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Keto-Enol Tautomerization of Quercetin in Solutions of a Cationic Surfactant, Miramistin

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Abstract—Tautomeric transformations of quercetin in solutions of a cationic surfactant, miramistin, have been spectrophotometrically studied. It has been established that, at pH ≥ 6, monoanions of enol-form quercetin are irreversibly transformed into keto-form monoanions, with the rate of this process depending on surfactant concentration and solution pH. It has been shown that the enol tautomer of quercetin is more stable in aqueous solutions, while the ketone form is stabilized in miramistin-containing media. The apparent dissociation constants have been determined for the enol ($pK_a^a = 6.60$) and ketone ($pK_a^a = 5.64$) tautomeric forms of quercetin in micellar solutions of miramistin.

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INTRODUCTION

Almost all medicinal plants contain a flavonoid, quercetin (3,5,7-trihydroxy-2-(3,4-dihydroxyphenyl)-chromen-4-one) [1]. This biologically active polyphenol belonging to the group of vitamins P is widely used in medicine [2] because of its antioxidant, chelating, capillary-anastaltic, anti-edematous, antispasmodic, antihistaminic, and diuretic properties, as well as high anti-inflammatory and antiviral activities. At present, it has been established that flavonoids affect processes in living systems via specific interactions with regulatory proteins [3]. At the same time, as has been known from the chemistry of bioregulatory processes [4], one of the basic structure-related factors of the biological activity of many compounds is their conformational variability under the action of specific intermolecular interactions. The results of quantum-chemical investigations [5–8] have shown the possibility of tautomeric transformations of quercetin molecules, with the enol-to-ketone form transition being most probable (Fig. 1).

Relative contents of the tautomeric forms in a solution depend on the structure of a molecule; the type of substituents in it; and the concentration, temperature, polarity, and acidity of a medium. However, the main role is played by a solvent, variations in the nature of which enable one to widely vary the concentrations of tautomers. According to current notions, organized microheterogeneous media are considered to be solvents of a fundamentally new type that can also affect the tautomeric transformations [9]. We were the first to experimentally confirm that, in organized media of biopolymers, such as human serum albumin (HSA) and poly(vinylpyrrolidone) (PVP), the enol form of quercetin is transformed into the ketone form, this process being accompanied by variations in the spectral characteristics of quercetin with time [10, 11]. It is known [12] that organized media of another type (surfactant micellar solutions) can also affect the tautomeric transformations of organic compounds. As the object for the study, we selected a cationic surfactant, miramistin (MR), which is, at present, one of the most efficient antiseptic agents with a broad spectrum of action with pronounced antimicrobial, antifungal, and antiviral activities [13] and, at the same time, may be used as a solubilizer of poorly soluble flavonoids in drugs. Moreover, amide group present in miramistin is typical of proteins and make it possible to use this surfactant for simulating biological systems. Note that the study of the effect of miramistin on the tautomerization and properties—in particular, acid-base properties—of quercetin is of both theoretical and applied significance for the development of formulations and production processes of drugs.

In this work, spectrophotometry was employed to investigate the effect of miramistin on the enol-to-ketone form transformation of quercetin as depending on solution pH, surfactant concentration, and time, as

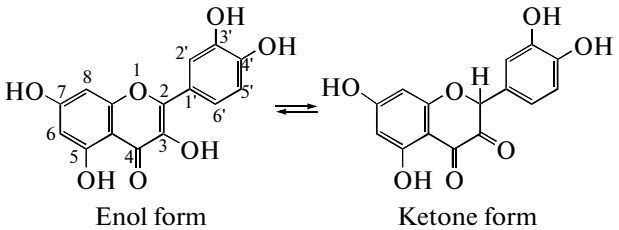


Fig. 1. Schematic representation of the keto-enol tautomerism of a quercetin molecule.

