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## Determination of molecular associations of some hydrophobic and hydrophilic bile acids by infrared and Raman spectroscopy

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**Abstract** The aim of this study was to analyse the Raman and infrared spectra of eight common mammalian bile acids in order to identify intermolecular interactions between hydroxyl and carbonyl groups. The results are considered in the light of the new hydrophilic/hydrophobic classification of bile acids. The alcohol OH group of the hydrophobic bile acids forms different intermolecular bonds. The most hydrophobic bile acid, lithocholic acid forms polymers, and this may explain its very low water solubility. The hydrophilic bile acids have some of their alcohol OH groups free of any intermolecular interaction. The strongly hydrophilic murideoxycholic acid also forms dimers, again consistent with a very low water solubility. The proposed structural arrangements are in agreement with published crystallographic studies.

**Key words** Hydrophobic · Hydrophilic bile acids

**Abbreviations** LC Lithocholic acid ( $3\alpha$  monohydroxy- $5\beta$ -cholan-24-oic acid) · C Cholic acid ( $3\alpha$ ,  $7\alpha$ ,  $12\alpha$  trihydroxy- $5\beta$ -cholan-24-oic acid) · DC Deoxycholic acid ( $3\alpha$ ,  $12\alpha$  dihydroxy- $5\beta$ -cholan-24-oic acid) · HDC Hyodeoxycholic acid ( $3\alpha$ ,  $6\alpha$ , dihydroxy- $5\beta$ -cholan-24-oic acid) · CDC Chenodeoxycholic acid ( $3\alpha$ ,  $7\alpha$  dihydroxy- $5\beta$ -cholan-24-oic acid) · UDC Ursodeoxycholic acid ( $3\alpha$ ,  $7\beta$  di-

hydroxy- $5\beta$ -cholan-24-oic acid) ·  $\beta$ -MC  $\beta$ -Muricholic acid ( $3\alpha$ ,  $6\beta$ ,  $7\beta$  trihydroxy- $5\beta$ -cholan-24-oic acid) · MDC Murideoxycholic acid ( $3\alpha$ ,  $6\beta$  dihydroxy- $5\beta$ -cholan-24-oic acid)

### Introduction

Bile acids are biologically important cholesterol end-products with a large variety of structures. They play a major role in intestinal lipid absorption. They are removed from the blood by the liver, secreted into bile and in man undergo an enterohepatic circulation 6–10 times a day.

The relative hydrophobicity of a bile acid is an important physicochemical feature and probably determines its biological function (Armstrong et al. 1982; Heuman 1989). This bile salt monomeric hydrophobicity index (HI) is based on the logarithms of bile salt retention factors in a reverse phase liquid chromatographic system (Heuman 1989). According to this system, LC is the most hydrophobic bile acid, DC and CDC are strongly hydrophobic while HDC, UDC,  $\beta$ -MC and MDC are hydrophilic.

Variations in chemical structure strongly influence the hydrophobicity index and metabolic properties of bile acids (Hofmann et al. 1984). For example, hydrophobic bile acids seem to recruit more cholesterol in the bile and appear to inhibit cholesterol- $7\alpha$ -hydroxylase, the key enzyme of bile acid biosynthesis (Heuman et al. 1988; Bilhartz et al. 1988). In patients with cholesterol gallstones, higher bile cholesterol secretion (Reuben et al. 1985) and lower cholesterol- $7\alpha$ -hydroxylase activity (Salem et al. 1975) have been observed. Moreover, some bile acids such as CDC or UDC are also currently used to dissolve cholesterol gallstones in man (Danziger et al. 1972; Makino et al. 1975).

In general, infrared spectroscopy has been used in this field as a method to identify unknown compounds (Small 1971). Since infrared and Raman spectroscopy measure vibrational properties of molecular bonds, they are also well suited for the study of the interaction between lipids. Be-

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fore using these techniques for studying the interaction of hydrophilic/hydrophobic bile acids with artificial biological membranes, it appeared necessary to obtain data on the possible intermolecular associations of the most common physiological bile acids which can be found in mammalian biles.

The aim of this study was to analyse these molecular associations by infrared and Raman spectroscopy in the light of a recent classification of physico-chemical properties (hydrophilic versus hydrophobic bile acids) which may also be important in different biological functions.

