GLYCOSYLATION OF o-ACYLAMINO DERIVATIVES OF 4-METHYL-7-HYDROXY-COUMARIN

I. K. Kozlova

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Certain glycopyranosides of o-acylamino-4-methyl-7-hydroxycoumarin were studied. They have potential application as fluorogenic substrates for a number of enzymes in biochemical diagnostics of hereditary diseases.

Glycosides of 4-methyl-7-hydroxycoumarin (4-methylumbelliferone) are widely used as synthetic fluorogenic substrates for a number of enzymes in biochemical diagnostics of human hereditary diseases. However, they are not suitable for use with other types of lysosome enzymes, which split only those glycosides which contain a fatty acid residue in the β-position relative to the O-glycoside residue. Glycopyranosides of 2-hexadecanoylamino-4-nitrophenol are synthetic substrates of the glycolipid type [1]. The disadvantage of substrates based on nitrophenol is the low sensitivity of the chromogenic method of determination of the activity of the enzymes, compared with the fluorogenic method based on the use of fluorescent tagged analogs of natural substrates of these enzymes [2]. Synthetic fluorogenic substrates with a glycolipid nature are not known up to the present time.

The present work deals with development of methods of synthesis of 1,2-trans- and 1,2-cis-glycopyranosides of o-hexadecanoylamino-4-methylumbelliferone (I-IV), which may be of interest as potential fluorogenic substrates for lipid-hydrolases in biochemical diagnostics of certain forms of glycolipidoses.

As the aglycones for synthesis of the glycosides, we used 6-hexadecanoylamino- (V) and 8-hexadecanoylamino-4-methylumbelliferones (VI), whose synthesis and properties are described in our preceding publication [3].

β-D-Galactopyranosides I and II were obtained by the Koenigs–Knorr synthesis, specifically modified for the synthesis of 1,2-trans-glycosides. One of its modifications consists in the reaction of the phenolate ion with an α-bromo derivative of a monosaccharide acetate [1]. Aglycones V and VI were converted into the corresponding sodium salts Va and VIa by treatment with an equimolar amount of NaOEt. The glycosylation of the aglycone salts proceeds in high yield, if a small amount of DMSO is added to the solvents, tetrahydrofuran or acetone, generally used for this purpose. It is known that an aprotic polar solvent, while increasing the nucleophilicity of the anion, favors the occurrence of the reaction by the S_N2 mechanism. The yields of the galactoside acetates VII and VIII are 60-65%. Galactopyranosides I and II were obtained by deacylation of the corresponding acetates VII and VIII by the action of sodium methylate in methanol.

It is known that in contrast to 1,2-trans-glycosides, there is no general method for the syntheiss of 1,2-cis-glycosides, including the α-L-fucosides [4]. To obtain α-L-fucopyranoside III, we used the Helferich method, consisting in fusing the phenols together with fully acetylated sugars in the presence of acid catalysts ($ZnCl_2$, H_2SO_4, p-toluenesulfonic acid, etc.) [5]. In general, two possible acetates of α- and β-glycosides are thus obtained, but their ratio in the reaction mixture is determined by the nature of the catalyst selected, and also by the difference in the thermal stability of the acetates of the stereoisomeric glycosides under the reaction conditions. Frequently, $ZnCl_2$ is used as catalyst for obtaining 1,2-dis-glycosides [6, 7].

The investigation showed that fusion of aglycone V with L-fucose tetraacetate in the presence of $ZnCl_2$ leads to a mixture of acetates of α- and β-anomeric fucopyranosides with the β-anomer predominating. Thus, at an overall yield of 22%, the ratio of the acetates of

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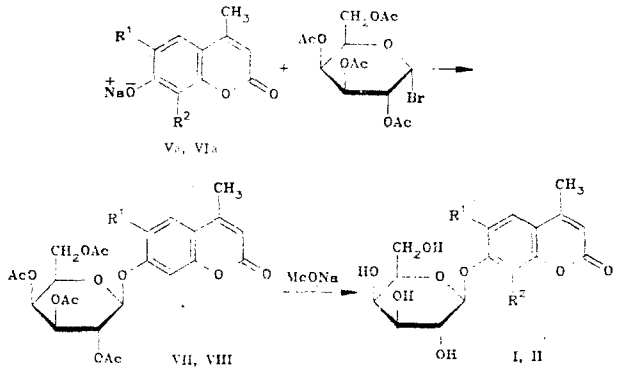
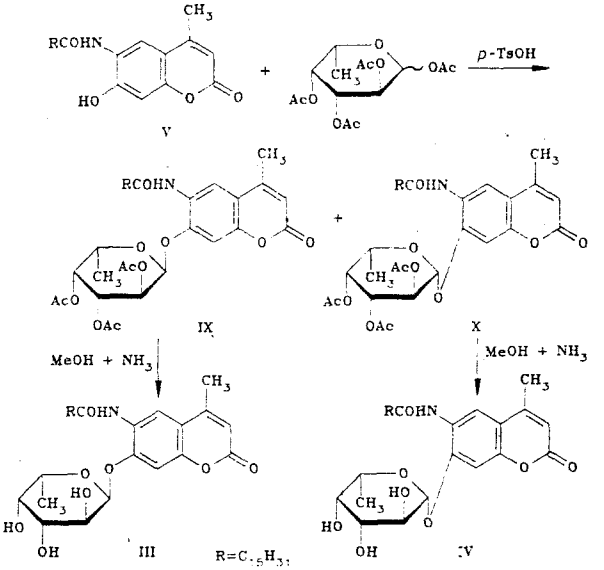


