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Complexing 7,3',4'-trioxyflavonol with cell phospatidylcholine

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Aim. *To investigate the complex formation of 7,3',4'-tryoxyflavonol of flavonoids group with cellular phosphatidylcholine.* ***Methods***. *Semi-empirical quantum chemistry, spectroscopy NMR.* ***Results***. *The changes in conformational status of 7,3',4'-tryoxyflavonol at complex formation have been shown.* ***Conclusions***. *The conformational changes in phosphatidylcholine take place under the 7,3',4'-tryoxyflavonol/phosphatidylcholine complex formation.*

Keywords: comlexing, tryoxyflavonol, phosphatidylcholine, NMR-spectroscopy, Overhauser effects, semi-empirical quantum chemistry.

Introduction. Flavonoids are biologically active compounds of plant origin with a wide spectrum of activity. Up-to-date over 6,000 molecules of this class have been extracted and identified, and their number is increasing constantly [1–3].

Over 40 types of biological activity of flavonoids have been described in literature [4–6]. Flavonoids have anti-inflammatory, anti-allergic, anti-viral, and anti-tumour effects. Besides, they are characterized by anti-oxidant properties, which provide protection from oxidation and damage of cells by free radicals. Nevertheless, little is known about the molecular mechanism of the impact of this group of compounds on cellular membranes, and their localization in the latter is a matter of discussion. Moreover, a complex structure of these molecules, responsible for numerous intramolecular interactions and conformational motility, is of great interest [7].

This work presents the results of investigations on the impact of 7, 3', 4'-trioxyflavonol, remarkable for its pronounced anti-allergic effect on cellular phosphatidylcholine (PC) (Fig. 1).

It was previously shown that coupled systems [8, 9], in particular, the molecules of flavonoid group, rutin and quercetin, form complexes with cellular phospholipids via the system of -electrons [10]. This mechanism of complexing allows explaining preservation of biological activity at considerable changes of substitutes when there appeared steric hindrances for the active centres approach close enough for the hydrogen bonds formation.

Materials and Methods. An interaction of trioxyflavonol and PC (lecithin) was studied by semi-empirical quantum chemistry and NMR-spectroscopy on nuclei ^{13}C and ^{1}H.

Numerous quantum-chemical calculations using a network approach with subsequent geometry optimization by a molecular mechanics method were performed to determine the geometry of complexes at

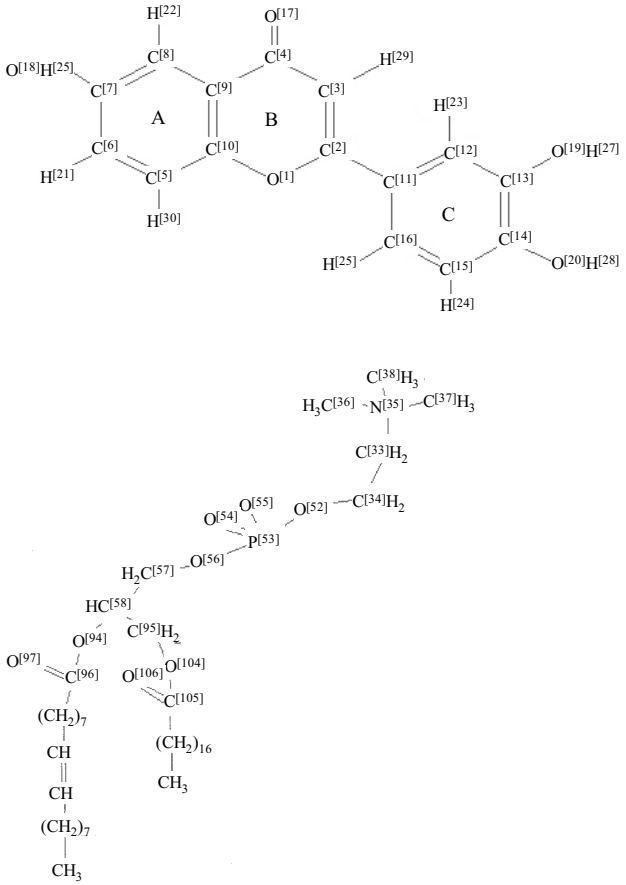

Fig. 1. Interaction of ring C of 7, 3', 4'-trioxyflavonol with phosphatidyl choline.

initial configurations of molecule centres, forming a complex. Then structures and electronic structure of molecules were specified by the MNDO and AM1 methods, using HyperChem 7.0 software package.