## I. Methods

### a. Materials

The solvents used for chromatography, extraction and synthetic procedures (analytical grade) were supplied by Merck (Darmstadt, Germany) and Prolabo (Paris, France). Chromium oxide ( $\text{CrO}_3$ ) and sodium borohydride ( $\text{NaBH}_4$ ) were obtained from Sigma (St. Louis, MO, USA) and Fluka (Buchs, Switzerland), respectively.

Lithocholic acid (LC), deoxycholic acid (DC), cholic acid (C), and ursodeoxycholic acid (UDC), were purchased from Steraloids (Wiltron, NH, USA) and Calbiochem (San Diego, USA). Hyodeoxycholic acid (HDC) and chenodeoxycholic acid (CDC) were a generous gift from the Roussel-Uclaf laboratories (Romainville, France). The purity of bile acids was verified by gas liquid chromatography to be better than 98%.

Murideoxycholic acid (MDC) (Hofman et al. 1992) (old name: Murocholic acid) was prepared chemically in two main steps as previously described (Parquet et al. 1990). Briefly, HDC was first methylated by diazomethane and oxidized according to two described procedures (Hoen et al. 1946; Ilda et al. 1988). The 6-oxo methyl ester formed was reduced with sodium borohydride in methanolic solution. Separation of methylated MDC was achieved by chromatography on a silica gel column prepared with chloroform/acetone 90:10 (v/v). Finally, MDC methyl ester was saponified with NaOH and crystallized in a solvent mixture of methanol/water.  $\beta$ -Muricholic acid ( $\beta$ -MC) was prepared according to Ilda et al. (1988) with some modifications.

### b. Spectroscopy

The spectra were recorded with an IFS 66 Bruker Fourier Transform Infrared spectrometer. The KBr beamsplitter limited the spectral range to  $6000\text{ cm}^{-1}$  to  $400\text{ cm}^{-1}$ . A conventional DTGS detector was used. In the solid state the samples were obtained by pressing into a KBr pellet. To overcome pressure effects, some recordings were made with a Diffuse Reflectance System (Gransby Corporation). In order to reduce intermolecular interactions some compounds have been studied in a solution of chloroform. Only

four bile acids (CDC, UDC, LC,  $\beta$ -MC) have sufficient solubility in a non-polar solvent.

For Raman spectroscopy, the same Fourier Transform Spectrometer was used with an FRA 106 equipped with a Nd:YAG laser emitting continuously at  $1.06\text{ }\mu\text{m}$  with a maximum power of 100 mW. A  $\text{CaF}_2$  beam-splitter was employed in the range  $11000$  to  $6000\text{ cm}^{-1}$ . A Ge-liquid-nitrogen cooled high sensitivity detector was operated for Raman detection. A narrow band pass filter allowed us to record Raman lines as close as  $70\text{ cm}^{-1}$  to the Rayleigh diffusion line. The usual limit of resolution was  $1\text{ cm}^{-1}$  for infrared and  $4\text{ cm}^{-1}$  for Raman spectra.

## II. Results

The vibrational spectra of bile acids are mainly defined by the CH, OH and C=O stretching and deformation of the functional groups of these molecules. The position of the vibrational bands in infrared have been studied in the past (Small 1971; Margareta et al. 1970). Our observations and conclusions are concerned with the position and the shape of the C=O stretching line around  $1700\text{ cm}^{-1}$ , observed by absorption infrared spectroscopy and Raman diffusion spectroscopy and on the OH stretching bands around  $3500\text{--}3200\text{ cm}^{-1}$  in infrared spectroscopy. This vibration gives no detectable line in Raman spectroscopy. Some information may also be obtained with the out-of-plane bending of hydroxyl in the spectral range  $1000\text{--}1100\text{ cm}^{-1}$ .

The main interest of these observations is the detection of extra lines due to intermolecular interactions deriving from van der Waals forces or hydrogen-bonding. These intermolecular interactions are important in a crystalline lattice and may be reduced when the compound is in solution in a non-polar solvent.

The results obtained from the infrared and Raman spectra are collected in Table 1. We focus our attention on some of them in order to obtain evidence of hydrophilic properties. In the following, comparisons are made on the observed spectra for two molecules having some similarity in their conformation.

### Lithocholic acid ( $3\alpha\text{ OH}$ ) and Deoxycholic acid ( $3\alpha$ , $12\alpha\text{ OH}$ )

LC is the most hydrophobic bile acid according to Heuman and in our own classification (Table 2).

