IR SPECTRAL STUDY ON NATURE OF 2–PYRIDINE ALDOXIME METHYL CHLORIDE INTERACTION WITH SOME STEROLS. III. LANOSTEROL AND 7-DEHYDROCHOLESTEROL

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Continuing our previous works, the aim of this spectral study was to investigate the character of the molecular interaction of 2-pyridine aldoxime methyl chloride (2-PAM-Cl) with two sterols: lanosterol and 7-dehydrocholesterol. A Vertex 70-Bruker spectrometer with high precision running parameters and the very versatile OPUS software package were employed to obtain infrared spectra. FTIR spectra of pure partners of interaction (2-PAM-Cl, lanosterol, 7-dehydrocholesterol) were compared with the spectra corresponding to remnants, obtained after under-vacuum solvent removal from equimolar partner mixtures in solution. The comparative FTIR spectra analysis, obtained using the KBr pellets technique, indicates that the investigated interaction is the hydrogen bonding mediated in a specific grade for each sterol.

INTRODUCTION

Preceding part of this paper series refers to the interaction of 2-PAM-Cl with cholestanol.[1]

The 2-pyridine aldoxime methyl chloride (2-PAM-Cl) (Fig. 1) has the unusual capacity to reactivate enzymes that have been inhibited by organophosphorus toxic compounds.[2] It is the most used antidote to cholinesterase inhibitors (or to organo phosphate chemicals) between pyridinium oximes.[3] Current understanding of its applications is based on proximity of the two specific substituents (methyl and oxime) in the pyridine cycle.
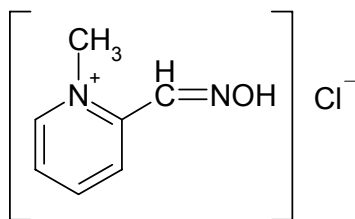

Fig. 1 – Molecular formula of 2-PAM-Cl.

Recent biological studies have demonstrated that sterols and sterol derivatives possess biological activities.[4,5] Considering their important roles in the human metabolism[6] we are interested in theoretical and spectral studies concerning the nature of molecular interactions at which these compounds can participate.[1,7-11]

In connection with our previous studies,[1,7-11] the main objective of the present study has been to investigate the interaction between 2-PAM-Cl and two sterols: lanosterol and 7-dehydrocholesterol (Fig. 2).

Lanosterol is a zoosterol found in the wool of sheep.[12] 7-Dehydrocholesterol is also a zoosterol. It is not only an intermediate of cholesterol biogenesis, but isolated from a photosynthetic precursor of vitamin D3. The presence of this compound in human skin enables humans to manufacture vitamin D3 in the sun light, via an intermediate isomer pre-vitamin D3. 7-Dehydrocholesterol and ergosterol are effective precursors for 20-hydroxyecdysone formation in plant cell suspension culture.[13] It is also found in the milk of several mammalian species.[14]

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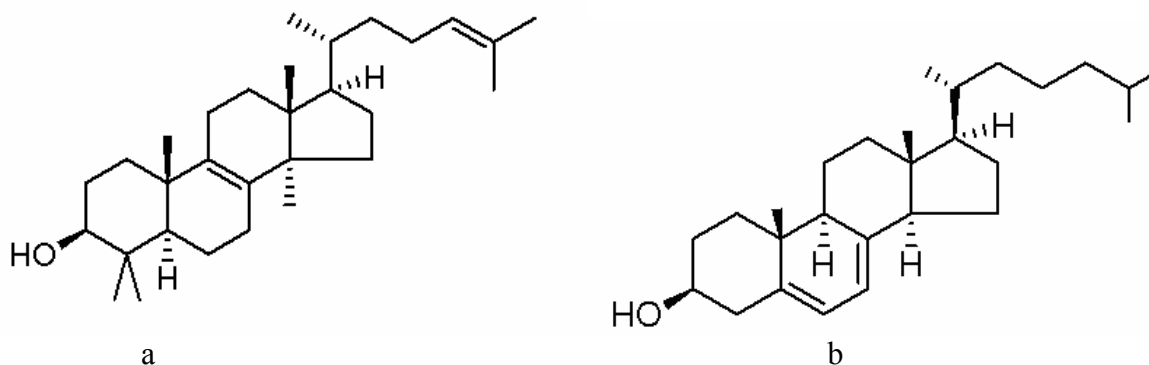
Fig. 2 – Molecular formula of lanosterol (a) and 7-dehydrocholesterol (b).

