NEW SYNTHESES OF METHYL ETHERS FROM METHYL α-L-FUCOPYRANOSIDE

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A method for obtaining methyl esters of methyl α-L-fucopyranoside based on the partial methylation of methyl α-L-fucopyranoside with the subsequent liquid and gas-liquid chromatography of the methyl ethers is described. Their properties are given.

Known methods for the synthesis of methyl esters of L-fucose, one of the main components of the oligosaccharide moieties of glycoproteins are laborious and involve several stages [1]. A promising approach to the synthesis of methylated sugars is based on the partial methylation of methyl glycosides followed by the chromatography of the methyl esters [2, 3]. We have synthesized individual ethers of methyl α-L-fucopyranoside by this method. They were identified with the aid of ^{1}H NMR spectroscopy.

The partial methylation of methyl α-L-fucopyranoside with methyl iodide and silver oxide gave the whole set of methyl esters, but the amount of the 4-mono- and 3,4-di-O-methyl ethers was small. To separate the mixtures of methyl ethers according to their degree of substitution we used liquid chromatography on silica gel. The fractions of mono- and di-O-methyl ethers were obtained in quantitative yield, and they were converted into their acetates and were rechromatographed on a column of silica gel. Below we give the chromatographic properties of the acetates of the methyl ethers (the retention times relative to the acetates of methyl 3-O-methyl-α-L-fucopyranoside, the retention time of which was 10.5 min):

	Position(s) of the methyl group(s)							
	2	3	4	2,3	2,4	3,4	2,3,4,	–
R_f	0.53	0.56	0.58	0.48	0.50	0.54	0.50	0.55
R_f^f	1.45	1.00	1.30	0.46	0.77	0.58	0.31	2.00

Under the conditions of liquid chromatography, complete separation of the acetates of the monomethyl esthers was observed. At the same time, when the acetates of the di-O-methyl ethers were chromatographed only that of the 2,3-di-O-methyl ether was isolated in the individual state. The two-component mixtures obtained in this process were separated by preparative GLC with a yield of 55% when the column was loaded with 100 mg of each mixture. Thus, all the methyl ethers of methyl α-L-fucopyranoside have been obtained and characterized in the individual state uncontaminated by isomers.

The positions of the methyl groups in the methyl ethers were established by ^{1}H NMR spectroscopy (Table 1). The chemical shifts of the carbon atoms for the acetates of the methyl ethers are given in Table 2. They agreed well with the known effects of methylation [4] and of acetylation [5].

The results of ^{13}C NMR spectroscopy of the methyl ethers are frequently used to identify the structures of oligo- and polysaccharides. Table 3 gives the values of the chemical shifts of the carbon atoms of the methyl ethers of methyl α-L-fucopyranoside found experimentally and calculated. The assignment of the signals was made with allowance for the effects of methylation found for methyl α-D-fucopyranoside [6].

EXPERIMENTAL

Melting points were determined on a Boëtius instrument. Specific rotations were determined on a Perkin–Elmer M 141 polarimeter using chloroform as solvent. ^{1}H NMR spectra were measured on a Bruker UX-90 E spectrometer. Chemical shifts are given in ppm relative to

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TABLE 1. Chemical Shifts of the Protons in the ^1H NMR Spectra of the Acetates of Partially Methylated Derivatives of Methyl α-L-Fucopyranoside

Position(s) of the methyl group(s)	H-1	H-2	H-3	H-4	H-5	3H-6	OMe	OAc
—	5,04	5,49	5,65	5,41	3,70	0,96	3,03	1,78; 1,77; 1,75
2	4,81	3,72	5,59	5,40	3,72	0,98	3,14;3,20	1,84; 1,70
3	5,02	5,48	3,69	5,28	3,69	1,08	3,04;3,25	1,70; 1,72
4	5,03	5,62	5,62	3,24	3,65	1,12	3,04;3,23	1,70; 1,73
2,3	4,76	3,30	3,30	5,29	3,35	1,09	3,15;3,32[2]	1,83
2,4	4,77	3,87	5,52	3,32	3,71	1,13	3,14;3,17 3,27;	1,77
3,4	5,04	5,64	3,65	3,03	3,64	1,21	3,08;3,20 3,37	1,73
2,3,4	4,76	3,81	3,62	3,03	3,68	1,22	3,20;3,28; 3,34;3,40	—