A broad band is observed at  $3385\text{ cm}^{-1}$  corresponding to an alcohol bonded OH and an acid bonded OH is observed at  $3305\text{ cm}^{-1}$ . One band at  $1705\text{ cm}^{-1}$  in infrared and  $1700\text{ cm}^{-1}$  in Raman, also indicates bonds to the carbonyl C=O. The out-of-plane vibration of the  $3\alpha\text{ OH}$  is observed at  $1041\text{ cm}^{-1}$ . As mentioned in all Tables (Margareta et al. 1970), the OH bending wave number varies according to the equatorial position and is around  $1050\text{ cm}^{-1}$ , while the axial position appears around  $1050\text{--}970\text{ cm}^{-1}$ . The observed line is broad which con-

**Table 1** Infrared and Raman spectra of common bile acids

Bile acids	Infrared $\nu$ O-H bond $\text{cm}^{-1}$	Infrared $\nu$ C-O-H bond $\text{cm}^{-1}$	Infrared $\nu$ C-H bond $\text{cm}^{-1}$	Raman $\nu$ C-H bond $\text{cm}^{-1}$
<b>Hydrophobic</b>				
3 $\alpha$ Lithocholic	3305 3385	1041	1705	1700
3 $\alpha$ , 7 $\alpha$ Chenodeoxycholic	3426	979 1048	1709	1706
3 $\alpha$ , 12 $\alpha$ Deoxycholic	3643 3400 3235	1042 1014	1702 1690	1710 1692
<b>Neutral</b>				
3 $\alpha$ , 7 $\alpha$ , 12 $\alpha$ cholic	3494 3408 3362	981 1014 1043	1716 1705	1717
<b>Hydrophilic</b>				
3 $\alpha$ , 6 $\alpha$ Hyodeoxycholic	3542 3436 3277	1010 1036	1737 1710	–
3 $\alpha$ , 7 $\beta$ Ursodeoxycholic	3500 3520 3346 3210	1043 1054	1696 1717	1694 1717
3 $\alpha$ , 6 $\beta$ murideoxycholic	3542 3376 3276	1036	1706	1689
3 $\alpha$ , 6 $\beta$ , 7 $\beta$ $\beta$ -muricholic	3555 3538 3465 3425	1050 1001	1695 1659	1695 1659

**Table 2** Hydrophobicity indices (HIX) of individual tauro conjugated bile acids

Bile acid	According to Heuman (1989)	This work
LC	+1	+1
DC	+0.69	+0.59
CDC	+0.53	+0.46
C	0	0
HDC	–0.35	–0.35
UDC	–0.47	n.d.
$\beta$ -MC	–0.78	n.d.
MDC	n.d.	–0.89

Hydrophobicity indices (HIX) were calculated from capacity factors ( $k_x$ ) determined by reverse phase HPLC (mobile phase methanol/water 70/30 (w/v), ionic strength 0.15 and pH 8.5–9.0, stationary phase octadecylsilane (C18)). HIX was calculated using the formula:

$$\text{HIX} = \ln(k_x/k_{tc}) / \ln(k_{tlc}/k_{tc})$$

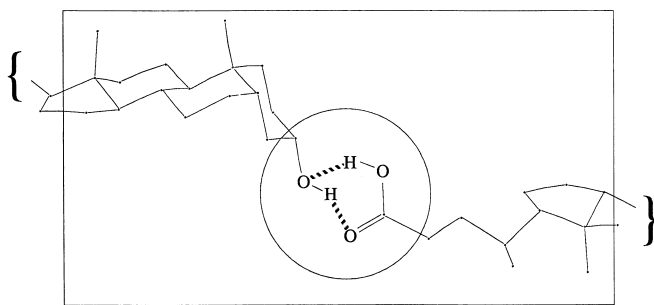
where  $k_{tc}$  and  $k_{tlc}$  refer to capacity factors of taurocholate and tauro lithocholate, respectively

firms the possibility of intermolecular bonding. In order to explain the strong intermolecular interactions, the strong hydrophobic character and the low water solubility of this compound, an intermolecular bond between OH in 3 $\alpha$  and C=O from another molecule can be assumed (Fig. 1). This results in a sort of polymeric sheet. This molecular struc-

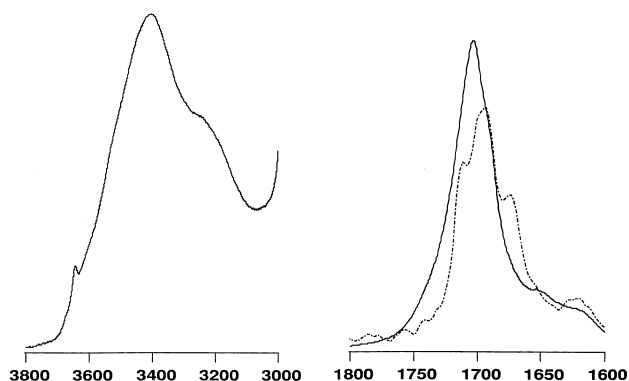
ture has been proposed from X-ray diffraction studies (Arora et al. 1975).

