Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2016, 8(4):661-669

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Synthesis of 3-oxo bile acids selected products in the biomedical research and testing their FT-IR spectroscopy

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ABSTRACT

Testing a 3-oxo of bile acids derivatives with method of infrared spectroscopy which is integrated with Fourier transformation (FT-IR spectroscopy) is used to obtain information about the interactions between the solute and solvent.. The goal of the work is better inside into the possibility of creating intermolecular interactions of bile acids in tests of associations and only−associations of bile acids and their use for medical purposes. Synthesis was performed a 12α−hydroxy–3−oxo–5β−cholanoic acid in 4 synthetic stages from deoxycholic acid. We have investigated the potential interactions of the carbonyl groups of the methyl ester of 7α,12α−dihydroxy −3−oxo–5β−cholanoic acid and 3−oxo−12α−hydroxy–5β−cholanoic acid with various solvents using Guttman model, Kirkwood−Bayer–Magat model and the Linear relationship of solvation energy. During the test, the samples were placed in sodium chloride cuvette thickness of 0.057 and 0.116 mm. By recording the FT-IR spectra on the apparatus Thermo Scientific NEXUS 670 using a detector with deuterated triglicinsulfat in range of 2000 to 1600 cm^{-1}, resolution from 2 cm^{-1} and using 50 scans performed, were obtained by the concentrations of Me−ester of 7α,12α−dihydroxy−3−oxo–5β−cholanoic acid and 3−oxo−12α−hydroxy−5β−cholanoic acid between 0.003 and 0.2 moldm $^{-3}$ .The ester carbonyl group is more sensitive to interactions with solvents compared to carbonyl from keto group, because of less steric of protection of said carbonyl of the ester functional groups. This is a consequence of higher polarity, which owned due to its inductive effect.

Keywords: 3−oxo bile acids, FT-IR spectroscopy

INTRODUCTION

Oxo derivatives of cholic acid, such as for example 3α, 7α-dihydroxy-12-oxo-5β-cholanoic acid (sodium salt and methyl ester) are the potential modifiers of the blood-brain barrier transport, and has been shown to promote the absorption of quinine, increase the analgesic effect of morphine and extend "sleeping time" (time sleepiness) induced by pentobarbital.[1] 12α−hydroxy−3−oxo−5β−cholanoic acid (3–oxo derivate of deoxycholic acid) is located in the colon. It is substrate for $CRAD_2$ (cis−retinol dehydrogenase 2). Cells expressing $CRAD_2$, produce 12α–hydroxy−3–oxo−5β−cholanoic acid. Possible applications of 3–oxo derivatives of bile acids are in the adsorption of appropriate drugs within the intestinal membrane. These effects of 3−oxo derivatives of bile acids, but mostly 7−oxo and 12−oxo derivatives of bile acids are in the adsorption of the study drugs, resulting in the formation of complexes with the maximum number of hydrogen bonds between the oxo derivatives of bile acids with drugs such as verapamil and lidocain. [2]. Oxo derivatives of bile acids, usually 7-oxo and 12-oxo derivatives of bile acids are used as absorption promoters and they have a role to help the intestinal, buccal, transdermal, ocular, nasal, rectal and pulmonary absorptions of various drugs in concentrations that are non-toxic. [3]. 3−Oxo derivatives of bile acids bind to PXR, FXR and other relevant members of the nuclear receptors of subfamily of NR1, which are used for the accumulation of bile acids in the liver of sinusoidal nonparenchymal cellular populations and billiary cells. The above mentioned receptors can be activated by 3−oxo derivatives of bile acids, which induce the expression of the

