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Dedicated to Professor Victor-Emanuel Sahini on the occasion of his 80th anniversary

IR SPECTRAL STUDY ON NATURE OF 2–PYRIDINE ALDOXIME METHYL CHLORIDE INTERACTION WITH SOME STEROLS. II. CHOLESTANOL

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The aim of this spectral study was to investigate the character of molecular interaction of 2-pyridine aldoxime methyl chloride (2-PAM), a cholinesterase activator, with a cholesterol derivative, cholestanol. For this were carried FTIR spectra at JASCO400/600 and Vertex 70-Bruker spectrometers for 2-PAM, cholestanol and remnant, obtained after under-vacuum solvent removal from equimolar partners mixture in $CHCl_3$ solution. The comparative analysis of the spectra obtained using KBr pellets technique indicates that investigated interaction is hydrogen bonding mediated. Used arguments are based on the wavenumber shifts and integrated intensities for the bands corresponding to OH stretching vibrations.

INTRODUCTION

The 2-pyridine aldoxime methyl chloride (2-PAM) (Fig. 1) owes an unusual capacity to reactivate enzymes that have been inhibited by organophosphorus toxic compounds.[1]

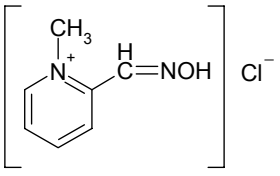

Fig. 1 – Molecular formula of 2-PAM.

For this reason it is used as an antidote to these compounds. Recent theoretical works[2,3] (based on *ab initio* method using Gaussian 98 program) evidenced interaction of 2-PAM with dehydroepiandrosterone and 2-PAM with cholesterol. These demonstrated the possibility that steroid compounds play the role of a carrier for 2-PAM to the receptor site, by forming electrostatic complexes mediated by hydrogen bonding.

Considering the important role of different sterols in human metabolism[4] we are interested in theoretical and spectral studies concerning nature of molecular interactions at which these compounds can participate.[5,6] Using a quantum semiempirical method (AM1) and a molecular mechanics method, (MM+), we calculated[7] some properties as energy of interaction and length of formed hydrogen bond for complex 2-PAM: cholesterol.

In this work we propose an IR spectral study of the interaction between 2-PAM and a minor, but very toxic sterol constituent in the human body, cholestanol. Recently, Seyama[8] pointed out that increase of cholestanol in serum concentration induces a pathological condition named *cerebrotendinous xanthomatosis.*

From chemical point of view, the cholestanol (Fig. 2) is a 5,6-dihidrocholesterol derivative, *i.e.* a monohydroxy alcohol, where hydrogen bonding is of central importance from the theoretical and practical stand points.

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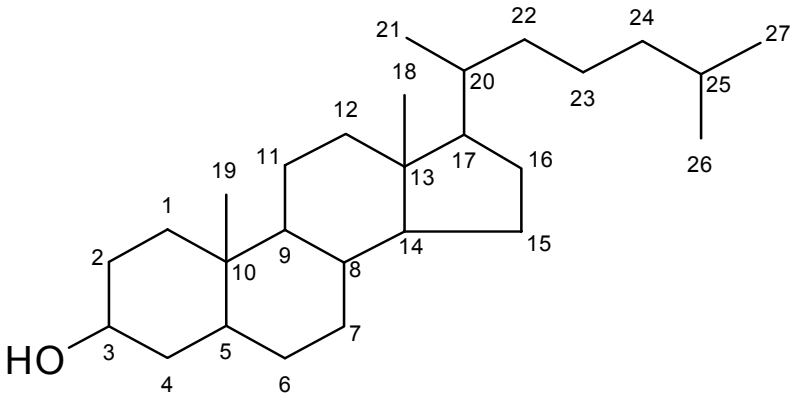

Fig. 2 – Molecular formula of cholestanol.