When this molecule is diluted in  $\text{CHCl}_3$ , the broad lines disappear and free OH lines are observed at  $3690 \text{ cm}^{-1}$  (alcohol OH) and  $3608 \text{ cm}^{-1}$  (acid OH), with a sharp extra line at  $3514 \text{ cm}^{-1}$  and a broad bound line at  $3059 \text{ cm}^{-1}$ . A free C=O line at  $1741 \text{ cm}^{-1}$  and a bound line of higher intensity at  $1707 \text{ cm}^{-1}$  are observed. The dilution does not affect the relative intensity of bound lines compared to free lines. This feature implies that all 3 $\alpha$  OH and C=O are bound to give strong interactions between several molecules. Some of them may be broken by solvent effects, but a kernel remains indestructible. This observation is in complete agreement with the molecular structure obtained from a crystallographic study (Arora et al. 1975).

For DC (3 $\alpha$ , 12 $\alpha$  OH), a sharp and low intensity band at  $3643 \text{ cm}^{-1}$  (Fig. 2) indicates a free OH bond, while strong intensity broad bands at  $3400 \text{ cm}^{-1}$  and  $3200 \text{ cm}^{-1}$  indicate bound OH and bound acid OH, respectively. Three bands around  $1700 \text{ cm}^{-1}$ , both in infrared and Raman indicate intermolecular bonds to the C=O. A structure was predicted (Candeloro et al. 1972) and gives channels which are able to be a host molecule in complex association. This work presents a study on an inclusion compound of DC. It confirms the occurrence of hydrogen-bonding between 3 $\alpha$  OH, 12 $\alpha$  OH and C=O.



**Fig. 1** Conformation of lithocholic acid (LC) ( $3\alpha$  OH). (Derived from Arora et al. 1975)



**Fig. 2** Some features of the absorbance IR (full line) and Raman (dotted line) spectra of deoxycholic acid (DC) ( $3\alpha$   $12\alpha$  OH)

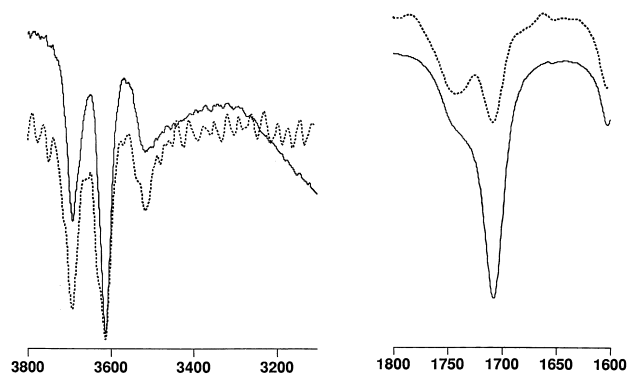
Apart from some OH bonds which may be free, the two bile acids are similar and may give very strong intermolecular associations, which explains their hydrophobic index.

#### Chenodeoxycholic acid and ursodeoxycholic acid

CDC is a hydrophobic bile acid while its epimer, UDC, is a hydrophilic one. The two bile acids have only a slight configurational difference due to the hydroxyl in position  $7\alpha$  or  $7\beta$ .

In the case of CDC, we observe an alcohol bound OH shifted to  $3426\text{ cm}^{-1}$  and a carbonyl band at  $1709\text{ cm}^{-1}$ . In the Raman spectrum, we observe only one peak at the same wavenumber. This may indicate that there is no dimeric association between the carboxyl groups of two molecules. Two broad peaks at  $979$  and  $1048\text{ cm}^{-1}$  correspond to the out-of-plane vibration of the axial  $7\alpha$  and equatorial  $3\alpha$  hydroxyl groups, respectively.