CYP3A4 regulatory gene at lower concentrations compared to those required to activate PXR receptor . [4]. To determine the relationship in vivo between the individual 3−keto derivatives of bile acids and physiologically adaptive $CYP3A_4$ regulatory gene, research was conducted on experimental animals (mice and rat models). Adaptive CYP3A4 enzyme is now known through the members of the nuclear receptor superfamily and function as a ligand−active factor of transcription. In particular pregnane X receptor (PXR, NR1I2) and constitutive androstane receptor (CAR, NR1I1) have involved as important sensors for potentially toxic compounds. PXR binding to 3−oxo derivatives of the bile acids regulate the whole program of the genes involved of detoxification and elimination of xenobiotics from the body [5]. In particular 3−oxo derivatives of bile acids treat a primary billiary cirrhosis and other human cholestasis liver disease, which is characterized by elevated levels serum of liver enzymes and prevent the heavy itching (pruritus), lethargy and jaundice. Currently there are no effective long−term treatment of this chronic disease of the liver, other than liver transplantation. Chemopreventive actions of PXR agonists may be useful in the treatment of human cholestasis. [6]. 3−Oxo derivatives act as selective modulators of vitamin D−receptor (VDR), even through their structure is fundamentally different from the natural hormones of 1α, 25−dihydroxy vitamin D_3 [1,25(OH)2D_3] [7].

Studying the effects of solvents on the vibrational spectra is used to obtain informations about the interactions between the solute (Me-ester of 7α,12α−dihydroxy−3−oxo−5β−cholanoic acid and 3−oxo−12α−hydroxy−5β−cholanoic acid) and solvent. Comparing the absorption spectra of the compounds in solutions of different polarity, it was found that the position, shape they intensity of the absorption of the bar changes, depending on the type of solvent. These changes are the result of intermolecular interactions dissolved sub-solvent (such as ion-dipole, dipole-dipole, dipole-induced dipole, hydrogen bond) which primarily tend to change the energy difference between the primary and the excited state of the absorbing species. The influences of the media, the absortion spectrum can be studied, studying spectral change: a transition from the vapor phase into the solution or changing the nature of the solvent. How often is not possible to obtain absorption spectrum, the observed compounds (solutes) in the vapor phase, the most commonly used other means to study the effect of solvents on the absorption spectrum [8].

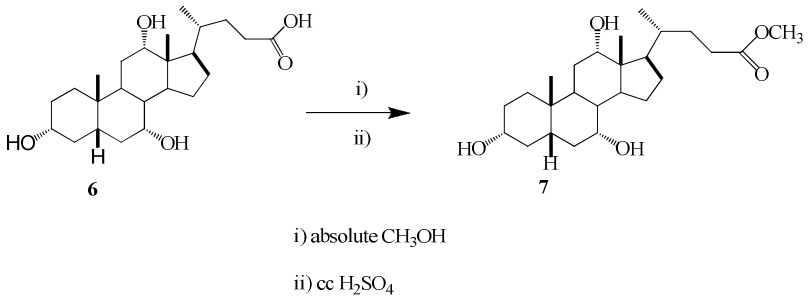
The aim of this paper is a synthesis of methyl ester of 7α,12α−dihydroxy−3−oxo−5β−cholanoic acid and 12α−hydroxy−3−oxo−5β−cholanoic acid, as well as consideration the possibility of creating intermolecular interactions between the carbonyl groups of Me−ester of 7α, 12α−dihydroxy−3−oxo−5β−cholanoic acid and 12α−hydroxy−3−oxo−5β−cholanoic acid with different solvents due process of association and selfassociation molecules of bile acids, the building of hydrogen bonds by appling IR spectroscopy as well as their application for medical purposes.

EXPERIMENTAL SECTION

All chemicals used in the experimental work were purchased from the manufacturer Sigma−Aldrich and before use are not further purified. The TLC chromatographic monitoring reactions were used tiles producers Merck (Kieselgel / UV 254), with a suitable developer. To elicit a TLC plate was used H_2SO_4 1:1 with heating in a hot plate. Chromatographic purification of the substances was performed by column “flash chromatography” making use of the manufacturer Merck silica gel (0.04−0.063 mm). NMR spectra were recorded on a Bruker AC−250 with an operating frequency assigned to the protons of 250.13 MHz and for the carbon−nucleus C_{13} 62.9 MHz. IR spectra were recorded on a FT−IR spectrometer NEXUS 670 SP−IR. FT–IR spectra were recorded on a Nexus Thermo Scientific apparatus 670 using a detector with deuterated triglicinsulfat (DTGS) is from 2000 to 1600 cm^{-1} with a resolution of 2 cm^{-1} and 50 scans. The concentrations of Me−ester 3−ketocholic acid ranged between 0.003 and 0.02 $moldm^{-3}$. Samples were during recording were placed into sodium chloride thickness of 0.057 and 0.116 mm at a temperature of 298K.