RESULTS AND DISCUSSION

In the Tables 1-3 are listed the pairs of values: the wave numbers (cm^{-1})- transmittance (%) of the observed bands in FTIR spectra for 2-PAM (Table 1), cholestanol (Table 2) and remnant, obtained after under vacuum solvent removal from 2-PAM:cholestanol equimolar mixture in $CHCl_3$ (Table 3). For the assignment of the observed bands we used EXP'AIR program, French version and reference data.[9-12]

Table 1

FTIR spectrum data for the 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

After analysis of the data listed 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, we can observe the following spectral characteristics. The weak intensity band number 1, positioned at 3694 cm^{-1}, can be assigned to vibrations specific for OH attached to –N=CH group, substituted in pyridine ring of 2-pyridine aldoxime methyl chloride. The six strong intensity bands numbered 2-7 (3079, 3022, 2949, 2908, 2837, 2736 cm^{-1}) can be assigned, in order, to vibrations corresponding to CH stretching vibrations in disubstituted alkenes, alkanes and pyridine ring. The band 9, 10 positioned at 2360, 2341 cm^{-1} are specific for NO bond and, probable, to a nitrate impurity present in 2-PAM.

In the spectral range 2000-400 cm^{-1} which is very important in organic functional analysis, we can observe all the other vibrations characteristic to structural group presented in this molecule. Particularly between 800-600 cm^{-1} appear the

bands, numbered 33-36 characteristic for C-Cl stretching vibrations.

The FTIR spectra of pure cholestanol (Table 2) can by interpreted in correlation with that of cholesterol[5-9] considering the structural *i.e.* spectral differences due to absence of double bond in position 5-6.

Table 2

FTIR spectrum data for the cholestanol

No.	$\tilde{\nu}$ (cm^{-1})	T%	No.	$\tilde{\nu}$ (cm^{-1})	T%
1	3443.28	48.49	14	1042.34	58.58
2	2930.31	5.66	15	1003.77	103.92
3	2865.7	21.12	16	955.55	90.93
4	2359.48	93.99	17	932.41	92.10
5	1634.38	87.00	18	733.78	90.15
6	1467.56	54.19	19	636.39	89.92
7	1381.75	66.53	20	500.44	83.28
8	1333.53	90.31	21	474.40	83.13
9	1303.64	88.99	22	460.90	83.44
10	1232.29	93.37	23	432.94	84.78
11	1170.58	91.37	24	419.44	83.63
12	1136.83	91.29	25	405.94	82.07
13	1078.01	86.48			

Thus in the range 4000-2000 cm^{-1} appears the medium intensity, large, nearly symmetric, band positioned at 3443 cm^{-1} characteristic for OH stretching vibrations, and the most intense band corresponding to CH stretching vibrations which have two differentiated peaks, corresponding to -CH_2, -CH and -CH_3 groups in alkanes.

In the range 2000-400 cm^{-1} we observe a reduced number of bands than in cholesterol[5] corresponding to OH bending and CH bending vibrations, characteristic for functional groups of cholestanol molecule.

The characteristic features of FTIR spectrum carried for equimolar mixture of interaction partners may be deduced from analysis of Table 3 data.

Table 3

FTIR spectrum data for the complex: 2-PAM:cholestanol

No.	$\tilde{\nu}$ (cm^{-1})	T%	No.	$\tilde{\nu}$ (cm^{-1})	T%
1	3399.89	62.26	15	1180.22	86.99
2	3080.73	75.54	16	1170.58	87.13
3	2931.27	5.43	17	1134.90	89.12
4	2865.70	18.72	18	1078.01	84.71
5	2849.31	19.09	19	1043.3	60.94
6	2737.46	76.82	20	1012.45	70.21
7	1627.63	84.46	21	955.55	89.09
8	1582.31	84.28	22	929.52	89.03
9	1505.17	73.68	23	788.74	81.85
10	1467.56	49.69	24	733.78	90.52
11	1445.39	59.76	25	658.57	92.47
12	1381.75	65.44	26	636.39	92.34
13	1324.86	77.70	27	494.65	87.71
14	1234.22	88.75	28	440.65	85.53

We observe that the medium intensity large band positioned to 3399 cm^{-1} has a asymmetric character now, specific for existence of more types of OH stretching vibrations.