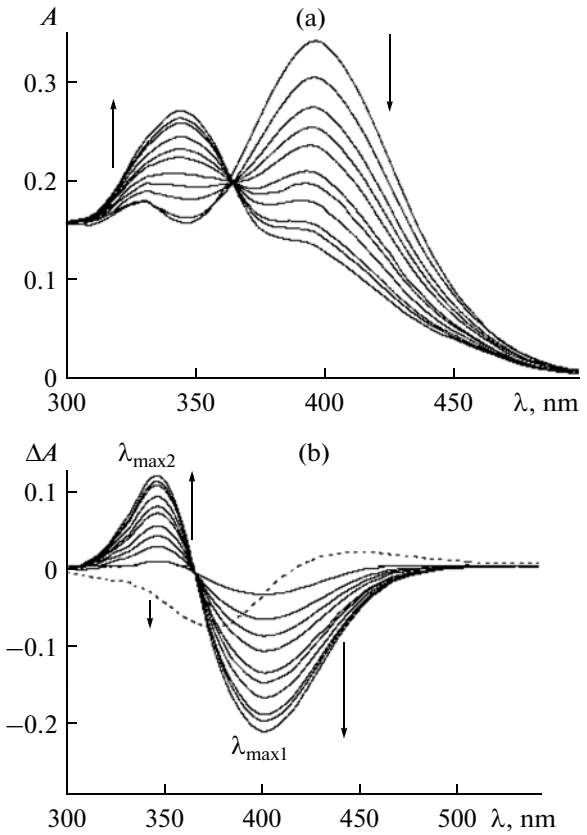
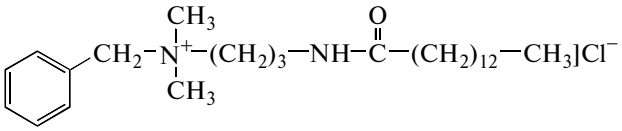


Fig. 2. (a) Absorption spectra of quercetin in the presence of miramistin and (b) their variations measured for 300 min. The dotted curve refers to the spectrum of a miramistin-free solution of quercetin measured in 300 min. $C_{MP} = 1.2 \times 10^{-3}$ M, pH 6.86, $l = 1$ cm, and the interval between successive measurements is 30 min.

well as to determine the acid-base characteristics of both tautomeric forms.

EXPERIMENTAL

Miramistin (benzyldimethyl[3-(myristoilamino)propyl]ammonium chloride) (Infamed) was used in this work.



Structural formula of miramistin

Initial solutions of quercetin, rutin (Sichuan Xieli Pharmaceutical Co. Ltd., Korea), and the cationic surfactant were prepared by dissolving precisely weighed portions of the preparations. In all experiments, the concentrations of flavonoids and ethanol were 2×10^{-5} M and 4%, respectively; the ionic strength was $\mu = 0.2$ M (NaCl).

The acidity of the solutions was preset in a pH range of 4–8 by adding phosphate buffers and controlled using a Hanna Instruments HI 221 universal ion meter equipped with a glass electrode. Electron spectra were recorded with a Specord M-40 spectrophotometer (Germany).

In order to quantitatively estimate the effect of miramistin on the acid-base properties of the enol and ketone forms of quercetin in a pH range of 3–8, apparent dissociation constants (pK_a^a) were measured spectrophotometrically [14]:

$$pK_a^a = pH + \log\{[ROH]/[RO^-]\}$$
$$= pH + \log([A_{RO^-} - A)/(A - A_{ROH})],$$

where A, A_{ROH}, and A_{RO^-} are the light absorption values by an analyzed solution and micellar solutions of miramistin that contain quercetin in the molecular (ROH) and deprotonated (RO^-) forms, respectively, and pH is the acidity of the analyzed solution.

RESULTS AND DISCUSSION

Previously, the study of the stability of solutions of supramolecular complexes of flavonoids with PVP and HSA [10, 11] showed that the absorption spectra of quercetin in these systems substantially varied with time, namely, the intensities of bands with λ_{max1} (385 nm (PVP) and 399 nm (HAS)) gradually decreased, while new bands with λ_{max2} (335 nm (PVP) and 327 nm (HSA)) simultaneously arose, which were attributed to the formation of the keto-tautomer of quercetin.