SYNTHESIS OF METHYL ESTER OF CHOLIC ACID

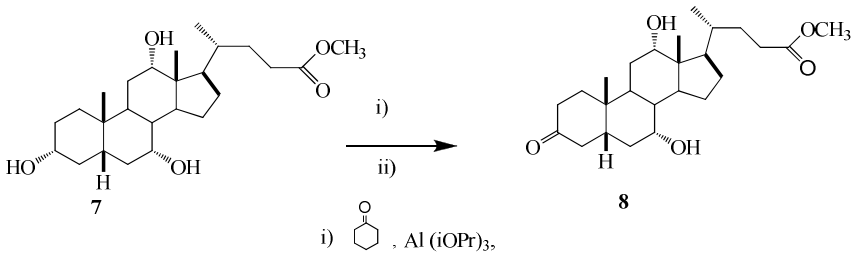
A solution of cholic acid (6) (10g, $2.45*10^{-2}$ mol) in absolute methanol (CH_3OH) (100ml) was heated with stirring the reaction mixture, as long as the substance does not dissolve. Then, the heating was interrupted and added 3 drops of cc H_2SO_4. Heating was carried out at reflux for 1h and 30 min. After completion of the reaction, the volume of the reaction mixture was reduced by evaporation of CH_3OH on the rotary evaporator. Then concentrated the reaction mixture was transferred into 200 ml of H_2O. NaCl is added, because of salting out of the reaction mixture. The crystals were separated by vacuum filtration and dried in the air, whereby a white crystals of Me-ester of cholic acid (7) were separated in a yield of 93.13%. (Scheme 1).



Sheme 1. Methylation reaction of cholic acid to give a Me−cholate

SYNTHESIS OF METHYL ESTER 7α,12α−DIHYDROXY−3−OXO−5β−CHOLANOIC ACID

To a solution of methyl ester of cholic acid (7) (5g, $1.85*10^{-2}$ mol) in cyclohexanone (50ml) was added aluminium isopropoxide $Al(iOPr)_3$ (3.63g, $1.78*10^{-2}$ mol). The reaction mixture was heated at 100^0C for 4 hours. After completion of the reaction, the reaction mixture was acidified with HCl (1:1) and excess cyclohexanone was removed by distillation with steam. The solid residue after the distillation in the balloon is separated from the aqueous layer and dissolved in chloroform. An aqueous phase in which the residual crystals Oppenauer oxidation (methyl ester 7α,12α−dihydroxy−3−oxo−5β−cholanoic acid) is filtered with a Büchner funnel, wherein the crystals from the filter paper had washed with chloroform and merged with the existing crystal products. $CHCl_3$ was evaporated on a rotary evaporator. The residue after evaporation was purified on a silica gel wherein the mixture used as the eluent toluene: aceton (3:1). The yield of methyl ester 7α,12α−dihydroxy−3−oxo−5β−cholanoic acid (8) was 66.4%. (Sheme 2).

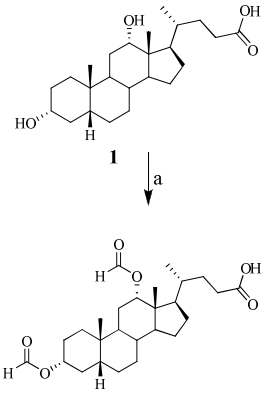


Sheme 2. Oppenauer oxidation of Me−cholate to Me−ester of 3−ketocholic acid

Further studies are based to the synthesis of 3−oxo−12α−hydroxy−5β−cholanoic acid from deoxycholic acid.

