In the range of concentrations from 0.002 to 0.1% the absorption of light by the colored solutions of the substance obeys the Bouguer–Lambert–Beer law.

As the result of observations made over a year, it has been established that in the storage process the optical density of solutions of the preparation rises. In view of this, in all cases the quantitative determination was made 30 min after the preparation of the solutions.

To plot a calibration graph we prepared standard solutions of the substance. With this aim, accurately weighed samples (0.0025,0.0075, 0.0125, 0.0185, 0.025, 0.0315, and 0.0375 g) were transferred to 50-ml measuring flasks and water was added to the mark in each case. The resulting solutions were subjected to photoelectric colorimetry as described above.

The zero point was determined with distilled water (Fig. 1). The metrological results for the concentration of 0.015% were as follows: mean of five determinations 0.0146; mean square error $7.953 \cdot 10^{-4}$; relative standard deviation from the mean result 0.0544.

ANALYSIS OF THE PRODUCTS OF THE ENZYMATIC HYDROLYSIS OF LAMINARIN WITH THE AID OF AN AUTOMATIC LIQUID ANALYZER

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Several examples of the separation of mixtures of neutral mono- and oligosaccharides using automatic analyzers are given in the literature. Thus, good results have been achieved in the separation of maltodextrins by gel filtration on Bio-Gel P-2 [1] and of α-linked oligosaccharides of glucose on anion-exchange resins using a borate buffer as eluent [2]. For the analysis of laminarin oligosaccharides we have used a Jeol JLC 6AH automatic liquid analyzer (Japan).

Figure 1 shows the pattern of the distribution on Bio-Gel P-2 of the mixture of oligosaccharides obtained by the enzymatic cleavage of laminarin by the endo-β-1,3-glucanase LIV from the marine mollusc Spisula sachalinensis [3]. The sharp fall in the amount of laminarin with the formation of a large amount of oligomers is typical for the action of an endo enzyme; on prolonged enzymatic hydrolysis, glucose and laminaribiose predominated in the reaction products. On Bio-Gel P-2 separation of the oligosaccharides according to their molecular weights took place, but the isomeric dimers and trimers were not separated. This problem was solved by the fractionation of the mixture of oligosaccharides produced by enzymatic hydrolysis on LCR-3 anion-exchange resin (Jeol, Japan). On using borate buffers with various pH values [2], it proved to be possible to separate laminaribiose and gentiobiose (Fig. 2). In comparing acid and enzymatic hydrolyzates of laminarin it is important to observe that gentiobiose is present in the former and is absent from the latter (see Fig. 2). Obviously, under the action of LIV enzymatic hydrolysis does not take place to the stage of the formation of gentiobiose. Thus, it is possible to use the automatic liquid analyzer in kinetic investigations of α-1,3-glucamase. The advantages of the proposed method are obvious: the use of small amounts of substance (250 μg of enzymatic hydrolysis products per separation), rapidity and accuracy of the analysis, and the possibility of the quantitative treatment of the results.

The carbohydrates were separated on LCR-3 ion-exchange resin under the conditions described by Torii and Sakakibara [2], but the passage of buffers 1, 2, and 3 was continued for 110, 120, and 240 min, respectively. The column (2 × 100 cm) of Bio-Gel P-2 (200-400 mesh) was eluted with water at the rate of 0.51 ml/min at 65°C. The analysis took 9 h. The sugars were determined colorimetrically after reaction with a 0.15% solution of orcinol in 70% H_2SO_4 at 95°C. The rate of feed of the reagent was 0.042 ml/min and of the eluate 0.21 ml/min.

