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ORIGINAL PAPER

Polymorphism and Intramolecular Proton Transfer in Fluoroquinolone Compounds

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Abstract Electronic absorption, luminescence, IR and Raman spectra of polymorphous forms of fluoroquinolones were investigated. Assignment of the band maxima due to π→π* and n→π* electronic transitions were done. The structural changes are responsible for the absorption band modifications. One-electron transitions in the long wavelength region, excitation wavelengths, oscillator strengths and involved molecular orbitals for the zwitter-ionic and cationic protonated forms for different fluoroquinolones were calculated with quantum-chemical and molecular dynamic methods. The electron density redistributions on the FQs separate fragments during the photoexcitation to the S_1*-state were carry out by Mulliken calculations. It was shown that the degree of neutral and zwitter-ion FQs penetration through the bacterium membrane is different.

Keywords Fluoroquinolone · Fluorescence · Quantum-chemical calculations · Electronic structure · Zwitter-ion · Penetration through the bacterium membrane · Electron density redistribution · Excitation levels

Abbreviations

FQ	Fluoroquinolone
fq^-	Anionic form of fluoroquinolone
fqH	Neutral form of fluoroquinolone
$fqH^{\pm}$	Zwitter-ionic form of fluoroquinolone
fqH_2^+	Cationic form of fluoroquinolone
nfqH	Norfloxacin
cfqH	Ciprofloxacin
pfqH	Pefloxacin
enfqH	Enoxacin
UV	Ultra-violet radiation
LUMO	Lowest unoccupied molecular orbital
HOMO	Highest occupied molecular orbital
DLoPC	1,2-Dilinoleoyl-sn-Glycero-3-phosphcholine

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Introduction

Fluoroquinolone (FQ) compounds belong to one group of antibiotics which are used in medicine [1–3] at present. They are widely used as drugs against gram-negative and gram-positive bacteria. Fluoroquinolones affect the bacterial topoisomerases: topoisomerase-IV, DNA-gyrase, ferments. Being located in active center they act as antiferments [4].

In aqueous solutions it is the dynamic equilibrium of several FQ protolytic forms, including anionic (fq^-), neutral

(fqH), zwitter-ionic ($fqH^{\pm}$) and cationic (fqH_2^+) forms [5] (Scheme 1).

Neutral and zwitter-ion forms prevail at pH 7 (Scheme 2).

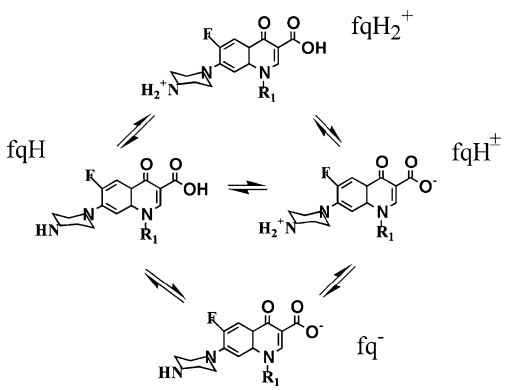
Discussed FQs of general structure (Scheme 2) are present below:

FQs	The location of substituent		
	R_1	X	R_2
norfloxacin (nfqH)	$-C_2H_5$	CH	H
ciprofloxacin (cfqH)		CH	H
pefloxacin (pfqH)	$-C_2H_5$	CH	CH_3
enoxacin (enfqH)	$-C_2H_5$	N	H

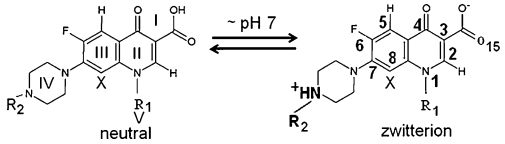
Crystal structures of zwitter-ion forms of FQs were described for norfloxacin [6], ciprofloxacin [7], and enoxacin [8]. The presence of several functional groups in the FQ crystal structures which are able to be protonated can strongly influence on the FQs penetrability when they diffuse trough the bacterium lipid bilayers [9].

Under UV-irradiation, different rearrangements in the FQ structures including the formation and opening of the piperazine ring and proton migration can take place. Photochemical reactions accompanied by intra- and intermolecular proton transfer [10, 11] are characteristic for FQs under UV. Similar elementary reactions connected with the H^+ transfer play a hub role in numerous fermentative reactions. Electron density redistribution between separate molecular fragments due to the intramolecular proton transfer reflects in the spectral-luminescent properties of FQs polymorphic forms.

