88
10.6			−1.09	1.86
11.8			−1.10	1.74
13.0			−1.13	1.39

Results and Discussion

1. Influence of pH upon the Polarograms It was deduced that the polarographic waves of cycasin were due to the azoxy group in its aglycone part. Macrozamin, which has the same group, showed accordingly just resembled appearances. The polarograms of macrozamin at various pH, and the relationships between pH, the half-wave potential, and the limiting current were shown in Fig. 1 and Table 1.

The following two points were the remarkable differences on the polarograms between cycasin and macrozamin. In the former, the two-stepped waves at pH 4 to 7, the half-wave potential of which were adjacent to each other, were drawn as a single wave. In the latter, however, the two waves could be distinguished clearly. Concerning to the first wave, observable at pH 1 to about 6, the limiting current gradually decreased and the half-wave potential shifted to a more negative potential with increasing pH value. The relationship between the half-wave potential and pH was a linear one, the slope of which was 95 mV per pH unit. The second wave was recognized from about pH 4. Its limiting current increased at the cost of the first one, and after the arrival to the maximum value at near pH 7, decreased again. The half-wave potential remained in approximately constant.

The second point of the differences was the phenomena of maxima. In cycasin, the round maxima which could be suppressed comparatively with ease by a small amount of gelatin, were observed only at about pH 7. In the case of macrozamin, nevertheless, they were exceedingly remarkable in the whole alkaline range above pH 6. Moreover, the more addition of gelatin, about five times of the case of cycasin, was necessary for the perfect suppression of these maxima.

It was also shown on macrozamin as well as on cycasin that being labile in an alkaline solution, it is subjected to an irreversible decomposition, rapidly even at room temperature. For example, the decrease of the limiting current at 25° C during 20 minutes amounted to 4 % at pH 10.6, 21 % at pH 11.8, or up to 49.5 % at pH 13.0, and furthermore, it was not recovered by the adjustment of pH to the acidic side. For a convenience, here was shown the polarograms which were observed 5 minutes after the preparation of the alkaline electrolytic solutions.

2. The Limiting Currents at pH 1 and 7 The electrode reaction mechanism deduced on cycasin was as follows. Involving 4 electrons and 4 hydrogen ions, the reduction at pH 1 gives a hydrazo compound, which is, at pH 7, further reduced with participation of 2 electrons. Six electrons are, therefore, involved in the latter reaction. An entirely resembled reaction mechanism can be assumed also on macrozamin. The diffusion constant, D, of macrozamin was calculated from the Ilkovič equation, $Id = 605\ n\ C\ D^{1/2}\ m^{2/3}\ t^{1/6}$, where Id, n, and C denote the diffusion current, electron number involved in the reaction, and the concentration of depolarizer, respectively. Inserting the known values in the equation, D amounted to 3.84×10^{-6} $cm^2\ sec.^{-1}$ at pH 1 and $3.57 \times 10^{-6}\ cm^2\ sec.^{-1}$ at pH 7. It is known on two substances that a relationship, $D_1/D_2 = \sqrt{M_2/M_1}$, is established between the diffusion constants, D_1 and D_2, and the molecular weights, M_1 and M_2.[5] With respect to macrozamin and cycasin, the second term of the equation equals to $\sqrt{384/252}$, i.e. 1.23. The first term, calculated from D_1 of cycasin described in the preceding report, were 1.12

at pH 1 and 1.38 at pH 7. For fear of high gelatin concentration in the case of macrozamin, this calculation was also carried out on *Id* obtained on cycasin under the same conditions, but it gave similar results.

It is thus considered to be approved by the comparative discussion on macrozamin, too, that the explanation, which was conducted on cycasin, for the electrode reaction mechanisms of an aliphatic azoxy group is sufficiently pertinent.

