

Synthesis and study of complexes of the novel Russian antiviral drug Camphecene with Plant's Flavonoids

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Abstract

Traditionally, glycyrrhizic acid has been used to form polydentate complexes. For the first time in the presented paper, the complexation of the Plant's Flavonoids (Quercetin (Qu) and its glycoside - Rutin (Rut)) with the novel Russian antiviral drug Camphecene (Camph) was investigated. The complexes obtained at different molar ratios were studied using UV/Vis spectroscopy. Formation of the host: guest complexes were registered: Qu and Rut molecular complexes (*Camph+2Qu*; *Camph+2Rut*) with a stability constant $K = 3.3 \cdot 10^8 \text{ M}^{-2}$. Comparison of the binding constants of the obtained complexes shows that the efficiency of Camphecene complexation with the participation of flavonoids is more efficient than with the participation of triterpenoids. Besides, it was found that the complexes of Camphecene with the quercetin and rutin are soluble in water, in contrast to the complexes with triterpenoids, which makes it possible to increase the bioavailability of both Camphecene and flavonoids. The obtained results demonstrate the high potential of flavonoids Qu and Rut to the development of novel pharmaceutical forms using the example of Camphecene in the form of molecular complexes, as the novel forms of delivery.

Keywords

Camphecene
Quercetin
Rutin
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1. Introduction

It is known that a decrease in therapeutic doses of medicinal substances and prolongation of action is possible when they are clathrate with plant glycosides. This property was used in the approach of Academician G. A. Tolstikov to reduce therapeutic doses of drugs and prolong the action [1-3]. In this regard, the presented work considers the possibilities of synthesizing new supramolecular complexes of Qu and Rut using the new antiviral drug Camphecene for the development of low-dose pharmaceutical substances on their basis. The authors consider that these pharmaceutical substances can be used to suppress the multiplication of viruses in the early stages. It is known that Camphecene **1** (Fig. 1), has a broad spectrum of antiviral activity. It is proved to be active against the influenza virus strains type A and type B [4].

The purpose of this work is to synthesize and study supramolecular complexes based on the scaffold monoterpene Camphecene and plant flavonoids to develop, in the

future, previously unknown low-dose pharmaceutical substances with antiviral activity. Influenza is known to be the most common and dangerous respiratory viral infection. It causes annual epidemics and pandemics, leading to significant increases in morbidity and mortality in all regions of the world. In connection with the growing number of cases of viral infections and especially resistant viral strains, it is necessary to improve the available therapeutic methods, complementing them with the discovery of new antiviral agents. On the other hand, it is widely recognized that the medical heritage of plants is a valuable resource for the treatment of infectious disorders. This indicates a growing interest in antiviral products based on secondary plant metabolites [5, 6].

One of the unique plant components used in traditional medicine is bioflavonoids quercetin and rutin (Fig. 1).

Plant flavonoids **2** and **3** are attracting more and more attention of chemists and pharmacologists due to the wide spectrum of their biological activity. Flavonoids have long attracted scientific interest as antiviral agents - in a few

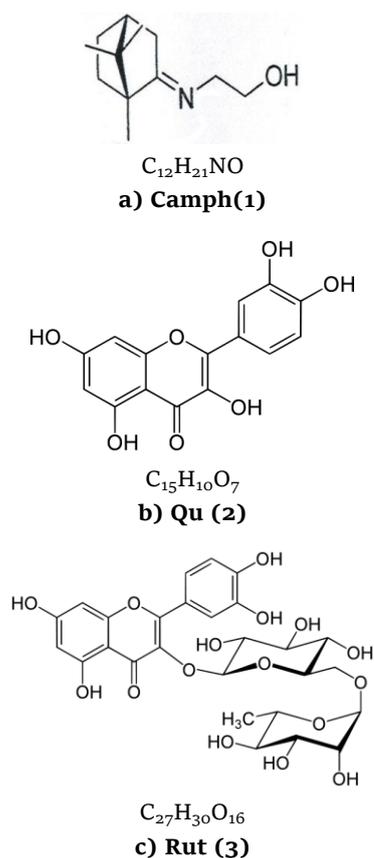


Fig. 1 Structures of a) Camphecene (1), $C_{12}H_{21}NO$; b) Quercetin (2), $C_{15}H_{10}O_7$; c) Rutin (3), $C_{27}H_{30}O_{16}$

studies, they have shown an inhibitory effect on proteases of various types of coronaviruses [7].

