Spectral Study of Some Cholesteryl Carbamates

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Cholesteryl carbamates were prepared by the reactions of cholesteryl chloroformate with amine derivatives from heterocycle compounds, in the presence of the pyridine as an acid acceptor. The compounds were characterised by FT-IR and NMR measurements.

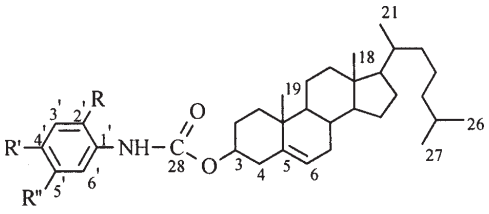
Keywords: cholesterol, cholesteryl carbamate, FTIR spectroscopy, attenuated total reflection

Cholesteryl carbamates have been reported as synthetic cationic lipids such as cholesterol polyamine carbamates [1] who posses some structural features: a hydrophobic moiety (steroid nucleus), a positively charged head group (nitro, halogeno) and a linker such as urethanic group to bond these two moieties together covalently; liniar polymers containing end-capped cholesteryl carbamate [2]; inhibitors of cholesterolesterase [3]; model for lipoplex formation with respect to gene delivery (lipofection), a key first step in gene therapy [4]; liquid crystals dyes with high melting point, deeply colored materials [5]; anticancer agents [6].

In the past few years years, many carbamates with cholesterol skeleton at 3β-position in A ring [7- 10], have been prepared. The mesogenic properties of steroids derivatives depends both structure of sterol and nature of the substituents in 3β-position. The phase transition (mesomorphic and polymorpic) [8, 11-13], interaction with antibiotics [14] and cyclodextrins [8, 13, 15] in these homologous series have been examined in detail.

This paper presents the synthesis and spectral characterization of four cholesteryl carbamates.

The cholesteryl derivative **I- IV** were prepared by condensation of cholesteryl chloroformate and substituted aniline in the presence of pyridine as proton scravenger [10].



I: R = I; R' = H; R" = H

II: R = CH_3; R' = H; R" = I

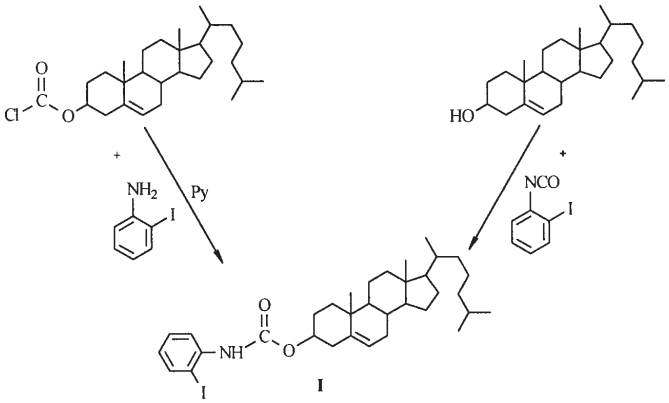
III: R = CH_3; R' = H; R" = NO_2

IV: R = NO_2; R' = OCH_3; R" =H

The cholesteryl carbamate **I** was obtained also by direct reaction of cholesterol with *o*-iodophenyl isocyanate in dry toluene.

Materials and methods

All solvents used in synthesis and recrystallization were purified by distillation before use. Cholesteryl



chloroformate, o-iodophenyl isocyanate were from Aldrich. Cholesterol (Ch) was Merck product. The purity of cholesteryl butyrates was checked by TLC silica gel plates 0.25 mm (Merck) using petroleum ether: ethyl ether 5:3 as eluent mixture.

The transition temperatures, clearing points were determined with MPA100 OptiMelt melting point system, using Meltview software, with ramp rates 1°/min.

IR spectra were recorded on a Jasco 6300 FT-IR spectrometer in the region of 4000 – 400 cm^{-1}, detector TGS, apodization Cosine. All spectra were baseline corrected using SpectraManager II software. ATR spectra were obtained with an attenuated total reflection attachment Gladi ATR, apodization Cosine. The instrument had a spectral resolution of 4 cm^{-1}, used in all spectra determinations.

