Synthesis and mesomorphic behaviour of lithocholic acid derivatives[†]

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Abstract. A series of liquid crystalline derivatives of lithocholic acid were prepared using simple chemical reactions involving the terminal functional group—hydroxyl at C-3 and/or carboxyl at C-24. Thus methyl 3-(3-carboxy propionyl) lithocholate (I), 3-(3-carboxy propionyl) lithocholic acid (II), 3-acetyl lithocholic acid (III), 3-propionyl lithocholic acid (IV), 3-benzoyl lithocholic acid (V), 3-(4-nitrobenzoyl) lithocholic acid (VI), 3-cinnamoyl lithocholic acid (VII), methyl-3-(4-nitrobenzoyl) lithocholate (VIII) and 1,4-bis [cholan-24-methoxy carbonyl-3-oxycarbonyl] butane (IX) were prepared in good yields and characterized by IR, NMR and polarizing optical microscopy. Compounds (I) and (IX) exhibited monotropic behaviour while the others were enantiotropic. Some of the compounds also showed a high tendency of super cooling. Compounds (V), (VI) and (IX) formed cholesteric phase while the remaining compounds displayed smectic phase.

Keywords. Mesomorphic; lithocholic acid; transition temperature.

1. Introduction

Tens of thousands of liquid crystalline materials are known (Kelkar and Hartz 1980). Several liquid crystalline compounds containing steroid unit have also been reported (Demus and Zaschke 1984). Studies on the liquid crystalline behaviour of homologous series of *p-n*-alkoxy benzoates (Dave and Vora 1970), *trans p-n*-alkoxy cinnamates (Dave and Vora 1971), *p-n*-alkoxy benzylidene-*p*-amino benzoates (Dave and Kurian 1973a) and 6-*n*-alkoxy-2-naphthoates (Dave and Kurian 1973b) of cholesterol have appeared in the literature. Cholesterol itself is isotropic and only replacement of the hydroxyl with an appropriate substituent in the sterol can induce liquid crystalline behaviour. Lithocholic acid, [cholan-24-oic acid, 3-hydroxy-(3a, 5b)], like all bile acids, possesses a steroid skeleton. It is a crystalline compound that melts to give clear isotropic liquid. In the present investigation several liquid crystalline aliphatic and aromatic ester derivatives of lithocholic acid were prepared. The derivatives prepared may be divided into four classes depending upon the nature and position of the substituent.

(I) Substituent at C-3 position that terminates the functionality. The lithocholic acid derivatives that belong to this category have a free carboxyl group at C-24 position. These derivatives can be used further for reaction with polymers containing free hydroxyl groups to confer liquid crystal-

line properties to the latter. The substituents used include acetyl, propionyl, benzoyl, *p*-nitrobenzoyl, and cinnamoyl.

(II) Substituent at C-3 position that creates a new functionality (–COOH). This lithocholic acid derivative has two-terminal carboxyl groups, one at C-24 position and the other at C-3 position (in fact, the substituent at C-3 is 3-carboxy propionyl). It is mesomorphic and can be used further to prepare main chain liquid crystalline polymers (Shaikh *et al* 1999).

(III) Substituent at C-24 position that terminates the functionality and a substituent at C-3 position that creates a new functionality (–COOH). This is a monofunctional lithocholic acid derivative with –COOH group (3-carboxy propionyl) attached at C-3 position while the –COOH group at C-24 is blocked.

(IV) Substituents at C-3 and C-24 positions that terminate the functionality. Both the functional groups, –OH at C-3 and –COOH at C-24, are blocked in this case and, therefore, cannot be further used either to attach to polymer backbone or to prepare main-chain polymers, but would provide more insight into the mesomorphic behaviour of lithocholic acid based materials.

2. Experimental

Lithocholic acid (Aldrich Chemical Co, USA), acetyl chloride, propanoic acid, benzoyl chloride, succinic acid, (S.D. Fine Chemicals, India), adipoyl chloride (E. Merk), cinnamic acid and *p*-nitrobenzoic acid (Loba Chemie) were purchased from commercial sources and used as such unless mentioned otherwise. Propionyl chloride, cinnamoyl chloride, *p*-nitrobenzoyl chloride and succinic anhydride were pre-

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pared from the respective acids as per the conventional procedures (Furniss *et al* 1978). Other reagents and solvents obtained from commercial sources were purified according to standard procedures (Perrin *et al* 1980).

