IR SPECTRAL STUDY ON THE NATURE OF 2-PYRIDINE ALDOXIME METHYL CHLORIDE INTERACTION WITH SOME STEROLS. I. CHOLESTEROL

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abstract: The aim of this spectral study was to investigate the character of the molecular interaction of cholesterol (Ch) with 2-pyridine aldoxime methyl chloride (2-PAM). For this purpose it was made a comparative analysis of the FTIR and conventional IR spectra carried for Ch, 2-PAM and the remnant obtained after solvent ($CHCl_3$) removal from an equimolecular mixture of 2-PAM:Ch. The analyses of the spectra, obtained using KBr pellets technique, indicates that the investigated molecular interaction is hydrogen bonding mediated.

key words: IR spectra, molecular interaction, cholesterol, cholinesterase activator.

Introduction

The cholesterol (Fig. 1), the most abundant sterol in human body, has a prominent, but not completely elucidated, role in metabolism [1÷3]. It is a major component of lipoproteins, a structural moderator of cell membranes permeability and a precursor in the biochemical synthesis of steroid hormones and D_3 vitamins.

Its beneficial or malefic role in metabolism, associated with the change of membrane permeability is much debated in literature [4].

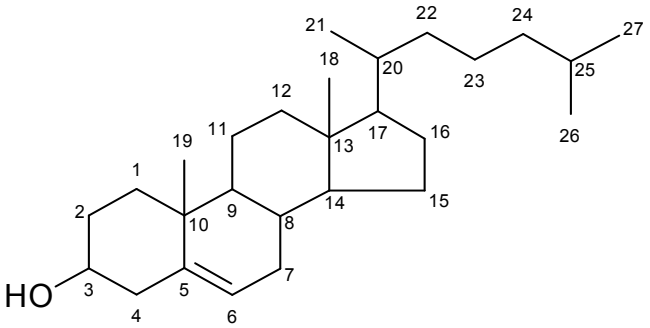

Fig. 1: *Molecular formula of cholesterol.*

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From structural point of view, cholesterol is a steroid *i.e.* a compound containing a ring system with three cyclohexane rings (A, B, C) connected as in phenanthrene, joined with a cyclopentane. At C_3 a -OH, at C_{10}, C_{13} a methyl and at C_{17} a 1-methyl,5-dimethyl-pentyl groups are substituted [4]

Cholesterol and related sterols [5] present particular properties of this class of compounds. Among other things the self associative properties of cholesterol based on hydrogen bond formation in non polar solvents have been studied with variable temperature IR spectroscopy [6] and theoretical methods [7] by our group.

The interaction of cholesterol with 2-pyridine aldoxime methyl chloride 2-PAM (Fig. 2) an antidote to cholinesterase inhibitors or to organophosphorous chemicals was studied in [8] using an *ab initio* method with Gaussian 98 program.

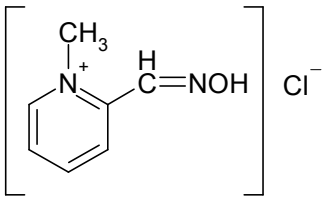

Fig. 2: *Molecular formula of 2-PAM* .

The heat of formation and the most energetic probable conformation of the complex cholesterol:2-PAM were determined. From the examination of the calculated [8] global atomic charges results that in the supposed cholesterol-2PAM complex, the six member A cycle with oxygen atom from the hydroxyl group of the cholesterol molecule is the most important in hydrogen bond established interaction. This point out that cholesterol can act an important function in the transport of the bonded 2-PAM molecule at that site where intoxication with organophosphorous compounds is developed [9]

We used in [10÷12] MM+ and AM1, as simplest modelling methods, to describe this interaction and to predict some energetic and geometrical properties (enthalpy of association, energy of interaction, length of hydrogen bonds) of the formed complex.

For this reason we consider opportune an IR spectral study to investigate the character of the molecular interaction between cholesterol and 2-PAM, because the IR spectroscopy is a high-resolution structural method.

Experimental part

Materials

In spectral study were used UC Belgium p.a. cholesterol and Merck 2 pyridine aldoxime methyl chloride (2-PAM) products.

The equimolar mixture 2-PAM:cholesterol was dissolved in $CHCl_3$ of spectral purity, also a Merck product. Solvent was removed under vacuum and KBr pellets were prepared for spectral study.

Methods

Conventional IR spectra were obtained using a SPECORD IR-75, Carl Zeiss Jena spectrophotometer within the range 4000-400 cm^{-1}; FTIR spectra were carried at JASCO 400/600 spectrometer in the range 7000-400 cm^{-1}, with a resolution of 4 cm^{-1} and a scanning speed of 2 cm^{-1}/sec.

As alternative method for assignment of the observed bands was used EXP'AIR program French version.

Results and discussion

In the Tables 1-3 are listed the pairs of wavenumbers (cm^{-1}) – transmission (%) values of the observed bands in FTIR spectra for 2-PAM (Table 1), cholesterol (Table 2) and remnant obtained after under vacuum solvent removal from their equimolar mixture in $CHCl_3$ (Table 3). The details concerning used materials and the conditions for obtaining of the IR conventional and FTIR spectra are given in the experimental part.

