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Fine Structure of Hydrogen Bonds in Cholic Acid Revealed by 2DIR Spectroscopy

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Abstract: Based on cryogenic FT-IR spectroscopic studies of hydrogen bonds in cholic acid，two-dimensional FT-IR spectroscopy was applied to enhance our understanding of the hydrogen bonds of cholic acid. Fine spectral structures were revealed by asynchronous 2D FT-IR spectra. The co-relationship among various bands was discussed according to the synchronous 2D FT-IR spectrum.

Keywords: 2D-IR, hydrogen bond, cholic acid.

Hydrogen bond, which occurs in diverse bio-molecules, plays an important role in regulating their conformation, molecular packing behavior as well as some biological functions related to their structure. Thus, extensive investigations on hydrogen bonds have lasted for several decades. Infrared spectroscopy, being sensitive to hydrogen bonds in molecular systems, was used as a major tool in the study of hydrogen bonds[1]. However, in many cases, the severe band-overlapping problems made the spectra too complex to get additional structural information of hydrogen bonds. Consequently, it can only provide qualitative information about hydrogen bonds[2]. The advent of 2DIR spectroscopy[3-10] brought about new hopes of studying hydrogen bonds in bio-molecules. Noda proposed a feasible way to construct 2DIR spectroscopy using perturbation-induced time-dependent fluctuation of IR signal[3,4]. In 1993, the mathematical formalism was proposed to produce 2D spectra. Thus, 2D correlated spectra could be constructed from systematic variation with arbitrary and complex waveform[5]. Since then 2DIR spectroscopy got a wide application in various research fields. In 2DIR spectra, the overcrowded bands were spread into a second dimension, thereby simplifying the spectra with overlapped bands. On the other hand, the 2DIR spectra can provide information concerning the interaction among various chemical groups. All the promising features mentioned above enhance the ability of FT-IR spectroscopic method. Ozaki *et al.* used 2DIR spectroscopy to study the molecular association and got very good results[7,8]. We

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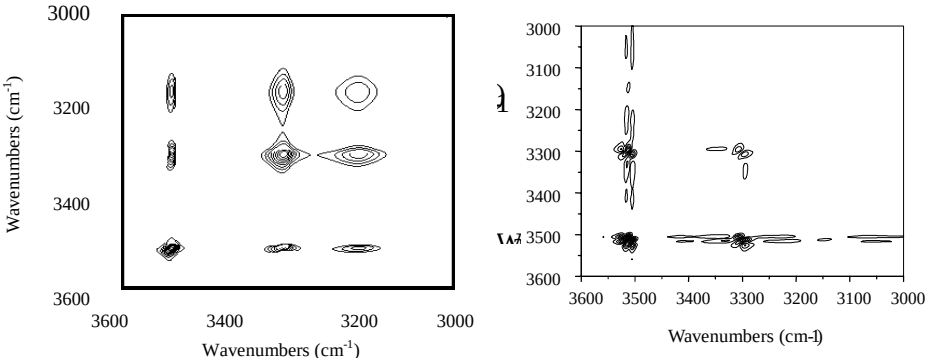
have also used 2D FT-IR spectroscopic technique to differentiate two states in orthorhombic crystalline region of polyethylene[10]. Bile acids are very important molecules in the digestion process of human being. Further understanding the structural-functional relationship of the molecules is important in understanding the mechanism of gallstone formation[11-21]. In our previous work, we have used cryogenic FT-IR spectroscopic method to investigate bile acids and cholesterol[20, 21]. In this paper, we used 2DIR spectroscopy to study the hydrogen bonding system in cholic acid. The temperature range was selected from room temperature to *ca* -150°C, since at low temperature, molecular thermal movement is significantly suppressed and bandwidths decrease accordingly and the resolution of spectra are enhanced.

Cholic acid was purchased from Fluka Company. Nujol mull method was used in the sample preparation. FT-IR spectra were recorded on a Nicolet Magna IR-750 spectrometer. MCT detector was used and 25 spectra were acquired. Each spectrum was obtained at a resolution of 4 cm^{-1} with 32 scans co-added. The temperature variable experiments were conducted using Perkin Elmer's p/n 21.000 temperature variable cell with NaCl windows. To prevent the condensation of moisture, the sample was sealed in the cell with NaCl window. The experiments were performed under vacuum. The 2DIR spectra were constructed from a series of temperature variable FT-IR spectra using the software in Win-Pro of Bio-Rad Company. Additionally, a program of computing generalized 2D correlation spectra according to Burie's work[9] was written in our lab. The same 2D asynchronous spectra were obtained.