The ^{1}H-NMR and ^{13}C-NMR spectra were performed on a Varian Gemini 300-BB instrument. All the NMR spectra were recorded at ambient temperature (ca. 295K) using deutherochloroform as solvent and TMS as internal standard; for ^{13}C-NMR spectrum the resonance of deutherochloroform at 77.00ppm was taken as reference.

Results and discussions

Syntheses of cholesteryl carbamates were performed in dry toluene, in the presence of pirydine as acid acceptor and all reactions were monitored by thin-layer chromatography (TLC). The products obtained in the usual manner were purified by column chromatography and thereafter crystallized.

The crystallization of new cholesteryl carbamates **I -IV** from mixture of benzene and ethanol leads to high purity compounds.

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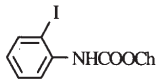
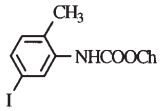
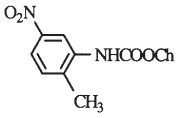
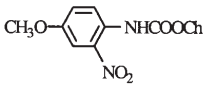
Table 1
THE PHYSICAL CONSTANTS OF COMPOUNDS **I-IV**

Type	Compound	Molecular formula	Molecular weight	Yield [%]	Clear point (°C)	TLC R_f
I		$C_{34}H_{50}INO_2$	631.69	90	146.7	85
II		$C_{35}H_{52}INO_2$	645.71	75	137.1	75
III		$C_{35}H_{54}N_2O_4$	566.83	77	155.4	75
IV		$C_{35}H_{54}N_2O_5$	582.83	78	123.2	70

where Ch is the rest of cholesterol

Compounds **I -IV** presented a selective reflection from solvents such as acetone, amyl alcohol, methyl-ethyl cetone.

The newly cholesteryl carbamates were characterized using FT-IR and NMR spectra.

FT-IR study

The objectives of FT-IR of cholesteryl carbamates **I /-IV** study were: 1) to characterize four cholesteryl carbamates by ATR technique; 2) to compare the ATR spectra of cholesteryl carbamates with FT-IR transmission spectra using KBr pellets (KBr spectra); 3) to evaluate the differences in spectra bands of CO and NH on cholesteryl derivatives with uretanic group at C-3 sterolic.

Attenuated total reflexion (ATR) is now the most common sampling technique in FT-IR spectroscopy. ATR-FTIR is an alternative to transmission mode used for infrared spectroscopy study. FTIR is usually performed in transmission mode. In the ATR-FTIR technique, the IR beam is guided in an IR transparent crystal by total reflexion. ATR occurs when a beam of radiation enters from a more-dense (with a higher refractive index) into a less-dense medium (with a lower refractive index). The IR beam penetrates a very short distance beyond the interface and into the less-dense medium before the complete reflection occurs. This penetration is called the evanescent wave and typically is at a depth of a few micrometers (mm). Its intensity is reduced (attenuated) by the sample in regions of the IR spectrum where the sample absorb [16].

However, ATR does present some challenges. An infrared spectrum of a sample obtained using an ATR accessory is not identical to the spectrum obtained by transmission. The ATR technique introduces relative shifts in band intensity and absolute shifts in frequency. The relative intensity shift is well-known and is readily corrected. The second characteristic feature is the shift of absorption bands to lower frequency. ATR intensity is given by equation (1), when the sample thickness is greater than the penetration depth.

$$A = \log_{10} e \frac{n_2}{n_1} \frac{E_0^2}{\cos\Phi} \frac{d_p}{2} \alpha \qquad (1)$$

where:

E_o and α are the electric fields of the evanescent wave at the boundary and the absorption coefficient per unit thickness of the sample, respectively;

dp, n_1, and n_2 - the penetration depth, ATR crystal refractive index, and sample refractive index, respectively.

As equation (1) indicates, the ATR spectrum is influenced by the refractive index of the sample. Also, according to equation (2), penetration depth, dp, depends on n_2. Thus, instead of α, $n_2 \cdot dp.\alpha$ determines the absorption band positions. Because of the anomalous dispersion of the refractive index, $n_2 \cdot dp.\alpha$ always introduces a band shift to lower frequency and this shift becomes particularly noticeable for strong bands. In other words, the presence of absorptions in the infrared spectrum represents a change in the refractive index of the sample across the band and results in the shift to lower frequency.

$$d_p = \frac{\lambda}{2\pi \cdot n_1 \sqrt{\sin^2\Phi - \left(\frac{n_2}{n_1}\right)^2}} \qquad (2)$$

where λ, Φ are wavelength, incident angle.

