

*Dedicated to the memory of
Professor Ecaterina Ciorănescu-Nenitzescu (1909–2000)*

NEW BIOCOMPATIBLE POLYESTERS BASED ON BILE ACIDS

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New biocompatible and biodegradable polyesters based on cholic acid or deoxycholic acid were synthesized by polycondensation reaction of 3-succinoyloxy-derivatives of bile acids with diethylene glycol. Chemical structure of the synthesized compounds has been proved by their elemental analysis, ^1H -, ^{13}C -NMR and IR spectra. Thermal behaviour and crystallinity of the polymers have been investigated.

INTRODUCTION

Bile acids (BA) are amphiphilic compounds from the steroid family biosynthesized from cholesterol in the liver and having an important role in biological processes.¹⁻³ BA are widely applied in synthetic chemistry due to their enantiomeric purity, high stability of the steroid nucleus, reactivity of the side groups, optical activity, self-assembling capacity, low cost and ready availability.^{4,5} Various biocompatible polymeric materials with bile acids residues on the main chain,⁶ as pendant groups⁷⁻⁹ and as chain-end groups¹⁰⁻¹³ have been reported.

Previous attempts to synthesize polyesters by BA homopolycondensation led to very rigid, non-degradable and insoluble polymers¹⁴⁻¹⁶. Therefore, the present study aimed at synthesizing new biocompatible and biodegradable polyesters by the polycondensation of cholic acid (CA) or deoxycholic acid (DCA) hemiesters with diethylene glycol (DEG). These polyesters could have possible biomedical applications as drug delivery systems. The procedure for the synthesis of polyesters is described in details and the polymers crystallinity and thermal stability were analysed.

RESULTS AND DISCUSSION

The synthesis of new biodegradable polyesters based on biocompatible monomers (DEG and BA) was carried out in two steps.

In the first step, 3 α -(succinoyloxy)-7 α , 12 α -dihydroxy-5 β -cholan-24-oic acid (**1**) and 3 α -(succinoyloxy)-12 α -hydroxy-5 β -cholan-24-oic acid (**2**) (Figure 1) were obtained by esterification of OH in C³ position of the bile acid (CA or DCA) with succinic anhydride under basic catalysis (triethylamine-TEA). The structure of the two hemiesters was confirmed by elemental analysis, IR, ^1H - and ^{13}C -NMR spectroscopy.

FT-IR spectra of **1** and **2** contained a splitted band at 1725 cm^{-1} and 1714 cm^{-1} for **1** and at 1725 cm^{-1} and 1704 cm^{-1} for **2**, assigned to the C=O group stretching vibrations of the ester and carboxylic acid, respectively.

^1H -NMR spectra for the hemiesters based on CA and DCA showed peaks at 4.48 ppm and 4.65 ppm, respectively, attributed to the proton of the C³ bound to the succinoyloxy ester group ($\text{CH-O-CO-CH}_2\text{-CH}_2\text{-COOH}$). The positions and the peak integrations of C⁷ H (3.61 ppm) and C¹² H (3.82 ppm) for **1** and C¹² H (3.85 ppm) for **2** remained unchanged compared with those of unmodified bile acids

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indicating that the hydroxyl groups at these positions did not react and only the OH group

bound to C³ position was selectively esterified with succinic anhydride.

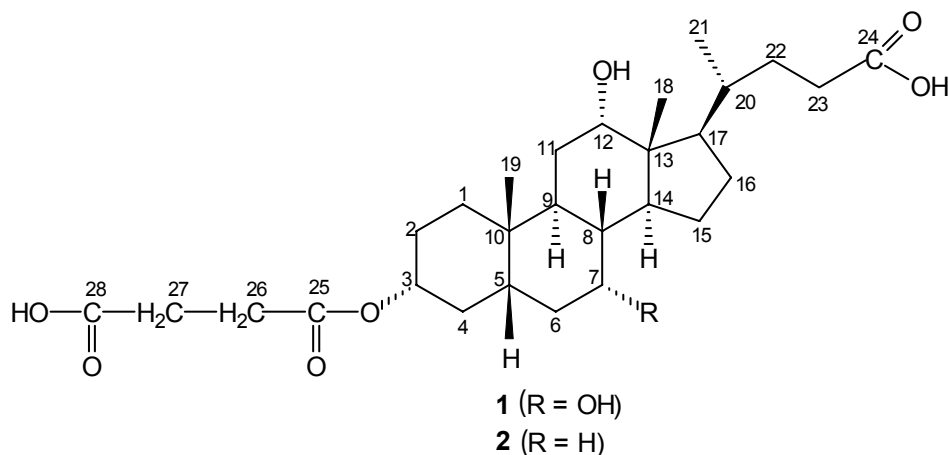


Fig. 1 – The chemical structures for compounds **1** and **2**.