The paper aims to estimate the influence of polymorph transformations on the spectral luminescent properties, electron structure and the diffusion degree of different FQs polymorphous forms.



Scheme 1 Dynamic equilibrium of different FQs protolytic forms in aqueous solutions



Scheme 2 Dynamic equilibrium of different FQs polymorphic forms in aqueous solutions

Experimental Section

Materials

Finely dispersed powders of norfloxacin (nfqH), ciprofloxacin (cfqH), enoxacin (enfqH) and pefloxacin (pfqH) were purchased from Sigma Chemical Co. (St. Louis, U.S.A.) and were used as received. The initial concentration of the compounds in water, 10^{-3} mol/l, was created via dissolving an accurately weighed sample of a substance in 100 ml of distilled water. The working concentration of the test substance was 5.10^{-5} mol/l.

Methods

Absorption spectra were recorded on a LOMO SF-258 UVI spectrophotometer in quartz cells (l=1 cm) and luminescence spectra were measured with a Shimadzu RF-1501 instrument. The pH was measured with a Thermo Orion 920A plus pH-meter.

Quantum-chemical calculations were carried out by the B3LYP functional and 6–31 G basis set within the GAMESS computational package [12]. For the molecular dynamic (MD) simulations it was used the B3LYP [13, 14] functional and SVP basis set within the Turbomole v.6.1 computational package [15]. The starting structures for the MD-DFT optimization were taken from classical molecular dynamics simulations by including also water molecules beyond the first solvation sphere. The water molecules within a distance of 2.5 Å surrounding the compounds were considered in the calculations after testing with smaller and larger values.

Results and Discussion

Absorption, Luminescence, IR and Raman Spectra

The absorption spectra of fqH are close. As an example, in Fig. 1 the absorption spectrum of norfloxacin (nfqH) is shown. It is characterized by several bands in the UV-region. The UV/vis spectra of quinolone have characteristic absorption bands. A first maximum is observed between 240 and 300 nm which is due to the $\pi \rightarrow \pi^*$ electronic transition in the aromatic ring. The longest wavelength maximum (between 300 and 380 nm) is due

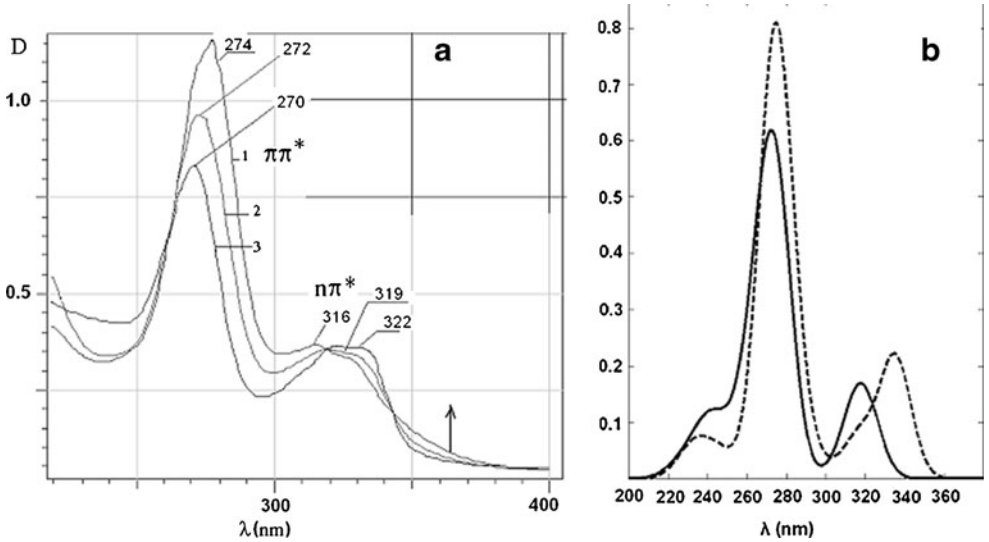


Fig. 1 **a** experimental absorption spectra of nfqH at 1–pH 2, 2–pH 7, 3–pH 9 ($c = 5\times10^{-5}$ mol/l), **b** calculated spectra of nfqH (*full line* zwitterionic form, *dotted line* cationic form)

to an n→π* electronic transition and consists of two subpeaks which are caused by an equilibrium of the quinolone molecules forming an intermolecular hydrogen bond with the solvent and quinolones forming an intramolecular hydrogen bond between the 4-keto and the 3-carboxylic acid group.

