15. R. Gigg and C. D. Warren, J. Chem. Soc., 2205 (1965).
16. T. J. Lucas and C. Schuerch, Carbohydr. Res., 39, 39 (1975).
17. P. Kovač and R. Brazný, Chem. Zvesti, 29, 544 (1975).
18. J. Kiss and F. Burkhardt, Helv. Chim. Acta, 53, 1000 (1970).

SYNTHESIS OF METHYL DERIVATIVES OF URONIC ACIDS. II. SYNTHESIS OF THE 2,3-, 2,4-, AND 2,4-DI- AND 2,3,4-TRI-O-METHYL ETHERS OF METHYL (METHYL α-D-GALACTOPYRANOSID)URONATE

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UDC 547.917

Unidirectional methods are proposed for the synthesis of the 2,3-, 2,4-, and 3,4-di-, and 2,3,4-tri-O-methyl ethers of methyl (methyl α-D-galactopyranosid)uronate by the oxidation (CrO_3—H_2SO_4—acetone) of the corresponding methyl O-benzyl-O-methyl-α-D-galactopyranosides having unsubstituted 6-OH groups to the corresponding (methyl O-benzyl-O-methyl-α-D-galactosid)uronic acids followed by esterification with CH_2N_2 and catalytic hydrogenolysis of the benzyl groups.

Continuing work on the synthesis of methyl derivates of uronic acids, we have synthesized all the di-O- and tri-O-methyl ethers of D-galacturonic acid.

Methyl (methyl 2,3-di-O-methyl-D-galactopyranosid)uronate is one of the main products of the methanolysis of completely methylated pectic substances [1]. However, up to the present time no synthesis of the pure α anomer of this compound has been proposed. A number of authors have obtained the crytalline β anomer [2] and an anomeric mixture of the 2,3-di-O-methyl ethers [1, 3]. We propose a synthesis of methyl (methyl 2,3-di-O-methyl-α-D-galactopyranosid)uronate from methyl 4,6-O-benzylidene-2,3-di-O-methyl-α-D-galactopyranoside by the following method: debenzylidenation, tritylation of the primary alcoholic 6-OH group, benzylation of the 4-OH group, detritylation, oxidation with a solution of chromium trioxide in acetone, esterification with diazomethane, and hydrogenolysis of the protective benzyl group.

Methyl (methyl 2,4-di-O-methyl-α-D-galactopyranosid)uronate was synthesized by the method of Kovač and Brezný [4], but we obtained the intermediate product methyl 3-O-benzyl-4,6-O-benzylidene-2-O-methyl-α-D-galactopyranoside in a simpler manner [5].

In the syntheses of methyl (methyl 3,4-di-O-methyl-α-D-galactopyranosid)uronate described in the literature, the difficultly accessible methyl (methyl 3,4-O-isopropylidene-α-D-galactopyranosid)uronate is used as the starting material; and, moreover, the alkaline conditions of methylation lead to the formation of unsaturated compounds [6], while on detosylation [7] deesterification of the methyl uronate takes place, which requires subsequent re-esterification with diazomethane. In the synthesis of the 3,4-di-O-methyl ether we started from methyl 2-O-benzyl-3-O-methyl-6-O-trityl-α-D-galactopyranoside [8], the methylation of which, with subsequent detritylation, oxidation, esterification, and hydrogenolysis of the benzyl group, gave the required product.

The synthesis of methyl (methyl 2,3,4-tri-O-methyl-α-D-galactopyranosid)uronate presents no fundamental difficulties and has been proposed by a number of authors starting from D-galacturonic acid [2, 9] and from methyl 2,3,4-tri-O-methyl-α-D-galactopyranoside by permanganate oxidation [10]. We have performed an alternative synthesis of this compound, starting from methyl 6-O-benzyl-α-D-galactopyranoside using the following scheme: exhaustive methylation, hydrogenolysis of the benzyl group, oxidation, and esterification.

M. V. Frunze Simferopol' State University. Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 283-285, May-June, 1982. Original article submitted December 31, 1981.

0009-3130/82/1803-0259$07.50 © 1983 Plenum Publishing Corporation

EXPERIMENTAL

Melting points were determined on a heated stage, and specific rotations on a SM-1 polarimeter (Na lamp). Refractive indices were determined on a IRF-22 refractometer. The measurements were performed at 20°C. Column chromatography was performed on silica gel L (40-100 μ) (Czechoslovakia). The results of the microanalysis of all the compounds synthesized agreed with the calculated figures.