The absorption spectra of quercetin in a miramistin solution measured with intervals of 30 min (Fig. 2) show that a new band with $\lambda_{max2} = 345$ nm arises in miramistin micellar solutions (Fig. 2a) similarly to those in organized solutions of PVP and HSA. Variations in these spectra (ΔA) relative to the spectrum of the solution measured immediately after the reagents are mixed, i.e., at $t = 0$ min (Fig. 2b), exhibit a reduction in the intensity of the band with $\lambda_{max1} = 400$ nm and simultaneous growth of the band with $\lambda_{max2} = 345$ nm. Under these conditions (pH 6.86), only a slight decrease in the intensity of the band with $\lambda_{max1} = 375$ nm and the appearance of a low-intensity band at 450 nm (Fig. 2b, dotted line) are observed in the spectrum of a quercetin solution per se over 300 min, with the latter band obviously corresponding to partial oxidation of quercetin with the formation of quinoid structures. Therefore, it may be assumed that the appearance of the band at $\lambda_{max2} = 345$ nm in the miramistin micellar solution is associated with the formation of keto-form of quercetin as a result of its tautomerization. Moreover, the absence of a rise in the absorption at 450 nm in this system indicates higher stability of quercetin solutions in the presence of the surfactant.

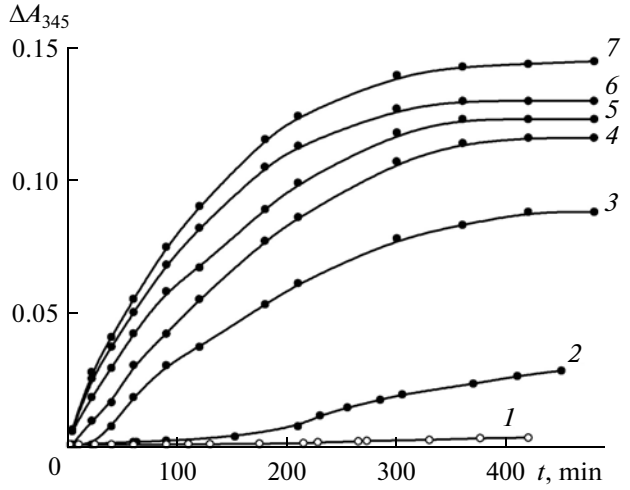


Fig. 3. An increase in the intensity of the band with $\lambda_{max2} = 345$ nm in the spectra of quercetin solutions with different miramistin concentrations as depending on time. C_{MR} (10^{-3} M): (*1*) 0.0, (*2*) 0.22, (*3*) 0.484, (*4*) 0.67, (*5*) 1.79, (*6*) 2.24, and (*7*) 5.15; pH 7.4; and $l = 1$ cm.

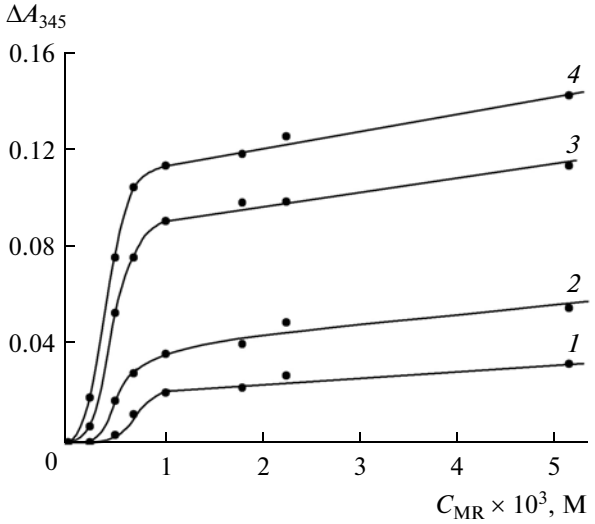


Fig. 4. An increase in the intensity of the band with $\lambda_{max2} = 345$ nm in the spectra of quercetin solutions as depending on miramistin concentration in different time periods (min): (*1*) 30, (*2*) 60, (*3*) 180, and (*4*) 400; pH 7.4, and $l = 1$ cm.

In order to confirm the above-proposed scheme of keto-enol tautomerization of quercetin in the medium of miramistin (Fig. 1), similar studies were performed with rutin, which represents quercetin the labile proton of the C_3–OH group of which is substituted by rutinose, which must hinder the keto-enol tautomerism. It has been experimentally established that, similarly to the cases of HSA and PVP [10, 11], in the presence of miramistin, the spectra of rutin remain unchanged with time (72 h); i.e., in the absence of the labile proton in the C_3–OH group, keto-enol tautomerism indeed does not take place. Thus, the interaction with miramistin facilitates the keto-enol tautomerization of a quercetin molecule, while the hypsochromic shift observed for the maximum in the absorption spectrum of keto-form of quercetin is due to a distortion of the system of π-conjugated bonds between γ-pyrone and phenol groups.