Table 1. FTIR spectrum of 2-PAM

No.	$\tilde{\nu}$ (cm^{-1})	T%	No.	$\tilde{\nu}$ (cm^{-1})	T%
1	3694.94	72.35	21	1323.89	8.63
2	3079.76	5.60	22	1293.04	32.40
3	3022.87	5.88	23	1240.00	37.10
4	2949.59	5.18	24	1181.19	20.08
5	2908.13	18.61	25	1165.75	61.39
6	2837.74	5.68	26	1115.62	96.92
7	2736.49	4.96	27	1051.01	108.06
8	2360.44	60.24	28	1013.41	0.75
9	2341.16	61.71	29	943.02	76.56
10	2042.25	75.82	30	925.66	52.48
11	2002.71	72.98	31	872.63	77.57
12	1947.75	78.51	32	812.85	30.61
13	1849.40	81.22	33	800.31	20.71
14	1723.09	84.68	34	787.78	11.62
15	1627.63	32.39	35	746.32	65.78
16	1594.84	22.82	36	658.57	64.69
17	1582.31	18.52	37	554.43	90.41
18	1504.20	2.67	38	521.65	71.39
19	1441.53	23.34	39	494.65	68.37
20	1401.03	71.33	40	440.65	46.61

For the assignment of the observed bands we used EXP'AIR program, French version and reference data [13÷16]. After examination of the data presented in Table 1 results that in the range 4000-2000 cm^{-1}, the most important in the present study for evidence of hydrogen bonding formation, the weak band 1, positioned at 3694 cm^{-1} can be assigned to a vibration specific for OH attached to ─N═ CH group, substituted in pyridine ring.

In 2-PAM spectrum the most intense bands (number 2-7) correspond to C─H asymmetric stretching vibrations in CH_3, CH═ and aromatic ring. The bands 9, 10 positioned at 2360, 2341 cm^{-1} are specific for N─O bond and probably, to a CO_2 impurity present in 2-PAM.

In the spectral range 2000-400cm^{-1}, which is very important in organic functional analysis, we can observe all the vibrations characteristic to structural groups present in this molecule. Particularly, in this domain between 600-800cm^{-1} appear the bands numbered 33-36 specific for C-Cl stretching vibrations.

Table 2. FTIR spectrum of Cholesterol

No.	$\tilde{\nu}$ (cm^{-1})	T%	No.	$\tilde{\nu}$ (cm^{-1})	T%
1	3693.98	108.07	18	1105.98	113.59
2	3444.24	113.02	19	1084.76	116.75
3	2935.13	21.60	20	1056.80	77.12
4	2900.41	42.00	21	1023.05	123.59
5	2866.67	40.65	22	1007.62	114.78
6	2840.63	63058	23	953.63	90.02
7	2360.44	96.21	24	925.66	96.44
8	1554.34	129.31	25	884.20	100.63
9	1499.38	111.79	26	839.85	93.15
10	1463.71	87.15	27	799.35	92.03
11	1376.93	81.03	28	740.53	105.42
12	1365.35	83.79	29	699.07	110.82
13	1332.57	98.70	30	593.00	104.05
14	1272.79	102.95	31	542.86	103.15
15	1235.18	99.34	32	476.33	104.35
16	1190.83	97.61	33	415.59	103.72
17	1132.97	108.01			

The large asymmetric band number 2 in table 2 for cholesterol with maximum positioned at 3444 cm^{-1} corresponds to stretching OH vibration in cholesterol.

Attribution of the other bands listed in Table 2 for cholesterol is based in principal on the literature data [14].

In the Table 3 where are listed the observed bands in the spectrum of the equimolar mixture 2-PAM:cholesterol (s. experimental part) we observe the asymmetric type band with number 1 positioned at 3463,53 cm^{-1} which can be assigned to hydrogen bonded OH group in the formed complex. The maximum of this band is shifted in rapport with that of pure

cholesterol with $\Delta\tilde{\nu}$ = 19 cm^{-1} (to shorter wavelength) and with $\Delta\tilde{\nu}$ = 231 cm^{-1} (to longer wavelength) in rapport with that of 2-PAM. These values are two types of arguments in favour of complex formation, mediated by hydrogen bonds. This is in accord with recent theoretical works [8] and [12] and other practical assignments for hydrogen bonding [17]. Surely, more precise data concerning nature of complex species formed in the tertiary systems 2-PAM-Ch-Solvent must be obtained working in solutions.

Table 3. FTIR spectrum of the mixture 2-PAM-Ch

No.	$\tilde{\nu}$ (cm^{-1})	T%	No.	$\tilde{\nu}$ (cm^{-1})	T%
1	3463.53	121.92	21	1293.04	82.785
2	3079.76	61.31	22	1239.04	84.81
3	3023.84	59.31	23	1181.19	75.05
4	2937.06	22.10	24	1132.97	109.20
5	2900.41	44.66	25	1105.01	114.82
6	2866.67	39.69	26	1055.84	96.41
7	2840.63	45.25	27	1012.45	40.47
8	2736.49	60.31	28	953.63	93.02
9	2360.44	96.79	29	925.66	89.57
10	2002.71	100.14	30	883.23	100.02
11	1722.12	95.64	31	800.31	73.79
12	1627.63	97.41	32	787.79	65.54
13	1595.81	92.91	33	744.39	98.71
14	1583.27	102.02	34	658.57	103.81
15	1555.31	122.13	35	593.00	108.85
16	1505.17	53.22	36	554.43	107.28
17	1459.85	86.63	37	520.69	102.14
18	1442.49	80.13	38	494.65	102.17
19	1376.93	88.40	39	440.65	94.18
20	1324.86	63.13			

Conclusions

1. It was made a comparative analysis of the observed FTIR and IR conventional spectra for 2-PAM, cholesterol and their equimolar mixture, obtained after under vacuum solvent removal from solution.
2. This spectral study reveals a complex formation between 2-PAM:cholesterol mediated by hydrogen bond.

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