IR SPECTRAL AND THEORETICAL STUDY CONCERNING THE NATURE OF OLIGOMERIC ASSOCIATION FORMS IN SOME STEROLS SOLUTIONS. II.CHOLESTANOL

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Abstract: The aim of this experimental and theoretical study was to investigate the character of molecular interactions in solutions of cholestanol, a minor sterol constituent in the human body, where it is synthesised in liver. We explored cholestanol behaviour in chlorinated and aromatic solvents using IR spectroscopy at constant and variable temperatures. Information concerning the existence of some oligomeric forms of association i.e. dimers, trimers, tetramers etc of cholestanol are first decrypted by decomvolution of experimental obtained complex spectra in the specific domain (3700-3200cm -1) of intermolecular H bonding study. Then we used numerical analysis to obtain values of association constants and other specific parameters for oligomeric cholestanol species.

Introduction

The special role of cholestanol in metabolism has been recently taken into discussion [1-3] but it has not been completely elucidated. Cholestanol is a minor, but very toxic [1] sterol constituent in the human body. Increase of cholestanol in serum concentration induces a pathological condition *cerebrotendinous xanthomatosis (CTX).* As chemistry is concerned, cholestanol (5,6 dehidrocholesterol) is a mono-hydroxi alcohol and presents the peculiar properties of this class of compounds. This is the tendency for self associations [4], working as a proton donor or proton acceptor molecule, its participation in hydrogen bonds in different processes of association, but also in non specifically interaction as dipole-dipole and van de Waals (vdW) complexes. It has been established that hydrogen bonds and vdW complexes are especially important in order to understand biological functions of cholesterol related compounds. Hydrogen bond arrangement is very important [5].

We consider that knowledge of cholestanol self-association in solutions can lead to a better understanding of it reducing membrane role permeability. We consider opportune use IR spectroscopy at constant and variable temperature, as a major investigation method,

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because it is a selective high-resolution method in this type of study [5,6]. According to IR data analysis in sterol there can be three types of OH vibration (see fig 1) [4].

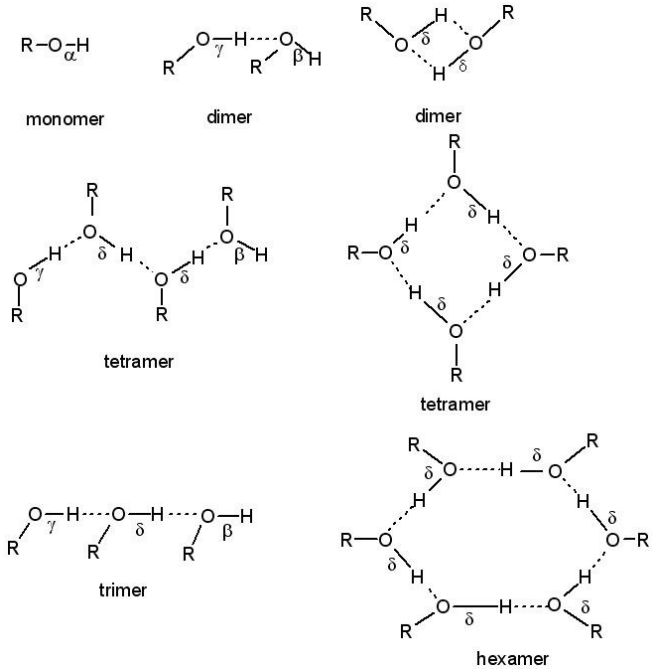

Fig. 1. *Classification of OH vibrations in sterols*

Experimental

Materials

Working solutions of Aldrich cholestanol p.a. in $CHCl_3$, C_6H_6, $C_6H_5CH_3$ (all solvents Merck products of spectral purity) were prepared in standardised water free conditions as follows: $CHCl_3$ - 0.25; 0.2; 0.175; 0.142; 0.107; 0.08 mol / l

C_6H_6 – 0.15; 0.12; 0.075; 0.0375; 0.0187 mol/ l

$C_6H_5CH_3$ – 0.15; 0.075; 0. 0375; 0.0187 mol / l

Note that solubility of cholestanol in CCl_4 is very reduced; it is not possible to study cholestanol solutions in this solvent.

Methods

IR spectra were obtained using a Specord IR 75 Carl Zeiss Iena spectrophotometer within the range 4000- 400 cm^{-1}. We used a variable temperature IR cell and an electrical heating systems produced by the same firm, which were integrated in a prototype made in our laboratory mobile installation [7] calibrated by as to ensure a suitable temperature control (to 1^0C) in the range of temperature – 180 to 250 ^0C. IR spectra of cholesatnol solution

were carried out only after stabilisation of thermal equilibrium [8]. The experiment is very laborious because of special process of heating, washing and drying of IR cell [8,9]. To transfer the IR spectra in numeric format we followed these stages:

- the spectra were scanned with Ben Q 500 scanner EUSB flatbed at resolution dpi and colour at 24 bits
- correction on rotation errors ($0.1\ ^0/\ A_4$) with scanning program Wide image 2.0 (Contex Scanning Technology) resulted images 25 MB in format bitmap
- using a program for digitising diagrams Get Data 2.04 which allows reading of co-ordinates x, y after author reference system obtaining the pairs of value transmitance-frequence of absorption for each spectrum.
- The determined points were exported in text files and recalculated to obtain absorbencies with the help of Microsoft Excel program
- File data were imported in program Peak feat 4.11 for separation of characteristic peaks. Two methods for peak separation were used:

Option Auto Fit Peaks I Residuals develop hide peak by residual analysis

Option Auto Fit Peaks III Deconvolution makes data analysis by Gauss deconvolution and filtering in frequencies domain. This second option leads to the best result.