Experimental spectra of nfqH (Fig. 1a) exhibit gradual quenching of the ~320 nm band with increasing acid (pH= 2) concentration. Simultaneously, a new band with a significantly red shifted maximum (~365 nm) appears. The band of the π→π* electronic transition is slightly red-shifted (Δλ≈4 nm) and its optical density is also gradually increasing. This general features are well reproduced in the calculated spectra presented in Fig. 1b.

Analysis of the experimental data coupled with the theoretical calculations shows that a stepwise increasing of the H^+ concentration results in a different protonation order of the oxygen and nitrogen atoms depending on the protolytic forms of the FQs. For instance, monoprotonated forms of $nfqH_2^+$, $pfqH_2^+$ and $cfqH_2^+$ is formed by protonation of the oxygen atom of carboxyl group that is H-bonded to the 4-keto group. Diprotonated form, when one of the oxygen atoms of –COOH group is occupied, is characterized by the protonation of the 4-keto group with intramolecular H-bond formed with the second oxygen atom carboxyl group. This results in the redistribution of the electron density along the π-conjugated system. The structural changes are responsible for the absorption band modifications. For instance, quenching of the n→π* electronic transition band at 300–320 nm due to closing six-membered pseudo-ring (Fig. 1a). The growth of the new absorption band at 365 nm which occurs in the acid nfqH solutions with a large proportion of diprotonated species, may be a reflection of the structural changes of –COOH group. Namely, one of its O atoms is protonated and the second one is bonded to the 4-keto group.

Generally, the n→π* type electronic transitions exhibit the blue-shifts of absorption bands because of the increased stability of the ground state compared with the excited state of the molecule mainly due to H-bonding of the lone-pair electrons.

IR- and Raman-spectra of neutral and zwitter-ion forms of fqH are sensible to the proton addition to the oxygen atom of carboxy group or to the nitrogen atom of piperazine ring [16]. Thus, the band in the region 1730–1700 cm^{-1} corresponds to the stretch of carbonyl group ν(C=O) of COOH for neutral form. IR- and Raman spectra of zwitter-ion $nfqH^{\pm}\bullet2H_2O$ are shown in Fig. 2. For zwitter-ion form of FQs, there is no band ν(C=O) of carboxyl group in its

