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Interconversion between the Azoxyglycosides by Cycad Emulsin*

Studies on Some New Azoxyglycosides of *Cycas revoluta* Thunb. Part III

Sir:

Previously, we reported the isolation of cycasin¹⁾ and two β -glucosyl-cycasin, neocycasin A²⁾ and B³⁾, which were proved to be β -laminaribiosyl- and β -gentiobiosyl-oxyazoxymethane, respectively. We adverted also to the existence of some other azoxyglycosides in the seed of Japanese cycad.

Here, the evidences are presented that these glycosides are interconvertible by catalysis of cycad emulsin, and further a new higher oligoglycoside, neocycasin C, is also produced. In this case, it is noteworthy that neocycasin A, containing a β -1.3 glucosyl linkage, is predominantly produced from cycasin as substrate, although in the hitherto reported transglucosylation^{4,5)} a β -1.6 or 1.4 linkage was mainly formed by emulsins obtained from various origins.

The cycad emulsin employed here is an acetone-dried preparation obtained from the cycad seeds by the tannic acid precipitation method⁶⁾.

(1) Enzymatic cleavage of neocycasin A or B into cycasin by cycad emulsin was examined as follows. The reaction mixture contained neocycasin A (0.012 M in final concentration), the enzyme (5 mg) and acetate buffer (pH 4.6) in a total volume of 2 ml, and was incubated at 30°C. Neocycasin B was examined in the

same way but on an one tenth scale. The intermediary products identified by paper chromatography were cycasin, glucose and laminaribiose or gentiobiose.

(2) The enzymatic transfer reaction was investigated with cycasin as substrate. The reaction mixture, containing 2.5 ml, of cycasin (0.15 M in final concentration), 2 mg of the enzyme and acetate buffer (pH 5.6) was incubated at 30°C under toluene. The samples were withdrawn at intervals, deproteinized and subjected to paper chromatography. As shown in Fig. 1, neocycasin A was the first major transfer product, which was deduced to be synthesized by transfer of β -glucosyl moiety from a donor cycasin to another one acting as acceptor. Laminaribiose, neocycasin B, and later gentiobiose also appeared. Accompanied by further decrease of cycasin or neocycasin A, an appearance of a new azoxyglycoside, neocycasin C, came to be recognized. During the course of this reaction, the accumulation of neocycasin A reached its maximum after nearly sixteen hours.

FIG. 1. Paper Chromatogram showing the Fate of Carbohydrates during the Course of Enzymatic Interconversion between the Azoxyglycosides.

Development, *n*-BuOH-AcOH-H₂O (4:1:1) 2 runs; Reagents, R, resorcin-HCl in aq. EtOH, A, aniline hydrogen phthalate in aq. *n*-BuOH.

For the conditions of reaction see text.

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(3) Isolation of the reaction products was attempted. A mixture, 10 ml, of the same composition as above was incubated for thirty hours. The deproteinized digest was chromatographed on carbon column (dia. 30×270 mm; carbon 40 g) with water, then aqueous ethanol of step-wise-increasing concentrations successively. The eluates were fractionated into 50 ml portions, of which the carbohydrates were examined by paper chromatography. In the aqueous fractions glucose was found, and in the same way, in the 10% ethanolic ones glucose and gentiobiose, in 15% ones cycasin, laminaribiose, and neocycasin B, in 30% ones neocycasin A, B and C, and in the 40% ones neocycasin C were found. The fractions were separately combined according to their constituents, and were preparatively rechromatographed on paper. The each component separated on the papers was eluted with water, lyophilized, and subjected to the following identification.

The obtained samples and their hydrolysates were all identified by paper chromatography with the respective authentic specimens.

i) Neocycasin A (33.2 mg). After partial acid hydrolysis ($0.2N$ H_2SO_4 , $100^\circ C$, 1.5 hrs.), the formation of cycasin, glucose and laminaribiose was proved. Acetylation of the powder gave its acetate in long prisms; m.p. $141.5^\circ \sim 143^\circ C$ alone or on admixture with authentic hepta-acetylneocycasin A.

ii) Laminaribiose (11.3 mg). In the digests with cycad emulsin, glucose was found. Its acetate was obtained as prisms, m.p. $165^\circ \sim 166^\circ C$

(micro hot plate) which showed no depression on admixture with authentic octaacetyl- β -laminaribiose.

iii) Neocycasin B (3.5 mg). This was cleaved by the partial acid hydrolysis into cycasin, glucose, or gentiobiose.

iv) Gentiobiose (1.9 mg). The degradation products by the complete acid hydrolysis ($1N$ HCl , $100^\circ C$, 1.5 hrs.) was glucose.

v) Neocycasin C (2.4 mg). The chromatographic behavior of this product was the same as that of one of the unidentified azoxyglycosides found in cycad seeds. When it was partially hydrolysed with acid, the spots of neocycasin A, glucose, laminaribiose, cycasin and an unknown sugar were recognized. The last one was considered to be composed of three or more glucose units from its coloration and R_F value. Since any biose or bioside other than the two above mentioned which have β -1,3 linkages was not found, this sugar, too, would have the same configuration. That is, it may be laminari-triose or -tetraose.

Accordingly, the constitution of neocycasin C, though it is not fully established, may be 3-O- β -glucosyl or laminaribiosyl neocycasin A. Further investigations are now in progress.

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