Polymer Bulletin 42, 41–46 (1999)

Polymer Bulletin

The ambient temperature synthesis and characterization of bile acid polymers

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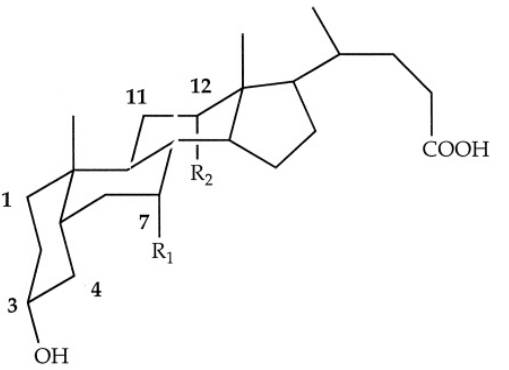
Received: 20 July 1998/Revised version: 16 November 1998/Accepted: 16 November 1998

Summary
Room temperature polymerization of three naturally occurring bile acids, cholic, lithocholic and deoxycholic, was carried out using a mixture of diisopropyl carbodiimide (DIPC), and a 1:1 salt of dimethyl amino pyridine and *p*-toluenesulfonic acid (DMAP/PTSA). The corresponding polymers were characterized by IR, NMR, Thermal Analysis and X-ray diffraction. Molecular weights were calculated by gel permeation chromatography in the range 50,000-60,000, using polystyrene standards. The polymers were also characterized by mass spectrometry, using matrix assisted laser desorption ionization-time of flight, MALDI-TOF.

Introduction

Bile acids are an interesting class of naturally occurring acids that contain a varying degree of hydroxylation, in which all the hydroxyl groups are on one side of the molecule, designated as the α face, shown in Figure 1.



Cholic Acid: $R_1, R_2 = OH$
Lithocholic Acid: $R_1, R_2 = H$
Deoxycholic Acid: $R_1 = H, R_2 = OH$

Figure 1. Structure of bile acids

This structural characteristic has been exploited in the study of inclusion complexes of bile acids with different substrates (1). Over fifty different organic

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substances are known to form inclusion compounds with methyl cholate (2). New synthetic hosts incorporating two molecules of cholic acid linked by a rigid diamine have been designed (3), as well as macrocycles consisting of two molecules of both cholic acid and benzylamine (4). These type of macrocycles, known as cholaphanes and cholanamides, respectively, were able to enclose a variety of organic substrates through hydrogen bond formation (5).

The biological role of bile acids has been well documented (6). Bile acids combine with glycine in mammals and with taurine in non-mammals to form amides which can be hydrolyzed by intestinal bacteria in the metabolic pathway. Therefore, it is expected that analogous poly(bile acids) would also have a tendency to be biodegradable, or exhibit other biological activity (6).

The first polymerization of bile acids in 1988 was carried out in toluene at 90-110°C, using p-toluenesulfonic acid as the catalyst (7). Although polymers with number average molecular weight of 4000-5000 were obtained, some degree of cross-linking via the hydroxy groups was observed at both the 7 and 12 carbon positions. The degree of cross-linking was found to be a direct function of reaction temperature. Another method of incorporation of bile acids in polymers has been to place a reactive functionality on the acid, followed by polymerization of that moiety. Previous work has included derivatization of bile acids with an acrylate functionality at carbon 3 and subsequently, polymerization of the olefin via a free radical route inhibited crosslinking (8). However, this route places the bile acid as a pendant group, and not within the backbone of the polymer.

An alternative route to linear, main chain poly(bile acids) is ambient temperature polyesterification. This approach minimizes cross-linking tendencies of the monomer by enhancing the polymerization ability of the hydroxyl group at carbon 3 through esterification. Previously, aliphatic and aromatic polyesters have been synthesized using diisopropylcarbodiimide (DIPC) and catalytic amounts of a 1:1 mixture of dimethylaminopyridine (DMAP) and p-toluenesulfonic acid (PTSA) at room temperature (9). Using this methodology, we have achieved the room temperature synthesis of linear, non-crosslinked poly(bile acids) of moderate molecular weight. The resulting polymers were characterized using several analytical techniques such as gel permeation chromatography, NMR, and IR. In addition, matrix assisted laser desorption (MALDI) (10,11) was used in the mass spectrometric analysis of polymers. In our work, MALDI-TOF spectra irrefutably confirmed the formation of oligomeric species from bile acids.