FORMYLATION OF DEOXYCHOLIC ACID

To a solution of deoxycholic acid (1) (5.00g, $1.27*10^{-2}$mol) in formic acid (35ml) was added perchloric acid (15 drops) and then the reaction mixture was heated at $50-55^0$C for 1.5 hours. The reaction was monitored by thin layer chromatography (TLC) on silica gel plates (Kieselgel⁄ UV 254) with the developer system: chloroform−acetone−1 drop of acetic acid, followed by 50% H_2SO_4 challenger and heating. After the expiry of the reaction time, in the cooled reaction mixture was added acetic anhydride (5ml) and then the reaction mixture was poured into the water (500 ml), wherein crystallized the 3α,12α−diformyloxy−5β−cholanoic acid (2) as white crystals (yield: 92.76%). (Sheme 3).



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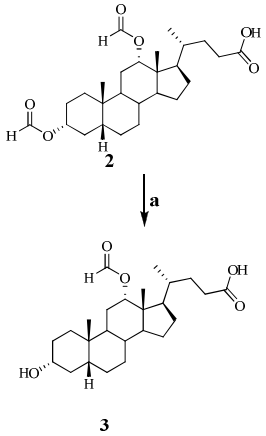
Sheme 3. . a) HCOOH, $HClO_4$, $(CH_3CO)_2O$, CH_3COCH_3, 50–55^{0}C, 1,5h

SELECTIVE DEFORMILATION OF 3α,12α−DIFORMYLOXY−5β−CHOLANOIC ACID

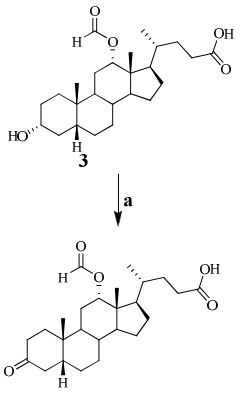
To a solution of 3α,12α−diformyloxy−5β−cholanoic acid (2) (2.00g, 4.43*10^{-3} mol) in acetone (22ml) was added dropwise 8M NaOH (52ml) and the reaction mixture stirred at room temperature for 7 hours. After the expiry of the reaction time, the reaction mixture was poured into aqueous solution of acetic acid (1:4), wherein crystallized the 3α−hydroxy−12α−formyl−5β−cholanoic acid. The mother liquor was extracted (4*20 ml) and the extract was dried with anhydrous Na_2SO_4, and afterwards the dried agent was removed and the solvent evaporated. Provides compound (3), wherein the total yield was 98.14%. The reaction was monitored by thin layer chromatography (TLC) on silica gel plates (Kieselgel / UV 254) with the system developer aceton–hloroform (1:6) – 1 drop of acetic acid. As the challenger was used 50% H2SO4 and heating.



Scheme 4. a) 8M NaOH, s.t. 4h, then CH_3COOH (1:4)

OXIDATION OF 3α−HYDROXY−12α−FORMYL−5β−CHOLANOIC ACID USING THE N−BROMOSUCCINIMIDE (NBS)

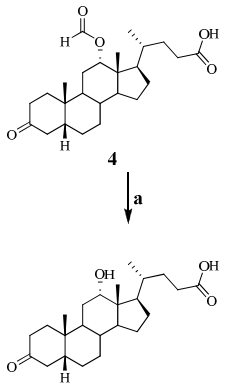
The solution of 3α−hydroxy−12α−formyl−5β−cholanoic acid (3) (1.963g, 4.67*10^{-3}mol) in t−butanol (40ml) in which was added N−bromosuccinimide (NBS, 2.50g) was dissolved in water (100 ml) and the reaction mixture was heated at boiling temperature for 90 minutes. After the expiry of the reaction time, the reaction mixture was cooled from room temperature and poured into water (800ml) and then acidified with HCl (1:1) to a pH of 7, wherein the crystallized holanka–5β–formil–12α–okso–3 acid (4) in a yield of 87.58%. (Sheme 5).