Figure 1 2DIR spectra of cholic acid in its OH stretching region
a synchronous spectrum **b** asynchronous spectrum



Cholic acid is a steroid compound with three OH groups at 3, 7, and 12 positions. The hydrocarbon chain with a carboxyl group at the 17 position. FTIR spectra showed three bands in the OH stretching region (3600 and 3000 cm^{-1}). As the temperature of the sample is decreased, these bands became stronger, sharper and exhibited red shift. The single crystal structure of cholic acid with no guest molecule[22] indicated 4 molecules packed within a unit crystal cell. The three OH groups and one carboxyl group of cholic acid formed four types of hydrogen bonds. Based on the O…O distance of the hydrogen bonds, a tentative assignment of the three OH stretching band in FT-IR spectrum has

been made. The geometric parameters of the hydrogen bond OH stretching bands at room and low temperature are shown in **Table 1**. **Figure 1a** shows the 2D synchronous spectrum of cholic acid in its OH stretching region. Three autopeaks corresponding to OH stretching bands could be observed. In addition, the presence of three pairs of positive cross peaks among the three OH stretching bands demonstrated the existence of strong interaction among the OH groups. The results listed in **Table 1** support this point. The cross peaks around (3170, 3310), (3310,3170) represent the hydrogen bonding between C_{12}-OH and C_3-OH groups, the cross peaks at (3310,3515), (3515, 3310) correspond to the hydrogen bonds between C_3-OH and C_7-OH. Although no hydrogen bond binds the C_{12}-OH and C_7-OH directly, the two OH groups are linked *via* the hydrogen bond chains: C_7-OH…O=C_{24}-O-H…O-C_{12}. One can observe the cross peak at (3515, 3170), (3170,3515). On the other hand, we found the cross peaks of all the OH stretching bands with the C=O stretching bands. The three OH groups and one COOH group of the cholic acid formed a hydrogen network. 2D asynchronous spectrum in the OH stretching region of cholic acid illustrated in **Figure 1b** reveals the fine spectral structure that seems not to be found by other methods. For the second OH stretching band whose peak position is around 3296 cm^{-1} at low temperature, a pair of cross peaks around (3296, 3307), (3307, 3296) were observed along the diagonal line. No elongate contour was found for the cross peaks. In addition, the position of the cross peak is not the same as the start and end points of the series of band shifting in the temperature variable FT-IR spectra. Consequently, according to the properties of asynchronous spectra, the cross peaks in the asynchronous spectra indicate that the 3299 cm^{-1} band is composed of the two overlapping bands around 3296 and 3307 cm^{-1}. Up to now, this fine spectral structure of hydrogen bonds has not been reported. Further studies are performed to deep our understanding of these two bands on the structural basis. Around 3507 cm^{-1} in low temperature spectrum, four cross peaks were found along the diagonal line. According to the properties of the asynchronous spectra, the presence of the 4 cross peaks suggested that the band was composed of at least three overlapping bands. However, in the 2D asynchronous spectrum, the feature of elongate contour was clearly observed in the cross peak. Gericke and Czarnecki reported[23, 24], such feature is characteristic for the cross bands caused by the band shifting rather than the multiple overlapping bands. Thus, the presence of the cross peaks for the 3507 cm^{-1} bands could not prove the existence of multiple overlapping bands.

The 2D asynchronous spectrum revealed that the 3299 cm^{-1} band is virtually composed of two overlapping bands. These results demonstrate that 2DIR spectroscopy provide us a new chance to get insight into the relationship between the molecular structure and its vibrational spectra.

Table 1 The geometric parameters of hydrogen bonds in cholic acid and corresponding OH stretching bands at room temperature and low temperature spectra

OH groups	Hydrogen bonds	O...O distance (A)	ν_{OH} in RT spectra (cm^{-1})	ν_{OH} in LT spectra (cm^{-1})
C_{24}-O-H	C_{24}-O-H....O-C_{12}	2.634(5)		
C_{12}-O-H	C_{12}-O-H...O-C_3	2.723(5)	3182	3158
C3-O-H	C3-O-H...O-C7	2.774(5)	3320	3299
C_7-O-H	C_7-O-H...O=C_{24}	2.856(6)	3522	3507

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