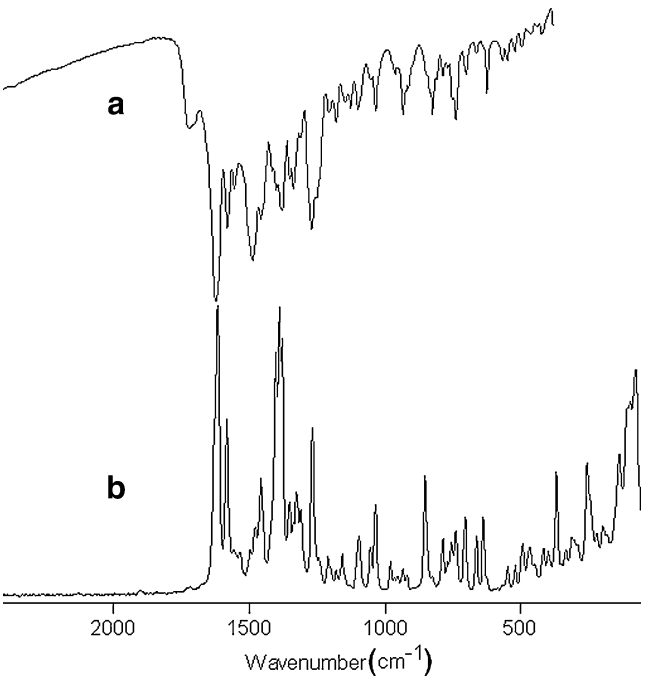


Fig. 2 IR (**a**) and Raman (**b**) spectra of $nfqH^{\pm}\bullet2H_2O$

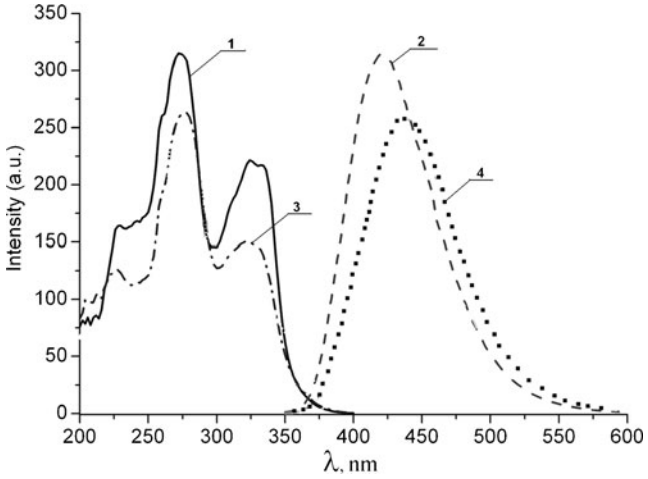


Fig. 3 Fluorescence excitation spectra (1, 3) and emission spectra (2, 4) of nfqH in D_2O (1, 2) and H_2O (3, 4); C = 1 0^{-5} M, pH=6.0

IR-spectra. The largest intensity bands in the Raman spectra of $fqH^{\pm}$ are assigned to the $\nu_s(COO^-)$ vibration in the region of 1380–1405 cm^{-1}. Its intensity in the IR-spectrum is low that corresponds to the selection rules. For the $nfqH^{\pm}$, both IR and in Raman bands of carboxylic-anion are active: $\nu_s(COO^-)$=1382 cm^{-1}, $\nu_{as}(COO^-)$=1586 cm^{-1}. We assigned the intensive band at 1625–1620 cm^{-1} to the stretch vibration ν(C=O) of carbonyl group in position 4 (see Scheme 2). It also appears in the Raman spectrum of similar intensity.

Spectral-luminescence characteristics of aqueous solutions of FQs with different number of H^+ were presented in [17]. The nfqH fluorescence excitation emission spectra of nfqH in D_2O and H_2O are shown in Fig. 3. The fluorescence excitation spectra correlate well with the absorption spectra shown in Fig. 1a. In deuterated water, the fluorescence intensity increases, and a spectral peak is blue-shifted on 15 nm. The increasing of fluorescence intensity of FQs in D_2O is due to decrease of nonradiative energy loss. The fluorescence intensity of crystalline $nfqH^{\pm}$•$2H_2O$ is low and its maximum lies at 460 nm.

Theoretical Investigations

Table 1 summarizes the vertical singlet excitations responsible for the long wavelength absorption of the zwitter-ionic and protonated forms of nfqH and enfqH.

The transition wave lengths in Table 1 reveal the fact that protonation of the nfqH zwitter-ion shifts the 320 nm absorption maximum by 16 nm. Simultaneously, its intensity increase is observed. Thus, until complete protonation is attained, the experimental spectrum will be a superposition of spectra from zwitter-ionic and protonated cationic forms which would explaining the gradual quenching of the 320 nm absorption and the rise of the longer wavelength peak.

Proton transfer among two heteroatoms along the H-bond system proceeds in ground and excited states with low activation energy and with high velocity constants (10^{10}–10^{11} l/mol*s) [18].

Mulliken calculations allow to trace the electron density redistribution on the FQs separate fragments during the photoexcitation to the S_1*-state. According to the calculations of donor-acceptor complexes with close structure, an electron structure of compounds changes during the photoexcitation. Electron density of the different FQs polymorphic forms is non-uniformly distributed on separate molecule fragments. The total charge transfer changes (i.e. the charge transfer number [Δ q(e)]) describing quantitatively the electron density transfer between the molecule fragments are presented in Table 2. The examples include pfqH and cfqH. The total energy of fqH in neutral form is lower than that of zwitter-ionic form (Table 2). The same regularities are the other antibiotics concerned.

The IUPAC numeration of the atoms in quinolones is given in Scheme 1 (right) while Scheme 1 (left) shows the fragment numbering used in theoretical calculations.