When the spectrum of this acid is measured in  $\text{CHCl}_3$  solution (Fig. 3), three sharp lines can be observed in the  $3700\text{--}3100\text{ cm}^{-1}$  range, one corresponding to a free alcohol OH bond at  $3690\text{ cm}^{-1}$ , another one at  $3611\text{ cm}^{-1}$  corresponding to hydrogen bond. Large dilutions do not affect the relative strength of the OH lines. The carboxyl



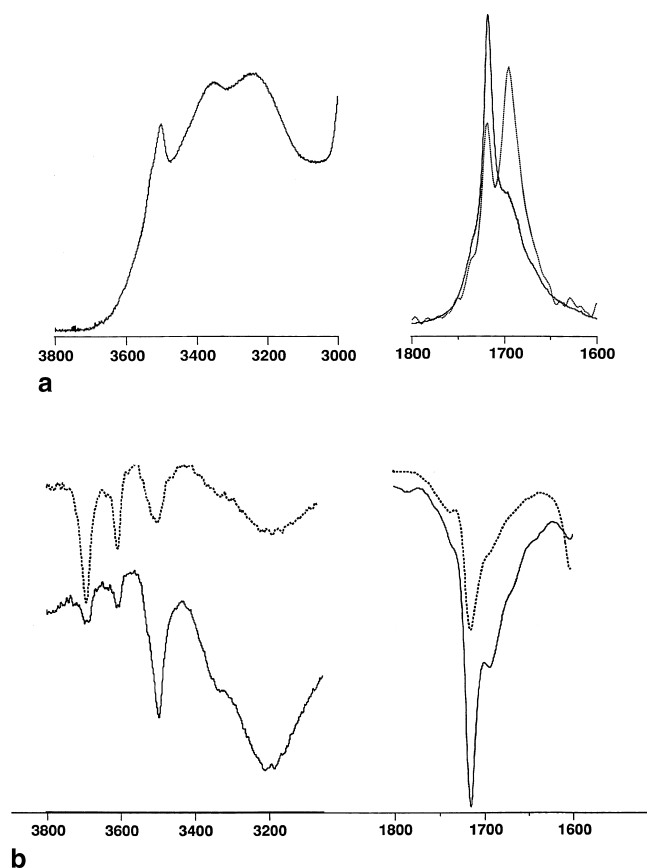
**Fig. 3** IR transmittance spectrum for a solution of chenodeoxycholic acid (CDC) ( $3\alpha$   $7\alpha$  OH) in  $\text{CHCl}_3$ . (Full line: higher concentration, dotted line: higher dilution)

group gives two lines, one at  $1708$  and one at  $1742\text{ cm}^{-1}$ , the latter corresponding to a free bond. The dilution spectra show that these intermolecular bonds are not easily broken by solvent effects. We conclude that there are strong intermolecular bonds between OH  $3\alpha$ ,  $7\alpha$  and the carboxyl group of neighbouring molecules. The relative position of atoms has been described (Lindley et al. 1980) and agrees with our observations.

The spectra of UDC show significant differences (Fig. 4a). In the solid state a sharp OH bond appears at  $3516\text{ cm}^{-1}$  with bound alcohol at  $3346\text{ cm}^{-1}$  and bound acid at  $3210\text{ cm}^{-1}$ . The carbonyl region shows two peaks at  $1717\text{ cm}^{-1}$  and  $1690\text{ cm}^{-1}$  corresponding to a dimeric association. The Raman spectrum shows two peaks at the same wavenumbers but the intensities of the components are reversed, as shown in Fig. 4 a. This behaviour is characteristic of a dimer association of two carboxylic acids, with asymmetric vibration of the two carbonyl functions at lower frequency and higher intensity for the Raman line, and an antisymmetric vibration at higher frequency and higher intensity in infrared. The out-of-plane vibrations give a broad line at  $1054\text{ cm}^{-1}$  and a shoulder at  $1043\text{ cm}^{-1}$  corresponding to the  $7\beta$  and  $3\alpha$  hydroxyl groups, respectively.

When we investigate this acid in  $\text{CHCl}_3$  solution (Fig. 4b), we observe three sharp lines at  $3691$ ,  $3608$  and  $3498\text{ cm}^{-1}$  and a broad line at  $3205\text{ cm}^{-1}$ . The dilution shows clearly a balancing of the intensity between the last two lines which slowly disappear and a growth of the first two lines. The analysis of the carbonyl line shows that the lines at  $1715\text{ cm}^{-1}$  and  $1693\text{ cm}^{-1}$  remain very strong. At higher dilution a faint line appears at  $1740\text{ cm}^{-1}$  indicating the appearance of a free carbonyl bond.