Results and discussion

Spectral characterisation of all investigated cholestanol systems by IR spectroscopy of solutions must go through three stages:

i) numerical resolution of cholestanol recorded spectra for gaussian absorption band corresponding to OH vibrations implicated in H bonding

ii) identification of cholestanol derived oligomers responsible for deconvolution of large OH band

iii) evaluation of formation constant and other thermodynamic functions of oligomeric cholestanol species.

After first stage we obtained specifically resolved spectra (s. fig. 2)

Passing to stage ii) we supposed that in the solution there is an equilibrium $nA \leftrightarrow An$ and a quantity of oligomers A_i which have the main contribution at deconvolution band, may be described by the relation:

$$\ln h_j = \ln\left(\frac{\beta_i e_\alpha^{/}}{e_j^{/}}\right) + i \ln h_\alpha^{\lambda}$$

where: h_j - high of peaks apartenent γ terminal or internal δ OH group

h_α - high (absorbance) of peak corresponding to monomers α OH group

β_i – constant for A_i formation

$e_\alpha = (\varepsilon_\alpha \ l)^{-1}$; $e_j = (\varepsilon_j \ l)^{-1}$

where: ε_α , ε_j – molar absorptivities for monomer and oligomer

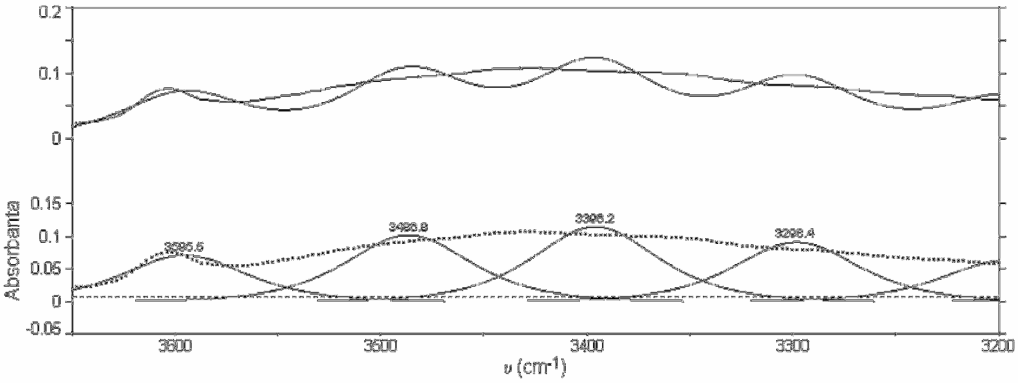

Fig. 2. *IR spectra of cholestanol solution în $CHCl_3$ (concentration 0,175 mol/l)*

Table 1. Characteristics of IR absorption for cholestanol solutions in $CHCl_3$ at 295 K (l_{cell}=0,04 cm)

No. crt.	c (mol/l)	$\widetilde{\nu}_\alpha$ (cm^{-1})	A	$\widetilde{\nu}_\gamma$ (cm^{-1})	A	$\widetilde{\nu}_\delta$ (cm^{-1})	A
1	0,075	3594,13	0,039	3482,28	0,051	3386,09	0,056
2	0,107	3595,76	0,048	3484,90	0,062	3389,96	0,068
3	0,142	3595,03	0,069	3486,39	0,098	3392,62	0,109
4	0,175	3595,49	0,071	3486,78	0,102	3396,15	0,115
5	0,2	3602,04	0,075	3493,01	0,104	3404,15	0,119
6	0,25	3600,49	0,086	3488,59	0,110	3399,49	0,125

Table 2. Characteristics of IR absorption for cholestanol solutions in benzene at 298 K (l_{cell}=0.06 cm)

No. crt.	c (mol/l)	$\widetilde{\nu}_\alpha$ (cm^{-1})	A	$\widetilde{\nu}_\gamma$ (cm^{-1})	A	$\widetilde{\nu}_\delta$ (cm^{-1})	A
1	0,0187	3578,22	0,288	3463,34	0,379	3384,94	0,375
2	0,0375	3578,52	0,336	3468,79	0,395	3384,09	0,375
3	0,075	3577,06	0,393	3471,17	0,405	3387,01	0,356
4	0,12	3577,67	0,563	3468,64	0,532	3378,61	0,426
5	0,15	3582,63	0,734	3465,68	0,647	3371,79	0,546

Table 3 **Characteristics of IR absorption for cholestanol solutions in toluene at 298 K (l_{cell}=0.06 cm)**