Melting point was determined by using electrically heated aluminium metal block melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin Elmer 883 IR spectrophotometer, calibrated with polystyrene. Samples were taken in the form of KBr pellet, nujol mull or as solution in carbon tetrachloride as the case may be. Spectra were recorded in 4000–600 cm^{-1} range. ^{13}C -NMR spectra were recorded on a Bruker 50 MHz spectrometer at room temperature in deuteriated chloroform or DMSO. The chemical shifts were referenced to either CDCl_3 or DMSO. ^1H -NMR spectra were recorded using a Bruker 200 MHz NMR spectrometer at 303 K in CDCl_3 (concentration, 40 mg/ml) using a 5 mm-diameter NMR tube.

The thermal properties were determined by using LEITZ LABORLUX 11 POL microscope with polarized transmitted and incident light provision. Leitz micro heating stage 350 was coupled to the microscope. WILD MPS 0-32 $\times$ camera was attached to record photographically the birefringence pattern observed under microscope. Each sample was heated up to isotropization, then cooled to room temperature and again heated up to isotropization in a single checking. Heating and cooling rates for each sample were maintained at $10^\circ\text{C}/\text{min}$. The samples were further characterized by differential scanning calorimetry (DSC) with a Mettler TA 4000 series instrument. It consisted of a DSC cell coupled to Mettler TC 11 TA processor. Three runs, first heating (from room temperature to isotropization), cooling (to room temperature), and second heating (from room temperature to isotropization) were performed for all the samples. The heating and cooling rates were $10^\circ\text{C}/\text{min}$.

Methyl 3-(3-carboxypropionyl) lithocholate, (I), was prepared by the reaction of succinic anhydride with lithocholic acid methyl ester (LAME). LAME was prepared using a reported procedure with a slight modification (Ahlheim *et al* 1986). A mixture of lithocholic acid (5 g), anhydrous methanol (25 ml), and concentrated hydrochloric acid (0.13 ml) was heated to reflux and stirred at reflux temperature for 2 h. The stirring was continued overnight at room temperature. LAME was isolated by filtration and purified by recrystallization from methanol (yield 93%, m.p. 125°C). A mixture of LAME (10 g), succinic anhydride (8 g), and pyridine (30 ml) was stirred in an inert atmosphere at 90°C for 24 h. The product, (I), was isolated by filtration, washed with copious water and dried. It was further purified by Soxhlet extraction and recrystallized from acetone and dried under vacuum (yield 56%, m.p. 163°C). 3-(3-Carboxy propionyl) lithocholic acid, (II), was prepared in a similar manner as (I), except that lithocholic acid was used in place of LAME (yield 59.3%, m.p. 228°C). 3-acetyl lithocholic acid, (III); 3-propionyl lithocholic acid, (IV); 3-benzoyl lithocholic acid, (V); 3-(4-nitrobenzoyl) lithocholic acid (VI); and 3-cinnamoyl lithocholic acid, (VII) were pre-

pared using identical procedure in which lithocholic acid was treated with the respective acid chloride viz. acetyl chloride, propionyl chloride, benzoyl chloride, *p*-nitrobenzoyl chloride or cinnamoyl chloride in dichloroethane in presence of pyridine at 60 – 70°C under nitrogen atmosphere for 24 h ((III) (yield 90%, m.p. 182°C), (IV) (yield 62%, m.p. 230°C), (V) (yield 54%, m.p. 260°C), (VI) (yield 76%, m.p. 218°C), and (VII) (yield 55%, m.p. 238°C)). Methyl 3-(4-nitrobenzoyl) lithocholate, (VIII), was prepared in similar manner as (VI) except that LAME was used in place of lithocholic acid (yield 81%, m.p. 205°C). 1,4-Bis [cholan-24-methoxy carbonyl-3-oxycarbonyl] butane, (IX), was prepared by treating LAME with adipoyl chloride (mole ratio 2 : 1) in presence of pyridine at room temperature for 24 h (yield 61%, m.p. 158 – 159°C).

3. Results and discussion

The general structure of the various lithocholic acid derivatives synthesized is shown in figure 1. IR spectra of the lithocholic acid derivatives except (VIII) and (IX) showed the $-\text{OH}$ stretching in the range 3300 – 3500 cm^{-1} due to the presence of $-\text{COOH}$ group. The peaks due to acid carbonyl and ester carbonyl were observed in all spectra around 1700 cm^{-1} and 1730 – 1753 cm^{-1} , respectively. In the compounds containing both acid as well as ester carbonyl (I–VII) these peaks were seen as doublets. Aromatic esters (V, VI, VII and VIII) showed peaks in 700 – 900 cm^{-1} region which may be attributed to C–H deformation of aromatic ring. In addition, (VI) and (VIII) showed absorbance at $\sim 1530\text{ cm}^{-1}$ which is a characteristic feature of $\text{N}=\text{O}$ stretching.