Experimental

Materials

Bile acids and other chemicals were purchased from Aldrich and used as received. All solvents used were reagent grade Mallinckrodt and were used as received. All glassware was rigorously dried before use.

Methods

Molecular weights, relative to polystyrene standards, were determined by Gel Permeation Chromatography using a system composed of a Waters 590 pump, a Rheodyne injector, a Polymer Laboratories Pgel 5 µ mixed-C column, and a Perkin-Elmer LC-25 refractive index detector. Chloroform with 2% of triethylamine (TEA) added was used as the solvent at a flow rate of 1.0 mL/min. Mass spectrometry using MALDI-TOF was performed on a Vestec LaserTec Bench Top, linear mode, 1.2 m flight tube, Tektronix TDS 520A digitizing oscilloscope, ZEOS 486-33 computer with GPIB running GRAMS/386

software and nitrogen laser, 337.1 nm, 250 microJoules pulse energy, and 3 ns pulse duration, accelerating voltage was 30 kV and 44 scans averaged. The matrix used for polylithocholic and polydeoxycholic acids was 1,8,9-anthracenetriol (dithranol) with THF as the solvent. In the case of polycholic acid, α-cyano-4-hydroxycinnamic acid (α-CHCA) was used as the matrix with pyridine as the solvent. NMR spectra were obtained on a Varian XL-300 or JEOL-90 spectrometer, 300 MHz and 90 MHz, respectively, using TMS as a reference. IR spectra were recorded on a Perkin-Elmer 281 spectrophotometer. A Siemens 5100 diffractometer with a High Star General Area Diffraction Detector (GADDS) was used for diffraction studies of the three polymers. The X-ray source was a stationary Cu tube, using the CuKα, 1.54 å radiation. Thermogravimetric Analysis was performed on a V5.1A DuPont 2000 system with a heating rate of 10°C/min under a nitrogen atmosphere. Differential Scanning Calorimetry (DSC) was performed on a Seiko model 220C instrument interfaced to a Seiko Rheostation 5200. Aluminum oxide was used as the reference material. The analyses were performed from -140°C to 200°C at a heating rate of 10°C/min under a nitrogen atmosphere.

Synthesis of poly(bile acids)

Lithocholic acid (0.51 g, 1.35 mmol) and a 1:1 salt of DMAP and PTSA (0.045g, 0.143 mmol), were mixed in a 100 mL, round bottom 3-necked flask under nitrogen. Using a syringe, 15 mL of dry CH_2Cl_2 was added slowly while stirring at 40°C. Diisopropyl carbodiimide (0.22g, 1.73 mmol) was added and the homogeneous solution was continuously stirred at room temperature for 30 h. The polymer product was precipitated by pouring the reaction mixture into a solution of 200 mL of dry CH3OH while stirring. After centrifugation and drying *in vacuo*, 0.45 g, (92.2% yield) of a white solid, poly(lithocholic acid) was obtained and characterized as follows: IR (cm^{-1}): 2950, 2880, 1750, 1450, 1380, 1170. ^{1}H NMR ($CDCl_3$, δ): 0.65 (s, 3H) [C18-CH_3]; 0.92 (overlapping s and d, 6H) [C19, C21-CH_3]; 1.0-2.4 (multitude of signals) [coprostane nucleus]; 4.75 (m, 1H) [C3-H]. MALDI-TOF m/z (daltons): range from 288.08 to 3087.43. TGA: decomposition beginning at 380°C, 1.3% residue. DSC: T_g = 19.3°C.

The synthesis of poly(deoxycholic acid) was carried out in a similar fashion, except for the solvent system used was a 5:1 mixture of CH_2Cl_2 and pyridine. A white solid (70.8% yield) was obtained, and was characterized as follows: IR (cm^{-1}): 3530, 2960, 2900, 1750, 1460, 1390, 1260, 1170. ^{1}H NMR ($CDCl_3$, δ): 0.68 (s, 3H) [C18-CH_3]; 0.92 (s, 3H) [C19 -CH_3]; 0.98 (d, 3H) [C21-CH_3]; 0.99-2.4 (multitude of signals) [coprostane nucleus]; 4.0 (m, 1H) [C12-H]; 4.7 (m, 1H) [C3-H]. MALDI-TOF m/z (daltons): range from 226.96 to 5288.4. TGA: decomposition beginning at 370°C, 0.6% residue. DSC: T_g = 21.3°C.