The study of variations in the intensity of the absorption band with $\lambda_{max2} = 345$ nm attributed to keto-form of quercetin in miramistin solutions with different concentrations has shown that it grows with time and depends on miramistin concentration. Corresponding $\Delta A_{345} = f(C_{MP})$ dependences measured in 30, 60, 180, and 400 min (Fig. 4, curves *1*–*4*, respectively) are S-shaped, thus indicating the occurrence of several processes. As the surfactant concentration increases, an abrupt inflection is observed in the curves at $C_{MP} = 1 \times 10^{-3}$ M, which is its critical micelle concentration (CMC) [15]. In the concentration range in which premicellar associates of this surfactant are formed, 0.22×10^{-3} M $< C_{MP} < 1 \times 10^{-3}$ M, growth of the λ_{max2} band of quercetin is more intense than in micellar solutions; i.e., the tautomeric transformation of the enol-form of quercetin into its ketone form essentially depends on the CMC value of miramistin.

It is known that the relative contents of the tautomeric forms in a solution depend, in particular, on the acidity of a medium [9, 12]. It has been revealed (Figs. 5, 6) that, in a micellar medium of miramistin, the absorption band of quercetin at 345 nm arises only at solution pH above 6, which may be related to the onset of the dissociation of this flavonoid ($pK_{a1} = 7.12$ [10]) and the formation of its monoanion. However, we have established [10, 11] that the formation of supramolecular complexes of quercetin with PVP and HSA is accompanied by a decrease in its dissociation constant. Therefore, based on variations in the spectra of quercetin in a miramistin micellar solution (Fig. 7, curve *1*) with an increase in pH (Fig. 7, curve *2*), apparent dissociation constant $pK^a_{a1} = 6.8$ was determined and the contents of the acid and base forms of quercetin was calculated.

The data exhibited in Fig. 6 show that the rate of the growth in the intensity of the band at 345 nm assigned to the keto-form ($\Delta A_{345}/\Delta t$) varies with an increase in pH (curve *1*) symbatly to the formation of quercetin monoanion (curve *2*). Hence, it is the enol-form quercetin anion that undergoes the tautomeric transformation and the keto tautomer formed under these conditions also occurs in the anionic form. Indeed, the acidification of this solution causes a hypsochromic shift of the band with $\lambda_{max} = 345$ nm (Fig. 7, curve *3*) to 300 nm (Fig. 7, curve *4*). This shift is reversible and characterizes the acid-base equilibrium of the ketone form of quercetin, which has resulted in the determination of the apparent dissoci-

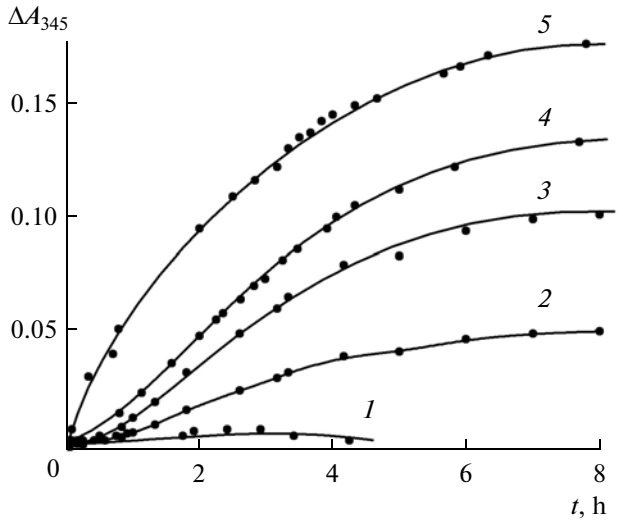


Fig. 5. An increase in the intensity of the absorption band with $\lambda_{max2} = 345$ nm in the spectra of quercetin solutions in the presence of miramistin at different pH values as depending on time. $C_{MP} = 2.0 \times 10^{-3}$ M; pH = (*1*) 6.00, (*2*) 6.20, (*3*) 6.52, (*4*) 6.86, and (*5*) 7.4; and $l = 1$cm.