Proton NMR spectrum of LAME (which was used to synthesize I, VIII, and IX) showed a strong singlet at 3.65 ppm due to acetoxy methyl groups in addition to the usual peaks of lithocholic acid. LA derivative (I) showed multiplet at 2.65 ppm due to two adjacent methylene groups of the succinoyl moiety in addition to the strong singlet at 3.65 ppm. Proton NMR spectrum of LA derivative (V) exhibited multiplets at 8.1, 7.5 and 7.1 ppm which were assigned to the three non-equivalent protons of the benzene ring. LA derivative (VI) showed multiplet at 8.25 ppm, which was not well resolved and was assigned to the two non-equivalent protons of *p*-nitrobenzoyl group of (VI). Proton NMR spectrum of (IV) showed a multiplet at 2.3 ppm due to the methylene group adjacent to $-\text{CH}_3$ and carbonyl. LA derivative (III) exhibited a sharp singlet at 2.05 ppm that was assigned to the three equivalent acetyl protons. ^{13}C -NMR spectrum of (III) revealed 26 lines for 26 carbons in the (III) molecule. The peaks due to COOH and OCOCH_3 were seen at 171.01 and 74.7 ppm, respectively. The remaining ^{13}C signals were similar to those observed in lithocholic acid. LA derivative (VIII) exhibited a sharp singlet at 3.65 ppm and a multiplet at 8.25 ppm assigned to methyl

protons of the ester group and the *p*-nitrobenzene moiety, respectively. LA derivative (IX) showed multiplets at 2.3 ppm and 1.65 ppm which could be assigned to the non-equivalent methylene protons of the adipoyl moiety in addition to a strong singlet at 3.65 ppm attributed to

the three equivalent methyl ester protons. The results of ^1H -NMR and ^{13}C -NMR studies of these compounds are summarized in table 1.

Mesomorphic behaviour of the lithocholic acid derivatives was studied by polarizing optical microscopy and

Figure 1. General structure of lithocholic acid derivatives. (I) $\text{R}^1 = -\text{CH}_3$, $\text{R}^2 = \text{HOOCCH}_2\text{CH}_2-$; (II) $\text{R}^1 = \text{H}$, $\text{R}^2 = \text{HOOCCH}_2\text{CH}_2-$; (III) $\text{R}^1 = \text{H}$, $\text{R}^2 = -\text{CH}_3$; (IV) $\text{R}^1 = \text{H}$, $\text{R}^2 = -\text{CH}_2\text{CH}_3$; (V) $\text{R}^1 = \text{H}$, $\text{R}^2 = -\text{C}_6\text{H}_5$; (VI) $\text{R}^1 = \text{H}$, $\text{R}^2 = -\text{C}_6\text{H}_4\text{NO}_2$; (VII) $\text{R}^1 = \text{H}$, $\text{R}^2 = -\text{CH}=\text{CH}-\text{C}_6\text{H}_5$; (VIII) $\text{R}^1 = -\text{CH}_3$, $\text{R}^2 = -\text{C}_6\text{H}_4\text{NO}_2$.

Table 1. Summary of NMR studies of lithocholic acid derivatives.

Lithocholic acid derivative	Substituent	Peak position (ppm) and nature of peak
LAMe	----COOCH ₃	S, $d = 3.65$
I	----COOCH ₃	S, $d = 3.65$
II	HOOC CH ₂ CH ₂ COO-	M, $d = 2.65$
	HOOC CH ₂ CH ₂ COO-	M, $d = 2.5$
	----CH ₂ COOH	
III	CH ₃ COO-	S, $d = 2.05$ (171.01, 74.7)*
IV	CH ₃ CH ₂ COO-	M, $d = 2.3, 1.2$
V	COO	M, $d = 8.1, 7.5, 7.1$
VI	O ₂ N- COO-	M, $d = 8.25$
VIII	-----COOCH ₃	S, $d = 3.65$
	O ₂ N- COO-	M, $d = 8.25$
IX	-OOC(CH ₂) ₄ COO-	M, $d = 2.3, 1.65$

----linked to C-24 of LA, -linked to C-3 of LA, * ^{13}C -NMR data.

differential scanning calorimetry. Transition temperatures of the compounds, determined by using polarizing microscope equipped with a controlled microheating stage, are given in table 2.

