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Bile Acids Bound to Polymers

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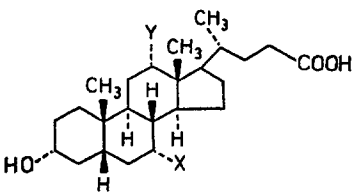
This paper is dedicated to Professor Georg Manecke on the occasion of his 70th birthday

Summary

Bile acids are steroids containing one - lithocholic acid, two - desoxycholic acid, and three - cholic acid hydroxyl groups, respectively. The hydroxyl group in position 3 of the steroid skeleton after esterification of the carboxyl group at C-24 under suitable conditions selectively reacts with methacryloyl chloride to give the corresponding methacrylic ester derivatives which undergo radically initiated uni- and copolymerizations to yield high molecular weight polymers.

Introduction

Lithocholic acid **1**, desoxycholic acid 2 and cholic acid 3 are steroids and known as the bile acids. The substances occur in bile as sodium salts of N-acyl derivatives of glycin and taurine. The biological function of the salts in bile is to aid in the solubilization and assimilation of fats and hydrocarbons, such as carotene. The bile acids are obtained by alkaline hydrolysis of the peptide bonds.



X = Y = H 1

X = H, Y = OH 2

X = Y = OH 3

As steroids the bile acids are closely related to cholesterol which, either free or in the form of esters, is actually widely distributed in the human body, particularly in nerve and brain tissue, and to ergosterol, a sterol of yeast, wich give vitamin D_2 when irradiated. Recently some work has been published on the synthesis of polymers containing steroids in the side chain and their potential use as polymer drugs (1-3). Cholesterol has been bound to a flexible spacer containing a methacrylic ester group (4). The aim of this work was to synthesize bile acid ester from 1 and 3 containing a polymerizable group attached

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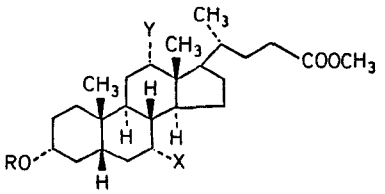
directly to the steroid in the C-3 position.



R = $COC(CH_3)CH_2$

X = Y = H 6

X = Y = OH 7

Experimental

Materials

Lithocholic acid **1** (99% Fluka) was recrystallized from chloroform. Cholic acid **3** (puriss.Fluka) was used as received. Methacryloyl chloride was prepared from benzoyl chloride and methacrylic acid. The product was isolated by fractional distillation into a Schlenck tube.

The methyl esters **4** and **5** were prepared by dissolving 50 g of the appropriate bile acid **1** or **3** in 250 ml of dry methanol containing 1.27 ml of conc.hydrochloric acid. The mixture was refluxed for 20 min and the ester was isolated by crystallization. After drying in vacuo at 328K, the substances were identified by their ^1H NMR spectra and their melting points.
Lithocholic acid methylester: yield 93%, F.: 398K
Cholic acid methylester : yield 81%, F.: 427K

Synthesis

3-Methacryloyl lithocholic acid methylester **6**: To a stirred solution of 30 g lithocholic acid methylester **4** and 13.8 ml dry triethylamine in 200 ml of dry chloroform the solution of 9.6 ml methacryloyl chloride in 30 ml of dry chloroform was added at 273K over a period of 45 min. The solution was slowly warmed to room temperature and reacted for further 24 h. The reaction mixture was added to ice/water containing 6 ml conc.hydrochloric acid. After filtration the organic layer was separated and washed with water several times, dryed and evaporated in vacuo at room temperature. Then ethanol was added and the crude product was crystallized from ethanol. After drying in vacuo at room temperature the product was identified by ^1H NMR spectroscopy.
^1H NMR in $CDCl_3$: δ = 1.9 ppm (s,3H,methacrylic-H), δ = 3.7 ppm (s,3H,ester-H), δ = 4.75 ppm (m,1H,C-3-H), δ = 5.5 and 6.1 ppm (m,1H each,vinyl-H) further absorbance see lithocholic acid (6)
yield: 67%, F.: 423-424K

3-methacryloyl cholic acid methylester 7: The preparation was carried out as described above methacryloyl chloride was added very slowly at 273K and the reaction mixture was stirred for

24 h. By ^{1}H NMR spectroscopy was demonstrated that esterification occurred at the C-3-OH exclusively.
^{1}H NMR in $CDCl_3$: δ = 1.9 ppm (s,3H,methacrylic-H), δ = 4.6 ppm (m,1H,C-3H), δ = 3.7 ppm (s,3H,ester-H), δ = 5.5 and 6.1 ppm (m,1H,each,vinyl-H) further absorbance see cholic acid (6)

Measurements

The ^{1}H NMR spectra were obtained on a Bruker 80 MHz FT-NMR spectrometer Typ WP 80 SY in $CDCl_3$ with TMS as internal standard at 310K, conc. 25 mg/ml.
M_n values were determined by membrane osmometry on a Knauer Typ 07.00 operating in toluene (puriss.Fluka) at 310K and in chloroform at 299K.

