THE SYNTHESIS OF CHOLESTERYL ALKYL ETHERS

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ABSTRACT

Seventeen cholesteryl alkyl ethers were synthesized through alcoholysis of cholesterol p-toluenesulfonate. This method was found superior to the etherification of sodium or potassium cholesterylate with alkyl halides or methanesulfonates, especially for the preparation of long-chain unsaturated alkyl ethers of [7(n)-^{3}H]cholesterol of high specific activity.

INTRODUCTION AND RESULTS

Cholesteryl hexadecyl and octadecyl ethers were isolated from bovine cardiac muscle by Funasaki and Gilbertson, who also synthesized these two compounds as well as the pentadecyl and heptadecyl ethers (1). The cholesteryl ethers were prepared by refluxing the respective alkyl p-toluenesulfonates with potassium cholesterylate in anhydrous benzene, using an adaptation of the Williamson etherification method (2). Similar procedures were described for etherification of glycerol derivatives with unsaturated alkyl chains without isomerization from the cis to trans form (3, 4, 5). In the present study two modifications of the Williamson reaction were applied for the preparation of hexadecyl (IX) and cis-9'-octadecenyl (XIV) ethers (Table 1), yielding 14% and 19% of the desired products, respectively. The methods,however, were found unsuitable for preparation of the [7(n)-^{3}H]cholesteryl ethers XIV or XVI of high specific activity.

Cholesteryl ethers were also prepared by Stoll (6), who heated cholesterol p-toluenesulfonate with methanol, ethanol, n-propanol or benzyl alcohol. This method was also applied for 3-methyl etherification of other 5-ene-steroids (6, 7). In the present work, a modification of this procedure was used to give long-chain saturated and unsaturated alkyl ethers in 47-67% yields (Table 1). Similar yields were also obtained when labeled alkenyl or alkadienyl ethers were prepared. Compounds IX and XIV synthesized by this method were identical to the corresponding compounds prepared by the modified Williamson reactions.

TABLE 1. Cholesteryl Alkyl Ethers Obtained by the Modified Stoll Procedure

No.	Alkyl	$[\alpha]_D^{25}$	M.p., °C	Yield[a], %	Molecular ion m/e	Relative retention times[b]	Calculated, %		Found, %	
							C	H	C	H
I	2'-Propenyl	-29.3	78-79	53.2	426	0.453[c]	84.5	11.7	84.3	12.0
II	Butyl	-27.7	81-82	64.1	442	0.574[c]	84.2	12.2	84.0	12.0
III	iso-Amyl	-27.9	88-90	52.9	456	0.060	84.2	12.3	83.9	12.1
IV	Hexyl	-31.9	69-71	57.5	470	0.080	84.3	12.3	84.0	12.4
V	Octyl	-28.1	97-98	60.7	498	0.122	84.3	12.4	84.1	12.3
VI	Decyl	-25.0	58-62	47.2	526	0.196	84.4	12.5	84.2	12.3
VII	Dodecyl	-28.2	71-73	58.9	554	0.279	84.5	12.6	84.2	12.6
VIII	Tetradecyl	-25.3	47-49	46.4	582	0.405	84.5	12.7	84.2	12.6
IX	Hexadecyl	-22.9	57-59	46.5	610	0.636	84.6	12.8	84.3	12.9
X	Octadecyl	-21.9	65	66.8	638	0.850	84.6	12.8	84.5	12.9
XI	Eicosyl	-20.5	61-64	45.8	666	1.225	84.7	12.9	84.5	12.8
XII	cis-9'-Hexadecenyl	-20.1	18-24	54.1	608	0.598	84.8	12.5	84.7	12.6
XIII	trans-9'-Hexadecenyl	-22.9	47-48	56.8	608	0.597	84.8	12.5	84.9	12.6
XIV	cis-9'-Octadecenyl	-23.4	39-41	63.6	636	0.853	84.9	12.6	84.9	12.4
XV	trans-9'-Octadecenyl	-22.3	48-49	60.2	636	0.830	84.9	12.6	85.0	12.4
XVI	cis,cis-9',12'-Octadecadienyl	-20.6	24-28	53.2	634	0.877	85.1	12.3	84.9	12.2
XVII	trans,trans-9',12'-Octadecadienyl	-21.9	57-58	50.2	634	0.853	85.1	12.3	84.8	12.1

a. Calculation based on crystallized ethers obtained from cholesterol p-toluenesulfonate.

b. Relative to cholesteryl hexadecanoate on 3% QF-1 at 260°C (absolute retention time 49.3 minutes).

c. Relative to cholesteryl hexyl ether on 3% SE-30, 270°C (absolute retention time 15.3 minutes).