RESULTS AND DISCUSSION

In Table 1 are presented the values of the observed bands in FTIR spectra for 2-PAM.

As can be seen from this table, 2-PAM-Cl presents a characteristic band at 3680 cm^{-1} that corresponds to vibrations specific for O-H bond, attached to –N=CH group, substituted in pyridine ring of 2-pyridine aldoxime methyl chloride. The bands between 3081 cm^{-1} and 2736 cm^{-1} (3081, 3019, 2947, 2835, 2736 cm^{-1}) can be assigned to stretching vibrations of C-H bonds in –CH$_2$-, -CH$_3$ groups and pyridine ring.

All the other vibrations characteristic to the organic structural group presented in this molecule appear in the spectral range 2000-400 cm^{-1}.

In the FTIR spectra of pure lanosterol (Table 2) can be observed the large band with asymmetric character positioned at 3423 cm^{-1}, due to OH stretching vibrations. The characteristic profile of the band corresponding to different CH stretching vibrations have the maximum at 2946 cm^{-1}, 2875 cm^{-1} and 2835 cm^{-1}. The more intense bands are due to C=C bending vibration in alkanes at 1372 cm^{-1} and 1450 cm^{-1}; in alkenes (with different substitutions) at 1085 cm^{-1}.

For equimolar mixture 2PAM-lanosterol (Table 2) it can be observed that OH stretching vibrations band are positioned at 3436 cm^{-1} and is more symmetric than in the spectrum of lanosterol. The maximum of this band is slightly switched ($\Delta \widetilde{\nu} = 13$ cm^{-1}) to the larger wave numbers.

All aspect of the spectrum in this case confirm the possibility of the complex formation between both species, but this complex can be due in great measure to a π-π associated with hydrogen bonding type interaction.

The FTIR spectra of pure 7-dehydrocholesterol (table 3) can by interpreted in correlation with that of cholesterol[8,9] considering the structural *i.e.* spectral differences due to presence of double bond in position 7-8.

Table 1

FTIR spectrum data for the 2-PAM-Cl

No.	$\widetilde{\nu}$ (cm^{-1})	T%	No.	$\widetilde{\nu}$ (cm^{-1})	T%
1	3680.23	85.23	13	1239.82	29.42
2	3081.41	8.24	14	1180.99	15.07
3	3018.88	8.71	151	1164.87	48.71
4	2947.09	4.48	6	1017.73	0.48
5	2834.69	3.30	17	925.76	53.65
6	2019.36	86.59	18	875.38	35.88
7	1628.56	24.95	19	800.55	12.71
8	1582.85	7.30	20	788.48	13.08
9	1505.04	1.01	21	746.74	75.53
10	1400.45	8.48	22	713.49	80.04
11	1324.52	3.77	23	658.53	58.12
12	1292.04	20.24			

Table 2

FTIR spectrum data for the lanosterol and 2-PAM-Cl:Lanosterol

	2-PAM-Cl:Lanosterol			Lanosterol	
No.	$\widetilde{\nu}$ (cm^{-1})	T%	No.	$\widetilde{\nu}$ (cm^{-1})	T%
1	3436.30	71.06	1	3422.61	58.22
2	3081.06	21.65			
3	2931.79	7.75	2	2946.08	16.19
4	2848.95	11.54	3	2874.78	35.18
5	2736.83	17.18	4	2834.75	56.16
6	2001.01	15.07			
7	1628.19	44.00	5	1628.99	82.14
8	1582.59	30.83			
9	1504.56	14.13			
10	1442.59	58.83	6	1449.96	61.17
11	1372.18	75.30	7	1371.80	44.32
12	1325.35	20.95			
13	1293.01	49.89	8	1289.50	82.66
14	1239.32	53.42			
15	1180.98	36.04	9	1085.48	81.12
16	1013.35	5.18	10	1054.34	51.18
17	925.86	69.41	11	932.59	90.21
18	788.81	24.01	12	850.67	88.37
19	746.15	75.06	13	818.05	89.90
20	658.35	71.30	14	654.22	91.18
21	520.83	72.24	15	551.79	90.54