I, Va, VII $R^1=NHCOC_{15}H_{31}$, $R^2=H$; II, VIa, VIII $R^1=H$, $R^2=NHCOC_{15}H_{31}$

α- and β-anomeric fucopyranosides IX and X, after their separation by the adsorption chromatography is 1:2. A much better result is obtained when p-toluenesulfonic acid is used as the catalyst. In this case, the overall yield of acetates IX and X reaches 50%, whereby the ratio between α- and β-fucosides after the chromatographic separation is 3:1, i.e., the yield of the 1,2-cis-glycoside acetate increases to 37% in this case. It should be noted that the time of fusion should not exceed a few minutes, since increase in the reaction time results in degradation of the hydroxycoumarin ring, which can be readily confirmed from the absence of fluorescence of the products in the reaction medium during TLC control of the course of reaction.



Due to the rapid occurrence of the glycosylation and, possibly, because of the presence of a bulky substituent at the amide nitrogen atom, the undesired transacylation, which is known to be observed frequently on heating N-acylated o-aminophenols in the presence of Lewis acids [8, 9], can be avoided.

Deacylation of the fucopyranoside acetates IX and X was carried out by the action of a 5% ammonia solution of in MeOH at 0°C; sodium methylate splits the O-glycoside bond, even in catalytic amounts, and cannot be used for deacylation.

As expected, a doublet in the 4.8-4.9 ppm range J_{12} = 8.0-8.3 Hz is characteristic of the PMR spectra of β-galactopyranosides I and II in the anomeric proton region. This conforms the axial position of the α-anomeric proton at the glycoside center. The PMR spectra of α- and β-isomeric fucopyranosides III and IV substantially differ in the anomeric proton region. A doublet at 5.0 ppm, J_{12} = 7.7 Hz, corresponds to the 1-H of β-fucoopyranoside IV, while the 1-H doublet in of α-fucopyranoside III spectrum is shifted to a weaker field (5.56 ppm, J_{12} = 4.1 Hz), which characterizes the equatorial position of 1-H at the glycoside center in fucopyranoside III (see Table 1).

TABLE 1. Characteristics of o-Hexadecanoylamino-4-methylumbelliferone Glycosides I-IV

Compound	mp, °C (from alcohol)	UV spectrum, λ_{max}, nm (log ε)	1-H PMR spectrum of glycoside, ppm (J_{12}, Hz)	Fluorescence spectrum*, nm		Found, %		Empirical formula	Calculated, %	
				λ_{exc}	λ_{emis}	C	H		C	H
I	205—206	320 (4.04)	4.80 (8.0)	312 (340)** 344***	413 417***	64,7	8,4	$C_{32}H_{49}NO_9$	65,0	8,3
II	186—187	319 (4.17)	4.90 (8.3)	320	430	64.8	8,1	$C_{32}H_{49}NO_9$	65.0	8,3
III	158—160	336 (4.04)	5.56 (4,1)	336	415	67,0	8,6	$C_{32}H_{49}NO_8$	66,8	8,5
IV	185—187	336 (4.04)	5,00 (7,7)	336	415	67,0	8,8	$C_{32}H_{49}NO_8$	66,8	8,5

*The fluorescence spectra are not corrected; λ_{exc} is λ of maximal fluorescence excitation, λ_{emis} is λ of maximal fluorescence emission.
**A shoulder in the spectrum.
***Spectrum in DMFA.

In the UV spectra of glycosides III and IV (see Table 1), a 16 nm bathochromic shift of the absorption maximum is observed relative to glycoside I at the same value of absorption (log ε 4.04).

The fluorescence quantum yields of β-galactopyranosides I and II (Q 0.38 and 0.002) are practically equal to the quantum yields of the initial aglycones V and VI, respectively (Q 0.27 and H 0.0046), i.e., the fluorescence is determined by the position of the N-acyl grouping relative to the pyrone ring of hydroxycoumarin, and is independent of the substitution of the hydroxyl group by the glycoside residue.

The author wishes to express his gratitude to G. I. Klebanov for taking the fluorescence spectra and determining the quantum yields.

EXPERIMENTAL

The PMR spectra were run on a Bruker WH-250 spectrometer (DMSO-D_6 and CD_3OH), the UV spectra on a Specord M-40 spectrophotometer (ethanol), and fluorescence spectra on a Hitachi MPF 4A spectrofluorimeter (ethanol). A Silpirl UV-254 silica gel was used for the absorption chromatography. The TLC was carried out on Silufol UV-254 plates in systems: chloroform—methanol, 9:1 (A), chloroform—methanol, 1:1; (B); ether (C), chloroform—acetone, 1:1 (D), chloroform—methyl ethyl ketone—methanol, 97:1:2 (E).

