REACTION OF CHOLESTEROL WITH ACETYLATED ALKYL ORTHOACETATES OF D-GLUCOPYRANOSE

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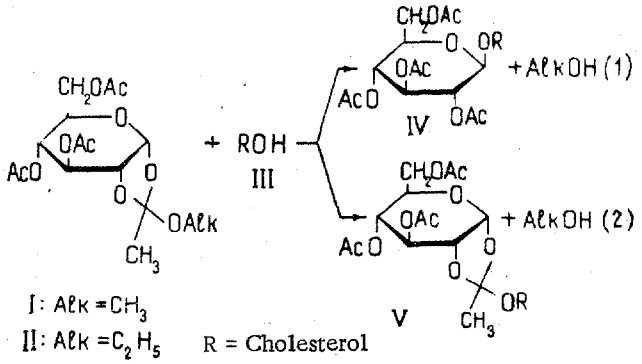
Reference has been made previously [1-4] to the orthoester method, a new method that we have developed for the synthesis of glucosides. The reaction upon which this method is based [1, 2] was discovered in an investigation of the condensation of acetylated alkyl orthoacetates of D-glucopyranose (I) and (II) with cholesterol (III). The present paper gives a more detailed account of the results obtained in a study of this reaction. They enable not only the optimum conditions of glycosylation to be selected but also some idea of the mechanism of this reaction to be obtained.

We have studied the condensation of the alkyl orthoacetates of glucopyranose with cholesterol in various solvents in the presence of acid catalysts (proton or Lewis acids). It was found that this condensation can take place in two directions depending on the nature of the solvent and the type and amount of the catalyst:



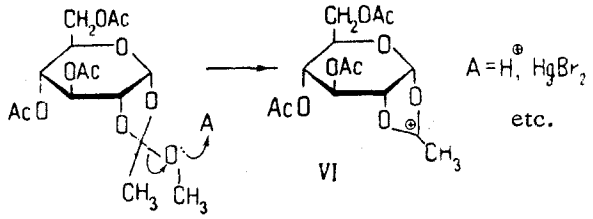
Under the conditions studied, the reaction catalyzed by p-toluenesulfonic acid (TsOH) takes place only in direction (2), regardless of the solvent. The same is observed in solvents of low polarity (dichloroethane or ethyl acetate) in the presence of mercuric bromide ($HgBr_2$) or a mixture of this agent with TsOH. In nitromethane, the direction of the condensation catalyzed by $HgBr_2$ or a mixture of $HgBr_2$ and TsOH ($HgBr_2 \gg$ TsOH) is determined by the amount of mercuric bromide. If the amount of this agent exceeds ~ 0.006 mole per mole of orthoester, glycosylation (1) is the main direction of the reaction. At $HgBr_2 \leq 0.001$ mole, transesterification (2) predominates. An analogous situation is found for reactions catalyzed by mercuric chloride ($HgCl_2$) or cubic bromide ($CuBr_2$) in nitromethane or by $HgBr_2$ in acetonitrile, but the change in the direction of the reaction (from transesterification to glycosylation) takes place at far higher amounts of catalyst (of the order of 0.1-0.5 mole).

Other catalysts that we investigated [titanium tetrachloride, $TiCl_4$; cupric acetate, $Cu(OAc)_2$; and mercuric acetate, $HG(OAc)_2$] in nitromethane caused only transesterification, the last agent having an extremely low activity. Consequently, glycosylation (1) takes place in highly polar media and is catalyzed by mercury or copper halides. For each combination of solvent and catalyst there is some minimum amount of catalyst below which transesterification (2), and above which glycosylation (1), predominates. On this basis the optimum standard method for the glycosylation of alcohols with orthoesters of sugars has been developed, allowing us to syntheize cholesterol β-D-glycopyranoside tetraacetate (IV) in high yield (45%). This method was subsequently used for the synthesis of various glycosides from complex aglycones [2-5].

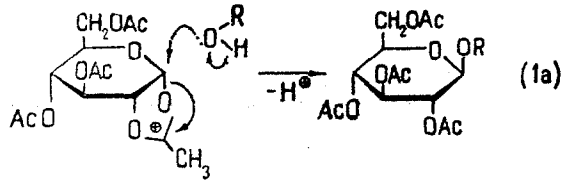
It must be emphasized that we did not observe the formation of an α-glucoside in any of the experiments on the glycosylation of cholesterol, which shows the high stereospecificity of the reaction, and we have subsequently confirmed this with other examples [3, 4].

