

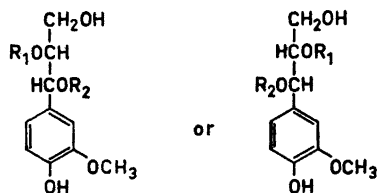
nents was amorphous ($[\alpha]_D^{25} - 16^\circ$) whereas the other (II), obtained in smaller amounts, was crystalline (m.p. $162-166^\circ$, $[\alpha]_D^{25} + 45^\circ$; Found: C 51.0; H 6.77; O 42.3; $C_{16}H_{24}O_{10}$ requires C 51.0; H 6.43; O 42.5). Treatment of either of the glucosides with β -glucosidase yielded D-glucose and a component, indistinguishable from guaiacyl glycerol [1-*C*-(4-hydroxy-3-methoxy-phenyl)-glycerol] by paper chromatography and UV absorption in neutral and alkaline solution. Paper electrophoresis in borate (pH 10) and sulphonated phenylboronic acid (pH 6.5) buffers distinguishes between *threo*- and *erythro*-guaiacyl glycerols.² Using this method both aglucones were identified as the *threo*-isomer. Furthermore the aglycone from I was transformed to the tetraacetate, which was identified by m.p., mixed m.p. and thin-layer chromatography with an authentic sample of D,L-*threo*-guaiacyl glycerol tetraacetate.

The intense colour reactions of the two glucosides with phenol reagent "Echtblausalz B" (Merck) and also the UV absorption in neutral and alkaline solutions indicated that the phenolic hydroxyl was free in both substances. Glucoside I gave a strong blue colour with 2,6-dibromo-N-chloroquinonimine (which is characteristic of *p*-hydroxybenzyl alcohols³); whereas II gave no colour. This indicated that glucose is linked at the β - or γ -hydroxyl of the glycerol chain in I and at the α -position in II. Neither vanillin nor formaldehyde was formed when I was treated with periodate under conditions by which these products are readily formed from guaiacyl glycerol,⁴ showing that glucose is linked in the β -position. On the other hand, formaldehyde (identified as methylene-bis-dimedone) but no vanillin was formed from glucoside II, in agreement with the formula below.

The extract also contains free guaiacyl glycerol, present in larger amount than the two glucosides. The guaiacyl glycerol is optically active ($[\alpha]_D^{25} - 18^\circ$) and has the *threo*-form. The aglucones isolated from the two glucosides also had negative rotations. The absolute configuration of the aglucones is not yet determined.

A preliminary study of the water-soluble fraction of cambium and newly formed needles collected in the spring by paper chromatography and electrophoresis indicated that glucoside I was present.

The isolation of these optically active compounds in pine needles may be of some importance in connection with the



I $R_1 = \beta$ -D-glucopyranose; $R_2 = H$

II $R_2 = \beta$ -D-glucopyranose; $R_1 = H$

biosynthesis of lignin and other wood constituents having phenyl propane carbon structures.

Traces of *threo*-guaiacyl glycerol were recently isolated in the form of its tetraacetate after treatment of *Pinus resinosa* Ait heartwood under acetylation conditions at $50-70^\circ$ ⁵ and the related ω -hydroxypropionguaiacone found in small amounts in the bark of *Pinus silvestris* L.⁶

Acknowledgements. The author wishes to thank Professor B. Lindberg for his interest in this work and Professor E. Adler for a sample of D,L-*threo*-guaiacyl glycerol tetraacetate.

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Received September 24, 1965.

Exclusion Chromatography of Barley β -Amylase on Sephadex G-75

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We have earlier reported the fractionation of barley β -amylases on Sephadex G-100 columns.¹ Both that and the

immunochemical² and ultracentrifugal³ analyses of β -amylases of different molecular size included preparative procedures⁴ before the fractionation and therefore it was considered desirable to study the β -amylases without any purification and concentration, since these procedures may cause changes in the real "picture" of the β -amylase of barley.

In order to obtain more information about β -amylases of smaller molecular size we used Sephadex G-75 instead of the Sephadex G-100 used in our earlier experiments. Fractionation was performed immediately after extraction so as to avoid changes in the β -amylase during storage.

Extraction. A) *Free β -amylase.* Finely ground barley was defatted with acetone (-15°C) and the soluble proteins extracted with distilled water. The centrifuged extract was buffered to pH 7.5 with a phosphate solution giving a final concentration of 0.010 M. 2.5 % NaCl was also added to the solution. The solution was used as such in the chromatography.

B) *Bound β -amylase.* 30 g of acetone-treated barley was treated as follows:

- 1) 8 washings with 200 ml of distilled water
- 2) 20 washings with 200 ml of 5 % NaCl
- 3) 3 washings with 200 ml of 10 % NaCl
- 4) 5 washings with 200 ml of distilled water

5) Extraction of the latent β -amylase for 40 h at $+4^{\circ}\text{C}$ with 60 ml of a 0.7 % thioglycolic acid solution containing 0.7 % NaCl, pH 7.0. 2 ml of the centrifuged extract was subjected to chromatography immediately after the extraction procedure.

Exclusion chromatography. The Sephadex G-75 column (4×130 cm) was packed with a 0.010 M phosphate buffer of pH 7.5, containing 2.5 % NaCl. All the runs were made on the same column.