The ¹³C chemical shifts for C³ of hemiesters are identical to those of unmodified cholic acid (71.27 ppm) and deoxycholic acid (71.1 ppm), respectively.

In the second step, polymers **3** and **4** (Figure 2) were obtained by polycondensation reaction between DEG and hemiesters **1** and **2**. Synthesis of polyester **3** was performed in the presence of

dicyclohexyl carbodiimide (DCC) as a coupling agent and an equimolar mixture of 4-(N, N-dimethylamino) pyridine (DMAP) and p-toluenesulfonic acid (PTSA) as a catalyst while polyester **4** was obtained by azeotropic distillation under acid catalysis (PTSA). The analysis of FT-IR, ¹H- and ¹³C-NMR spectra prove the polymer structures presented in Figure 2.

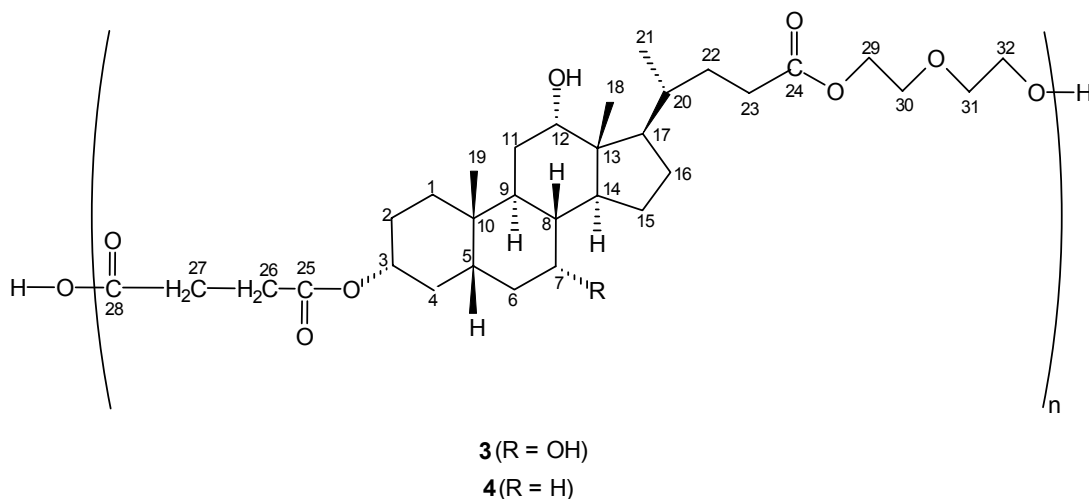


Fig. 2 – The chemical structures for products **3** and **4**.

The infrared spectra for both polyesters showed the absorption band assigned only to the stretching vibrations of the ester carbonyl group (at 1734 cm⁻¹ for **3** and 1736 cm⁻¹ for **4**) and not for the carboxylic acid.

¹H-NMR signals for compounds **3** and **4**, corresponding to the protons of $\text{CH}_2\text{-O-CH}_2$, $\text{COO-CH}_2\text{-CH}_2\text{-O}$ and $\text{CH}_2\text{-OH}$ (end group), could be

seen for both polyesters at 3.69 ppm, 4.25 ppm and 3.61 ppm, respectively.

The positions and the peak integrations of the protons at C⁷H (3.85 ppm) and C¹²H (3.98 ppm) for **3** and C¹²H (3.98 ppm) for **4** were not modified compared with the corresponding protons of the hemiesters **1** and **2** showing that OH in these positions were not implied in polycondensation.

^{13}C -NMR spectra of **3** and **4** proved also polyester formation by the presence of the peaks assigned to DEG (29.18; 29.76; 171.75; 174.32).

The number-average molecular weight, M_n , weight-average molecular weight, M_w and polydispersity index, PDI, were determined by gel

permeation chromatography with THF as solvent (Table 1).

The TG/DTG curves for the two polyesters are presented in Figure 4. DTG curves show one step of mass loss with one event for both compounds.