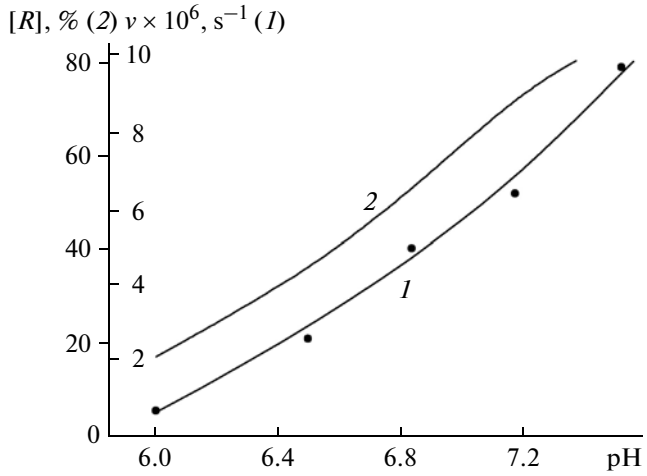
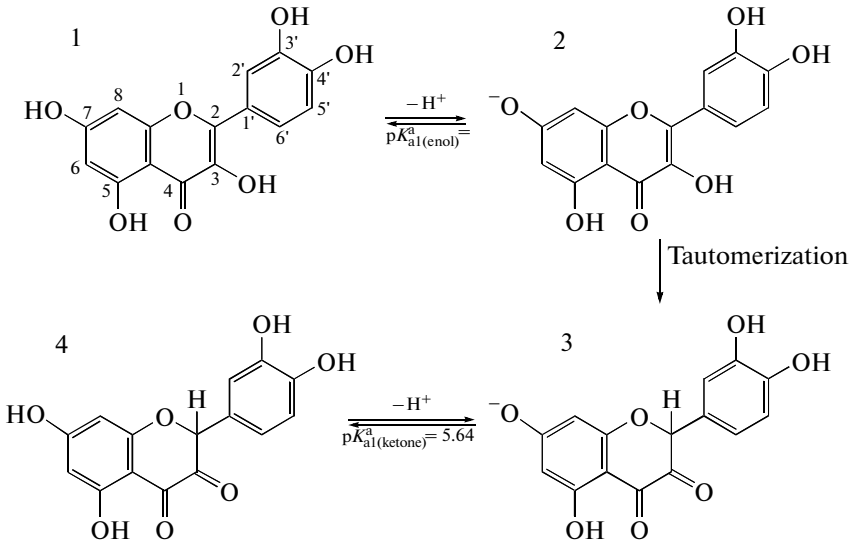


Fig. 6. The pH dependences of (*1*) initial rate of variation in the light absorption by quercetin at $\lambda_{max2} = 345$ nm ($v = \Delta A_{345}/\Delta t$) and (*2*) the calculated concentration of the deprotonated form of quercetin in a miramistin micellar solution at $C_{MP} = 2.0 \times 10^{-3}$ M.

ation constant of keto of form quercetin, $pK^a_{a1(ketone)} = 5.64$. Thus, based on the obtained experimental data, the absorption spectra presented in Fig. 7 have been identified as the spectra of the enol (curves *1*, *2*) and ketone (curves *3*, *4*) tautomers of quercetin occurring in the molecular (*1*, *4*) and anionic (*2*, *3*) forms in miramistin micellar solutions. The data obtained have led us to represent the protolytic equilibria of the enol and ketone forms of quercetin and the corresponding tautomeric transition in a miramistin micellar solution as follows:



Thus, it has been experimentally established that, in miramistin solutions, the enol form of quercetin passes to the ketone form, with the rate of this process being dependent on surfactant concentration and solution pH. The enol form is the more stable tautomer of quercetin in the case of its molecular solutions in water, while the ketone form is stabilized in organized media. The results obtained are of practical