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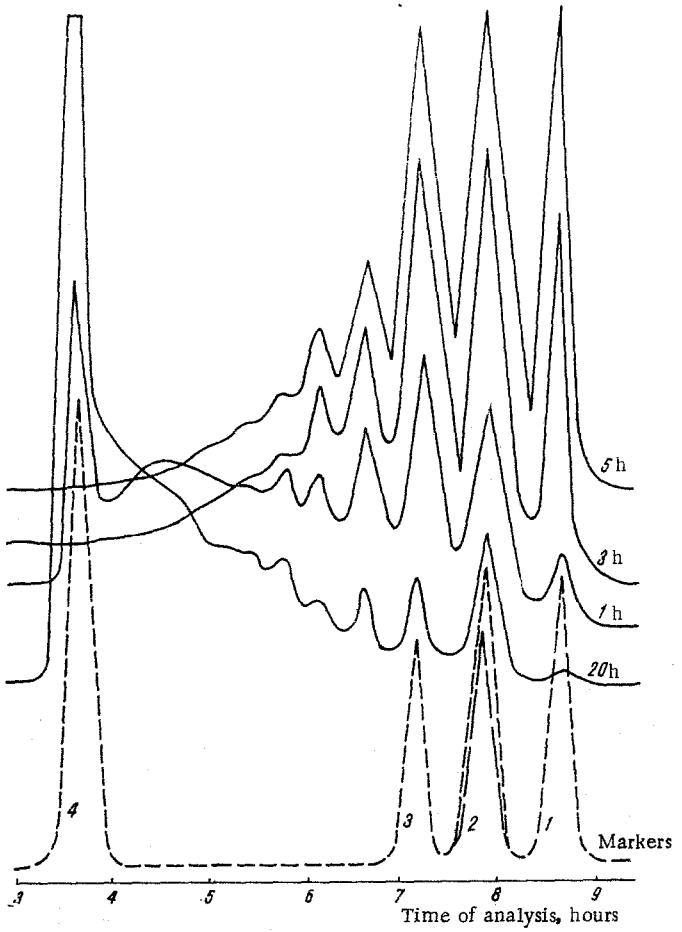

Fig. 1. Separation on Bio-Gel P-2 (200-400 mesh) of the products of the enzymatic hydrolysis of laminarin (for conditions, see text). Markers: 1) glucose; 2) laminaribiose; 3) laminaritriose; 4) laminarin: dashed line – gentiobiose.

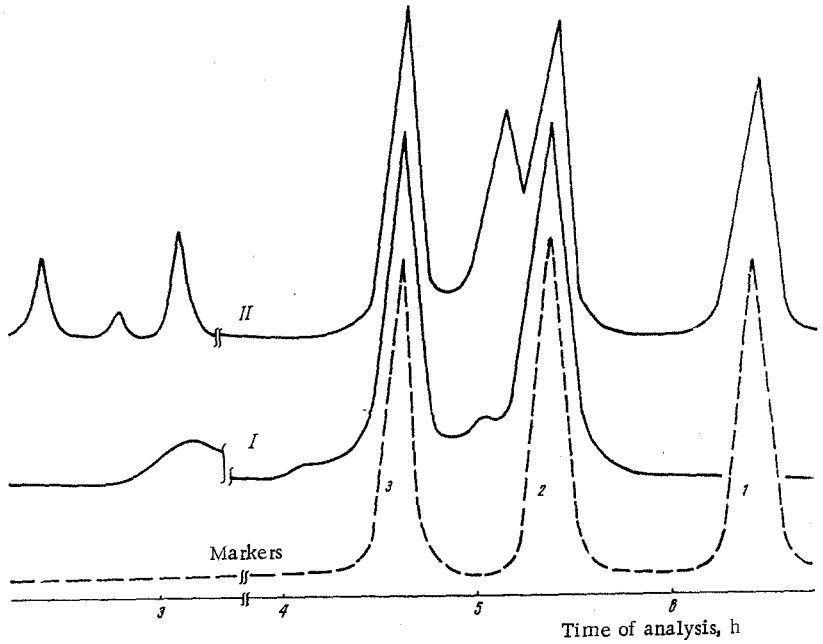

Fig. 2. Chromatographic separation [2] on LCR-3 ion-exchange resin of the products of the enzymatic (I) and acid (II) hydrolyses of laminarin. Markers: 1) gentiobiose; 2) glucose; 3) laminaribiose.

The concentration of the enzyme was determined by Lowry's method [4] and the amounts of carbohydrates in the samples by the phenol–sulfuric acid method [5]. Enzymatic hydrolysis was performed in the following way. To 27 ml of a solution (1 mg/ml) of laminarin from L. cychariodes [6] in water was added 0.85 ml of LIV (50 μg/ml) in 0.05 M (pH 5.6) succinate buffer with 0.05 M NaCl, and the mixture was incubated at 37°C. After predetermined intervals of time, aliquots (3 ml) were taken and they were boiled for 5 min to stop the enzymatic reaction and were analyzed. The partial acid hydrolysis of the laminarin from L. cychariodes (5 mg/ml) was performed with 0.25 M sulfuric acid at 100°C for 175 min. After neutralization the mixture obtained was first chromatographed on a carbon–Celite column [carbon–Celite (1.5 : 1)] with analysis of the fractions by paper chromatography on FN-1 paper in the butanol–pyridine–water (4 : 6 : 3) system: the fractions containing bioses were chromatographed (see Fig. 2).

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