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MOLECULAR INTERACTION OF A CHOLINESTERASE ACTIVATOR WITH ERGOSTEROL STUDIED BY HIGH RESOLUTION FTIR SPECTROMETRY

Mihaela Manea *, R. Gheevarghese ** and Valentina Chiosa*

abstract The aim of this spectral study was to investigate the character of the interaction of 2-pyridine aldoxime methyl chloride (2-PAM), a cholinesterase activator with ergosterol (Egl). It was realised a comparative analysis of high resolution FTIR spectra, carried out for 2-PAM:Egl and the remnant obtained after under vacuum solvents removal from equimolar mixture 2-PAM:Egl solution. The analysis of spectra, obtained with a Bruker Vertex 70 FTIR spectrometer and KBr pellets technique, indicate a π -π bonding molecular interaction between the both partners.

key words FTIR spectra, molecular interaction, 2-PAM/cholinesterase activator, ergosterol/pro-vitamin D

Introduction

The 2-pyridine aldoxime methyl chloride (2-PAM) represented in Fig 1 is the most used antidote to cholinesterase inhibitors (or to organo phosphate chemicals) between pyridinium oximes [1]. Current understanding of its applications is based on proximity of the two specific substituents (methyl and oxime) in the pyridine cycle.

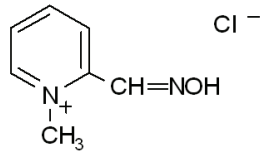

Fig. 1 *Molecular formula of 2-PAM*

The ergosterol represented in Fig. 2 i.e. is a 5,7,22-ergostatrien-3β-ol. Its synonym Provitamin D_2 is due to the fact that ergosterol is converted to ergocalciferol (vitamin D_2) upon irradiation by ultraviolet light or electronic bombardment [2]. Ergosterol is an unsaponifiable lipids found in ergot, yeast and other fungi [2]. The fungal biomass present on grains can be estimated by determination of level of ergosterol, a fungus specific marker

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molecule, which is the predominant compound of cell membrane, but is either absent or is merely as a minor constituent in the higher plants [3].

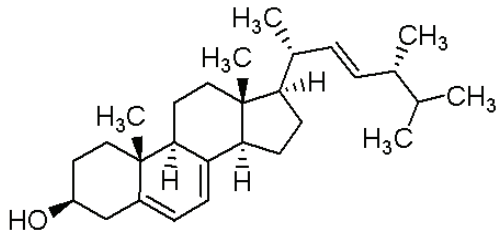

Fig. 2 *Molecular formula of ergosterol*

As an phitosterol, ergosterol must present the peculiar properties of the sterol class of compounds. Trends to association, based on hydrogen bond formation, is the most important for the characterisation of its interaction with other molecules in the membrane transfer [4]. Aim of this work is the FTIR spectral study of interaction of 2-PAM with ergosterol, because FTIR spectrometry is a very selective method for the molecular interaction detection [5].

Experimental

Materials: In this spectral study were used Sigma-Aldrich (Fluka) p.a. ergosterol and Sigma-Aldrich (Sigma) 2-PAM products. KBr, the solvents methanol (for 2-PAM) and $CHCl_3$ (for ergosterol) were Merck products spectral purity. The equimolar mixture was prepared by addition of two components: 2-PAM dissolved in methanol and ergosterol dissolved in $CHCl_3$. The solvents under vacuum were removed and remnant was used for preparation of KBr pellets for the spectral study.

Method: 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.

Results and Discussion

In Figs. 3÷5 are presented the FTIR spectra corresponding to 2-PAM, ergosterol and their equimolar mixture. For the assignment of the observed bands, we used EXP'AIR program, french version and reference data [6÷8] we dispose of FTIR, Raman and RMN spectra for the pure partners of interaction. Firstly, we compare the characteristics of IR spectrum for the 2-PAM, ergosterol and equimolar mixture in the range 4000–400cm^{-1}, where appear the stretching vibrations of the OH and CH bonds.

Starting with the spectrum of 2-PAM in this range we observe the 5 intense bands which can be assigned to CH stretching antisymmetric vibration in disubstituted alkenes, alkanes and pyridine ring.