A number of general methods (alkylation, detritylation, oxidation, esterification, and hydrogenolysis) that have been described in the preceding communication [8] were used.

Methyl (Methyl 2,3-Di-O-methyl-α-D-galactopyranosid)uronate (VII). A solution of 10.5 g of methyl 4,6-O-benzylidene-2,3-di-O-methyl-α-D-galactopyranoside (I) [11, 5] in 150 ml of dioxane–water (2:1) was treated with 10 ml of a 1 N solution of sulfuric acid. The reaction mixture was kept at 100°C until the benzylidene group had been hydrolyzed completely. The acid was neutralized with barium carbonate, the precipitate was filtered off, and the filtrate was evaporated to dryness. This gave 7.1 g (94%) of methyl 2,3-di-O-methyl-α-D-galactopyranoside (II), $[\alpha]_D$ +171° (c 1.9; chloroform), n_D 1.474. According to the literature [11]: $[\alpha]_D^{15}$ +173.7°, n_D^{15} 1.472.

A solution of 7.0 g of (II) in 7 ml of pyridine was treated with 9.6 g of chlorotriphenylmethane. The mixture was kept at 80°C until the reaction was complete, and the pyridine was distilled off at reduced pressure. The residue was dissolved in chloroform, and the solution was washed with water, with sulfuric acid solution, with water, with sodium bicarbonate solution, and with water again. It was then dried with anhydrous sodium sulfate and evaporated, and the residue was freed from the excess of chlorotriphenylmethane on silica gel with elution by benzene and benzene–acetone (15:1). The resulting methyl 2,3-di-O-methyl-6-O-trityl-α-D-galactopyranoside (III) was recrystallized from methanol. This gave 11.7 g (79%) of pure (III) with mp 74-76°C, $[\alpha]_D$ +73° (c 2.0; chloroform). According to the literature [12]: mp 77-80°C, $[\alpha]_D^{25}$ +68°.

The benzylation of 11.4 g of (III) gave 11.7 g (86%) of methyl 4-O-benzyl-2,3-di-O-methyl-6-O-trityl-α-D-galactopyranoside (IV), $[\alpha]_D$ +61° (c 2.1; chloroform), n_D 1.574.

The detritylation of 11.2 g of (IV) led to methyl 4-O-benzyl-2,3-di-O-methyl-α-D-galactopyranoside (V); 6 g (95%), $[\alpha]_D$ +77° (c 2.2; chloroform), n_D 1.5095.

The oxidation of 5.5 g of (V) followed by esterification gave 5.3 g (88%) of methyl (methyl 4-O-benzyl-2,3-di-O-methyl-α-D-galactopyranosid)uronate (VI), $[\alpha]_D$ +89° (c 1.2; chloroform), n_D 1.506.

The hydrogenolysis of 4.8 g of (VI) permitted (VII) to be obtained (3 g, 85%), $[\alpha]_D$ +126° (c 1.9; chloroform), n_D 1.469.

Methyl (Methyl 2,4-Di-O-methyl-α-D-galactopyranosid)uronate (IX). By the method of Kovač and Brežný [4], 10.0 g of methyl 3-O-benzyl-4,6-O-benzylidene-2-O-methyl-α-D-galactopyranoside (VIII) [5] yielded 2.1 g of (IX), mp 116-118°C, $[\alpha]_D$ +126° (c 1.4; chloroform). According to the literature [4]: mp 118.5-119°C, $[\alpha]_D^{22}$ +131°.

Methyl (Methyl 3,4-Di-O-methyl-α-D-galactopyranosid)uronate (XIV). The methylation of 6.1 g of methyl 2-O-benzyl-3-O-methyl-6-O-trityl-α-D-galactopyranoside (X) [8] led to methyl 2-O-benzyl-3,4-di-O-methyl-6-O-trityl-α-D-galactopyranoside (XI) (6 g, 96%), $[\alpha]_D$ +18° (c 2.8; chloroform), glass.