We may therefore conclude that UDC has a high hydrophilicity because OH bonds are associated with weak intermolecular short distance forces, while the carbonyl bond is involved in a very strong dimeric association. The behaviour is opposite for CDC. The alcohol OH bonds are locked by strong intermolecular hydrogen bonds while intermolecular forces acting on  $\text{C}=\text{O}$  are only weak and may be easily broken in solution.



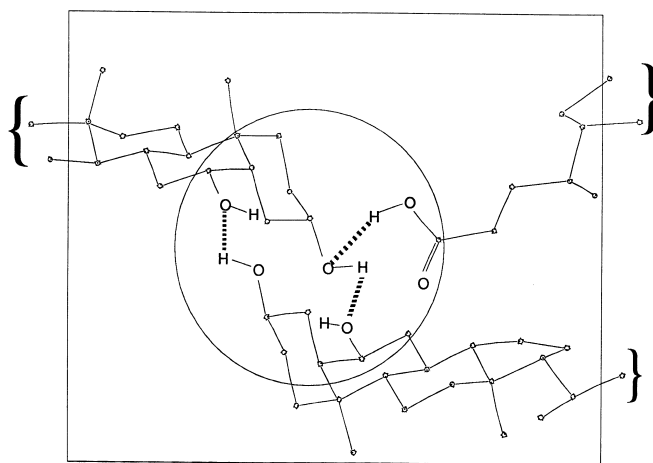
**Fig. 4** **a** Some features of the absorbance IR (full line) and Raman (dotted line) spectra of ursodeoxycholic acid (UDC) ( $3\alpha, 7\beta$  OH). **b** IR transmittance spectrum for a solution of ursodeoxycholic acid (UDC) ( $3\alpha, 7\beta$  OH) in  $\text{CHCl}_3$ . (Full line: higher concentration, dotted line: higher dilution)

#### Chenodeoxycholic acid and cholic acid

The first compound is hydrophobic while the second one is neutral according to Heuman's classification.

The main features are similar but we observe a weak line at  $3494\text{ cm}^{-1}$  in the infrared spectrum of C on the shoulder of the broad bound OH line. This line may result from the free  $12\alpha$  OH. Another difference is observed in the  $\text{C}=\text{O}$  region which shows a doublet for C at  $1705$  and  $1716\text{ cm}^{-1}$ . This observation is in complete agreement with the molecular structure obtained from a crystallographic study (Johnson et al. 1972). In the range of the out-of plane vibrations of the hydroxyl groups the same pattern is observed for the two molecules, corresponding to the bound  $3\alpha$  OH and  $7\alpha$  OH, and one sharp line at  $1014\text{ cm}^{-1}$  corresponding to the axial  $12\alpha$  OH in C.

The free  $12\alpha$  OH implies that C is more hydrophilic than CDC. Finally, this acid is considered as neutral in the index of hydrophobicity which implies that the  $12\alpha$  OH has only a weak influence on the index.



**Fig. 5** Conformation of hydoxycholic acid (HDC) ( $3\alpha, 6\alpha$  OH). (Derived from Hall et al. 1974)

#### Murideoxycholic acid and hydoxycholic acid

These two bile acids are classified as hydrophilic. HDC (Fig. 5) and MDC show a difference owing to the hydroxyl group in position 6. We observed (Table 1) significant differences which are reflected in their infrared spectra.

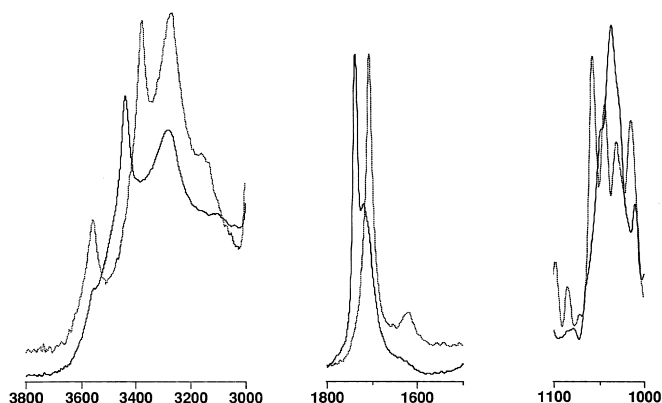
At  $3542\text{ cm}^{-1}$ , we detect a free OH bond in MDC, while this band is very weak in HDC (Fig. 6). The bands at  $3376\text{ cm}^{-1}$  are assumed to be due to weak intermolecular OH bonds. On the other hand, we notice that the  $\text{C}=\text{O}$  bands are completely different: for MDC, a dimer band appears at  $1706\text{ cm}^{-1}$  while a free  $\text{C}=\text{O}$  band is detected for HDC at  $1737\text{ cm}^{-1}$  and a bound carbonyl band at  $1710\text{ cm}^{-1}$ . In all other bile acids investigated the free band appears only in solution. The evidence of a free  $\text{C}=\text{O}$  bond in HDC has also been demonstrated by X-ray diffraction. (Hall et al. 1974). Nevertheless the possibility of bond formation exists.