PC, isolated from hen's eggs [12], and standard samples of trioxyflavonol («Aldrich», USA) were used in the experiments. Lecithin was purified by column chromatography. The purity was controlled using NMR spectrum and methods of thin-layer chromatography. Of 0.005 M solutions of lecithin with different concentrations of 7, 3', 4'-trioxyflavonol were used for investigation. Deutero-substituting chloroform of «Deutero GmbH» Company (Germany) was used as a solvent

The spectra were taken at relatively low concentrations of lecithin and flavonoids. Though a small concentration leads to an increase in the time of signal accumulation, its raise leads to a decrease in the accuracy of calculating chemical shifts (CS) due to the formation of micelles causing the extension of absorption line. Simultaneously, at increased concentration the number of points of flavonoid binding drops, as they get inside micelles, thus, the intensity of spectral lines becomes lower. In this case flavonoids form chain structures via hydrogen connections, which hinder the analysis of NMR-spectra. The plateau of titration curve at the accepted concentrations was not reached.

The value of medium Pd was maintained equal to 7.1, being controlled by OP-156/3 device with the accuracy up to 0.01. At the mentioned pD value the dependency of CS spectrum of NMR ^{13}C on pD is the weakest [13].

The interaction of 7,3', 4'-trioxyflavonol with PC results in the formation of several kinds of complexes, some of which are formed at binding of lecithin with different rings of 7,3',4'-trioxyflavonol. In their turn, flavonoids are localized at separate sites of lecithin, forming the complexes via hydrogen bonds. That is why the spectra were taken in conditions when the concentration of flavonoids exceeded that of PC and amounted to 0.08 M. The increase in lecithin concentrations regarding flavonoids had no significant effect on the PC value.

The NMR spectra of ^{13}C of flavonoids, PC and their mixtures were recorded by standard methods at 30°C using AM-300 spectrometer («Bruker», Germany) with the working frequency of 75 MHz for ^{13}C. The temperature of investigated samples was kept with the accuracy of 0.2°C. Tetramethylsilane was used as an internal standard.

45°-impulses were used with the delay of 1.5s between them. The number of accumulations was increased to 20,000 thus providing the signal/noise ratio no less than 60. The accumulation was performed at 64–128 k dots with the scanning width of 100–150 ppm.

At 100 ppm scanning width, 128k dots, and sampling time of 8.65 s, the digital resolution is 0.06 Hz and PC value is defined with the accuracy of up to 0.001 ppm. The averaging of the results of several PS measurements gives the accuracy not exceeding 0.005 ppm.

The changes in flavonoids conformational state at the complex formation were determined by the

Overhauser method using the same spectrometer. As the nuclear Overhauser effect (NOE) method is the only one applicable for determination of internuclei distances in solutions, it is of special significance for investigations on bioactive molecules activity in aqueous solutions. The method is based on direct interaction of magnetic nuclei, exposed as the change in intensity of one spectral line at irradiation of the other due to varying density of energetic levels, which results in an additional channel of relaxation.

If the intensity of spectral line in the absence of saturation of another line is marked as I_0, NOE S) at saturated I may be presented as follows:

$$(S) \quad \frac{I \quad I_0}{I_0} \quad 100\%.$$

Direct dipole-dipole interaction between nuclei, dependent on internuclei distance r, is described with [the] equation [14]

$$\frac{1}{} \quad \frac{p}{c} r^6,$$

where p_x – additional relaxation; $_c$ – correlation time.

The NOE experiments require high accuracy of measuring line intensity. To increase it, the method of differential spectra is used, which implies the summation of several spectrum scans without saturation and the same amount of passages with saturation. The averaging in this method decreases the effect of random changes in temperature, signal phases and frequencies on the measurement accuracy and improves the signal/noise ratio. The results of measurements are affected by paramagnetic admixtures, oxygen, in particular. Gas was eliminated by barbotage via dried helium. About 24 accumulations were performed, repeating this process 32 times. The relaxation time was in the range of T_1 = 1–2 s. The impulse of 90° was used for decoupler.