Scheme 5. a) t–BuOH, NBS, 30^{0}C, 3h

DEFORMYLATION OF 3−OXO−12α−FORMYL−5β−CHOLANOIC ACID

3−Oxo−12α−formyl−5β−cholanoic acid (4), (1,719g, 4.09 • 10^{-3}mol) was dissolved in acetone (18,5ml) and gradually added to an aliquot of the NaOH 8M (62ml). The reaction mixture was stirred at room temperature for 4 hours, and then poured into an aqueous solution of acetic acid (4:1, 300ml), wherein the crystallized holanska–5β–hidroksi–12α–okso–3 acid in the form of white crystals (yield: 77, 86%). (Sheme 6). The reaction was monitored by thin layer chromatography (TLC) on silica gel plates (Kieselgel / UV 254) with the system developer aceton–hloroform (1:2) – 1 drop of acetic acid. As the challenger, was used 50% H2SO4 and heating.



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a

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Scheme 6. a) 8M NaOH, s.t. 4h, then CH_3COOH (1:4)

RESULTS AND DISCUSSION

The positions of the vibrating strip of the keto groups, K_1 (carbonyl of the ester group) and K_2 (carbonyl from oxo groups) as well as empirical parameters used are shown in table 1. Table 2 shows the results of the regression analysis for the obtained correlation between the position on the carbonyl group and the parameters of KBM equation. Figure 1 shows IR spectra of the carbonyl of the ester group from the tape (K_1) and the keto group (K_2) in the area of valence vibration of carbonyl groups, K_1−tape carbonyl group originating from the ester group is at higher frequencies, K_2−tape carbonyl group originating from the keto group is located on the lower frequencies. Figure 2 shows Graphic correlation obtained KBM equation. Table 3 presents parameters obtained using Guttman equation. Figure 3 shows graphic correlation equation obtained by Guttman. Table 4 presents parameters obtained by LSER equation. Figure 4 shows overview of the correlation equation obtained LSER. Table 5 presents location of the vibrating strip carbonyl groups in 3−oxo−12α−hydroxy−5β−cholanoic acid and used empirical parameters. Figure 5 graphically shows the dependence of the position of the carbonyl strips of Gutmann acceptor number, while figure 6 graphically shows the dependence of the position of the carbonyl strips of α and π LSER parameters. The corresponding equations and linear regression coefficients are also fitting given in graphs.

Table 1. Position the vibratory strip carbonyl groups and used empirical parameters for the methyl ester 7α,12α−dihydroxy−3−oxo−5β−cholanoic acid

Solvents	Carbonyl 1	Carbonyl 2	AN	a	b	d	p	n	e
Acetonitrile	1735.04	1708.9	18.9	0.19	0.4	0	0.75	1.3441	37.5
Benzene	1740.08	1714.3	8.2	0	0.1	0	0.59	1.5011	2.3
Dichloroethane	1732.07	1708.55	16.7	0	0	0.5	0.81	1.4448	10.4
CCl_4	1741.08	1716.03	8.6	0	0	0.5	0.28	1.4612	2.2
CH_2Cl_2	1731.3	1708.41	20.4	0.3	0	0.5	0.85	1.4241	8.9
Chloroform	1728.75	1707.08	23.1	0.44	0	0.5	0.58	1.4458	4.8
Dioxane	1738.43	1712.68	10.8	0	0.37	0	0.55	1.4224	2.25
Duethyl ether	1745.38	1717.54	3.9	0	0.47	0	0.27	1.3524	4.2
Hexane	1748.83	1722.18	0	0	0	0	-0.11	1.3749	1.9
Tetrahydrofuran	1741.08	1713.97	8	0	0.55	0	0.58	1.4072	7.6
Toluene	1740.95	1715.04	3.3	0	0.11	0	0.54	1.4969	2.4

*Legend: CCl_4−carbon tetrachloride, CH_2Cl_2−methylene chloride, **AN**−acceptor number, **α**−measure of the ability of a compounds to act as a proton donor, **β**−measure of the ability of a compounds to act as a proton acceptor, **δ**−chemical shift in a particular solvent, **π**−measure of the polarizability, ie. dipolarity specific solvent, **n**−solvent refractive index, **ε**-electric permittivity solvent, K_1−tape carbonyl group originating from the ester group is at higher frequencies, K_2−tape carbonyl group originating from the keto group is located on the lower frequencies.*