Results and Discussion

The monomers were obtained by esterification of the C-3-HO-group of lithocholic acid methylester **4** and cholic acid methylester **5**, respectively, with methacryloyl chloride. In cholic acid methylester **5** the hydroxyl group at C-3 is in equatorial position and therefore more reactive than the hydroxyl groups at C-7 and C-12 (7). Futhermore the positions at C-7 and C-12 are more sterically hindered than at C-3. Thus, applying suitable reaction conditions cholic acid methylester **5** reacts with methacryloyl chloride exclusively at C-3-OH and no protecting groups are necessary to be attached at both the other alcoholic residues.

The monomers 3-methacryloyl lithocholic acid methylester **6** and 3-methacryloyl cholic acid methylester **7** are crystalline substances at room temperature which readily undergo radically initiated uni- and copolymerization with methylmethacrylate **8** and styrene **9**, respectively, in toluene and mixtures of toluene with THF. Although monomer concentration was low because of the low solubility of **6** and **7** in toluene, high molecular weight polymers were obtained in all cases. Reaction conditions and polymerization results are given in Table 1. The polymer formation was proven by membrane osmometry, by GPC which showed significant lowered elution volume, and by ^{1}H NMR spectra from which the copolymer compositions were determined. In copolymer **15** this procedure failed to give exact results because of extreme signal broadening in this particular case. The ease of polymer formation was quite surprising because the very rigid and bulky steroid skeletons are closely attached to the polymer main chain causing high sterically hindrance and restriction of polymer chain flexibility. Furthermore it is quite remarkable, that all uni- and copolymers prepared show good solubility in serveral organic solvents.

In copolymerization experiments with styrene **9** and methyl methacrylate **8** as comonomers the copolymer composition is not far from the composition of the starting comonomer mixture.

Table 1: Radical uni- and copolymerization of **6** and **7** with MMA **8** and styrene **9**

Monomer/ Comonomer	Polymer/ Copolymer	Solvent	Vol. (ml)	$m_{steroid}$ (g)	$m_{styrene,MMA}$ (g)	Reaction time (h)	Conversion (%)	Composition steroid/styrene steroid/MMA	M_n (g/mol)
Unipolymerization									
6	**10**	Toluene	30	5.0	-	16.5	58	-	87.700 *)
7	**11**	Toluene/THF 8/1	45	4.0	-	21.5	14	-	296.000 *)
Copolymerization									
6/8	**13**	Toluene	10	2.0	0.349	16.5	12	1.25:1 ***)	88.700 *)
6/9	**12**	Toluene	10	2.0	0.363	16.5	24	1 :1 ***)	37.700 *)
7/8	**15**	Toluene/THF 3/1	13.5	2.0	0.323	13	13.6		88.800 **)
7/9	**14**	Toluene/THF 3/1	13.5	2.0	0.337	19	7.4	1 :1 ***)	23.800 **)

*) by membrane osmometry in toluene T = 310K; **) in chloroform T = 298-300K; ***) by ^1H NMR c_{AiBN}: 5 mol%; T = 333K; comonomer molar ratio steroid/MMA, steroid/styrene: 1.25/1

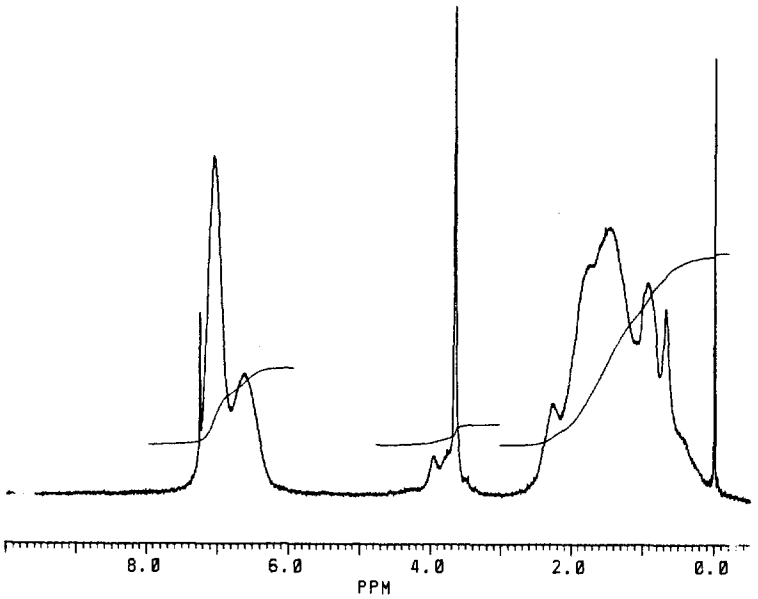


Fig. 1: ^{1}H NMR of copolymer from **7** and **9**, comonomer ratio in copolymer = 1/3.55; solvent $CDCl_3$

For copolymer **14** containing **7** and styrene **9** the copolymerization parameters were determined from ^{1}H NMR spectra to be $r_1 = 0.80 \pm 0.08$ and $r_2 = 0.91 \pm 0.08$.

Acknowledgement

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