The detritylation of 6 g of (XI) gave 3.2 g (95%) of methyl 2-O-benzyl-3,4-di-O-methyl-α-D-galactopyranoside (XII), $[\alpha]_D$ +45° (c 2.0; chloroform), n_D 1.512.

The oxidation of 2.8 g of (XII) followed by esterification gave 2.5 g (81%) of methyl (methyl 2-O-benzyl-3,4-di-O-methyl-α-D-galactopyranosid)uronate (XIII), $[\alpha]_D$ +38° (c 2.2; chloroform), n_D 1.503. According to the literature [6]: $[\alpha]_D^{27}$ +44°.

The hydrogenolysis of 1.4 g of (XIII) gave 0.95 g (92%) of (XIV). Crystallization from ether yielded pure (XIV), mp 112-114°C, $[\alpha]_D$ +165° (c 1.0; chloroform). According to the literature [6]: mp 114-115°C, $[\alpha]_D^{16}$ +168°.

Methyl (Methyl 2,3,4-Tri-O-methyl-α-D-galactopyranosid)uronate (XVIII). [The methylation of 5 g of 6-O-benzyl-α-D-galactopyranoside] (XV) [13] led to 5.5 g (96%) of methyl 6-O-benzyl-2,3,4-tri-O-methyl-α-D-galactopyranoside (XVI), $[\alpha]_D$ +89° (c 1.8; chloroform), n_D 1.498.

The hydrogenolysis of 3.2 g of (XVI) gave 2.1 g (91%) of methyl 2,3,4-tri-O-methyl-α-D-galactopyranoside (XVII), $[\alpha]_D$ +125° (c 2.6; chloroform), +147° (c 3.6; methanol), n_D 1.4595. According to the literature [4]: $[\alpha]_D^{27}$ +161° (methanol), n_D^{25} 1.4626; [15]: $[\alpha]_D^{28}$ +132.2° (methanol), n_D^{25} 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° (c 1.4; chloroform). According to the literature [9, 10]: mp 70.2-70.3°C, $[\alpha]_D^{26}$ +142.1° (chloroform); mp 73°C, $[\alpha]_D$ +169° (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 α-D-galactopyranosid)uronate are proposed.

LITERATURE CITED

1. S. Luckett and F. Smith, J. Chem. Soc., 1106 (1940).
2. S. Luckett and F. Smith, J. Chem. Soc., 1506 (1940).
3. R. E. Gill, E. I. Hirst, and J. K. Jones, J. Chem. Soc., 1025 (1946).
4. P. Kovač and R. Brezný, Chem. Zvesti, 29, 544 (1975).
5. V. I. Grishkovets and V. Ya. Chirva, Khim. Prir. Soedin., 28 (1982).
6. P. Kovač, Carbohydr. Res., 22, 464 (1972).
7. R. A. Eddington and E. E. Percival, J. Chem. Soc., 2473 (1953).
8. V. I. Grishkovets, A. E. Zemlyakov, and V. Ya. Chirva, Khim. Prir. Soedin., 279 (1982).
9. P. A. Levene and L. C. Kreider, J. Biol. Chem., 120, 597 (1937).
10. F. Smith, M. Stacey, and P. I. Wilson, J. Chem. Soc., 131 (1944).
11. G. J. Robertson and R. A. Lamb, J. Chem. Soc., 1321 (1934).
12. A. B. Foster, R. Hems, and J. H. Westwood, Carbohydr. Res., 15, 41 (1970).
13. C. E. Ballou and H. O. L. Fischer, J. Am. Chem. Soc., 76, 3188 (1954).
14. P. A. Levene, R. S. Tipson, and L. C. Kreider, J. Biol. Chem., 122, 199 (1937-1938).
15. P. A. Levene and L. C. Kreider, J. Biol. Chem., 121, 155 (1937).

CHARACTERISTICS OF A β-1,3-GLUCANASE FROM *Spisula sachalinensis* AS A GLYCOPROTEIN

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UDC 577.154

The nature of the carbohydrate-peptide bond and the composition of the carbohydrate chain in a β-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 β-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 β-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;

Pacific Ocean Institute of Bioorganic Chemistry, Far Eastern Scientific Center, Academy of Sciences of the USSR, Vladivostok. Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 286-291, May-June, 1982. Original article submitted July 8, 1981.

0009-3130/82/1803-0261$07.50 © 1983 Plenum Publishing Corporation