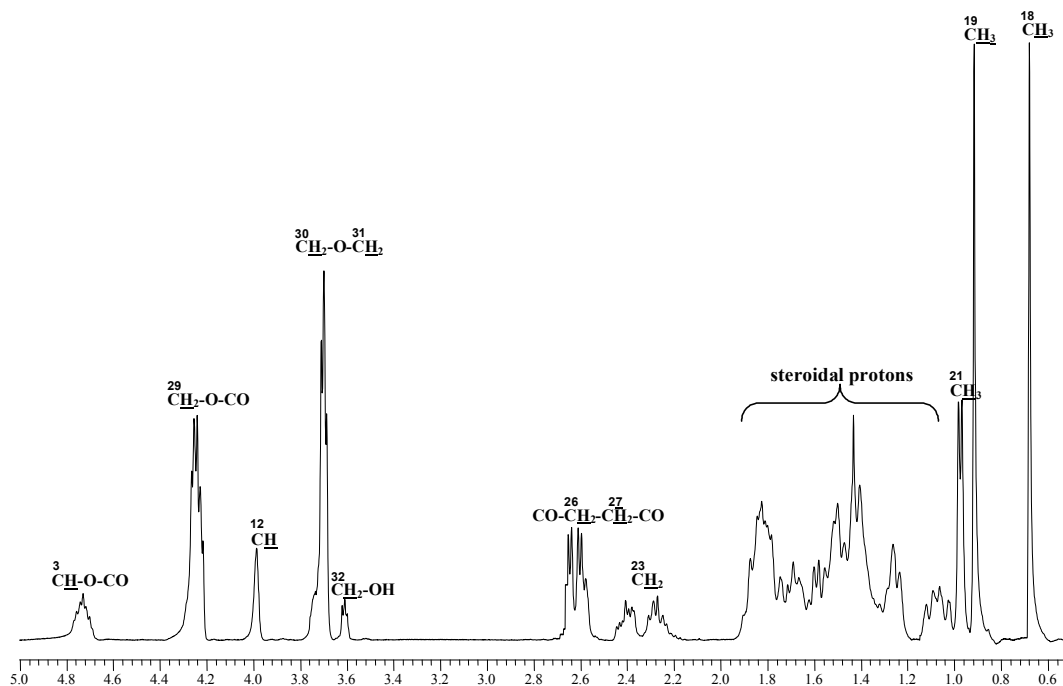


Fig. 3 – ^1H -NMR spectrum of compound **4**.

Table 1

M_n , M_w and PDI for polyesters **3** and **4**

Compound	M_n	M_w	PDI
3	6532	7761	1.18
4	10829	22525	2.08

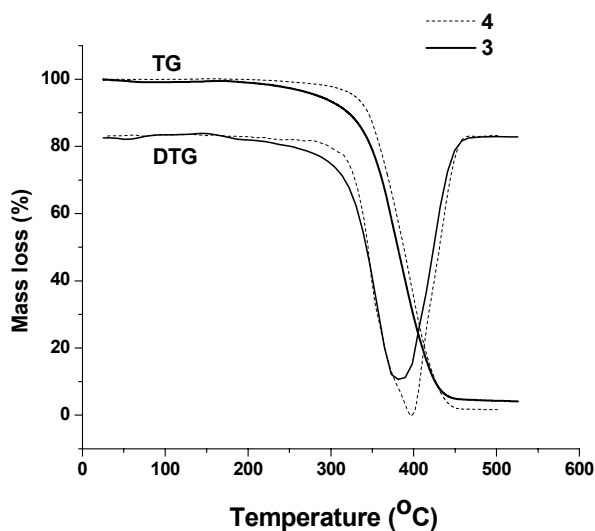


Fig. 4 – TG and DTG curves for polyesters based on CA (**3**) and DCA (**4**).

The mass loss (ΔW , %), the characteristic temperatures: the temperature corresponding to 5% weight loss (T_5), the temperature corresponding to the maximum mass loss (T_{max}), the temperature for the end of the decomposition (T_f) as well as the activation energies (E_a) for both polyesters are

collected in Table 2. E_a were determined by using Coats-Redfern (CR)¹⁷ and Urbanovici-Segal (US)¹⁸ methods. Thermogravimetric data showed higher thermal stability, mass loss and E_a for DCA-based polyester compared with CA-based polyester.

Table 2

Characteristic temperatures, mass loss and energy activation for samples 3 and 4 (see Table 1)

Compound	T_5 (°C)	T_{max} (°C)	T_f (°C)	ΔW (%)	E_a (KJ/mol)	
					E_a (CR)	E_a (US)
3	287.13	406.6	446.25	95.18	21.62	21.65
4	332.23	415.17	463.42	98.08	23.87	23.88

The X rays diffraction spectra at room temperature for polymers 3 and 4 indicate that their structure is partially crystalline and partially amorphous (Figure 5). The crystalline parts give sharp narrow diffraction peaks and the amorphous

component gives a very broad peak (halo). The ratio between these intensities is used to calculate the amount of crystallinity in the polymers. The crystallinity percent is 25 % for 3 and 27.4% for 4.