The sharp lines at  $1044\text{ cm}^{-1}$  and  $1014\text{ cm}^{-1}$  correspond to the  $3\alpha$  and  $6\beta$  OH groups, respectively. We conclude that the carbonyl groups are not associated in a dimeric form in HDC, while the  $3\alpha$  and  $6\alpha$  OH groups are associated by weak intermolecular bonding. MDC has few possibilities of intermolecular bonding but it can form a dimer between the carbonyl groups. All these features may explain why MDC is more hydrophilic than HDC.

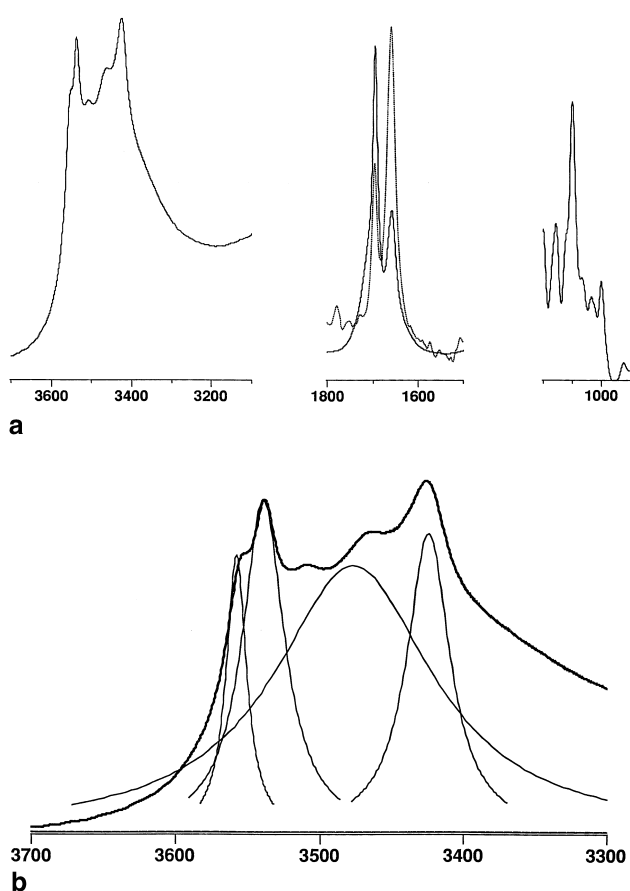
#### $\beta$ -Muricholic acid and ursodeoxycholic acid

These two compounds are classified as hydrophilic. We have already described the infrared spectra of UDC.  $\beta$ -MC is one of the most hydrophilic of all bile acids (with MDC) which we have studied. The structural difference is in an additional hydroxyl group at position  $6\beta$  in UDC.

Figure 7a shows the main features of the spectra with a decomposition of the profile in  $3300\text{ cm}^{-1}$  range (Fig. 7b). Sharp hydroxyl bands at  $3555\text{ cm}^{-1}$ ,  $3538\text{ cm}^{-1}$



**Fig. 6** Some features of the absorbance IR spectrum of murideoxycholic acid (MDC) ( $3\alpha$   $6\beta$  OH) (dotted line) and hydoxycholic acid (HDC) ( $3\alpha$   $6\alpha$  OH) (full line)