EXPERIMENTAL

Ultraviolet spectra were determined in a Varian Techtron spectrophotometer in isopropanol. Infrared spectra were measured in KBr disks using a Perkin Elmer spectrophotometer (Model 337). Nmr spectra were recorded on a Jeol C-60-H high resolution spectrometer in $CDCl_3$ solutions with tetramethylsilane as an internal standard. Mass spectra were taken through the direct inlet of the LKB 2091 Gas Chromatograph-Mass Spectrometer. Optical rotations were determined with a Perkin Elmer 141 polarimeter in chloroform solutions. TLC was performed on Silica Gel G (E.Merck, Darmstadt); 100 mesh silicic acid (Mallinckrodt) was used for column chromatography. 2-Propenol was purchased from Merck-Schuchard. The long-chain unsaturated alcohols and methanesulfonates were obtained from Nu-Chek Prep.Inc. All the other alcohols, hexadecyl bromide and p-toluenesulfonyl chloride were purchased from Sigma Chemical Co: and [7(n)-^{3}H]cholesterol (9.5 Ci/mmol) from The Radiochemical Centre, Amersham.

Alcoholysis of cholesterol p-toluenesulfonate. Cholesterol p-toluenesulfonate (8) (200 mg) and 500 mg of fatty alcohol (or 0.5 ml if liquid at room temp.) were placed in a 20 ml constricted glass tube. The tube was flushed with a stream of dry N_2, sealed and kept in an oven at 110°C for 150 min. The tube was opened, $NaHCO_3$ (0.5 g) and hexane (10 ml) were added, the mixture was stirred and the hexane phase was applied to a column containing 15 g of silicic acid (9). The procedure was repeated three more times with 10 ml portions of hexane and the silicic acid column was eluted with 50 ml each of the following solvents: hexane; 5%, 10%, 15% and 15% benzene in hexane; chloroform and methanol. Aliquots of the fractions were analyzed by TLC (petroleum ether:ether 98:2), using iodine vapor for visualization of the spots. The ethers were generally recovered from the first 15% fraction; in some cases a part of the compound was also found in the 10% or the second 15% fraction (R_f 0.31). The crude product was crystallized from acetone which was cooled to 0°C before filtration or to -40°C in the case of XII or XVI. The 2'-propenyl ether was prepared by heating the p-toluenesulfonate in 1 ml of 2-propenyl alcohol and was crystallized from methanol without chromatographic purification.

The parent peak (Table 1) and M^+-CH_3 ion fragments were found in the mass spectra of all the ethers studied. The following fragmentations were previously elucidated (1): m/e 368, 369, 329; M-113 (C_{17-20} fragmentation) was not formed by III; and $C_{1'-2'}$, side chain cleavage (m/e 399) was not found in the alkenyl or alkadienyl ethers. No significant differences were observed between the cis-trans isomers of the pairs XII-XIII, XIV-XV or XVI-XVII. The uv spectra of XVI and XVII did not exhibit the absorption near 230 nm associated with conjugated double bond systems. The 965 cm^{-1} ir band (trans-CH=CH-) (4) was present in XII, XV and XVII. Nmr δ (ppm) 0.64, 0.70, 0.90 and 1.0 (18-CH_3, 19-CH_3, 21-CH_3 and 26,27-CH_3, also found in cholesterol); 1.2 (broad s, CH_2-groups of alkyl moieties, not shown by I); 3.4-3.6 (m, 3α-H and 1'-CH_2) (10,11); 5.36 (m, 6-CH and -CH=CH-). 2'-Propenyl ether I: ir 915 cm^{-1} (CH_2=CH-); nmr 3.96 (s,1,3α-H), 4.06 (s,2,1'-CH_2), 5.05, 5.24 and 5.36 (three centered m, 6-CH and CH_2=CH-) ppm.

Preparation of [7(n)-^{3}H]cholesteryl cis,cis-9',12'-octadecadienyl ether (XVI). A solution of [7(n)-^{3}H]cholesterol (0.4 mCi, 0.023 mg) in toluene (0.4 ml) was plaeed in a tube and the solvent was evaporated at 40°C under a stream of N_2. Dry pyridine (1 ml) and p-toluenesulfonyl chloride (0.3 g) were added and the tube was kept at 38°C for 20 h (8). Ice (approx. 0.5 g) was added and after 30 min the mixture was extracted with hexane. The organic layer was successively washed with H_2O, a saturated solution of $NaHCO_3$ and water, dried over Na_2SO_4 and evaporated. The residue was heated with cis,cis-9,12-octadecadienyl alcohol (0.4 ml), in a sealed tube, as outlined above to give 0.13 mCi of labeled XVI (32.5% yield). The purity of the ether (>97%) was analyzed by TLC (12). The yields for labeled XIV were 29.6%, 49.8% and 66.9% when 0, 10 and 15 mg of unlabeled cholesterol were added, respectively, to the reaction mixtures.

Preparation of cholesteryl-cis-9'-octadecenyl (XIV) and hexadecyl (IX) ethers by the Williamson method. A. Cholesterol (5 g) in dry benzene solution was treated with Na (700 mg) and cis-9-octadecenyl methanesulfonate (3.3 ml) (4). The analytical XIV (1.5 g) was obtained after chromatographic purification and two successive crystallizations. B. Cholesterol (200 mg) and K (40 mg) were refluxed in dry diisopropyl ether (10 ml) for 20 minutes (4). Hexadecyl bromide (0.4 ml) was added and refluxing continued for another 15 min. The product was extracted with hexane, chromatographed and crystallized to give 60 mg of IX.

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