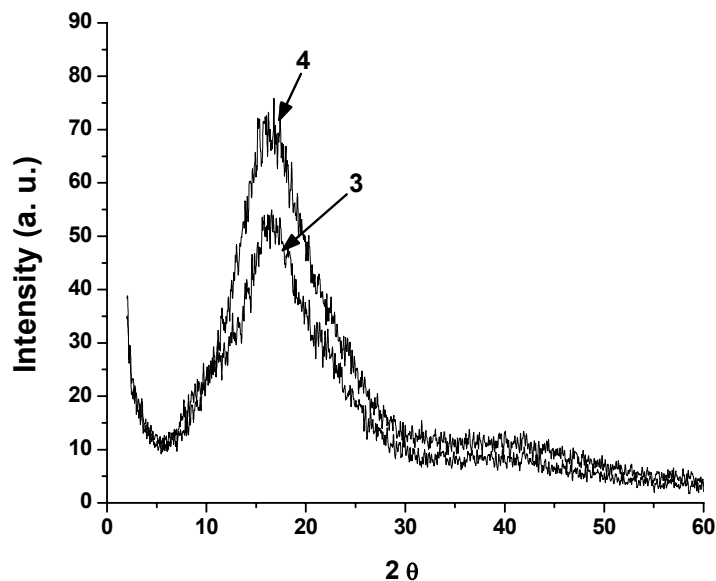


Fig. 5 – Diffractograms for polymers 3 and 4 at room temperature.

EXPERIMENTAL

The FT-IR spectra were recorded with a Bruker Vertex 70 spectrophotometer on KBr pellets. ¹H and ¹³C-NMR spectra were obtained in CDCl₃ or DMSO-d₆ with a Bruker Advance DRX 400C spectrometer (400 MHz (¹H) and 100 MHz (¹³C)) using SiMe₄ as reference. Molar mass averages were determined by using a PI-EMD 950 instrument equipped with an evaporative mass detector. Polystyrene standards were used for calibration. Thermal decomposition of the products were

examined with Perkin Elmer Pyris Diamond thermogravimetric analyzer. The X-ray power diffraction data were obtained with a Bruker AD8 Advance diffractometer.

Synthesis of 3α-(succinoyloxy)-7α, 12α-dihydroxy-5β-cholan- 24-oic acid (1)

CA (20 g, 0.0489 mol) was dissolved in 200 ml dry CHCl₃. Succinic anhydride (5.39 g, 0.0534 mol) and dry TEA (7.5 mL, 0.0538 mol) were then added and the mixture was stirred under reflux for 24 h. The solvent was removed by

distillation and the residue was re-dissolved in chloroform. The obtained solution was extracted with 0.1 N HCl and water and dried on MgSO₄. A light brown solid (19.87 g, 80 % yield) with m.p. 249.5 °C was obtained. IR (cm⁻¹): 1725.26 (ester C=O stretching band), 1714.66 (acid C=O stretching band); ¹H-NMR (400MHz, DMSO-d₆), δ (ppm) = 0.59 (s, 3H, C¹⁸H₃); 0.84 (s, 3H, C¹⁹H₃); 0.93 (d, 3H, C²¹H₃); 2.45-2.5 (dd, 4H, OC-CH₂-CH₂-CO); 3.62 (s, 1H, C⁷H); 3.79 (s, 1H, C¹²H); 4.48 (m, 1H, C³H-O-CO); Selected ¹³C-NMR resonances (100MHz, DMSO-d₆) = 28.89 (C²⁶); 29.19 (C²⁷); 71.27(C³); 171.73 (C²⁵); 172.35(C²⁴); 174.12(C²⁸). Elemental analysis for C₂₈H₄₄O₈, calculated (found)(wt%): C 66.14 (65.97); H 8.66 (8.54).