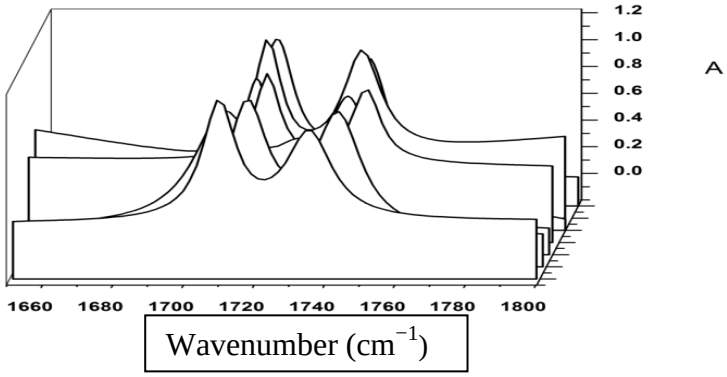


Figure 1. IR spectra of the carbonyl of the ester group from the tape (K_1) and the keto group (K_2) in the area of valence vibration of carbonyl groups

Table 2. Parameters obtained by KBM equation studied the system Me−ester 7α,12α−dihydroxy−3−oxo−5β−cholanoic acid

	n_0	f(e)	f(n)	R
Carbonyl 1	1766.8	-44.65	-84.93	0.418
Carbonyl 2	1732.8	-50.04	-36.09	0.512

Legend: ν_0−starting frequency of carbonyl tape, **f(ε)**−function electric permittivity solvent, **f(n)**−function of solvent refractive index, **R**−coefficient of determination.

Table 3. Parameters obtained using Guttman equation to test system Me−ester of 7α,12α−dihydroxy−3−oxo−5β−cholanoic acid

	n_0	a	R
Carbonyl 1	1746.9	-0.771	-0.9596
Carbonyl 2	1719.6	-0.575	-0.9507

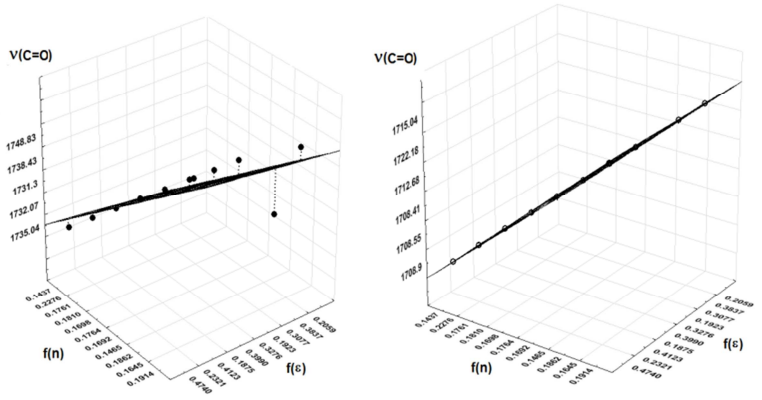


Figure 2. Graphic correlation obtained KBM equation studied the system Me−ester of 7α,12α−dihydroxy−3−oxo−5β−cholanoic acid

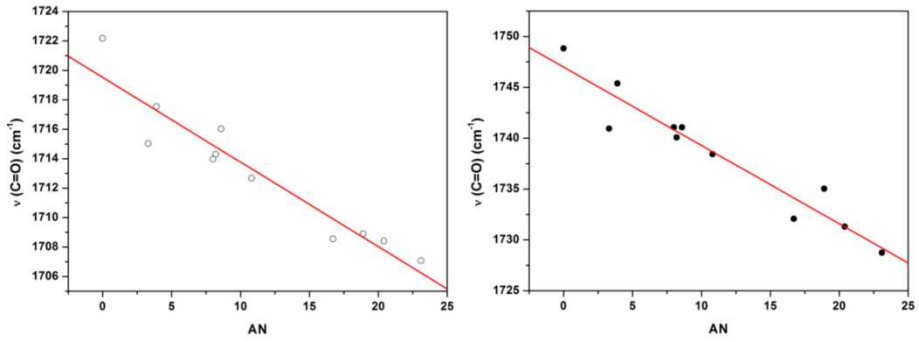


Figure 3. Graphic correlation equation obtained by Guttman to test system Me−ester of 7α,12α−dihydroxy−3−oxo−5β−cholanoic acid