As seen from Table 2, all FQs have strong donor-acceptor interaction. Methyl group and the ring fragment IV are the electron density donors, while the rings I, II, III are the electron density acceptors during the photoexcitation of neutral and cation forms of compounds. The

Table 1 One-electron transitions in the long wavelength region, excitation wavelengths λ (nm), oscillator strengths *f*, involved molecular orbitals and weights (%) for the zwitterionic (ZW) and cationic protonated (CT) forms of nfqH and enfqH calculated at B3LYP/SVP levels of theory

	nfqH				enfqH			
	λ	*f*	One-electron transition	Weight	λ	*f*	One-electron transition	Weight
ZW	320	0.13	H → L; H-1 → L+1	81; 11	322	0.23	H → L	90
	312	0.05	H-1 → L; H → L+1	80; 13	307	0.09	H-1 → L; H → L+1	84; 8
CT	336	0.21	H → L; H → L+1; H-1 → L+1	80; 9; 8	325	0.21	H → L; H → L+1	85; 9
	316	0.08	H → L+1; H-1 → L; H → L	69; 21; 7	310	0.03	H-1 → L; H → L+1	47; 39

Table 2 Charge transfer changes (Δq (e)) on different FQs fragments during the $S_0 \rightarrow S_1^*$ transition

Fragment	$pfqH^-$	pfqH	$pfqH^{\pm}$	$pfqH_2^+$	cfqH
I	0.14	−0.19	0.4	−0.13	−0.13
II	0.03	−0.13	0.05	−0.13	−0.12
III	−0.02	−0.35	0.08	−0.09	−0.19
IV	−0.06	0.47	0.03	0.41	0.56
V	−0.03	−0.08	0.06	0.07	−0.07
CH_3	0.00	0.22	−0.26	0.23	–
O_{15}	0.16	0.03	0.16	0	−0.03
Total energy, Ha	−1148.0803	−1148.6644	−1148.5240	−1149.0568	−1147.4249

quantity of transferred electron density changes depending on substituent. The maximal electron density transfer is observed for pefloxacin (0.69e) and a minimal one is for ciprofloxacin (0.56e). Such appreciable charge transfer leads to the increasing of molecule dipole moments, and the maximal increasing of dipole moments is also for pefloxacin (Δμ=23.7 Db).

The electron density delocalization (distribution) is significantly different in the case of anion and zwitter-ionic state: the excitation of pfq^- and $pfqH^{\pm}$ leads to electron density transition to the piperazinyl ring. As a result, the H-atom becomes negatively charged. The molecule fragment consisting of the ring **I** and $COO^{—}$ -group becomes an electron density donor while methyl group and H-atom of the piperazinyl ring are acceptors (Δq (e)=0.8) for this forms of compounds. Such electron transfer causes the great decrease of the dipole molecule moment (Δμ=−37.7 Db).

The orbital analysis of FQs showed that all boundary orbitals are principally localized on certain molecule fragments.

Boundary orbital's shapes of the different protolytic forms of pfqH are shown in Fig. 4. It is seen that each FQs fragment participates in the electron transition and in the charge transfer. The $S_0 \rightarrow S_1$ transition is the transition from HOMO to LUMO for both of pefloxacin protolytic forms. Molecular orbitals character of neutral FQs is sharply different from that of zwitter-ionic ones. For neutral FQs, HOMO is localized on the ring IV, and LUMO is localized on the rings I, II, III while for the zwitter-ionic molecule, HOMO is localized on COO^- -group and LUMO is mainly localized on the hydrogen atom of the piperazine group. The characters of the $pfqH^-$ LUMO and HOMO are similar to those of $pfqH^{\pm}$. This correlates with the charge transfer numbers of electron density between molecular fragments. The localization of boundary MOs and the electron density transfer have the same character in the structurally related compounds cfqH and nfqH.