Synthesis of

3α-(succinoyloxy)-12α-hydroxy-5β-cholan-24-oic acid (2)

Product **2**, obtained under similar conditions as **1**, resulted as a light brown solid (21.33 g, 85 % yield) with m.p. 235.5 °C. IR (cm⁻¹): 1725.26 (ester C=O stretching band), 1704.05 (acid C=O stretching band); ¹H-NMR (400MHz, DMSO-d₆), δ (ppm) = 0.68 (s, 3H, C¹⁸H₃); 0.92 (s, 3H, C¹⁹H₃); 0.98 (3H, d, C²¹H₃); 2.59- 2.64(dd, 4H, OC-CH₂-CH₂-CO); 3.84 (s, 1H, C¹²H); 4.65 (m, 1H, C³H-O-CO); Selected ¹³C-NMR resonances (100MHz, DMSO-d₆) = 28.72 (C²⁶); 29.05 (C²⁷); 71.1(C³); 171.73 (C²⁵); 172.35(C²⁴); 174.12(C²⁸). Elemental analysis for C₂₈H₄₄O₇, calculated (found)(wt%): C 68.29 (67.92); H 8.94 (8.92).

Synthesis of polyester with DEG of 3α-(succinoyloxy)-7α,12α-dihydroxy-5β-cholan-24-oic acid (3)

1 (1 g, 0.0019 mol) and DEG (0.208 mL, 0.0022 mol) were dissolved in 10 mL DMSO. DCC (0.82 g, 0.0039 mol) and an equimolar mixture of 4-DMAP (0.082 g, 0.00068 mol) and PTSA (0.116 g, 0.00068 mol) were then added and the mixture was stirred at room temperature for 48 h. The mixture was filtered for removing the precipitated dicyclohexylurea and the filtrate was precipitated into water. The precipitate was re-dissolved in ethyl acetate, extracted with water and dried on MgSO₄. After ethyl acetate evaporation, a brown solid was obtained. IR (cm⁻¹): 1734.91 (ester C=O stretching band); ¹H-NMR (400MHz, CDCl₃), δ (ppm) = 0.69 (s, 3H, C¹⁸H₃); 0.91 (s, 3H, C¹⁹H₃); 0.99 (d, 3H, C²¹H₃); 2.59- 2.63 (dd, 4H, OC-CH₂-CH₂-CO); 3.61 (1H, s, CH₂-OH); 3.69 (m, 4H, CH₂-O-CH₂); 3.85 (s, 1H, C⁷H); 3.99 (s, 1H, C¹²H); 4.25 (t, 2H, OC-O-CH₂); 4.59 (s, 1H, C³H-COO); Selected ¹³C-NMR resonances (100MHz, CDCl₃) = 29.18 (C²⁷); 29.76 (C²⁶); 63.28 (C³²); 63.62 (C²⁹); 68.88 (C³¹); 69.07 (C³⁰); 72.85 (C³); 171.75 (C²⁵); 172.35 (C²⁴); 174.32 (C²⁸).

Synthesis of polyester with DEG of

3α-(succinoyloxy)-12α-hydroxy-5β-cholan-24-oic acid (4)

2 (1g, 0.002 mol), dissolved in 10 mL toluene, was mixed with DEG (0.2 mL, 0.0021 mol) and PTSA (0.013 g, 0.000076 mol). The reaction mixture was refluxed at 110 °C for 24 h and the resulting water was removed by azeotropic distillation. Toluene was removed by distillation, the residue was re-dissolved into a mixture of DMSO/THF (3/3) (v/v) and then precipitated in water. Finally, the polyester **4** was collected on the filter and washed with water. The compound **4** was found as a light brown solid. IR(cm⁻¹): 1736.84 (ester C=O stretching band); ¹H-NMR (400MHz, CDCl₃), δ (ppm) = 0.68 (s, 3H, C¹⁸H₃); 0.92 (s, 3H, C¹⁹H₃); 0.98 (d, 3H, C²¹H₃); 2.59- 2.64 (dd, 4H, OC-CH₂-CH₂-CO); 3.61 (1H, s, CH₂-OH); 3.69 (m, 4H, CH₂-O-CH₂); 3.99 (s, 1H, C¹²H); 4.25 (t, 2H, OC-O-CH₂); 4.73 (s, 1H, C³H-COO); Selected ¹³C-NMR resonances (100MHz, CDCl₃) = 28.96 (C²⁶); 29.11 (C²⁷); 63.36 (C³²);

63.69 (C²⁹); 68.99 (C³¹); 69.13 (C³⁰); 73.05 (C³); 171.8 (C²⁵); 172.25 (C²⁴); 174.19 (C²⁸).

CONCLUSIONS

New polyesters of 3-succinoyloxy-bile acid (CA and DCA) with DEG were obtained and characterized by means of qualitative analyses and spectral measurements, as well as by crystallinity and thermal analysis. The new polyesters have a good solubility in many organic solvents, high thermal stability and crystalline/amorphous properties. These biocompatible polymers can be used as biodegradable matrices for drug delivery systems, either as such or after their grafting to a polysaccharide backbone.

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