6-Hexadecanoylamino-4-methylumbelliferyl-β-O-tetraacetylgalactopyranoside (VII). A 23.3-ml portion of 0.1 N solution of sodium ethylate in ethanol is added, with stirring, to a suspension of 1 g (2.33 mmoles) of aglycones V [3] in 5 ml of ethanol, the solution is evaporated to dryness, and the residue is dried for 10 h over KOH. The sodium salt Va obtained is suspended in 50 ml of dry THF, 5 ml of a solution 0.96 g (2.33 mmoles) of D-galactose α-bromotetraacetate [5] in DMSO are added, and the mixture is boiled for 22 h. It is then filtered, the filtrate is evaporated, 120 ml of water are added to the residue, and the mixture is extracted by 5 × 20 ml of ether. The extract is dried over $CaCl_2$, filtered through a 2-cm layer of SiO_2, and evaporated. Yield, 1.1 g (62%), mp 69°C, R_f 0.65 (A).

6-Hexadecanoylamino-4-methylumbelliferyl-β-D-galactopyranoside (I). A 0.12-ml portion of 0.5 N solution of sodium methylate in methanol is added to a solution of 0.4 g (0.52 mmole) of acetate VII in 10 ml of absolute acetonitrile, the mixture is held at 20°C for 36 h, and the precipitate is filtered off. Yield, 0.18 g (58%), R_f 0.24 (B).

8-Hexadecanoylamino-4-methylumbelliferyl-β-O-tetraacetylgalactopyranoside (VIII). A 23.3-ml portion of 0.1 N solution of sodium ethylate in ethanol is added with stirring to a solution of 1 g (2.33 mmoles) of aglycone VI [3] in 5 ml of ethanol, the solution is evaporated to dryness, and the residue is dried for 10 h over KOH. The Na salt obtained is suspended in 50 ml of dry acetone, and a solution fo 0.96 g (2.33 mmoles) of D-galactose α-bromotetraacetate in 5 ml of DMSO is added. The mixture is boiled for 24 h, filtered,

the filtrate is evaporated, 75 ml of water are added to the residue, and the mixture is extracted by 5 × 20 ml of ether. The extract is dried over $CaCl_2$, filtered through a 2-cm layer of SiO_2, and evaporated. Yield, 1.2 g (68%), an oily substance, R_f 0.4 (C).

8-Hexadecanoylamino-4-methylumbelliferyl-β-D-galactopyranoside (II). A 2-ml portion of a 5% NH_3 solution in absolute methanol is added at 20°C to a solution of 0.6 g (0.8 mmole) of acetate VIII in 10 ml of absolute methanol, and the precipitate that separates is filtered off after 5 h. Yield 0.23 g (50%), R_f 0.3 (D).

Glycosylation of 6-Hexadecanoylamino-4-methylumbelliferone (V) by L-Fucose Tetraacetate in the Presence of p-TsOH. A mixture of 0.62 g (1.44 mmole) of aglycone V and 0.48 g (1.44 mmole) of l-fucose tetraacetate [10] is stirred with 8 ml of 0.1% solution of p-TsOH in ether. The mixture is evaporated to dryness and fused under a 15 mm Hz vacuum at 185-190°C for 2 min. The reaction mixture is cooled to 20°C, extracted by 7 × 10 ml of ether, the extract is filtered through a 2-cm layer of SiO_2, evaporated, the residue is dissolved in 5 ml of chloroform, and chromatographed on a column with SiO_2 using chloroform as solvent. Yield 0.5 g (50%) of a mixture of fucopyranoside acetates IX and X. Preparative TLC is used (system E) to isolate the individual acetates IX and X. Yield 0.36 g (36%) of α-fucopyranoside acetate IX, mp 90-92°C, R_f 0.65 (E), and 0.13 g (13%) of β-fucopyranoside acetate X, mp 114-118°C, R_f 0.48 (E).

6-Hexadecanoyl-4-methylumbelliferyl-α-L-fucopyranoside (III). A 3-ml portion of a 5% NH_3 solution in methanol is added to 0.24 g (0.34 mmole) of fucopyranoside acetate IX, and the mixture is held at 20°C for 24 h. The mixture is then evaporated and the residue is chromatographed on a column with SiO_2 (system A). Yield, 0.08 g (40%) of α-fucopyranoside III, R_f 0.68 (A).

6-Hexadecanoyl-4-methylumbelliferyl-β-L-fucopyransoide (IV). A 4-ml portion of a 5% NH_3 solution in methanol is added at 0°C to a suspension of 0.35 g (0.5 mmole) of acetate X in 5 ml of methanol, and the mixture is held at the same temperature for 24 h. Subsequent treatment was as in the preceding procedure. Yield 0.09 g (30%), R_f 0.68, (C).

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