In order to apply the advanced ATR correction, four inputs are required:

- refractive index of the sample;
- refractive index of the ATR crystal (or internal reflection element, IRE);
- angle of incidence;
- number of bounces.

With these four inputs, the software automatically calculates the corrected ATR spectrum. The ATR spectrum itself is used to calculate the optical properties of the sample that lead to frequency shifts predicted by equation 2. Of the four parameters, three are determined by the accessory itself. The IRE refractive index is determined by the material of the crystal, and the angle and number of bounces by the design of the accessory. The index of refraction of the material depends, of course, upon the material. However, most organic materials have an index around 1.5; use of this value generally provides excellent performance of the correction [17].

Table 2 shows the IR values obtained in KBr and by ATR technique for compounds **I – IV**.

Compound	$\nu_{C=O}$	$\nu_{C-O(asim)}$	$\nu_{C-O(sim)}$	ν_{N-H}
$\mathbf{I}_{KBr}$	1738.51	1215.9	1038.48	3403.74
$\mathbf{I}_{ATR}$	1734.66	1206.26	1037.52	3402.78
$\mathbf{I}_{ATRc}$	1737.55	1216.86	1038.48	3403.74
$\mathbf{II}_{KBr}$	1737.55	1215.90	1037.52	3451.96
$\mathbf{II}_{ATR}$	1734.66	1214.93	1035.59	3449.06
$\mathbf{II}_{ATRc}$	1736.58	1216.86	1037.52	3450.03
$\mathbf{III}_{KBr}$	1738.51	1250.61	1051.01	3447.13
$\mathbf{III}_{ATR}$	1732.73	1249.65	1049.09	3447.13
$\mathbf{III}_{ATRc}$	1732,73	1252.54	1051.01	3447.13
$\mathbf{IV}_{KBr}$	1734.55	1250.67	1036.23	3446.17
$\mathbf{IV}_{ATR}$	1733.69	1225.54	1035.59	3446.17
$\mathbf{IV}_{ATRc}$	1735.62	1250.61	1035.59	3446.17

KBr-transmission, ATR- attenuated total reflection, ATRc – with advanced correction

Table 2
IR VALUES (cm^{-1}) FOR CHOLESTERYL CARBAMATES **I – IV**

In IR, the most important vibration of all compounds synthesized was carbonyl group vibration at 1734-1738 cm^{-1} (strong) for C=O bond, 1206 - 1250 cm^{-1} (very strong) for C-O bond and 3402 -3450 cm^{-1} (strong) for N-H bond.

The nucleus of the sterol is more difficult to interpret by the IR spectrum. The vibrations at 2931-2970 cm^{-1} ν_{asim}) and 2866-2875 cm^{-1} (ν_{sim}) characterize the methyl groups from steroid nucleus.

The spectra of cholesterylcarbamates as run by transmission and ATR are presented in figure 1 for compound **I** and figure **2** for compound **II**, together with the corrected spectrum. Distorssions are eliminated by the advanced ATR corrections (fig. 3 and fig. 4).

