

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

## Part IX. Cleavage into Cycasin from Macrozamin.

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The main glycoside found in the Japanese cycad is a glucoside, cycasin<sup>(1,2)</sup>, whereas that in the Australian cycads is a primeveroside, macrozamin<sup>(3,4)</sup>, and their aglycones are the same constitution.

In the preceding report<sup>(5)</sup>, where the velocity constants of the decomposition of cycasin and macrozamin were examined polarographically, a lag period observed in the acid hydrolysis of the latter was deduced to be resulted from the two glycosidic linkages in it. A tentative proof was also given by a paper chromatographic identification of cycasin as an intermediate in the course of the acid cleavage of macrozamin.

For the purpose of a trial to substantiate the cleavage of macrozamin into cycasin, its isolation by a column chromatography of the partial acid hydrolysate of macrozamin was carried out.

### Experimental and Results

Experimental methods are shown in Table 1. A solution of macrozamin in 0.1 N hydrochloric acid was refluxed on a boiling water bath for one hour, and the hydrolysate

Table 1. Preparation of cycasin from macrozamin.

Macrozamin 796 mg	
	-added 40 ml of 0.1 N HCl, refluxed on boiling water bath for 1 hr.
	-treated with Diaion A (anion exchange resin)
	-vac. evaporated
	-chromatographed on cellulose column (30 g, dia. 2.5×50 cm), developed with <i>n</i> -BuOH saturated with H <sub>2</sub> O, and fractionated for every 10 ml
Effluents 220~600 ml	
	-combined and vac. evaporated
	-recrystallized from EtOH
Cycasin 50.7 mg	

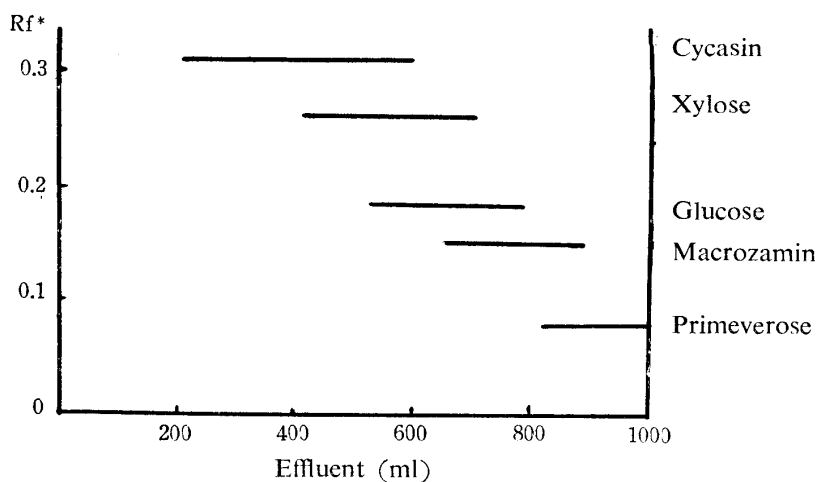


Fig. 1. Chromatographic separation into the components of the cleavage products from macrozamin.

\* Rf value in paper chromatography using BuOH : AcOH : H<sub>2</sub>O (4 : 1 : 1) as solvent

was neutralized by a treatment with anion exchange resin and then was evaporated in vacuo to dryness. The sample was put on the top of a cellulose column (30 g, dia. 2.5 × 50 cm), developed with *n*-butanol saturated with water, and was fractionated for every 10 ml. Each fraction was examined by the spot test with aniline hydrogen phthalate and resorcinol hydrochloric acid reagents, and the positive ones as for the carbohydrates were further examined by paper chromatography.

As shown in Fig. 1, cycasin and primeverose were found as intermediates in the course of cleavage, in addition to the unaltered macrozamin or the final products, glucose and xylose. The fraction in which cycasin was involved (effluents 220~600 ml) were combined and evaporated in vacuo to dryness, then the crystalline residues were recrystallized from ethanol in order to remove the contaminated xylose and glucose, and fine needles were obtained. The yield was 9.7% of the theoretical amount; its m. p. was 143° ~ 143.5° (decomp. uncorr.) undepressed on admixture with authentic cycasin, and further it was pure paper chromatographically, too. Elementary analysis was as follows:

Found: C, 38.05%; H, 6.36%; N, 11.11 %.

Calcd. for C<sub>8</sub>H<sub>16</sub>O<sub>7</sub>N<sub>2</sub>: C, 38.07 %; H, 6.39 %; N, 11.11 %.

Thus the possibility is established that macrozamin which has primeverose as its sugar component cleaves into cycasin which has glucose. It has been little known as for the decomposition of a glycoside which has a component of disaccharide. Bridel<sup>(6)</sup> reported that the primeverosides, for example gentiocaloside, monotropitoside, rhamnucoside, primeverin or primuraverin, were hydrolyzed with the specific enzyme, primeverase, into primeverose and the respective aglycone. On the other hand, he showed that in the case of acid hydrolysis the division of primeverose into xylose and glucoside further proceeded. Here is presented another process of acid hydrolysis in which a primeveroside, that is 6-O-β-D-xylosyl-D-glucoside, gives first xylose and glucoside, here cycasin, and then the latter successively liberates glucose and the aglycone. These results also support the con-

firmation of the structure of cycasin together with that of macrozamin which was proposed by Lythgoe et al.<sup>(7,8)</sup>

### Summary

Cycasin was identified by an isolation in the acid partial hydrolysate of macrozamin, and the correlations were proved between the sugar components of these two glycosides of the same aglycone.

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### References

- (1) K. NISHIDA, A. KOBAYASHI, and T. NAGAHAMA, *Bull. Agr. Chem. Soc. Japan*, **19**, 77 (1955).
- (2) K. NISHIDA, A. KOBAYASHI, T. NAGAHAMA, and T. NUMATA, *ibid.*, **23**, 460 (1959).
- (3) J. M. COOPER, *Proc. Roy. Soc. N. S. W.*, **74**, 450 (1940), (C. A., **35**, 4917 (1941)).
- (4) N. V. RIGGS, *Australlian J. Chem.*, **7**, 123 (1954), (C. A., **48**, 6510 (1954)).
- (5) K. NISHIDA, A. KOBAYASHI, T. NAGAHAMA, and J. NAWATA, *Mem. Fac. Agr. Kagoshin a Univ.*, vol. **III**, p. 6 (1957).
- (6) M. BRIDEL, *Compt. rend.*, **179**, 991 (1924); **180**, 1421 (1925).
- (7) B. LYTHCOE and N. V. RIGGS, *J. Chem. Soc.*, **1949**, 2716.
- (8) B. W. LANGLEY, B. LYTHGOE, and N. V. RIGGS, *ibid.*, **1951**, 2309