TABLE I. GLYCOSIDES AND SUGARS IN EACH ELUATE FROM THE CARBON COLUMN FOUND BY PAPER CHROMATOGRAPHY

| Spots | | | | | | | $R_F$ | | colors revealed with reagents | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | R. | A. |
| ± | + | ⧻ | ⧺ | ⧺ | + | | .55 | cycasin | y | — |
| ⧻ | + | | | | | | .46 | fructose | rb | pb |
| | | | | | + | ± | .40 | $A_1$ | y | — |
| ⧻ | ⧺ | + | + | | | | .35 | glucose | — | b |
| | | | | | + | ⧻ | .33 | $A_2$ (neocycasin A) | y | — |
| | | | | | + | ± | .27 | $A_3$ | y | — |
| + | ⧺ | + | + | ± | | | .23 | sucrose | rb | b |
| | | | | | ⧺ | ± | .20 | $A_4$ | y | — |
| | | | + | ⧺ | + | | .19 | L(laminaribiose) | — | b |
| | | | | | ± | ± | | tailing $A_x$ | y | — |
| | | | | | + | | .11 | | rb | — |
| | | + | | | | | .10 | | — | b |
| | | | | | + | | .07 | | rb | — |
| + | | | | | | ± | .00 | | rb | b |
| a | b | c | d | e | f | g | | | | |
| Eluates | | | | | | | | | | |

Filter Paper: Tôyô No. 2. Solvent: *n*-BuOH : AcOH : $H_2O$ (4 : 1 : 1).

Development: multiple ascending (2 runs). Reagents: R, Resorcin-HCl EtOH soln. A, Anilline hydrogen phthalate BuOH soln.

Eluates: a, with 4.7 l $H_2O$. b, 6.4 l followed after a. c, 7.2 l after b. d, with 3.2 l 2% EtOH. e, 4.0 l 5% EtOH. f, 5.0 l 10% EtOH. g, 4.0 l 40% EtOH.

Colors: b, brown. y, yellow. r, reddish. p, pale.

under reduced pressure at 50° to a thick syrup, 500ml.

The syrup was treated with 5 volumes of methanol to precipitate the gummy impurities, which were redissolved in their own volume of water and treated with methanol as above, repeatedly. The methanolic supernatants were concentrated and treated with lead acetate and hydrogen sulfide as usual. The filtrate was concentrated to syrup (Syrup I, 450 ml).

In order to separate the glycosides, Syrup I was chromatographied on a carbon column (300 g, dia. 5 × 42 cm). Water, and then 2,5,10, and 40% aqueous ethanol was successively passed through the column. The composition of carbohydrates in each eluate was examined by paper chromatography. As represented in Table I, the spot A group, assumed to be azoxy glycosides, in 10 and 40% ethanolic eluates was exceedingly characteristic. The last fraction which contained the substance visualized as spot $A_2$ alone, comparatively, was carefully concentrated to syrup. The syrup was treated with ethanol, set aside in a refrigerator, and resulted in crystallizing. After recrystallization from aqueous ethanol or methylcellosolve, 1.6 g of a colorless fine needle crystal (Fig. 1), neocycasin A, was obtained.

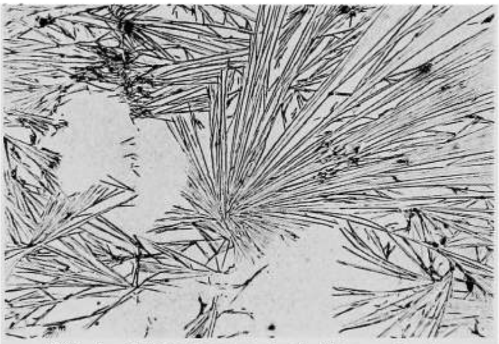

FIG. 1. Microphotograph of Neocycasin A.

### Properties of Neocycasin A

Neocycasin A thus obtained showed m.p. 162°～163° (decomp.) and $[\alpha]_D^{29} -35.1°$ (c. 1.0 in water). *Anal.* Found: C, 38.59, 38.78; H, 6.76, 6.55; N, 6.31. Calcd. for $C_{14}H_{26}O_{12}N_2 \cdot H_2O$: C, 38.88; H, 6.52; N, 6.48%. MW, Found: 411 (cryoscopic), Calcd: 432. It was soluble in hot aqueous ethanol or methycellosölve, readily soluble in water, sparklingly in ethylacetate, and not soluble in chloroform. It gave a positive Fehling or Molish reaction and a greenish blue coloration with anthrone. On paper chromatograms it gave a single spot yellowish colored with a resorcin-hydrochloric acid reagent.