Quercetin (Qu) is one of the most important plant molecules, showing pharmacological activity such as antiviral and anti-inflammatory effects. It has also been demonstrated to have a wide range of anti-cancer properties, and several reports indicate its efficacy as a cancer-preventing agent [8]. Quercetin **2** (Fig. 1), chemical name 2-(3,4-dihydroxy phenyl)-3,5,7-trihydroxychromen-4-one or 3,3',4',5,7-pentahydroxyflavone, is classified as a flavonol, one of the six subcategories of flavonoid compounds, and is the major polyphenolic flavonoid found in various vegetables and fruits, such as berries, dill, apples, and onions [9].

According to research results [10], Qu and other several substances exhibited better potential inhibition than Hydroxy-Chloroquine against COVID-19 main protease active site and ACE2. Based on the results obtained by computational methods on molecular docking, it is anticipated that Qu could affect SARS-CoV-2 by interacting with 3CLpro, PLpro, and/or S protein [8, 11].

Thus, Qu is currently promising as a biologically active substance of natural origin, capable of exerting a nonspecific complex effect on inflammatory and destructive processes in the body, and Qu be considered promising in the treatment of allergic pathology, inflammatory and non-inflammatory diseases (Alzheimer's). One of the special effects of quercetin is its protective effect on the vascular endothelium, which is important in COVID-19 since endo-

thelial dysfunction inevitably develops in this pathology [7].

2. Experimental

The following reagents were used in this study: Quercetin (purity 98.2%) from DIA-M (Russia) and Rutin (99.4% purity) from Sichuan Xieli Pharmaceutical Co., Ltd. (China), and locally produced chemicals of the chemically pure grade.

Camphecene - 2-(E)-((1R,4R)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-ylidene-aminoethanol was synthesized at the Novosibirsk Institute of Organic Chemistry (NIOCH SB RAS) (Fig. 2): a mixture of (1R)-(+)-camphor (1.0 equiv.), the appropriate amine (2.5 equiv.), and anhydrous $ZnCl_2$ (0.1% mol on camphor) was reflux for 5–12 h. Diethyl ether was added to the reaction mixture, after completion of the reaction. The organic layer was washed with brine, dried (Na_2SO_4), and evaporated. The crude product was subjected to vacuum distillation [12].

The complex of Camph and Qu and Rut was formed by mixing 96% alcohol solution. The process of complex formation between Qu and Rut and Camph was analyzed using electron-optical UV-Vis spectroscopy (a SPEKS SSP 705-PC spectrometer (CJSC Spectroscopic Systems, Russia).

The complexes of Qu/Rut and Camph were formed by mixing an alcohol solution of Qu/Rut and Camph at the molar ratio of 1:1 and 2:1 (using ethanol as alcohol). The stoichiometry of the complex was evaluated by the dependence of optical density of a Camph solution (measured at 201 nm) on Qu/Rut concentration. A contribution of Qu/Rut to absorbance was corrected by subtracting the absorbance spectrum of Qu/Rut from the total absorbance spectrum. Measurements were carried out in a quartz cell.

3. Results and Discussion

Following the objectives of the work, sets of complexes of bioflavonoids Qu and Rut with an antiviral drug Camphecene at different molar ratios "guest: host" were synthesized.

For a detailed study of the processes of complexation of Qu and Camph, the absorption spectra of Camph in a mixture with different concentrations of Qu were investigated. In the first step, complexes of Qu with Camph were obtained at molar ratios: 1:1 and 2:1 and studied by UV/Vis spectroscopy. The binding of Camph and Qu after mixing of their solutions was accompanied by changes in the

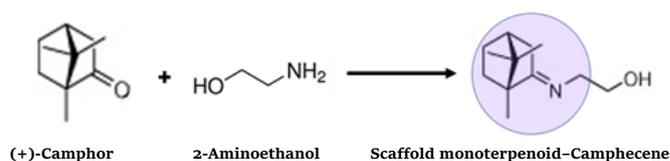


Fig. 2 Scheme of obtaining the scaffold monoterpeneid - Camphecene [12]

absorbance spectrum of Camph indicating the complex formation of these compounds (Fig. 3). The UV-VIS spectrophotometric analysis of the mixture of Camph and Qu has shown that the increase in Qu concentration is accompanied by the change in the shape of the Camph spectrum due to complex formation (its maximum of absorbance becomes lower). As demonstrated in Fig. 3, with an increase in the concentration of Qu from 0 to 0.125 mM, a bathochromic shift of the absorption maximum of Camph (201 → 215 nm) is recorded in the UV/Vis spectra, and a decrease in optical density is observed.