Concerning to the effect of the height, h, of the mercury reservior, upon the limiting current of macrozamin, a linear relation was obtained between $\sqrt{h}$ and the value of the current as shown in Table 2. The slopes at the point of $h = 60$ cm showed in both cases almost equal value at pH 1 and 7. Therefore, these limiting currents have not the character of so-called kinetic current but are diffusion-controlled.[6]

Table 2. Dependence of the limiting current on the height, h, of the mercury reservior

2.5×10^{-4} M Macrozamin, at 25° C.

h	$\sqrt{h}$	pH 1		pH 7	
		i	$i/\sqrt{h}$	i	$i/\sqrt{h}$
80 cm	8.94	2.28 μA	0.26	3.15 μA	0.35
70	8.36	2.04	0.24	2.90	0.35
60	7.75	1.91	0.25	2.68	0.35
50	7.07	1.74	0.25	2.43	0.34
40	6.32	1.56	0.25	2.22	0.35

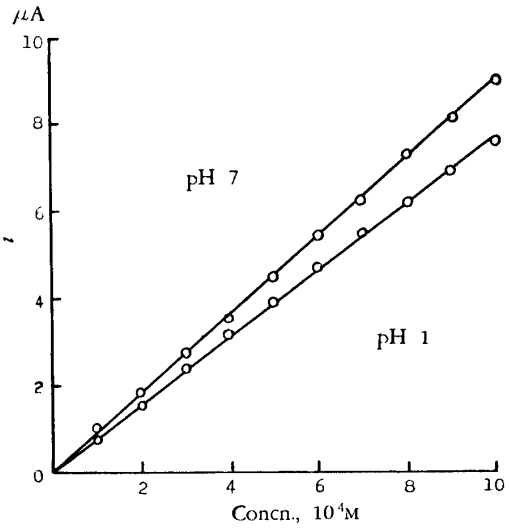

Fig. 2. Relationships between the limiting current and the concentration of macrozamin

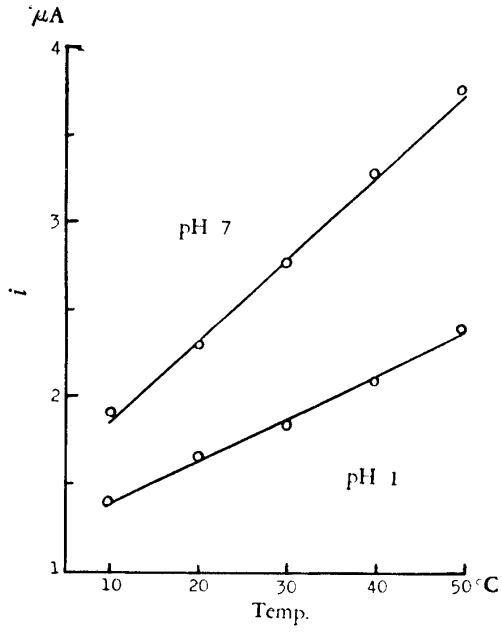

Fig. 3. Influence of temperature on the limiting current of 2.5×10^{-4} M macrozamin

As shown in Fig. 2, the limiting current stands in a linear proportionality with the concentration of macrozamin in the range of 10^{-4} to 10^{-3} M. The equations calculated by the least square method are shown as follows, where *Id* is the limiting current in μA and *C* is the concentration $\times 10^{1}$ M.

$$Id = 0.7536\ C + 0.0893 \qquad \text{pH } 1$$

$$Id = 0.8912\ C + 0.0647 \qquad \text{pH } 7$$

The effect of temperature upon the limiting current was shown in Fig. 3. The temperature coefficient at pH 1 and 7 are respectively 1.43 and 1.86 % deg.$^{-1}$, which are reasonable as compared with those of the usual ones.[7]

Summary

Polarographic studies on macrozamin was carried out and discussed comparing with that of cycasin. Being due to the aliphatic azoxy group in the aglycone, the polarograms of both glycosides differed scarcely except for an extraordinary maximum or two-step waves in that of macrozamin. With the comparison of the diffusion constants of two glycosides, the previously reported deductions on the electrode reaction mechanisms were given a further ascertainment.

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