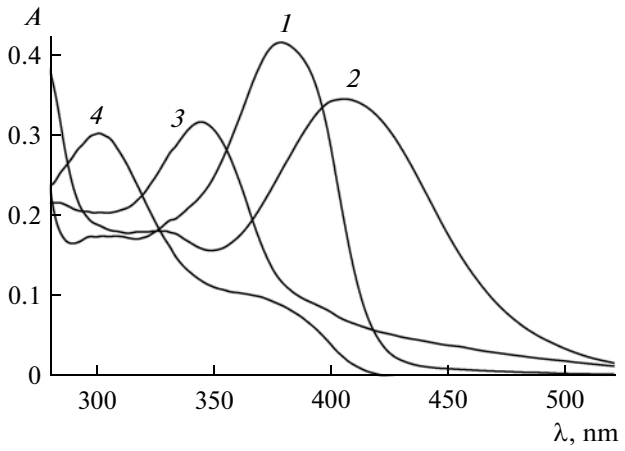


Fig. 7. Absorption spectra of (*1*, *2*) enol and (*3*, *4*) ketone tautomers of quercetin in the (*1*, *4*) molecular and (*2*, *3*) anionic forms in miramistin micellar solutions at $C_{MP} = 2.0 \times 10^{-3}$ M, pH = (*1, 4)* 3.0 and (*2, 3*) 7.4, and $l = 1$ cm.

significance for the development and standardization of formulations and dosage forms of drugs based on the quercetin–miramistin system.

REFERENCES

1. *Flavonoids. Chemistry, Biochemistry and Applications*, Andersen, O.M. and Markham, K.R., London: CRC, 2006.
2. Mashkovskii, M.D., *Lekarstvennye sredstva* (Drug Products), Moscow: Novaya Volna, 2005.
3. Williams, R.J., Spencer, J.P.E., and Rice-Evans, C., *Free Radical Bio. Med.*, 2004, vol. 36, p. 838.
4. *Khimiya bioregulyatornykh protsessov* (Chemistry of Bioregulatory Processes), Kukharya, V.P. and Luik, A.I., Eds., Kiev: Naukova Dumka, 1991.
5. Gritsenko, O.M., Degtyar'ov, L.S., and Pilipchuk, L.B., *Farm. Zh.*, 1999, no. 2, p. 34.
6. Kazakova, O.A., Gun'ko, V.M., Lipkovskaya, N.A., et al., *Colloid J.*, 2002, vol. 64, p. 412.
7. Potyagailo, A.L., Pilipchuk, L.B., and Govorun, D.M., *Biopolim. Kletka*, 2001, vol. 17, p. 256.
8. Gritsenko, O.M., Pilipchuk, L.B., Bogdan, T.V., et al., *Farm. Zh.*, 2003, no. 1, p. 62.
9. Shtykov, S.N. // *UNIVERSITATES*, 2003, no. 2, p. 20.
10. Barvinchenko, V.M., Lipkovska, N.O., Fedyanina, T.V., et al., in *Nanomaterials and Supramolecular Structures: Physics, Chemistry, and Applications*, Shpak, A.P. and Gorbyk, P.P., Eds., Dordrecht: Springer, 2009, p. 303.
11. Lipkovskaya, N.A., Barvinchenko, V.N., Pogorelyi, V.K., et al., in *Fiziko-khimiya nanomaterialov i supramolekulyarnykh struktur* (Physical Chemistry of Nanomaterials and Supramoleuclar Structures), Shpak, A.P. and Gorbik, P.P., Eds., Kiev: Naukova Dumka, 2007, vol. 2.
12. Mchedlov-Petrosyan, N.O., *Differentsirovanie sily organicheskikh kislot v istinnykh i organizovannykh rastvorakh* (Differentiation of Organic Acid Strength in True and Organized Solutions), Kharkov: Khar'kovsk. Nats. Univ. im. V.N. Karazina, 2004.
13. Instruction for Application of Miramistin Preparation, registration no. R 001926/01-2002 of 13.12.07.
14. Bernshtein, I.Ya. and Kaminskii, Yu.L., *Spektrofotometricheskii analiz v organicheskoi khimii* (Spectrophotometric Analysis in Organic Chemistry), Leningrad: Khimiya, 1986.
15. Barvinchenko, V.N., Lipkovskaya, N.A., Chepel', L.I., et al., in *Khimiya, fizika i tekhnologiya poverkhnosti. Mezhved. sb. nauchnykh trudov* (Surface Chemistry, Physics, and Technology), Kiev: Naukova Dumka, 2009, no. 15, p. 181.

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