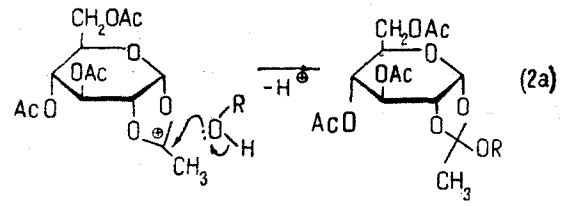
The results obtained enable some preliminary ideas on the mechanism of the new glycosylation reaction to be expressed which, it is considered, still require rigid experimental proof. It is quite clear that the formation of the glycoside (IV) or the new orthoester (V) must be due to a nucleophilic exchange at the glycosidic or the orthoester carbon atom of the original orthoester. However, the stability of the orthoesters even to such powerful nucleophiles as MeO^- (cf. [6]) excludes the possibility of a nucleophilic attack of the alcohol on the neutral molecule of the orthoester. Consequently, the first stage of the reaction is apparently the splitting off of an alkoxide under the influence of a proton or a metal

cation and the formation of a cyclic carbocation (VI):



The reaction of cations of this type with alcohols can take place in two directions [7]: by nucleophilic attack of the alcohol on the glycosidic center (1a) with the formation of a glycoside, and by nucleophilic attack of the alcohol on the electrophilic center of the cation (VI) (2) with the formation of a new orthoester:





In polar media, partial dissociation of mercuric bromide or analogous catalysts may be assumed with the formation of a complex anion of the type $[HgBr_3]^-$, taking part in the creation of a close ionic pair with the cation (VI). Under these conditions screening of the electrophilic center of the cation (VI) must take place, directing the attack of the nucleophilic reagent to the glycosidic center of the sugar with reversal of the configuration and the formation of a glycoside by scheme (1a). Under conditions where the complex anion is not formed (non-polar solvents) or exists in low concentration (small amounts of catalyst), the screening of the cation (VI) does not take place or becomes ineffective, so that the reaction takes place by scheme (2a), which leads to a new orthoester. The rearrangement of the benzoylated methyl orthobenzoate of D-glucopyranose into the isomeric methyl β-D-glucopyranoside described by Helferich [8] probably takes place by an analogous mechanism with the preliminary splitting out of methanol and its subsequent reaction with a cyclic carbocation of type (VI) screened by a complex anion.

Thus, it may be assumed that both directions of the reaction, rearrangement of the orthoester and glycosylation, take place with the same intermediate, i.e., a carbocation of type (VI). In both cases, the alcohols are glycosylated by a cation of type (VI). In actual fact, we have observed the conversion of orthoesters into isomeric glycosides under conditions similar to the glycosylation conditions.

The considerations expressed on the mechanism of glycosylation are based on some general ideas on the reactivity of the glycosidic center of a sugar (cf., for example, [7]) and agree well with the available experimental data. A more complete confirmation of the mechanism proposed can evidently only be obtained by carrying out a special investigation.

Experimental

The halogen-containing solvents were freed from acidic impurities by double distillation over calcium carbonate. The nitromethane was distilled at 100-200 mm over urea and then twice over phosphorus pentoxide. The acetonitrile was twice distilled over P_2O_5. The ethyl acetate was washed with water, with saturated aqueous sodium hydrogen carbonate, and with saturated aqueous calcium chloride, and was dried with $CaCl_2$ and twice distilled over P_2O_5. The alumina was neutralized by being boiled in distilled water 50 times, and was then brought to Brockmann activity grade III. The thin-layer chromatography was carried out on Al_2O_3 in the chloroform-methylethylketone (98.5:1.5) system [9]. Columns with a section of 1 cm^2 for each 0.5-1.0 g of substance were used for preparative chromatography on alumina. Gradient elution (discrete gradient) from CCl_4 to $CHCl_3$ was used. The fractions obtained from the column were analyzed by thin-layer chromatography.

Condensation of Cholesterol (III) with Acetylated Alkyl Orthoacetates of α-D-Glucopyranose (I) or (II)

Expt. No.	Orthoesters (I) or (II) mmole	Cholesterol mmole	Solvent, ml	Reaction time, hr	Catalyst, mmole per 1mmole of orthoester	Cholesterol recovered, %	Yeild of reaction products, %	Method of isolation*	Identification of the reaction products**
1	(I) 0.43	0.43	DCE ***, 4	4	TsOH 0.0005	—	(V)	—	chrom.
2	(I) 0.33	0.31	DCE 3.5	2.5	$HgBr_2$ 0.001	—	(V) 18	chrom.	chrom., mp
3	(I) 0.33	0.36	DCE 3.5	4	$HgBr_2$ 0.0006 + TsOH 0.0005	—	(V) 20	chrom.	chrom.
4	(II) 1.5	1.0	DCE 10	2	$HgBr_2$ 0.33	—	(V) 59	cryst.	chrom., mp., $[\alpha]_D$
5	(I) 0.36	0.28	CH_3NO_2, 3	2	TsOH 0.0004	—	(V)	—	chrom.
6	(I) 0.36	0.36	CH_3NO_2 4	2	$HgBr_2$ 0.001	57	(V)	—	chrom.
7	(I) 1.5	1.5	CH_3NO_2 16	4	$HgBr_2$ 0.001 + TsOH 0.0005	40	(IV) 15 + (V) 26	chrom.	chrom., mp., $[\alpha]_D$
8	(I) 1.0	1.0	CH_3NO_2 8	2	$HgBr_2$ 0.008 + TsOH 0.00025	41	(IV) 45	cryst.	chrom., mp., $[\alpha]_D$
9	(II) 1.0	1.0	CH_3NO_2 8	2	$HgBr_2$ 0.008 + TsOH 0.00025	37	(IV) 45	cryst.	chrom., mp., $[\alpha]_D$
10	(I) 0.5	0.5	CH_3NO_2 5	3	$HgBr_2$ 0.0002 + TsOH 0.0004	48	(V)	—	chrom.
11	(II) 2.0	2.0	CH_3NO_2 15	1.5	$Hg(OAc)_2$ 0.1	91	(V)	—	chrom.
12	(II) 1.55	1.55	CH_3NO_2 15	1.5	$Cu(OAc)_2$ 0.1	—	(V) 30	cryst.	chrom.