Synthesis of poly(cholic acid) was achieved by the same procedure, obtaining a light brown solid (25.2%). It was characterized as follows: IR (cm^{-1}): 3440-3360, 2950, 2890, 1750, 1470, 1390,1180. ^{1}H NMR (pyridine-d_5, δ): 0.80 (s, 3H) [C18-CH_3]; 1.0 (s, 3H) [C19-CH_3]; 1.18 (d, 3H) [C21-CH_3]; 1.0-2.5 (multitude of signals) [coprostane nucleus]; 4.08 (m, 1H) [C7-H]; 4.25 (m, 1H) [C12-H]; 4.8 (m, 1H) [C3-H]. MALDI-TOF, m/z (daltons): range from 1187.8 to 6307.2. TGA: decomposition beginning at 365°C, 92.9% loss up to 390°C, 2.4% residue. DSC: T_g = 58.8°C.

Results and discussion

The room temperature polymerization of three members of the bile acid family, lithocholic, deoxycholic, and cholic, with one, two, and three hydroxyl groups respectively, was successfully accomplished using a system of DIPC and a 1:1

complex of DMAP/PTSA. The increased reactivity of this system enabled room temperature polymerization of the bile acids, thereby eliminating any undesired cross-linking of the product. As a result, the mild reaction conditions employed allowed selective polymerization at the hydroxyl group at carbon 3 without affecting other, more sterically hindered OH groups (Figure 2). Ester formation was monitored by IR spectroscopy; the carbonyl stretching band occurred at 1710 cm^{-1} from the free acid group in the monomers. Upon polymerization, the peak shifted to 1750 cm^{-1}, indicative of an ester group. Selective polyesterification at carbon 3 was evident upon comparison of the IR spectra of the polymers of the three members of the series. Poly(lithocholic acid) did not show an O-H stretching signal after conversion of the only hydroxyl group in the monomer to an ester. However, poly(deoxycholic acid) and poly(cholic retained a signal at 3530 cm^{-1}, corresponding to the O-H stretching frequency of the unreacted hydroxyl groups carbon 7 and/or carbon 12 of the starting materials. More conclusive evidence of the selective polymerization was shown in the ^{1}H NMR spectra. The resonance shift for the proton on carbon 3 was changed from 3.60 ppm in lithocholic acid to 4.75 ppm in the polymer, indicative of the formation of an ester. Analogously, the proton signal on carbon 3 of deoxycholic acid was shifted from 3.4 ppm to 4.70 ppm in its polymer, while the resonance signal for the proton attached to carbon 12 at 4.0 ppm remained unchanged. This evidence confirmed selective reaction at C3. The same results were achieved for cholic acid and polycholic acid, which showed no change in the signals corresponding to C12 and C7 protons while the C3 proton signal moved from 3.65 to 4.80 ppm. Although this data indicates

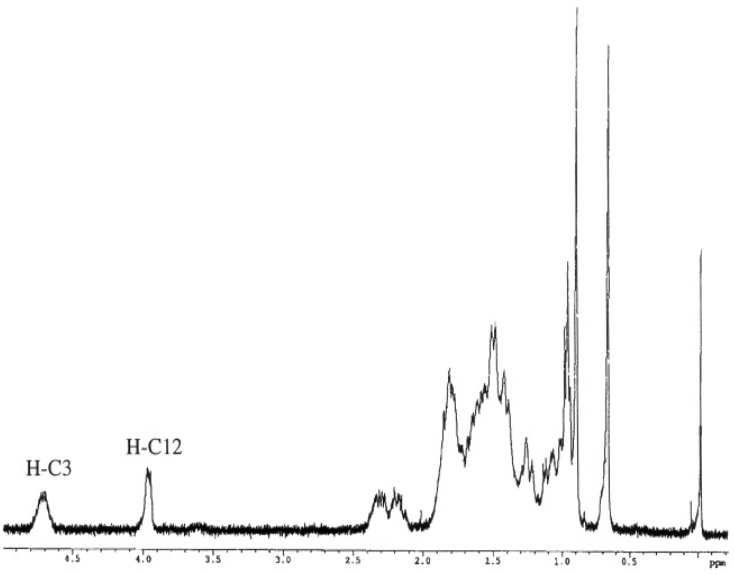

Figure 2. ^{1}H NMR spectrum of poly(deoxycholic acid)

selective conversion of the hydroxyl group to an ester group in all three cases, it does not confirm the formation of the bile acid polyester.