Fig. 3 shows the Camph spectra, which are the difference absorption spectra of the mixture of Camph and Qu and the spectrum of Qu at a given concentration.

An increase in the concentration of Qu (from 0.25 mM and higher) leads to the disappearance of the maximum absorption of Camph, which indicates the complete binding of Camph molecules in the presence of Qu (Fig. 4).

Fig. 4 demonstrates from the dependence of the optical density on the concentration of Qu at the value of the maximum absorption band of Camph $\lambda_{max1} = 201$ nm: the optical density sharply decreases its values depending on the concentration of Qu in the mixture.

The study of Rut (Fig. 5) demonstrated similar changes in the UV/Vis spectra of the obtained complexes. When the

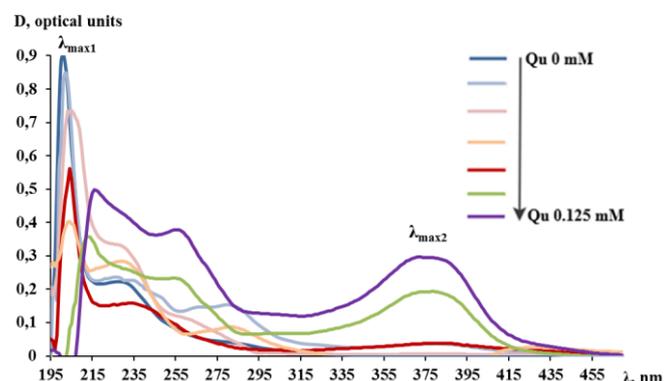


Fig. 3 Absorption spectrum of Camph (C_{Camph} = 0.5 mM) at different concentrations of Qu* (in 96% alcohol solution). The upper spectrum line corresponds to the absorbance spectrum of Camph in the absence of Qu (C = 0.0 mM).

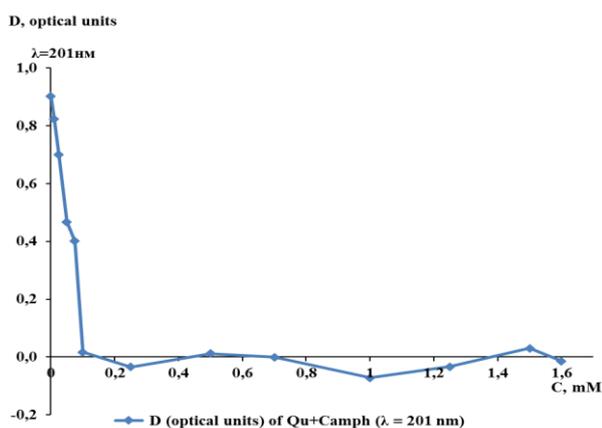


Fig. 4 Dependence of optical density on the concentration of quercetin

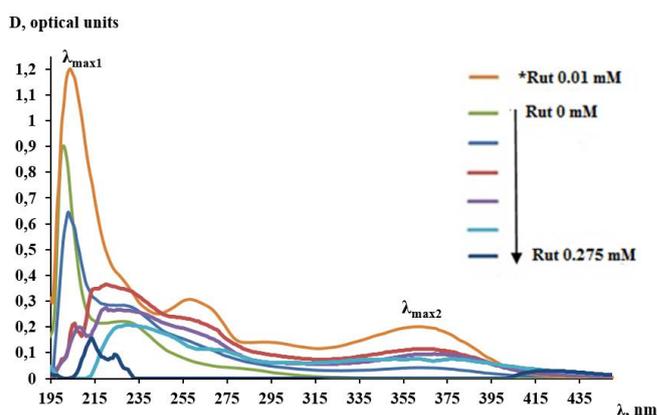


Fig. 5 Absorption spectrum of Camph (C_{Camph} = 0.5 mM) at different concentrations of Rut (*Rut 0.01 mM - standard solution of rutin)

Rut concentration changes in the range from 0.05 to 0.275 mM, a bathochromic shift of the absorption maximum of Camph is recorded (201 → 213 nm).

In this study, the stability constants for the complex of Camph and Qu/Rut were analyzed by changes of optical density of Camph solutions (with its constant concentration, C_{Camph} = 0.5 mM) with variable concentrations of Qu/Rut. To calculate the stability constant of the complexes, we used the Benesi-Hildebrand plot (1) [13]. Eq. (1) is applicable for certain experimental conditions (Camph concentration < Qu/Rut concentration).