### Acetylate of Neocycasin A

A solution of 500 mg of neocycasin A in 10 ml of pyridine was treated with acetic anhydride at room temperature as usual. The resultant acetylate, recrystallized from ethanol, was long prisms (FIG. 2), 540 mg, and m.p. 142°~143°, $[\alpha]_D^{29}-55.5°$ (c. 1.0 in chloroform), *Anal.* Found: C, 47.76; H, 5.85; N, 4.35. Calcd. for $C_{14}H_{19}O_{12}N_2$-$(CH_3CO)_7$: C, 47.46; H, 5.69; N, 3.95%. MW, Found: 711 (Rast), Calcd.: 708. $CH_3CO$, Found: 6.8 mols. per mol. of acetylate.

Deacetylated with one drop of 0.5N sodium methylate in 3 ml of ice cold chloroform-methanol, 98 mg of the heptaacetylate was reconverted into the original glycoside, 15 mg.

FIG. 2. Microphotograph of Heptaacetyl Neocycasin A.

### Acid hydrolysis

**1) Identification of glucose and formaldehyde in the complete acid hydrolysate** With 1N hydrochloric acid, 97 mg of neocycasin A was hydrolysed at 100° for 2.5 hours. The hydrolysate being vacuum evaporated repeatedly in order to expell the produced formaldehyde, glucose in the remains was determined by the Hanes method and found to be 2.0 mols. per mol. of the glycoside. In the above distillate, formaldehyde was identified as crystalline formaldomedone.

During these hydrolysis, it was shown paper chromatographically that the spot of neocycasin A was only substituted by glucose. The phenylosazone prepared from the hydrolysate as usual, was a yellow needle, m.p. 203°~204° (decomp.) alone or on admixture with authentic glucose-phenylosazone.

**2) Cycasin and laminaribose as the products of partial acid hydrolysis** Neocycasin A was hydrolysed partially with 0.2 N sulfuric acid at 100° for two hours. In this partial hydrolysate were detected four spots, three of which were identified to be cycasin, glucose, and unaffected neocycasin A, respectively. The remaining one, spot L, was, according to the literature[7], closely adjacent in $R_F$ value to that of laminaribiose or nigerose. After multiple development (3 runs) being applied in a mixture of *n*-butanol-pyridine-water (3:2:1.5 by vol.), spot L was found to be identical with that of authentic laminaribiose.

### Degradation with emulsin

Degradation of neocycasin A with cycad emulsin[2] was examined in acetate buffer, pH 4.6, at 30°. Thereby, the glycoside was finally split into glucose, which was proved paper chromatographically. On the other hand, this emulsin preparation did not act on maltose under the same condition.

### Action of alkali

**1) Isolation of octaacetyl-β-laminaribiose from heptaacetyl neocycasin A** A solution of 250 mg of heptaacetylate in 5 ml of chloroform with 2 ml of 2N methanolic sodium methylate, was kept for one hour at room temperature. The white substance, 140 mg, which precipitated with proceeding of the reaction, was collected, washed with a small amount of cold chloroform, and dried. About the contamination with some glucose due to an over reaction, it was chromatographied on carbon column (dia. 1.8×2.5 cm). After glucose was washed out with a sufficient amount of water, the fraction which was eluted with 35% ethanol was evaporated to dryness. The white powder obtained, 65 mg, showing a single spot corresponding to laminaribiose, was treated with sodium acetate and acetic anhydride at 110°~120°, and the resultant mixture was extracted with chloroform. After expulsion of the solvent, the syrup was treated with ethanol and subsequently evaporated in vacuo, repeatedly, and crystallized.

The needles, recrystallized from ethanol, were 13 mg in yield, m.p. 159°~160°, which accorded with that of octaacetyl-β-laminaribiose described in the general references. *Anal.* Found: C, 49.39; H, 5.70. Calcd. for $C_{28}H_{38}O_{19}$: C, 49.56; H, 5.64%.

**2) Detection of formaldehyde and hydrocyanic acid** In the above alkaline reaction mixture, the presence of formaldehyde and hydrocyanic acid which were derived from the aglycone was proved; that is formaldomedone

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[7] K. Aso and F. Yamauchi, *J. Ferment. Technol. Japan*, **33**, 194 (1955).