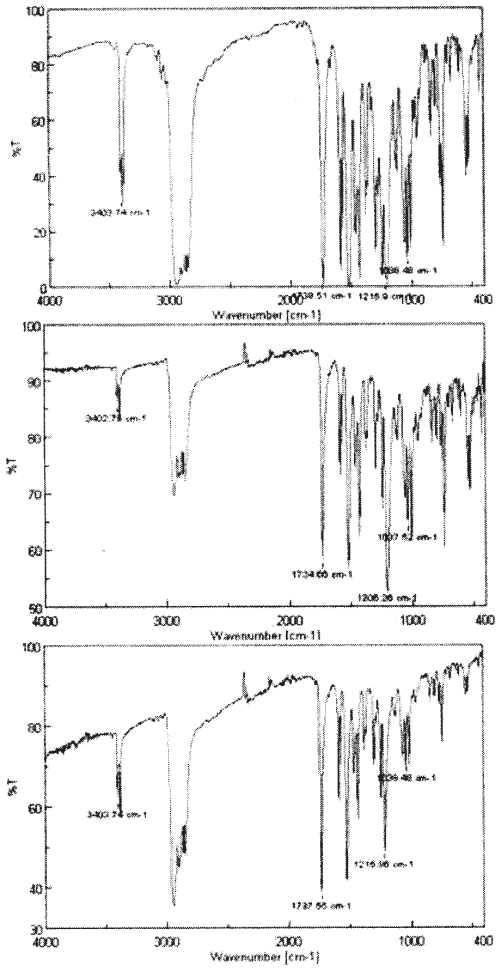


Fig.1. Cholesterylcarbamate **I** FT-IR spectra run by transmission (upper), by ATR (center), and by ATR correction (lower)

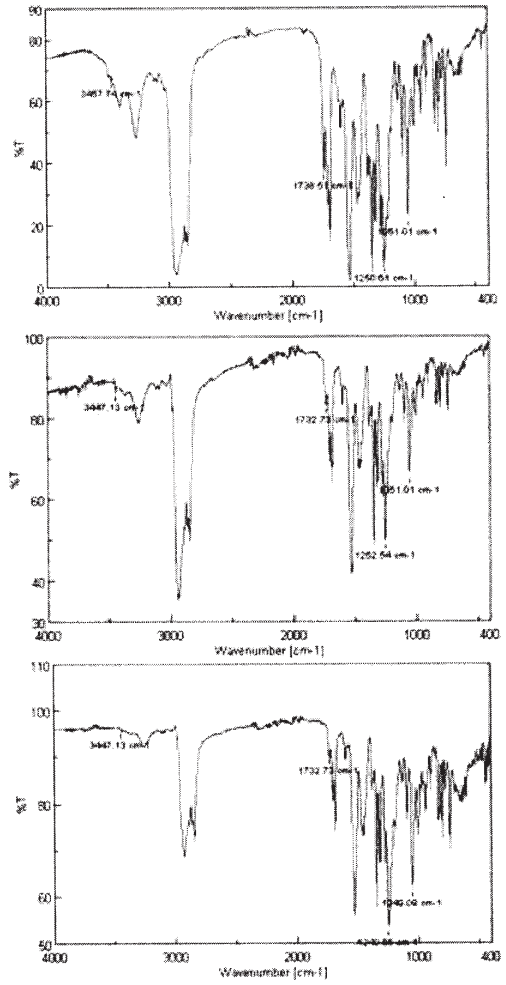


Fig.2. Cholesterylcarbamate **III** FT-IR spectra run by transmission (upper), by ATR (center) and by ATR correction (lower)

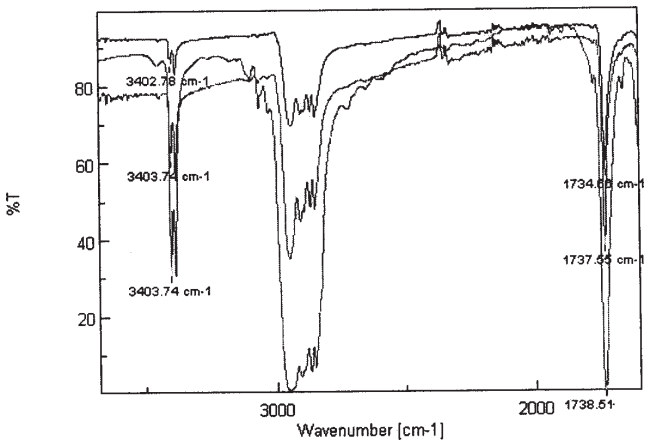


Fig. 3. Cholesterylcarbamate **I** FT-IR spectra run by transmission (low), by ATR (up) and by ATR with advanced ATR correction(middle)