The mesophases observed were compared with standard plates before assigning the texture (Demus and Richter 1978). LAMe is not mesomorphic, it melts at 125°C but does not exhibit mesophase. All the other LA derivatives (I to IX) are mesomorphic. Most of them also show a high tendency of super cooling. LA derivative (I) displayed monotropic smectic behaviour. Mesophase was observed at 109°C while cooling the isotropic melt. DSC thermogram, in heating mode, showed a single endotherm at 157°C representing isotropization. During cooling run also it showed one exotherm at 109–110°C which was attributed to mesophase formation as judged by observation under polarizing microscope. The absence of two distinct exotherms is, probably, because the mesophase is retained up to room temperature. LA derivative (II) also showed mesomorphic properties similar to (I). It melted directly during first heating around 236°C with slight brightness just before melting. The sample was heated 5° above its melting point, held at this temperature for 5 min, and then cooled at a rate of 10°C/min. Highly coloured birefringence was observed from 170–167°C while cooling. A comparison of this texture with

standard specimen suggested that it most probably was smectic G, (S_G), mosaic texture. This texture remained up to 135°C then changed into a new, different, unidentifiable texture which was retained up to room temperature. On second heating, smectic A, (S_A), batonnets were observed from 120°C onwards. The birefringence disappeared to give isotropic melt at 236°C.

LA derivative (III) showed smectic phase with broken fan texture at 130°C on heating as well as on cooling the isotropic melt (figure 2). During cooling, the texture changed at lower temperature into an unidentifiable texture. LA derivative IV, a higher homologue of (III), showed smectic phase of similar broken fan shaped texture but, as expected, at higher temperature, 195°C. However, mesophase was not observed during cooling, probably because of the super cooling. These observations are corroborated by DSC results shown in figure 3. The heating curve shows two endotherms corresponding to crystal–mesophase and mesophase–isotropic transitions, while the cooling curve is featureless.

The aromatic derivatives of LA, (V) and (VI) showed cholesteric phase at 235°C and 180°C, respectively. On cooling mesophase appeared in case of (V) at 220°C indicating some tendency for super cooling. On reheating, the birefringence increased from 180°C onwards. Heating and cooling cycles were repeated with similar observations. The super cooling was more prominent in case of (VI) which did not reveal mesophase formation on cooling the isotropic melt. This inference was further substantiated from the DSC results. The heating curve showed two endotherms corresponding to crystal–mesophase and mesophase–isotropic transitions, while the cooling curve was featureless. LA derivative (VII) showed a mesophase without clearly identifiable texture at 215°C on first heating and from 220°C on cooling. The mesophase–isotropic transitions were found to be 260°C, 237°C and 218°C for (V), (VII), and (VI), respectively. This is not expected as the average mesophase–isotropic transition temperature of *p-n*-alkoxycinnamates of cholesterol (251.8°C) has been reported to be higher than that of *p-n*-alkoxybenzoates of cholesterol (224°C) (Dave and Kurian 1973b). However, the observed mesophase–isotropic transition temperatures for (V) 260°C, (VII) 237°C, and (VI)

Table 2. Transition temperatures of lithocholic acid derivatives.

Lithocholic acid derivatives	Transition temp. °C		Phase
	C-LC	LC-I	
I	(109)	157	S
II	120, (167), (135)*	236	S
III	95, 125*, (130), (120)*	176	S
IV	195	220	S
V	235, (220)	260	Ch
VI	180	218	Ch
VII	220	237	not clear
VIII	115	213	S
IX	(133)	163	Ch

C-LC crystal-to-liquid crystal transition, LC-I liquid crystal-to-isotropic transition, *texture change, () cooling.

Figure 2. Photomicrographs (crossed polars) of III (a. heating, 120°C; b. cooling, 130°C).

Figure 3. DSC thermogram of IV (— first heating; ---- cooling; - - - second heating).

218°C in the present investigation correlate with the electron-withdrawing ability of the substituents (benzoyl < cinnamoyl < *p*-nitrobenzoyl). Also it should be noted that the cholesterol derivatives (*p*-*n*-alkoxy cinnamates or *p*-*n*-alkoxybenzoates) have no free polar functional group while the LA derivatives V, VI and VII have a free carboxyl group at C-24 position.

LA derivative (VIII) showed mesophase formation from 100°C and mesophase–isotropic transition occurred at 213°C which are lower than the corresponding temperatures (180°C and 218°C) for (VI). This probably is because the latter has a terminal –COOH group while the former has –COOCH₃. LA derivative (IX) showed monotropic behaviour, mesophase appearing at 135°C on cooling the isotropic melt.

4. Conclusion

Non mesomorphic lithocholic acid can be converted into liquid crystalline compounds by appropriate derivatiza-

tion. This was achieved by introducing terminal substituents in lithocholic acid.

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