The stability constant of the *n*Qu-Camph complex was estimated from the change in the optical density of Camph ($\lambda_{max1} = 201$ nm) at its fixed concentration in solutions in which the Qu (or Rut) concentration was varied. Eq. (1) allows, within the framework of one experiment, not only to estimate the stability constant of the complex (*K*) but also to determine the stoichiometry ratio "host: guest" (*n*) in the complex:

$$\Delta D/D - 1 = 1/[Qu]^n \cdot 1/K \quad (1)$$

where $\Delta D = \Delta \epsilon \cdot [Camph]$ - change in the optical density of the solution, *K* - the constant of stability of the complex, determined for the reaction $Camph + nQu \rightleftharpoons Camph-nQu$:

$$K = \frac{[Camph - nQu]}{[Camph] \cdot [nQu]} \quad (2)$$

The absorption spectrum of Camph was recorded at a wavelength of 201 nm, while the Camph concentration

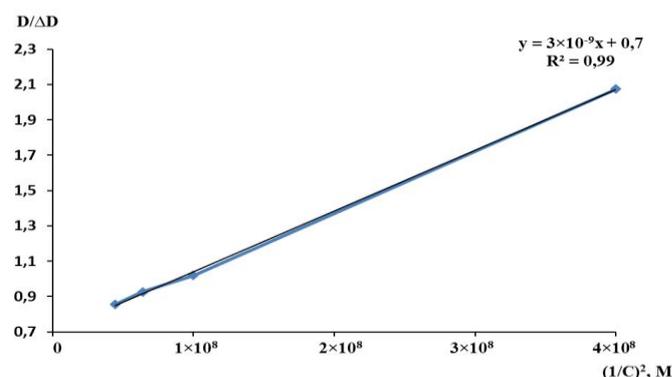


Fig. 6 Dependence of the slope of the straight line $D/\Delta D$ on $1/[Qu]^2$

was constant and amounted to 0.5 mM. The obtained dependence of the absorption intensity of Camphene ($\lambda = 201$ nm) on the concentration of Qu is shown in Fig. 6.

From the slope of the straight line $\Delta D/D$ depending on $1/[Qu]^2$ (Fig. 6), the stability constant of the complex was calculated using Eq. (1). The stability constant for the *Camph+Qu* complex is $1/K = 3 \cdot 10^{-9} M^2$ or $K = 3.3 \cdot 10^8 M^{-2}$.

Recognizing the value of the binding constant, the change in the Gibbs energy was calculated. Obtained from the binding constant, the change in Gibbs's energy $\Delta G = -47.8$ kJ. Based on the obtained negative value, it can be concluded that the reaction proceeds spontaneously during the formation of Qu and Camph complexes. Similarly, according to Eq. (1), the stability constant of the complex *Camph+Rut* was calculated, $1/K = 3 \cdot 10^{-9} M^2$ or $K = 3.3 \cdot 10^8 M^{-2}$. Using the value of the binding constant, the change in Gibbs's energy was calculated. The change in the Gibbs energy $\Delta G = -47.8$ kJ, which allows us to conclude that the reaction proceeds spontaneously during the formation of a complex of Rut with Camph.

Thus, the values of the binding constant Camph for Qu and Rut are comparable, and the same conclusion can be drawn about the change in the Gibbs energy $\Delta G = -47.8$ kJ.

Comparison of the binding constants of the obtained complexes shows that the efficiency of Camphene's complexation with the participation of flavonoids is more efficient ($K = 3.3 \cdot 10^8 M^{-2}$ for the *Camph+2Qu*; *Camph+2Rut* complexes) than with the participation of triterpenoids ($K = 6.94 \cdot 10^6 M^{-2}$ for *Camph+2GA*) [14]. Also, it was found that the complexes of Camphene with the quercetin and rutin are soluble in water, in contrast to the complexes with triterpenoids, which makes it possible to increase the bioavailability of both Camphene and flavonoids.

4. Conclusions

For the first time, the complexation of the Plant's Flavonoids (Quercetin (Qu) and its glycoside - Rutin (Rut)) with the novel Russian antiviral drug Camphene (Camph) was investigated.

The complexes obtained at different molar ratios "host: guest" - 1:1 and 2:1 were studied using UV/Vis spectroscopy.

Formation of the "host: guest" complexes were registered: Qu and Rut molecular complexes (*Camph+2Qu*; *Camph+2Rut*) with a stability constant $K = 3.3 \cdot 10^8 M^{-2}$.

The obtained results demonstrate the high potential of flavonoids Qu and Rut to the development of novel pharmaceutical forms using the Camphene in the form of molecular complexes, as the novel forms of delivery.

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