Remark. The assignment of the signals of the protons in the spectrum of the acetate of methyl α-L-fucopyranoside was made by the INDOR method. The assignment of the signals in the spectra of the partially methylated derivatives was made on the basis of the general laws of the upfield shifts of the signals of the skeletal protons when an acetyl group is replaced by a methyl group and on the basis of the spin–spin coupling constants of the protons $J_{1,2}$ = 3.6 Hz; $J_{2,3}$ = 11.0 Hz; $J_{3,4}$ = 3.4 Hz; $J_{4,5}$ = 1.4 Hz; $J_{5,6}$ = 6.7 Hz (for the acetate of methyl α-L-fucopyranoside).

tetramethylsilane, which was used as internal standard. The solvent used was C_6D_6. The assignment of the signals of the protons was made by the INDOR method. The ^{13}C NMR spectra were obtained on a Bruker WM-250 spectrometer. The solvent for the acetates of the methyl ethers was C_6D_6, δ_{TMS} = 0, and that for the partially methylated derivtives was D_2O. The chemical shifts were measured relative to CH_3OH taking as standrd CH_3 = 49.6 ppm. TLC was performed on silica gel L 5-40 μm(Chemapol) in the petroleum ether (bp 70-100°C)–acetone (1:1) system. The spots were revealed with 30% sulfuric acid in methanol. Silica gel L 100-160 μm(Chemapol), previously fractionated, was used for column chromatography. Fractions with a volume of 25 ml each were taken. GLC was performed on a Tsvet-106 instrument fitted with a flame-ionization detector and double columns (200 × 0.3 cm). The liquid phase was 1.5% of NPGS on Chromaton N-AW-HMDS (0.125-0.160 mm). The rate of flow of argon was 60 ml/min and the thermostat temperature was 160°C. For preparative GLC we used a PAKhV-0.7 instrument fitted with a katharometer and a glass column (200 × 1.4 cm). The liquid phase was 10% of NPGS on Chromaton N-AW-HMDS (0.20-0.25 mm, Chemapol). The rate of flow of helium was 400 ml/min. The temperature of the column was 170°C, that of the evaporator 300°C, and that of the collector 120°C. Glass tubes (80 × 5 mm) were used as receivers without cooling.

Synthesis of the Methyl Ethers of Methyl α-L-Fucopyranoside. The methylation of methyl α-L-fucopyranoside (7.0 g) with methyl iodide (14 ml) and silver oxide (28 g) in methanol (60 ml) at room temperature for 2 h gave 7.2 g of a mixture of methyl ethers.

Preparative Liquid Chromatography of the Methyl Ethers of Methyl α-L-Fucopyranoside. The mixture of methyl ethers (6.9 g) was deposited on a column (45 × 3 cm) of silica gel and was eluted with a gradient of methanol in chloroform. The yield of the 2,3,4-tri-O-methyl ether was 0.5 g, mp 94-95°C. $[\alpha]D^{20}$ −188.1° (c 1.3). According to the literature [1]: mp 97-98°C, $[\alpha]D^{20}$ −209°.

The fractions of di- and monomethyl ethers were obtained with yields of 3.6 and 2.7 g, respectively. The mixture of the acetates of the monomethyl ethers (1.9 g) was deposited on a column (35 × 2 cm) of silica gel and they were eluted successively with a solvent gradient using 0.3 liter each of 5, 10, 20, and 50% ethyl acetate in petroleum ether (bp 70-100°C). The results of the separation were as follows:

Methyl ether	Yield, g	mp, °C	$[\alpha]D^{20}$, deg
4	0.04	94-95	−169.8 (c 0.8)
3	0.32	118-119	−177.0 (c 1.2)
2	1.40	52-53	−181.1 (c 1.8)

TABLE 2. ^{13}C Chemical Shifts for Acetates of Methyl Ethers of Methyl α-L-Fucopyranoside

Position(s) of methyl groups	C-1	C-2	C-3	C-4	C-5	C-6	MeO-1	MeO-2	MeO-3	MeO-4	OAc	
—	97,3	68,3	68,0	71,3	64,3	15,9	55,4				20,7	
2	98,0	75,5	70,2*	71,6*	64,2	15,9	55,4	58,8			20,6	169,8
											20,8	170,3
3	97,4	70,1	75,7*	69,8*	64,5*	16,2	55,4		57,7		20,7	170,5
											21,0	
4	97,5	68,8	71,3	80,4	65,8	16,1	55,3			61,8	20,9	170,3
2,3	98,1	77,3[a]	78,0[a]	70,1*	64,5	16,1	55,4	58,9	57,6		20,8	170,6
2,4	97,9	75,8	73,4*	80,5*	65,6	16,2	55,2	58,9		61,8	21,1	170,6
3,4	97,7	71,1*	78,9[a]	79,6[a]	66,3*	16,4	55,2		58,3	61,7	21,0	170,4
2,3,4	98,1	77,3	80,8	79,7	66,0	16,4	55,2	58,9	58,3	61,7		

*The assignment of the signals was made by selective decoupling from protons; a – assignment of the signals ambiguous.