Table 4. Parameters obtained by LSER equation studied the system Me−ester of 7α,12α−dihydroxy−3−oxo−5β−cholanoic acid

	ν_0	α	β	δ	π	R
Carbonyl 1	1747.5	-13.8	0.75	-6.45	-13.39	0.952
Carbonyl 2	1720.5	-9.5	0.401	-2.18	-12.2	0.9484

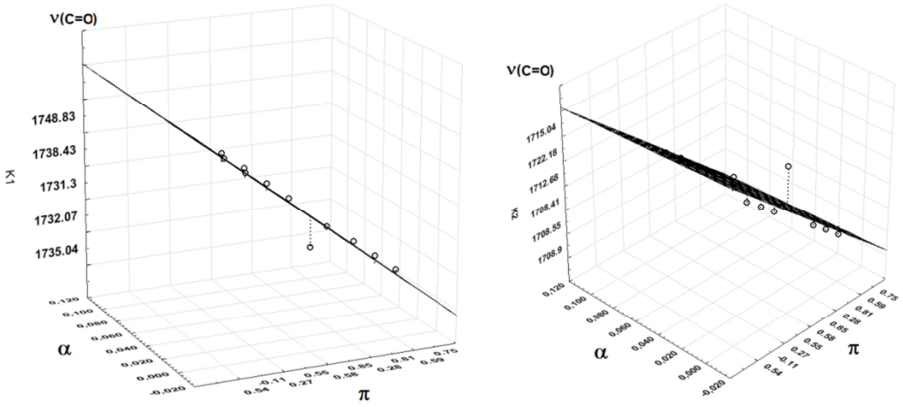
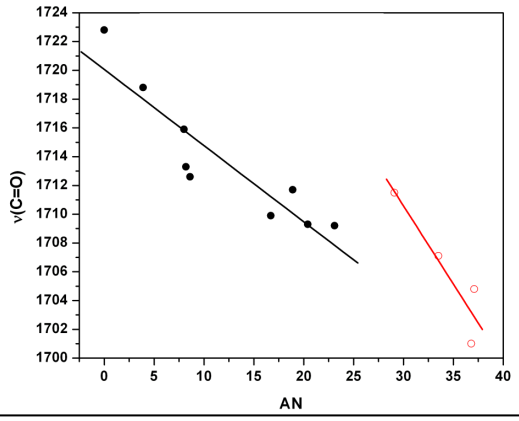


Figure 4. Overview of the correlation equation obtained LSER

Table 5. Location of the vibrating strip carbonyl groups in 3−oxo−12α−hydroxy−5β−cholanoic acid and used empirical parameters

Solvents	υ (C=O)2	AN	α	β	δ	π
Hexane	1722.8	0	0	0	0	-0.11
Carbon tetrachloride	1712.6	8.6	0	0	0.5	0.28
Chloroform	1709.2	23.1	0.44	0	0.5	0.58
Methyl chloride	1709.3	20.4	0.3	0	0.5	0.85
1.2 dichloroethane	1709.9	16.7	0	0	0.5	0.81
Benzene	1713.3	8.2	0	0.1	0	0.59
Tetrahydrofuran	1715.9	8	0	0.55	0	0.58
Diethyl ether	1718.8	3.9	0	0.47	0	0.27
Acetonitirle	1711.7	18.9	0.19	0.4	0	0.75
Ethanol	1704.8	37.1	0.86	0.75	0	0.54
Iso propanol	1707.1	33.5	0.76	0.84	0	0.48
Butanol	1701	36.8	0.84	0.84	0	0.47
tert− butanol	1711.5	29.1	0.42	0.93	0	0.43



Alcohols, ν (C=O)=1720.6−0.53AN, R=0.913,
other solvents: ν (C=O)=1743.2−1.1AN, R=0.922

Figure 5. Dependence of the position of the carbonyl strips Guttman acceptor number