Results and Discussion. The energies of complexing via the formation of -connection between the polar head of PC and rings A, B, and C of 7, 3', 4'-trioxyflavonol, were calculated using AM1 method and presented below (kJ/mol):

Ring An	18
Ring B	21
Ring C	30

As seen, the energy of complexing is maximal under binding lecithin to the ring C of 7, 3', 4'-trioxyflavonol. It is noteworthy that the energy of complexing is calculated as a small difference between high values and therefore is defined with a considerable error. The data presented are of qualitative character, however, the ratio of energy values obtained by other semi-empirical methods, DFT method, in particular, is the same.

The comparison of energy values of 7, 3', 4'-trioxyflavonol and benzilpenicillin (allergic) complexing with lecithin reveals that the complex with 7, 3', 4'-trioxyflavonol is more stable. Therefore, one of the possible mechanisms of anti-allergic activity may be the blocking of points of benzyl penicillin binding to lecithin.

The complexing of lecithin with the C ring of flavonoids is accompanied with the change in CS of C^{13} nuclei of the ring. At complexing with participation of electrons -system, CSs of aromatic ring carbohydrates draw closer [15, 16]. In this case PC introduction into 0.008 M trioxyflavonol solutions results in decrease of CS for $C_{[16]}$ by 0.116 ppm and the increase for $C_{[15]}$ by 0.019 ppm. At the same time double bond $C_{[11]}$-$C_{[12]}$ of the C ring increases by 0.002 Å while singular bond $C_{[16]}$-$C_{[11]}$ decreases by 0.004 Å.

CS also changes at the complexing of choline group carbohydrates. While for free lecithin CS equals 53.23 ppm, its value for $C_{[1]}$, $C_{[2]}$, $C_{[3]}$ shifts into a weak zone by 1.574 ppm at complexing with trioxyflavonol (Fig. 2).

Complexing is accompanied with the change in linear angles of phosphatidylcholine. The angle $O_{[56]}$-$P_{[53]}$-$O_{[52]}$ decreases by 10° and angle $C_{[34]}$-$C_{[33]}$-$N_{[35]}$ increases by 12°.

The values of CS changes obtained are in good qualitative agreement with the calculated values of changes in electronic density, if the interaction of -system electrons of flavonoids with choline and phosphate groups of lecithin is taken into account.

Quick exchange between numerous complexes, formed in the interacting system of 7, 3', 4'-trioxyflavonol-lecithin, is observed in the NMR time scale. Therefore, NMR method for a given temperature

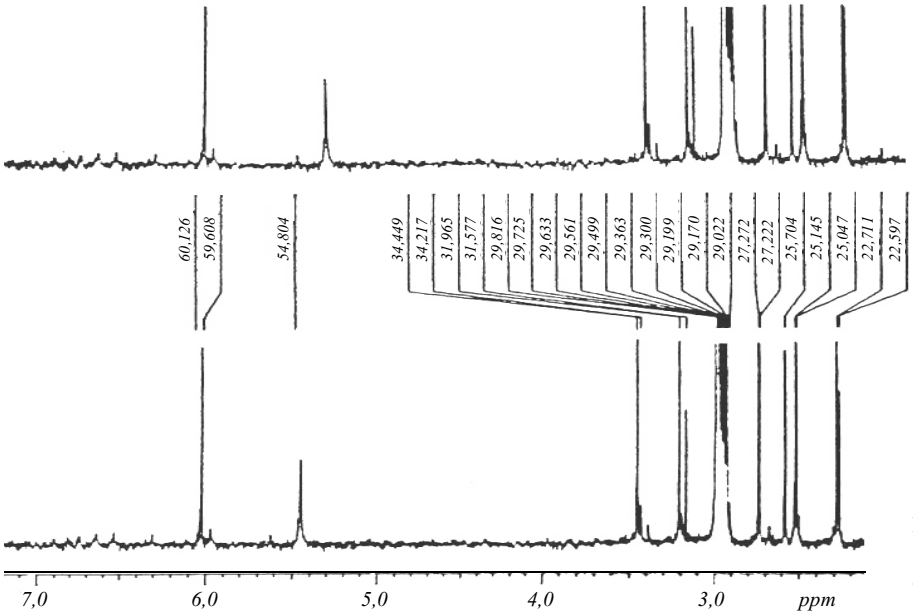

Fig. 2. Fragments of NMR spectra of ^{13}C 7, 3', 4'-trioxyflavonol: *a* – free; *b* – in the complex with lecithin