This is an argument in favor of new hydrogen bonds formation in the equimolar mixture of 2-PAM: cholestanol. Comparison with the spectrum of pure cholestanol evidences a shift ($\Delta\tilde{\nu}$ = 43 cm^{-1}) to the longer wavelength range of the maximum of the band corresponding to OH stretching vibrations. Comparison with the spectrum of 2 –PAM reflects a shift ($\Delta\tilde{\nu}$ = 295 cm^{-1}) to the longer wavelengths range of the same maximum**.** The order of magnitude of these shifts are in agreement with a complex formation between 2-PAM–cholestanol, mediated by the hydrogen bond, as results from

theoretical works[2,3,6,7] but concerning cholesterol**.** The integrated intensities calculated by us with software package OPUS 6.0, version mode A of Vertex 70 spectrometer, for the bands corresponding to OH stretching vibrations in the three molecular species are given in the Table 4.

Table 4

Values of OH stretching vibrations band integrated intensities.

No.	Sample	Interval of integration (cm^{-1})	Integrated intensity
1	2-PAM	3782.39 – 3498.47	4.90
2	cholestanol	3561.72 – 3153.28	11.99
3	2 PAM : cholestanol	3563.62 – 3261.14	12.43

It is obvious that the difference between integrated intensities of the sample 3 and 2, which have a comparable interval of integration, is in favor of sample corresponding to equimolar mixture of 2-PAM-cholestanol. The increase in intensity is also an argument in favor of complex formation between 2-PAM and cholestanol. The probable structure of this complex with hydrogen bond is presented in Figure 3.

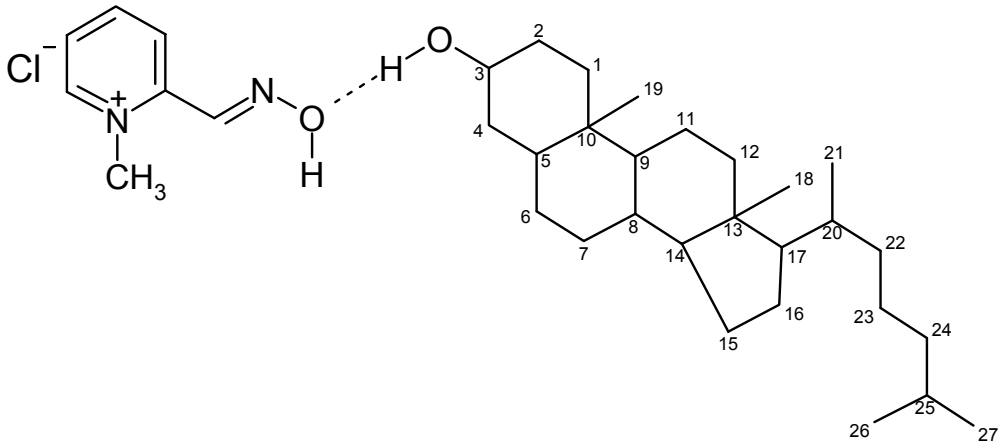

Fig. 3 – Hydrogen bond mediated structure of the complex between 2-PAM and cholestanol.

EXPERIMENTAL

Substances: In spectral study was used Aldrich cholestanol p.a., 2-PAM and KBr were Merck products p.a. The equimolar mixture 2-PAM:cholestanol was solved in $CHCl_3$, also a Merck product. Solvent was after then under vacuum removed. Obtained KBr pellets for spectral study were maintained in water free conditions. The internal reference method, based on KSCN, can not be used in this case, because of high reactivity, just in pellets, of one of studied partners (2-PAM).

Method and apparatus: FTIR spectra were carried out on 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. To obtain the integrated intensities of the bands, corresponding to OH stretching vibrations for the three mentioned species, we used the same KBr pellets and carried out spectra at the new Vertex 70 – Bruker spectrometer. The higher precision running parameters (spectral resolution better than 0.4 cm^{-1}, wavenumber accuracy better than 0.01 cm^{-1}, photometric accuracy better than 0.1% T) were used. Using OPUS software package of Vertex 70 the integrated intensities in A mode were obtained.

CONCLUSION

It was made a comparative analysis of the observed FTIR spectra for 2-pyridine aldoxime methyl chloride, cholestanol and their equimolar mixture, obtained after *in vacuo* solvent removal.

The spectral study reveals a complex formation between 2-PAM: cholestanol, mediated by hydrogen bonding.

This affirmation is sustained both by observed shifts of OH stretching vibrations band and by calculated integrated intensities.

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