7-dehydrocholesterol present a medium intensity, large, nearly symmetric band positioned at 3453 cm^{-1} corresponding to O-H stretching vibrations, and the most intense band which have two differentiated peaks corresponding to C-H stretching vibrations of -CH$_2$, -CH and -CH$_3$ groups in alkanes.

In Table 3 are presented the data for the remnant, obtained after under vacuum solvent removal from 2-PAM-Cl:7-dehydrocholesterol equimolar mixture. As can be seen in this table, the FTIR spectrum present a medium intensity large band positioned at 3347 cm^{-1}. This band has an asymmetric character, corresponding to O-H stretching vibrations.

Table 3

FTIR spectrum data for the 7-dehydrocholesterol and 2-PAM-Cl:7-dehydrocholesterol

	7-dehydrocholesterol			2-PAM-Cl:7-dehydrocholesterol	
No.	$\widetilde{\nu}$ (cm^{-1})	T%	No.	$\widetilde{\nu}$ (cm^{-1})	T%
1	3453.39	77.37	1	3346.87	76.48
2	3038.33	87.43	2	3079.89	25.40
3	2953.87	14.52	3	3035.70	22.20
4	2870.13	30.06	4	2954.41	3.04
5	1654.15	80.57	5	2868.48	11.48
6	1489.02	2.87	6	1628.46	63.27
7	1427.05	35.09	7	1596.01	55.02
8	1396.29	2.80	8	1581.58	53.00
9	1227.08	70.52	9	1504.48	16.50
10	1193.31	76.81	10	1469.88	9.88
11	1169.00	76.92	11	1412.64	19.92
12	1063.01	54.52	12	1380.05	53.23
13	1037.25	60.15	13	1323.96	35.66
14	986.29	86.74	14	1239.52	68.52
15	946.63	87.20	15	1181.20	55.06
16	876.24	15.02	16	1063.75	50.04
17	832.91	77.37	17	1012.53	19.47
18	801.15	87.89	18	832.45	60.01
19	713.90	76.01	19	800.44	68.29
20	602.86	92.23	20	788.53	30.99
21	508.51	95.89	21	744.63	73.96

Comparison of the mixture spectrum with the spectrum of pure 7-dehydrocholesterol evidences a shift ($\Delta\tilde{\nu}$ =106 cm^{-1}) to the longer wavelength range of the maximum of the band corresponding to OH stretching vibrations. Comparison of the mixture spectrum with the spectrum of 2–PAM-Cl reflects a shift ($\Delta\tilde{\nu}$ = 333 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-Cl:7-dehydrocholesterol, mediated by the hydrogen bond, as results from theoretical works[1,7-11,15,16] but concerning other sterols (cholesterol, cholestanol and ergosterol).

The integrated intensities for the bands corresponding to OH stretching vibrations, calculated by OPUS 6.0 software package, version mode A of Vertex 70 spectrometer, are presented in the Table 4.[17]

The change observed between integrated intensity is an argument in favor of complex formation between 2-PAM-Cl and lanosterol, indicate the possibility of π-π associated with hydrogen bonding type interaction. For the case of interaction of the 2-PAM-Cl with 7-dehydrocholesterol, a complex mediated by hydrogen bond is evidenced (Fig. 3).

Table 4

Values of OH stretching vibrations band integrated intensities measured for absorbance spectra.