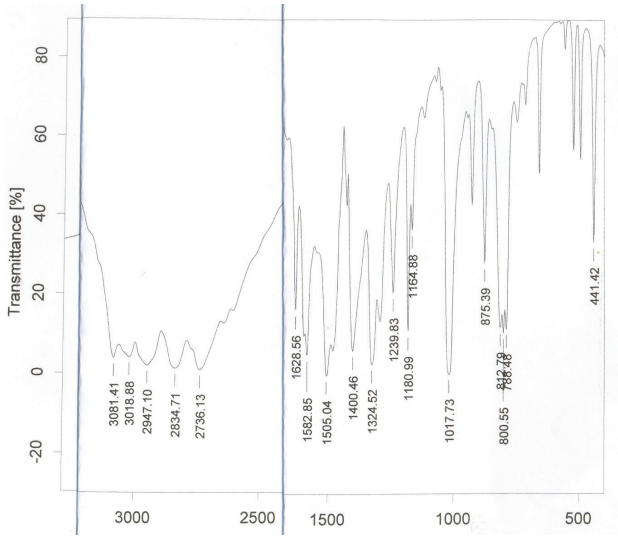

Fig. 3 *FTIR spectrum of 2-PAM*

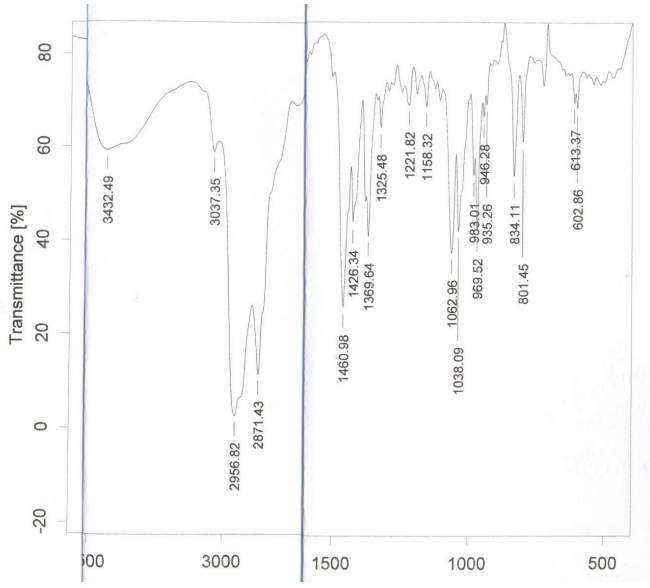

Fig. 4 *FTIR spectrum of ergosterol*

For ergosterol in the same range we observe the large with the asymmetric character band, specific for the associated ergosterol molecules by hydrogen bond, positioned at 3432.4 cm^{-1}, due to OH stretching vibrations. The characteristic profile of the band

corresponding to different CH stretching vibrations have the maximum at 3037.35 cm^{-1}, 2955.82 cm^{-1} and 2871.43 cm^{-1}.

For equimolar mixture (Fig 5), in the same range we observe that OH stretching vibrations band are positioned at 3433.67 cm^{-1} and is more symmetric than in the spectrum of ergosterol (Fig. 4). This fact reflect that a part from hydrogen bridges, existing between ergosterol molecules, are destroyed as consequence of the homogenate solutions formation with 4 components (2-PAM, CH_3OH, Egl, $CHCl_3$), but the maximum of this band is slightly switched ($\Delta\nu$=1.33 cm^{-1}) to the larger wave numbers. The aspect of band, due to CH vibrations, is also changed and reflects the appearance of the corresponding bands in the pyridine ring.

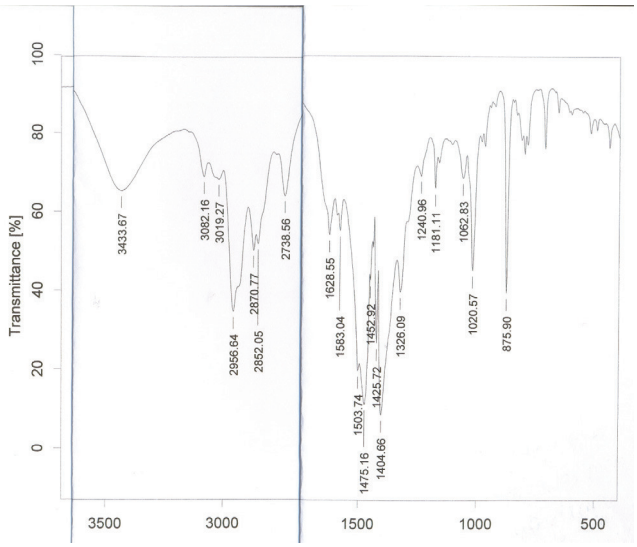

Fig. 5 *FTIR spectrum of mixture 2-PAM: ergosterol*

In the range 2000–400 cm^{-1} in the spectrum of 2-PAM (Fig. 3) we observe the bands at 1628.56 cm^{-1}, 1582 cm^{-1}, 1505 cm^{-1} assigned to C = N and C = C stretching vibrations and between 800–600 cm^{-1} for C – Cl stretching vibrations.

In the spectrum of ergosterol (Fig. 4) in this range, the more intensive bands are due to C=C bending vibration in alkanes at 1460.98 cm^{-1} and 1427.34; in alkenes (with different substitutions) at 1325.48 cm^{-1}, 834.11 cm^{-1} and to C–O stretch vibration at 1240.99 cm^{-1}.

In the case of spectrum carried out for the equimolar mixtures of both partners (Fig. 5) we observe surely appearing the characteristic bands to 2-PAM at 1628.55 cm^{-1}, 1583.04 cm^{-1}, 1503.74 cm^{-1} as band components with the maximum at 1475.16 cm^{-1} originated from ergosterol spectrum. This band is now comparable as intensity and aspect form with the band whose maximum is positioned at 1404.66 cm^{-1} originated from 2 PAM. All aspect of the spectrum in this case confirm the possibility of the complex formation between both species, but this fact can be due in great measure to a π-π interaction between pyridine ring of 2-PAM and that part of ergosterole molecule which content two double bond at carbons 5,7. This idea needs the IR spectral continuation study and some modelling computations

concerning two considered species. These actions will be considered for future of this research.

Conclusions

1. It was effectuated a first analysis by the high resolution FTIR spectroscopy of 2-PAM, a cholinesterase reactivator and ergosterol /provitamin D_2.
2. The analysis based on the comparative search of the spectra, obtained for the interaction partners and for the remnant obtained after under vacuum solvents removal from equimolar partners mixture 2–PAM:ergosterol solution, indicate the possibility of π-π type interaction complex formation.

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