13	(II) 1.5	1.0	CH_3NO_2 15	1.5	$HgCl_2$ 0.013	28	(IV)+(V)	—	chrom.
14	(II) 1.0	1.0	CH_3NO_2 10	2	$HgCl_2$ 0.2	31	(IV) 42	cryst.	chrom.
15	(II) 1.5	1.0	CH_3NO_2 15	1	$CuBr_2$ 0.013	20.5	(V) 22 +(IV)	cryst.	chrom., mp
16	(II) 1.0	1.0	CH_3NO_2 10	2	$TiCl_4$ 0.02	44	(V)	—	chrom.
17	(II) 1.0	1.0	CH_3CN 12	2	$HgBr_2$ 0.008 + TsOH 0.00025	50	(V)	cryst.	chrom., mp
18	(II) 1.0	1.0	CH_3CN 12		$HgBr_2$ 0.1 + TsOH 0.0025	—	(IV) 12,5 +(V)	cryst.	chrom., mp
19	(II) 1.3	1.0	CH_3CN 15	1	$CuBr_2$ 0.0385	60	(V)>(IV)	—	chrom.
20	(II) 2.0	1.3	$CH_3COOC_2H_5$ 15	1	$HgBr_2$ 0.1	—	(V)	—	chrom., mp, $[\alpha]_D$
21	(II) 1.0	—	CH_3NO_2 10	3	$HgBr_2$ 0.005	—	Ethyl β-D-glucopyranoside tetraacetate, 33	chrom.+ cryst. (ether-petroleum ether)	
22	(V) 0.3	—	CH_3NO_2 2	3	$HgBr_2$, 0.05	—	(IV)		chrom.

* Chrom. = chromatography on alumina with subsequent crystallization from methanol; cryst. = crystallization from methanol.

** Chrom. = chromatographic identification; mp, $[\alpha]_D$ indicate that the respective constants correspond to literature data.

*** DCE = 1, 2-dichloroethane.

The initial glucose orthoesters (I) and (II) were obtained as described previously [10]. The condensation technique was the same in all experiments and was as follows. A solution of cholesterol and the orthoester was boiled for a few minutes and fresh solvent was added simultaneously at such a rate that the volume of the reaction mixture remained constant. After 2-3 ml of solvent had been distilled off, the catalyst was added (generally as a solution in dichloroethane) and boiling under the same conditions was continued for a determined period. A few drops of pyridine was added, and the mixture was cooled and evaporated to dryness under vacuum. The unchanged cholesterol was removed by recrystallization from nitromethane, and the mother liquor was evaporated to dryness and analyzed by thin-layer chromatography. The reaction products were isolated by chromatography on alumina with subsequent crystallization from methanol or by direct crystallization (table). Two typical experiments are described below.

Experiment 4. The reaction mixture was evaporated with pyridine and the residue was crystallized from methanol. Substance (V) crystallized in the form of friable spherical nodules, yield 0.42 g (59%). After recrystallization, 0.30 g of (V) was obtained with mp 95-98°C, $[\alpha]_D$ + 1° (c 5.0; chloroform). A mixture with an authentic sample of (V) gave no depression of the melting point. The sample was chromatographically homogeneous and identical with an authentic sample. Literature data: mp 98-100°C, $[\alpha]_D$ + 2° (chloroform [10].

Experiment 8. The reaction mixture was cooled and left overnight. The crystals of cholesterol were separated (0.16 g, 41%), the mother liquor was evaporated with pyridine, and the residue was crystallized from methanol. Yield of substance (IV): 0.32 g (45%), mp 157-159°C, $[\alpha]_D$ −27.3° (c 0.92; chloroform). A mixture with an authentic sample gave no depression of the melting point. Chromatographically the sample was homogeneous and identical with an authentic sample. Literature data: mp 157-159°C, $[\alpha]_D$ −25° (chloroform) [11].

Found, %: C 68.69; H 9.01. Calculated for $C_{41}H_{64}O_{10}$, %: C 68.86; H 9.00.

Summary

The condensation of acetylated alkyl orthoacetates of D-glucose with cholesterol has been studied. It has been shown that either transesterification or glycosylation may take place, according to the reaction conditions. Conditions have been found under which the sole direction of the reaction is glycosylation, leading to the 1, 2-trans-glycoside. A standard method for glycosylating complex alcohols with sugar orthoesters has been proposed.

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