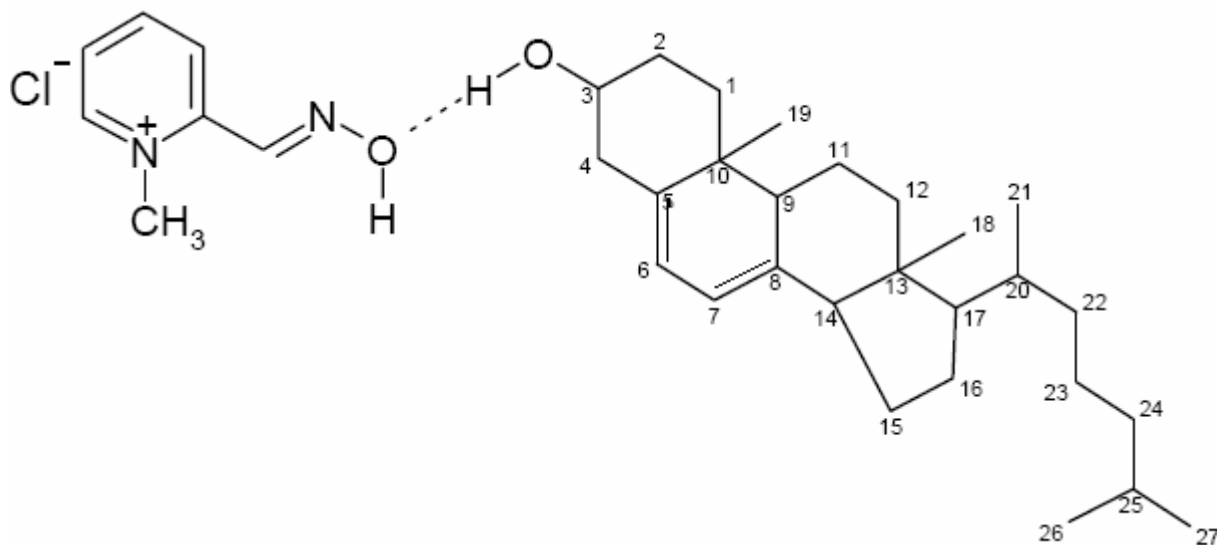| No.	Sample	Interval of integration (cm^{-1})	Integrated intensity
1	2-PAM	3779.38-3450.57	5.77
2	Lanosterol	3668.87-3070.19	63.68
3	2-PAM-Cl:Lanosterol	3687.32-3279.60	19.86
4	7-dehydrocholesterol	3568.98-3088.89	23.24
5	2 PAM:7-dehydrocholesterol	3562.37-3266.44	9.81

Fig. 3 – Hydrogen bond mediated structure of the complex between 2-PAM-Cl and 7-dehydrocholesterol.

In the future we consider opportune to realize molecular modeling studies concerning nature of molecular interaction in these systems.

EXPERIMENTAL

Substances: In this spectral study Sigma-Aldrich (Fluka) p.a. lanosterol, 7-dehydrocholesterol and Sigma-Adrich (Sigma) 2-PAM products were used. KBr, the solvents methanol for 2-PAM-Cl and CHCl$_3$ for lanosterol and 7-dehydrocholesterol were Merck products spectral purity.

The equimolar mixture was prepared by addition of two components: 2-PAM dissolved in methanol and sterol dissolved in CHCl$_3$. After under vacuum solvents removal and remnant for preparation of KBr pellets for the spectral study was used.

Method and apparatus: FTIR spectra were carried out on Bruker Vertex 70 spectrometer in the range 4000-400 cm^{-1} using transmittance (%) – wavenumber (cm^{-1}) dependence and higher precision running parameters: spectral resolution better than 0.01 cm^{-1}, photometric accuracy better than 0.1% T and OPUS v 6.0 software package version mode A for the spectra acquisition.

CONCLUSION

It was made a comparative analysis of the observed FTIR spectra for the pure 2-pyridine aldoxime methyl chloride, lanosterol, 7-dehydro-cholesterol and their equimolar mixture, obtained after *in vacuo* solvent removal.

The spectral study carried out has shown a shift of O-H stretching vibration and an increase in their integrated intensities that evidence a complex formation between 2-PAM-Cl and lanosterol (with the possibility of π-π associated with hydrogen bonding type interaction) and between 2-PAM-Cl and 7-dehydrocholesterol (hydrogen bonding interaction).

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