TABLE 3. ^{13}C Chemical Shifts for the Methyl Ethers of Methyl α-L-Fucopyranoside*

Position of methyl groups	C-1	C-2	C-3	C-4	C-5	C-6	MeO-1	MeO-2	MeO-3	MeO-4
—	100,2	68,7	70,3	72,5	67,2	16,0	55,8			
2	97,5(97,8)	78,0(78,2)	69,6(70,1)	72,4(72,2)	67,0(67,1)	16,0	55,5	58,3		
3	100,1(100,1)	67,6[a] (67,7)	79,6(80,4)	68,1[a] (67,9)	67,0(67,0)	16,1	55,8		56,6	
4	100,2(100,2)	69,0(69,1)	70,7(71,4)	83,3(82,1)	67,3(67,5)	16,1	55,8			62,6
2,3	97,2(97,8)	76,8(77,3)	78,8(80,0)	68,0(68,2)	66,8(67,0)	15,0	55,1	58,6	56,3	
2,4	97,5(97,7)	78,4(78,5)	70,1(70,6)	83,2(82,2)	67,0(67,4)	16,0	55,6	58,5		62,0
3,4	100,0(100,3)	68,0(68,3)	80,3(81,6)	78,9(78,1)	67,3(67,5)	16,2	55,8		57,6	62,1
2,3,4	97,3	77,3	79,6	78,8	67,0	16,1	55,5	58,2	57,4	62,1

*The values of the chemical shifts calculated with allowance for α-, β-, and γ-effects of methylation from [6] are given in partentheses; a – assignment of the signals not unambiguous.

The mixture of acetates of the dimethyl ethers (0.9 g) was deposited on a column (35 × 2 cm) of silica gel and was eluted in the same way as the monomethyl ethers. The yield of the 2,3-di-O-methyl ether was 0.2 g, $[\alpha]D^{20}$–183.8° (c 0.8). The yields of the fractions containing mixtures of the 3,4- and 2,4-di-O-methyl ethers and the 2,4- and 2,3-di-O-methyl ethers were 0.17 and 0.36 g, respectively. The preparative GLC of the first mixture gave in one cycle 23 mg of the 3,4-di-O-methyl with mp 74-75°C $[\alpha]D^{20}$ –182.5° (c 0.5), and 32 mg of the 2,4-di-O-methyl ether, $[\alpha]D^{20}$ –210.1° (c 0.9).

When the second mixture was chromatographed, the yields of 2,4- and 2,3-di-O-methyl ethers were 26 and 30 mg, respectively.

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OPTIMIZATION OF THE PRECIPITATION OF PLANTAGLYUTSID FROM EVAPORATED AQUEOUS EXTRACTS

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The optimization of one of the stages of the technology of Plantaglyutsid – its precipitation from the evaporated aqueous extract with ethanol – has been optimized by the methods of mathematical statistics. Information has been obtained on the optimum parameters for performing the process with the aim of achieving the maximum yield of preparation and lowering the consumption of raw material.

Plantaglyutsid is the total preparation obtrained from the leaves of Plantago major L. (rippleseed plantain) and it contains a mineralized complex of polysaccharides. It is used in medical practice as an antigastritis agent [1]. The technology of the isolation of Plantaglyutsid was developed by the Khar'kov Scientific-Research Institute of Pharmaceutical Chemistry. In 1978 its production was begun at the Tashkent Pharamecutical Chemical Plant.

There is no information in the literature on the optimization fo the technological stages in the production of Plantaglyutsid.

One of the main stages is the isolation of Plantaglyutsid from the leaves of the rippleseed plantain is its precipitation with ethanol from the evaporated aqueous extract. Hitherto, this process has been performed by treating the still residue after the evaporation of the aqueous extract with ethanol in a ratio of 1:3. The treated solution is kept under static conditions for 3-4 h. The precipitated Plantaglyutsid is separated by filtration, washed with ethanol, and dried.

EXPERIMENTAL

In order to itensify the precipitation stage, to shorten the technological cycle, and to raise the yield of desired product we have studied the main factors affecting the process and have performed its optimization by using the mathematical methods of experimental planning.

On the basis of preliminary experiments we determined as the main factors:

X_1 – the ratio of the volumes of still residue and precipitant;

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