Table 1
Distribution of electron density on atoms of free phosphatidyl choline and complexed with 7, 3', 4'-trioxyflavonol obtained by methods AM1 and MNDO

Atom	AM1		MNDO	
	Free	Bound	Free	Bound
P[53]	2,468	2,457	2,465	2,455
N[35]	4,956	4,955	4,951	4,955

Table 2
Electron structure of free phosphatidyl choline and complexed with 7, 3', 4'-trioxyflavonol obtained by methods AM1 and MNDO

Atom	Type of orbital and charge	AM1		MNDO	
		Free	Bound	Free	Bound
P[53]	S	0,911	0,906	0,913	0,904
	P_x	0,510	0,501	0,505	0,501
	P_y	0,524	0,522	0,521	0,521
	P_z	0,521	0,526	0,524	0,525
	q	2,466	2,455	2,463	2,451
N[35]	S	1,486	1,486	1,486	1,485
	P_x	1,125	1,123	1,120	1,119
	P_y	1,170	1,171	1,174	1,176
	P_z	1,180	1,177	1,176	1,178
	q	4,961	4,957	4,956	4,958

registers average CS values. The calculated structures of 7, 3', 4'-trioxyflavonol-lecithin complex, are close to those described in the work [17].

The calculations demonstrate that reviewed complexes are formed due to Coulomb interaction, which is notable for the charge re-distribution between complex components. The value of transferred charge changes; its maximum, equal to 0.12 atomic units, is registered in the complex of PC with C cycle of 7,3',4'-trioxyflavonol. The distance between the centre of aromatic ring and the atom of nitrogen of lecithin is 6.31 Å, between atoms $H_{[30]}$-$O_{[54]}$ - 5.35 Å. Noteworthy that the use of MNDO and AM1 methods for calculations of such complexes causes systematic overrating of the length of intermolecular connections] [14]. It should be emphasized that the structural data and distribution of electron density, obtained by these methods, almost coincide (Table 1).

The analysis of NMR spectra of ^{13}C nuclei allows the assumption that investigated group of molecules is most likely characterized by their interactions with -system of the cycle electrons alongside with simultaneous formation of the hydrogen bond between the A ring –OH-group and PC phosphate group. This complex is rather strong; it blocks the active centres of PC.

Table 2 presents the results of quantum-chemical calculations of changes in the charge for trioxyflavonol - PC complexes.

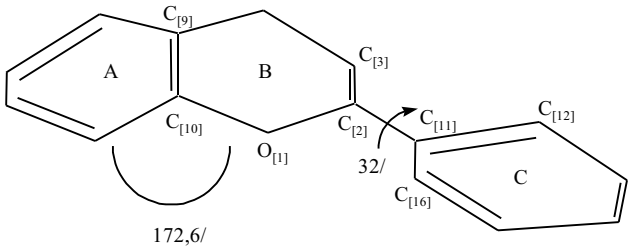

Fig. 3. Conformational changes in 7, 3', 4'-trioxyflavonol

The calculations also show that complexing results in the deformation of flavonoid plane. The structure formed by rings A and B becomes non-flat and the angle between their planes is 172.6°. The ring C turns around the single bond $C_{[2]}$-$C_{[11]}$ by 32°relative to the plane of ring A (Fig. 3).

The study on the flavonoid conformational states, measured using Overhauser's effect, demonstrate that at irradiation of $H_{[29]}$ solution of 7,3',4'-trioxyflavonol the integral intensity of spectral lines of protons $H_{[25]}$ and $H_{[23]}$ increases by 2.8 and 3.2% respectively (error 0.2%) (Fig. 4).

Such change in signal intensity indicates the hydrogen atoms approach due to the rotation of ring C of 7, 3', 4'-trioxyflavonol around the bond $C_{[2]}$-$C_{[11]}$. The addition of lecithin solution results in the formation of complex of C cycle with choline group of lecithin, hindering this rotation. Meanwhile, the line of proton $H_{[25]}$ was not observed in the same conditions, and the spectral line of proton $H_{[23]}$ was notable for a small increase in signal intensity if the signal from proton $H_{[29]}$ was irradiated. These data qualitatively confirm the calculated results about the turn of ring C relative to the plane of 7, 3', 4'-trioxyflavonol molecule.