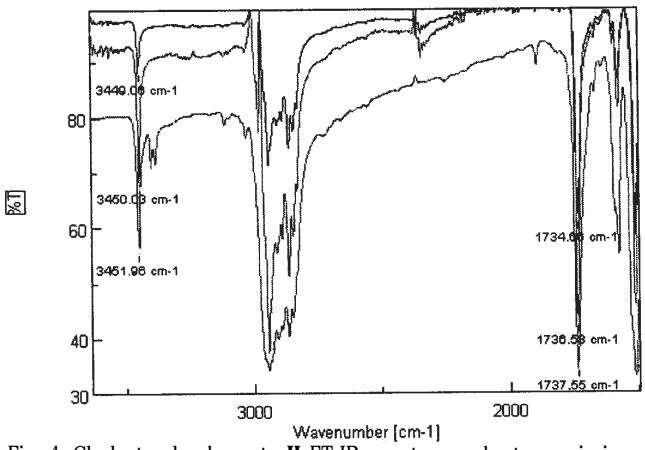


Fig. 4. Cholesterylcarbamate **II** FT-IR spectra run by transmission (low), by ATR (up) and by ATR with advanced ATR correction(middle)

Table 3

^{1}H-NMR FEATURES ($CDCl_3$, δ ppm, J in Hz) OF **I – IV**

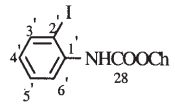
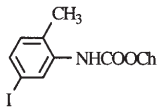
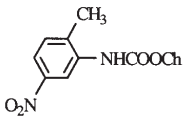
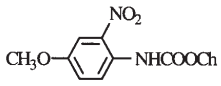
Type	Compound	H-3	H-6	-NH	H-3'	H-4'	H-5'	H-6'
I		4.62m	5.41m	6,67s	6.94 – 7.12 m (4H)			
II		4.60m	5.40m	6.32s	7.26d 8.5	7.50d 8.5 ; 2.9	-	7.70m
III		4.69m	5.42m	6.61s	7.31d 8.4	7.85 dd 8.4 ; 2.3	-	8.83dl 1,9
IV		4.66m	5.45m	7.41s	7.70d 3.0	-	7.03	8.52dl 9.4

Table 4

^{13}C-NMR FEATURES ($CDCl_3$, δ ppm) OF **I – IV**

Type	C-3	C-5	C-6	C-28	C-1'	C-2'	C-3'	C-4'	C-5'	C-6'
X	75.35	139.50	130.25	152.76	139.68	153.00	114.70	125.38	139.56	123.80
XI	75.22	139.27	130.90	152.98	138.81	138.77	119.47	136.01	135.78	122.81
XII	75.68	139.36	130.72	152.83	147.18	133.73	118.22	122.99	147.18	137.13
XIII	75.65	139.48	128.29	154.14	136.39	142.53	108.22	152.83	122.27	122.83

NMR Study

In the 1H-NMR spectra, sterol nucleus presents a multiplet at 0.90-2.10 ppm for saturated part and characteristic singlets for the methyl groups (CH_3 -18 and CH_3 -19) at 0.66-0.68 ppm and 0.89 ppm respectively.

The C-26 and C-27 methyl groups are doublets at 0.86 and 0.87 ppm with 6.6-6.8 Hz coupling constant. The C-21 methyl is a doublet at 0.90 ppm with 6.5-6.7 Hz coupling constant and 3α-H gives in all compounds a multiplet at 4.60-4.69 ppm. The protons of C-30 presents a quadruplet at 1.92 ppm with 7.1 Hz coupling constant.

In ^{13}C-NMR spectra, the discrimination between the various carbon atoms, was carried out by Attached Proton Test (ATP) [18]. The rest of the steroidic skeleton presents characteristic shifts for 27 atoms identical with those from the literature [19]. Tables 3 and 4 shows NMR characteristics obtained in $CDCl_3$ for compounds **I - IV**.

Hydrogen atoms C**H$_3$** at C-2' from compounds **II** and **III** appears as singlet at 2.20 ppm and 2.35 respectively and from OC**H$_3$** at C-4' in compound IV at 3.89 ppm as singlet.

Conclusions

Four cholesteryl carbamates were synthesized and spectrally characterized.

Structural similarity of these cholesteryl carbamates is confirmed by experimental analysis results.

ATR-FTIR spectra are equivalent to FTIR spectra, except the ATR-FTIR techinique requires smaller amount of sample compared with FTIR and allows precision control of samples. Distorssions are eliminated by the advanced ATR corrections.

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