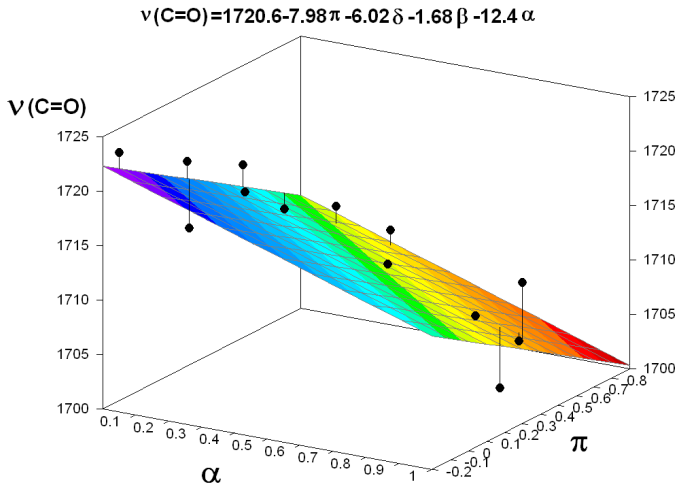


Figure 6. The position of the carbonyl strips tested LSER correlation

The reason for the higher susceptibility of ester carbonyl in the solvent, could be a rotation of the side chain at which the group is located. If there is a smooth rotation of the side chain, the ester carbonyl group has an additional degree of freedom relative to the keto group at the 3−position and therefore , more easily enters into interaction with solvents. The ester carbonyl group is more susceptible to interaction with the solvent as compared to the carbonyl of the keto group, which can be explained on the basis of less steric of protection of said carbonyl from the ester functional groups, and based on the more polar, which possess due to its inductive effect. Given that the coefficients of determination [R] low, KBM equation indicates that in addition to polarization and ruled interactions for the position of carbonyl group of test compounds responsible some specific interactions (hydrogen bonding, hydrophobic interactions or a steric effects). With the increase in electrophilicity solvent (higher value AN number) using spectrum shift to lower frequencies. The solvent in which the value for a parameter of the solvent different from zero: chloroform, methylene chloride and acetonitrile can assume the possibility of forming hydrogen bonding CH….O type. Two different carbonyl groups have different sensitivity to the solvent effect. In our case, carbonyl (1) of the ester group has a higher pitch, and also greater sensitivity to the solvent effects compared with the carbonyl (2) of the keto group. On the basis of the value coefficient front parameters α and π obtained in LSER model can be concluded that the greatest contribution to the position of the carbonyl strip the ability of the solvent to donate a proton (α) and dipolar interactions and polarizability. (π) Alcohols, such as proton donors show a different correlation with respect to the non−alcoholic solvent, the reason for this behavior is probably forming a hydrogen bond between the hydroxyl group to alcohol and a carbonyl group of 3−oxo−12α−hydroxy−5β−cholanoic acid. On the basis of the value coefficients from parameters α and π obtained in LSER model can be concluded that the greatest contribution to the position of the carbonyl strip the ability of the solvent to donate proton (α=4.12) as well as dipolar interactions and polarizability (π=7.98). For non−alcoholic solvents, in which the values for the parameter of the solvent other than zero: chloroform, methylene chloride and acetonitrile can assume the ability to form hydrogen bonds CH…O type.

CONCLUSION

In the case of an intermolecular cyclization of the keto group at the 3−position would be more sensitive to the interaction with different solvent in comparison to the ester−carbonyl group. The purpose of the inhibitor to have better insight into the possibility of creating intermolecular interactions bile acid would be very interesting and important to examine compounds similar structure with different substituents. Information obtained in spectroscopic studies can be very useful in testing self association and association of molecules of bile acids and their application in medicine to produce beneficial effects on sexually transmitted diseases, primary biliary cirrhosis, gallstones, cystic fibrosis, cancer, diabetes and leukemia.

Acknowledgments
Great thanks to Momir Mikov for his assistance in preparing the research. This work has been supported by the Ministry of Education, Science and Technology of Republic of Serbia Grant No. 41012.

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