The hydrogenolysis of 3.2 g of (XVI) gave 2.1 g (91%) of methyl 2,3,4-tri-O-methyl-\(\alpha\)-D-galactopyranoside (XVII), \([\alpha]_D^{27} +125^\circ \) (c 2.6; chloroform), \(+147^\circ \) (c 3.6; methanol), \(n_D^{20} 1.4595\). According to the literature [4]: \([\alpha]_D^{27} +161^\circ \) (methanol), \(n_D^{20} 1.4626\); [15]: \([\alpha]_D^{20} +132.2^\circ \) (methanol), \(n_D^{20} 1.4608\).

The oxidation of 1.5 g of (XVII) followed by esterification gave 1.3 g (77%) of (XVIII). Crystallization from ether yielded pure (XVIII), mp 71°C, \([\alpha]_D +134^\circ \) (c 1.4; chloroform). According to the literature [9, 10]: mp 70.2-70.3°C, \([\alpha]_D^{20} +142.1^\circ \) (chloroform); mp 73°C, \([\alpha]_D +169^\circ \) (water).

**SUMMARY**

Unidirectional syntheses of the 2,3-, 2,4-, and 3,4-di- and 2,3,4-tri-O-methyl ethers of methyl (methyl \(\alpha\)-D-galactopyranosid)uronate are proposed.

**LITERATURE CITED**


**CHARACTERISTICS OF A \(\beta\)-1,3-GLUCANASE FROM *Spisula sachalinensis* AS A GLYCOPROTEIN**

O. M. Myashtovskaya, V. V. Sova, and L. A. Elyakova

The nature of the carbohydrate-peptide bond and the composition of the carbohydrate chain in a \(\beta\)-1,3-glucanase from the marine mollusk *S. sachalinensis* has been investigated. According to the results of the phenol-sulfuric acid method, the neutral sugars amounted to 6.5% of the molecular weight of the enzyme. The composition of the neutral sugars (Glc : Gal : Man = 5:2:1) was determined by the GLC method. It was shown that the \(\beta\)-1,3-glucanase molecule contains no uronic or sialic acids. The amount of amino sugars (15% with equal amounts of glucosamine and galactosamine) was established by amino acid analysis. Alkaline degradation led via the \(\beta\)-elimination reaction to the splitting out of 50% of the neutral sugars and showed the existence of an O-glycosidic bond in the enzyme molecule. Various actions on the carbohydrate moiety (periodate oxidation and treatment with glycosidases) caused no appreciable change in the hydrolyzing capacity of the enzyme.

In the study of the primary structure of enzymes, many of which are glycoproteins, one of the interesting aspects is the investigation of the carbohydrate moiety of the molecule;
Fig. 1. Peptide map of a tryptic hydrolysate of the reaction product of the β-1,3-glucanase LIV with labeled methylamine: I) electrophoresis, pH 5.6, V 800 V, time 50 min; II) chromatography in the butanol-pyridine-acetic acid-water (60:40:12:48, v/v, system). The peptides were detected with a 0.3% solution of ninhydrin in acetone (1); 2) label at an O-glycosidic bond; 3) detection of neutral sugars.

the elucidation of its role in activity and the determination of the type of carbohydrate-protein bond.

The endo-β-1,3-glucanase L IV that we are studying, which was isolated from the crystalline style of the marine mollusk S. sachalinensis in the homogeneous state [1], contains a covalently bound carbohydrate component which is not separated on ion-exchange chromatography or prolonged dialysis. The results of high-voltage electrophoresis of a sample of the β-1,3-glucanase L IV hydrolysed by formic acid showed the absence of uronic acids [2]. No sialic acids were detected by Warren's method [3]. The amino sugar content (about 15%, with equal amounts of glucosamine and galactosamine) was determined with the aid of amino acid analysis. The amount of neutral sugars was 6.5% according to the results of the phenol-sulfuric acid method [4]. The qualitative composition of the neutral sugars in L IV hydrolyzed with 2 N HCl was determined by paper chromatography. The quantitative ratio of the monosaccharides (Glc:Gal:Man, 5:2:1) was established by the GLC method.

It is known that branched polysaccharides having α-D-mannosyl, α-D-glucopyranosyl, and D-fructopyranosyl residues at the nonreducing ends are capable of being precipitated by concanavalin A (Con A). Linear α-glucans and mannans are not precipitated by Con A, although they may be bound.

It has been established that yeast β-1,3-glucanases are glycoproteins [5, 6]. Using homogeneous forms of three enzymes — endo-β-1,3-, exo-β-1,3-1,6-, and exo-β-1,3-glucanases, indications of different degrees of glycosylation in them have been obtained. All the enzymes were close in amino acid composition and differed in the ratios of glucose and mannose in the molecules and their capacity for being bound by Con A [7].

Under the conditions of chromatography on a column containing (Con A)-Sepharose 4B, weak binding of the β-1,3-glucanase L IV with the Con A was observed. On immunodiffusion in 1.5% agar containing Con A [8], precipitation was observed which disappeared on washing with 0.01 M phosphate buffer, pH 7.5, containing 0.15 M NaCl. Precipitation in homogeneous solution took place at high concentrations of the enzyme (3 mg/ml) and of Con A (5 mg/ml). It may be assumed that the observed interaction is nonspecific or weak, i.e., the carbohydrate moiety of L IV is probably not branched or does not contain glucose and mannose residues at the reducing ends.