No. crt.	c (mol/l)	$\widetilde{\nu}_\alpha$ (cm^{-1})	A	$\widetilde{\nu}_\gamma$ (cm^{-1})	A	$\widetilde{\nu}_\delta$ (cm^{-1})	A
1	0,0187	3575,41	0,268	3479,16	0,290	3395,62	0,279
2	0,0375	3578,38	0,305	3471,40	0,290	3382,72	0,265
3	0,075	3580,46	0,403	3474,25	0,315	3388,35	0,275
4	0,15	3580,88	0,562	3468,19	0,381	3382,32	0,316

Table 4 **Characteristics of IR absorption for cholestanol solution (0,075 mol/l) in benzene at different temperatures (l_{cell}=0,06 cm)**

No. crt.	c (mol/l)	$\widetilde{\nu}_{\alpha}$ (cm^{-1})	A	$\widetilde{\nu}_{\gamma}$ (cm^{-1})	A	$\widetilde{\nu}_{\delta}$ (cm^{-1})	A
1	297,00	3556,43	1,119	3449,52	0,791	3359,70	0,608
2	308,17	3559,99	0,645	3460,78	0,339	3381,13	0,286
3	319,80	3567,23	0,467	3464,73	0,148	3390,63	0,166
4	327,60	3565,81	0,455	3459,88	0,115	3391,11	0,149
5	338,70	3564,66	0,455	3449,05	0,162	3372,11	0,113
6	347,00	3561,17	0,446	3435,54	0,123	3355,84	0,062

Table 5 **Characteristics of IR absorption for cholestanol solution (0,0187 mol/l) in toluene at different temperatures (l_{cell}=0,06 cm)**

No. crt.	c (mol/l)	$\widetilde{\nu}_{\alpha}$ (cm^{-1})	A	$\widetilde{\nu}_{\gamma}$ (cm^{-1})	A	$\widetilde{\nu}_{\delta}$ (cm^{-1})	A
1	297,00	3562,38	0,278	3433,61	0,165	3353,57	0,107
2	309,70	3548,60	0,155	3462,00	0,058	3375,02	0,090
3	314,70	3566,31	0,149	3462,78	0,056	3372,68	0,085
4	319,79	3539,44	0,115	3450,17	0,054	3371,76	0,085
5	330,00	3539,30	0,110	3453,43	0,056	3371,52	0,088
6	335,30	3569,21	0,144	3457,52	0,053	3363,23	0,083

When A_j is the predominant absorbent species at frequence ν_j *(j = γ, δ)* and A_1 is absorbent species at frequence ν_α representation of *ln* h_j as function of *ln* h_α must be linear with the slope *i* giving the number of monomers units (this is *diagnosis diagram*)

Then, the constant of oligomer formation, β_i can be given by minimisation of residual function:

$$S = \sum_{k=1}^{N} \left[\left(A_S / h_\alpha \right)_{k_{\exp}} - \left(A_S / h_\alpha \right)_{k_{\mathrm{mod}\,el}} \right]^2$$

were $(A_s / h_\alpha)_k$ – stoichiometric ratio between cholestanol concentration A_s and absorbance h_α of monomer in all *N* probes and indices *exp* and *model* correspond to experimental ratios. The last ratio is described by the relation:

$$\left(A_S / h_\alpha \right) = e_\alpha + \sum i \beta e^i h_\alpha^{i-1}$$

The parameters β_i and e_α can be improved by least squares iterative method based on linear accepted dependence on temperature of ε_α and dependence of *ln* β_i as function of 1/T [10]. Starting from β_1, ΔG_i, ΔH_i, ΔS_i for A_i corresponding species can be calculated, according to very well known basic thermodynamic relations. Considering the resolved spectra for all cholestanol solutions we obtained the data systematised in table 1-5.

Considering these data and applying the already described algorithm for computation we find in $CHCl_3$ in the range of concentration of 0.0075- 0.25 mol/ l a dimer model of association for cholestanol molecules is credible (s. table 6).

Table 6. Calculated value for dimer associations of cholestanol in $CHCl_3$ solutions

Studied properties	Value
Concentrations (mol dm $^{-3}$)	0.1- 0.4
Temperature (K)	295
Number of samples	5
$\Delta\nu_\gamma$ (cm^{-1})	183± 4
Slop i_γ	0.54
ε_α (mol^{-1} dm^2)	216
ε_γ (mol^{-1} dm^2)	378
β_2 (mol^{-1} dm^3)	8.94
ΔG_2 (kJ mol^{-1})	-5.37

This represents the first published data in literature on cholestanol oligomers. But the data obtained for cholestanol in benzene and toluene solutions needs more extended experiments because the calculated diagnosis diagrams are not credible and the value of ΔG is positive for postulated models.

Conclusions

1. The association of cholestanol in four solvents was investigated by IR spectroscopy at constant and variable temperatures.

2. The obtained IR complex spectra were deconvoluted in range of OH stretching vibration and attributed to different forms of association.

3. In case of $CHCl_3$ solutions open dimer type of association is verified.

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