Gel permeation chromatography and mass spectrometry were used to confirm the polymerization of the three monomers. The molecular weights obtained by GPC were 59,000 for poly(lithocholic acid), 64,000 for poly(deoxycholic acid) and 2,800 for poly(cholic acid). These results are not necessarily a reliable estimate of the true molecular weight of the polymer, since the polystyrene standards are very different in chemical structure to the bile acid polymers. The use of MALDI-TOF gives irrefutable evidence of the formation of the polymers, showing signals for the different molecular ions of the oligomers formed in the reaction as well as demonstrating the existence of a bile acid repeat unit. In addition, MALDI-TOF results are influenced by the difficulty of getting higher molecular weight materials out of the matrix support. It is highly possible that the high molecular weight fractions of the polymers were never ionized, thereby significantly lowering the molecular weight determination. Figure 3 shows the MALDI-TOF mass spectrum for polycholic acid.

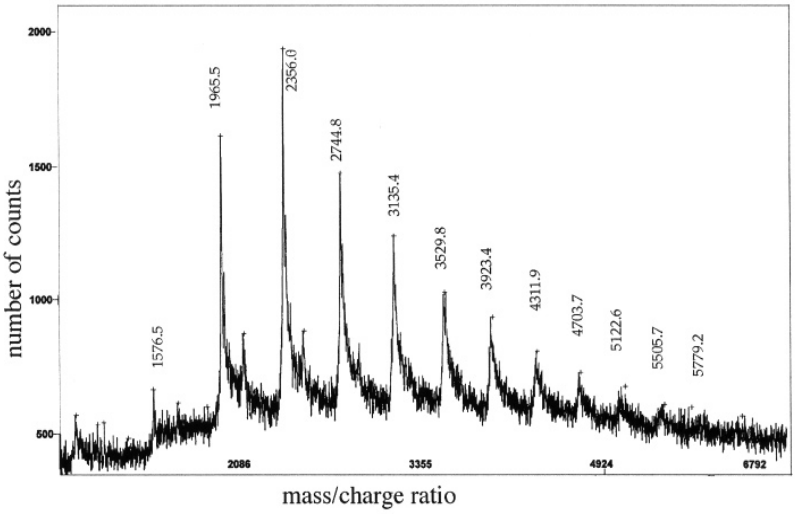

Figure 3. MALDI-TOF spectrum of poly(cholic acid)

The matrix used was α-cyano-4-hydroxycinnamic acid dissolved in pyridine. A low mass gate pulse was used to suppress ions which greatly attenuates all initial ions up to 1900 Daltons (m/z). Signals are seen in the form of the repeat unit, $(390)_n + H_2O$. For example, the signal at 2358 Daltons corresponds to six bile acid repeat units plus water. The increments in mass are clearly evident up to m/z 5.505.7. Small discrepancies of the mass/charge ratio with the actual mass of the repeat unit are due to limitations in resolution of the instrument. Small satellite peaks in the spectra correspond to adducts of pyridine solvent with the ions formed.

Partial crystallinity of the samples was investigated by powder X-ray diffraction, showing 30.2% crystalline regions for polylithocholic acid, 28.8% for polycholic acid and 15.9% for polydeoxycholic acid. Thermal analyses of the

same samples indicate all three polymers are stable up to 380°C, above the temperature range of their potential uses.

Conclusions

We have shown that bile acids with differing degrees of hydroxylation can be step polymerized at room temperature, using a coupling system of DIPC with DMAP/PTSA complex. The conditions employed allow the selective polyesterification of the monomer at carbon 3 with the terminal acid group, thereby avoiding cross-linking seen in higher temperature polymerizations.

Acknowledgments

This project was supported by COLCIENCIAS and by the Fundación de Apoyo of the Universidad del Valle, and was part of a collaboration program with the Chemistry Department of the University of Florida. We are grateful to Dr. D. Powell and D. Schoenfeld for MALDI-TOF spectra, D. Cameron for GPC analyses, T. Herod for X-ray diffraction studies, F. Gomez for NMR spectra, Dr. K. Williams for Thermal Analysis assistance, and Dr. J. Pawlow for editing this manuscript.

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