Measurement of β -amylase activity. The β -amylase activities were determined colorimetrically, using 3,5-dinitrosalicylic acid as reagent.⁵ Instead of the acetate buffer (pH 4.2) a phosphate buffer (pH 7.0) was used.¹

Results and discussion. Fig. 1 shows the fractionation of barley β -amylase on Sephadex G-75. In this case 10 g of barley was extracted for 2 h at $+4^{\circ}\text{C}$ with 30 ml of distilled water. After centrifugation 3 ml of the supernatant was immediately analysed on the column. The β -amylase was resolved into four fractions: A_{1a} , A_{1b} , A_2 , and A_{3+4} . The fractions A_{1a} and A_{1b} together corresponded to the fraction A_1 and the fraction A_{3+4} to the fractions A_3 and A_4 yielded by chromatography on

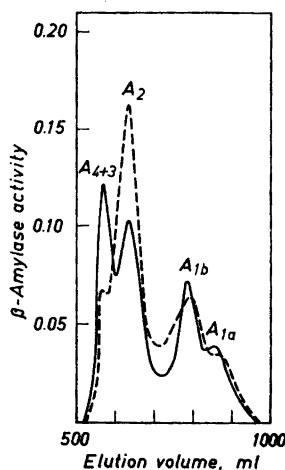


Fig. 1. Chromatography on Sephadex G-75 of untreated and oxidized β -amylase extracts of barley, — = β -Amylase activity of untreated extract, - - - = β -Amylase activity of oxidized extract. β -Amylase activity = mg maltose/min \times g of barley.

Sephadex G-100. The A_2 fractions were the same.

Our earlier observations^{1,2} and the hypothesis that the β -amylases of larger molecular size are formed by oxidative aggregation of the fundamental components A_{1a} and A_{1b} led us to study the effect of oxidation and reduction of the untreated extract.

For this purpose 50 g of acetone-treated barley was extracted for 2 h with 100 ml of distilled water at $+4^{\circ}\text{C}$. The centrifuged solution served as a sample pool for oxidations and reductions and a part of it was set aside to reveal the effect of standing.

After 23 days' standing a sample of the original extract was subjected to chromatography (Fig. 2). The fraction A_{3+4} had decreased, whilst the fractions A_{1a} had increased correspondingly during storage in the refrigerator ($+4^{\circ}\text{C}$).

10 ml of the extract was oxidized for 24 days at $+4^{\circ}\text{C}$ with 3 ml of a solution containing 5 M urea, 0.5 M ferricyanide, 2.5 % NaCl, 0.010 M phosphate, pH 7.0.

After 24 days the oxidized sample was analysed on the same column (Fig. 1). Fraction A_2 had increased remarkably

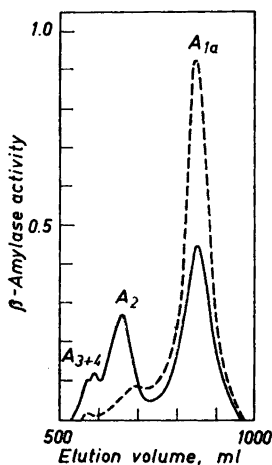


Fig. 2. Chromatography on Sephadex G-75 of reduced and stored β -amylase extract of barley. — = β -Amylase activity of stored extract, - - - = β -amylase activity of reduced extract. β -Amylase activity = mg maltose/min \times g of barley.

whilst fraction A_1 , surprisingly, did not show any significant change.

20 ml of the extract was reduced for 25 days at $+4^\circ\text{C}$ with 1 ml of a solution containing 10% thioglycolic acid, 0.010 M phosphate, pH 7.0.

The reduced sample was analysed in the same way as the previous samples. It was found that nearly all the amylase is eluted (Fig. 2) as a single peak at the site of A_{1a} , a small amount of the activity being left in front of the main peak.

The elution curve of the latent β -amylase is shown in Fig. 3. 2 ml of the centrifuged extract of bound proteins was subjected to chromatography immediately after the extraction procedures. The elution curve shows that the latent β -amylase is eluted at the site of the larger fundamental component, A_{1b} , of the free β -amylase.

When the β -amylase is extracted directly with thioglycolic acid or reduced immediately after extraction, only the peak A_{1b} is obtained.

Malt was also analysed in the same way as barley. The result can be seen in Fig. 3. The main peak is eluted at the site of A_{1a} . During the malting process the larger amylases have either dissociated or been

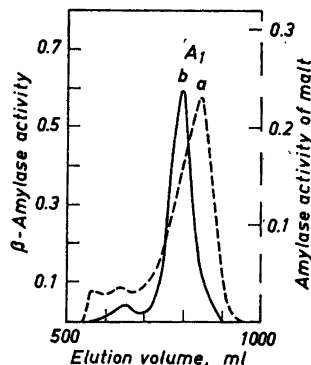


Fig. 3. Chromatography of the latent β -amylase of barley and the amylases of malt. — = β -Amylase activity of the latent enzyme, - - - = amylase activity of malt. β -Amylase activity = mg maltose/min \times g of barley.

split enzymatically into the smallest component.

Our data indicate that there is no danger of association or dissociation in the preparation of β -amylases, the different β -amylases existing as such in the extracts of barley. In all probability there are at least these four different β -amylases in intact barley and maybe even aggregates of still larger molecular size which are insoluble because of their high molecular weight. It is also possible that the latent β -amylase of barley is composed of these very large, insoluble aggregates, which are split and thus liberated by reductive extraction procedures.

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Received September 20, 1965.