**Fig. 7** **a** Some features of the absorbance IR (full line) and Raman (dotted line) spectra of  $\beta$ -muricholic acid ( $\beta$ -MC) ( $3\alpha$   $6\beta$   $7\beta$  OH). **b** Decomposition of the profile of the OH pattern of  $\beta$ -muricholic acid ( $\beta$ -MC) ( $3\alpha$   $6\beta$   $7\beta$  OH)

and  $3425\text{ cm}^{-1}$  corresponding to free alcohol or acid OH are observed. Two carbonyl bands at  $1694\text{ cm}^{-1}$  and  $1659\text{ cm}^{-1}$  corresponding to a very strong dimeric-bonding are well observed in infrared and Raman. The patterns for the C=O stretching are similar for both compounds. In

the range of the out-of-plane bending frequency of hydroxyl groups the only difference between the two acids is one sharp peak at  $1001\text{ cm}^{-1}$  corresponding to the axial  $6\beta$  hydroxyl in  $\beta$ -MC.

$\beta$ -MC has a low solubility in  $\text{CHCl}_3$ . We observed only two sharp lines at  $3694$  and  $3609\text{ cm}^{-1}$  and a broad weak line at  $3300\text{ cm}^{-1}$ . On the other hand only one free C=O line remains at  $1736\text{ cm}^{-1}$  with two weak dimeric lines at  $1695$  and  $1660\text{ cm}^{-1}$ . Consequently the crystalline lattice can be easily broken in solution.

$\beta$ -MC has two free OH bonds, which provide a higher hydrophilic character than UDC which has only one free OH bond.

### III. Discussion

The detailed analysis of spectra by infrared spectroscopy of bile acids furnishes information about the intermolecular forces acting on OH and C=O bonds. Raman spectroscopy gives no information on OH bonds but it confirms the observations made by infrared spectroscopy concerning the C=O bonds. This technique has the advantage of giving very high intensity lines for symmetrical vibrations in dimeric carbonyl bonds, as may be observed in the spectra of several bile acids.

A comparison of the different spectra with the Heuman classification of hydrophobic/hydrophilic bile acids (Heuman 1989), shows that hydrophobic acids have their OH bound in very strong intermolecular interactions. This is the case for the most hydrophobic bile acid, LC, which gives a polymeric association of ( $3\alpha$  OH) with carbonyl, thus forming sheets in which the OH group is hindered. This hydrophobic bile acid is practically insoluble in water at room temperature (Small 1971). Its low solubility may be directly related to a polymeric association of several molecules.

DC forms a cage in which the additional  $12\alpha$  OH is also involved in hydrogen bonds. This compound forms a hydrophobic cage.

CDC ( $3\alpha$ ,  $7\alpha$  OH) shows mainly intermolecular bonds between  $3\alpha$ ,  $7\alpha$  OH and the carboxyl group.

Most hydrophilic bile acids possess a free OH. However, their carboxylic group is generally involved in a dimeric association, with the exception of HDC which possesses a non-bonded carbonyl group.

A hydrophilic acid has some OH bonds free of any interaction. These bonds appear in the infrared spectrum as sharp lines superimposed on a strong absorption background. This behaviour is evident when we consider LC – no sharp line, DC – one weak sharp line, UDC – one intense sharp line, MDC – two sharp lines, and  $\beta$ -MC – three sharp and intense lines. This classification is in agreement with the hydrophilicity index.

The infrared spectrum in solution gives information on the strength of the intermolecular bonds. It appears that dissolution is able to break the bonds. Nevertheless, for a hydrophobic acid, a further dilution has no effect on the

remaining associated kernels. It is clear that the line at  $3529\text{ cm}^{-1}$  which is interpreted as an intermolecular bonded line, vanishes for hydrophilic acids such as UDC, and remains for hydrophobic acids such as LC. This character is also in agreement with the hydrophobicity index.

Moreover, this study suggests some interesting new questions concerning the capacity of bile acids to interact with neighbouring molecules, particularly hydrophilic ones. Using HPLC techniques, the measurement of the bile acid hydrophobicity index reflects the interaction of bile acids both with a hydrophilic phase (water/methanol) and a hydrophobic phase (aliphatic chains grafted onto the solid phase of the column). Although in both techniques (IR and/or Raman and HPLC) the phases containing bile acid (KBr,  $\text{CHCl}_3$  or water/methanol) are different, it is interesting that we find a link between the existence of a free OH group after intermolecular association of bile acids and the value of the corresponding hydrophobicity index. It is the first time that such a relationship has been demonstrated. Further, similar studies performed under more physiological conditions are now necessary. They could allow one to check the effectiveness of such associations and may contribute to an understanding of the detergent or protective effects of these compounds on biological membranes.

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