Conclusions. The methods of quantum chemistry and ^{13}C NMR spectroscopy revealed the formation of complex between 7, 3', 4'-trioxyflavonol and lecithin.

During formation of this complex the conformational changes of 7, 3', 4'-trioxyflavonol occur.

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Комплексообразование 7,3',4'-триоксифлавонола с клеточным фосфатидилхолином

Резюме

Цель. *Исследовать комплексообразование молекулы группы флавоноидов 7,3',4'-триоксифлавонола с клеточным фосфатидилхолином.* ***Методы***. *Структуру комплекса определяли методами полуэмпирической квантовой химии и спектроскопии ЯМР.* ***Результаты***. *Установлено изменение конформационных состояний 7,3',4'-триоксифлавонола при комплексообразова-*

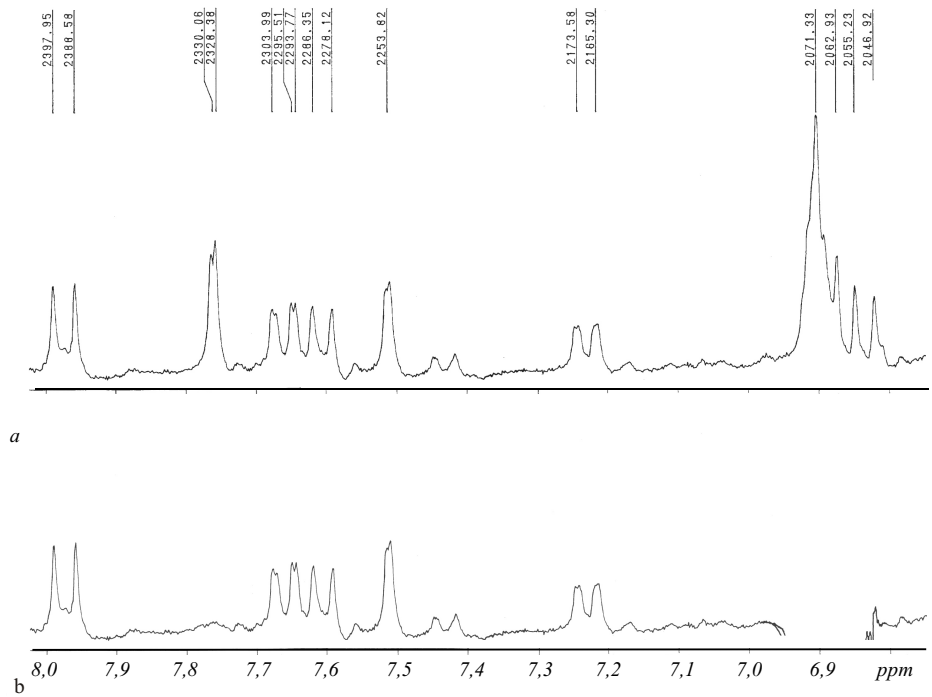

Fig. 4. NMR spectrum of ^1H 7,3',4'-trioxyflavonol (*a*) and fragment of NMR spectrum of ^1H NOE-dif 7,3',4'-trioxyflavonol (*b*)

нии. ***Выводы.*** *Формирование данного комплекса вызывает конформационные изменения фосфатидилхолина.*

Ключевые слова: комплексообразование, триоксифлавонол, фосфатидилхолин, ЯМР-спектроскопия, эффект Оверхаузера, полуэмпирическая квантовая химия.

Р. С. Насібуллін, Е. Р. Фахретдинова, В. М. Нусратуллін, Р. І. Галєєва

Комплексоутворення 7,3',4'-триоксифлавонолу з клітинним фосфатидилхоліном

Резюме

Мета*. Дослідити комплексоутворення молекули групи флавоноїдів 7,3',4'-триоксифлавонолу з клітинним фосфатидилхоліном.* ***Методи****. Структуру комплексу встановлено методами напівемпіричної квантової хімії і спектроскопії ЯМР.* ***Результати****. Визначено зміни конформаційних станів 7,3',4'-триоксифлавонолу при комплексоутворенні.* ***Висновки****. При формуванні цього комплексу відбуваються конформаційні зміни фосфатидилхоліну.*

Ключові слова: комплексоутворення, триоксифлавонол, фосфатидилхолін, ЯМР-спектроскопія, ефект Оверхаузера, напівемпірична квантова хімія.

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