The electron density transfer influences the bond strength and length changes. Our calculation results show insignificant interfragment bond strength and length

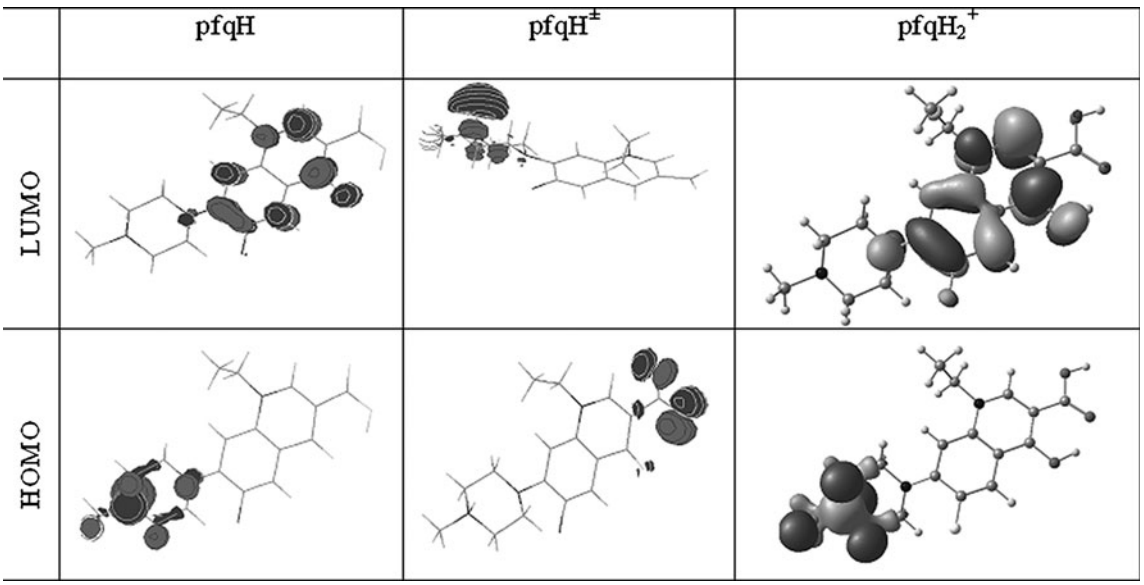


Fig. 4 Highest occupied (HOMO) and lowest unoccupied (LUMO) molecular orbitals of different protolytic forms of pefloxacin

changes with a little degree of charge transfer. Vice versa, the bond strength and length changes significantly in the fragments where charge transfer is large.

The main differences of electronic structure between the zwitter-ionic and the neutral and cationic forms of FQs reflect in the different diffusion degrees of the compounds through the biologic membrane. For example, it has been reported in [9] that the drug protonation conditions play a fundamental role in the kind of interaction between ofloxacin and membrane bilayers. Authors of [9] demonstrated experimentally that ofloxacin is able to interact preferentially with the negatively charged component of a biological membrane through the piperazine moiety of its molecule.

MD simulations were employed to study the diffuse translocation of cfqH as representative of the FQs across a model hydrated DLoPC bilayer. According to our calculations, while cfqH and $cfqH^{\pm}$ coexist at physiological pH, MD reveals that only the neutral form is readily entering the lipid DLoPC bilayer within the considered 300 ns simulation time. In turn, $cfqH^{\pm}$ has no opportunity for the penetration through the membrane bilayer because of the H-bonding of its negative COO^{-}-group—hydrogen bonds are involved in the interaction between $cfqH^{\pm}$ and headgroup region. It comes to agreement with the results of [9].

Conclusions

The comparable analysis of the experimental, quantum chemical and MD simulation data of different forms of the antibiotics FQs shows that the proton position in molecule influences on their spectral luminescence properties and electron density redistributions.

Molecular orbitals character of neutral FQs is sharply different from that of zwitter-ionic ones. The characters of the fqH^{-} LUMO and HOMO are similar to those of $fqH^{\pm}$. This correlates with the charge transfer numbers of electron density between molecular fragments.

Formation of the intra- and intermolecular H-bonds in the strong acid solutions leads to the redistribution of the electron density on some atoms of the studied compounds. It may result in the additional competition of the heteroatoms for the possessing proton. The spectral changes (absorbance and luminescence) in strong acid solutions are due to several different factors including competitive protonation of the oxygen and nitrogen atoms of the FQs compounds.

It was shown that the degree of neutral and zwitter-ion FQs penetration through the bacterium membrane is different. For neutral form it is higher. But after the FQs penetration to the intracellular space the balance among the protolytic forms restores [9].

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