

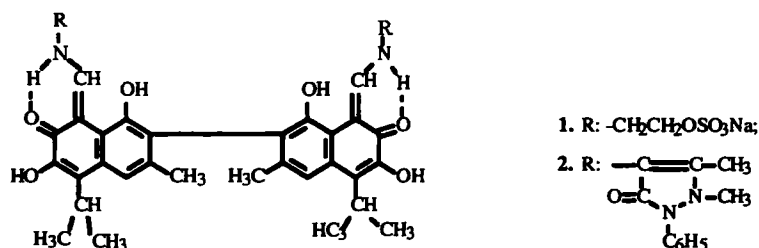
## A STUDY OF THE HYDROLYSIS OF AMINO DERIVATIVES OF GOSSYPOL AT VARIOUS pH VALUES

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UDC 576.656:615.281:615.37

*The results are given of a study of the hydrolysis of alkyl- and heterylimines of gossypol at various pH values.*

Among the numerous Schiff bases obtained from the cottonplant dialdehydonaphthol gossypol (Gp), two substances have been found that possess antiviral and interferon-inducing activities [1], these being products of condensation with the sodium salt of  $\beta$ -aminoethyl sulfate — megosin (1) — and with 4-aminoantipyrine — ragosin (2) [1].



Megosin is already being used in medical practice as an antiherpetic agent for adults. So far as concerns ragosin, at the present time its normative-technological documentation is being prepared for submission to the Pharmaceutical Committee of the Ministry of Health of the Republic of Uzbekistan with the aim of obtaining authorization for its clinical study as an interferon inducer for the prophylaxis and treatment of viral hepatitis, influenza, and other diseases.

In the present paper we give the results of a comparative study of the behavior of megosin and ragosin in buffer solutions modeling physiological fluids.

Aqueous solutions of megosin are unstable, while ragosin is practically insoluble in water and, therefore, for the comparative consideration of the spectra of Gp, megosin, and ragosin we used their solutions in a (1:10) mixture of methanol and water, which dissolves all three compounds well. On the storage of solutions of Gp and ragosin, some decrease in optical density took place, with retention of the general pattern of the spectrum (Fig. 1). In the case of megosin, the maximum at 390 nm disappeared and the pattern of the spectrum changed, witnessing structural changes in the megosin molecule, which is explained by its known hydrolysis to Gp and further transformation in an alkaline medium [2].

Ragosin also proved to be more stable than megosin in a buffer mixture with pH 2.5. While after ragosin had been stored for 120 h there was some decrease in the optical density (from 0.41 to 0.33) although the pattern of the spectrum had scarcely changed, in the case of megosin immediately after its dissolution in a buffer solution with pH 2.5 an intense absorption maximum was observed in the UV spectrum at 390 nm the optical density of which fell during 24 h and then changed no further during 120 h (Fig. 2).

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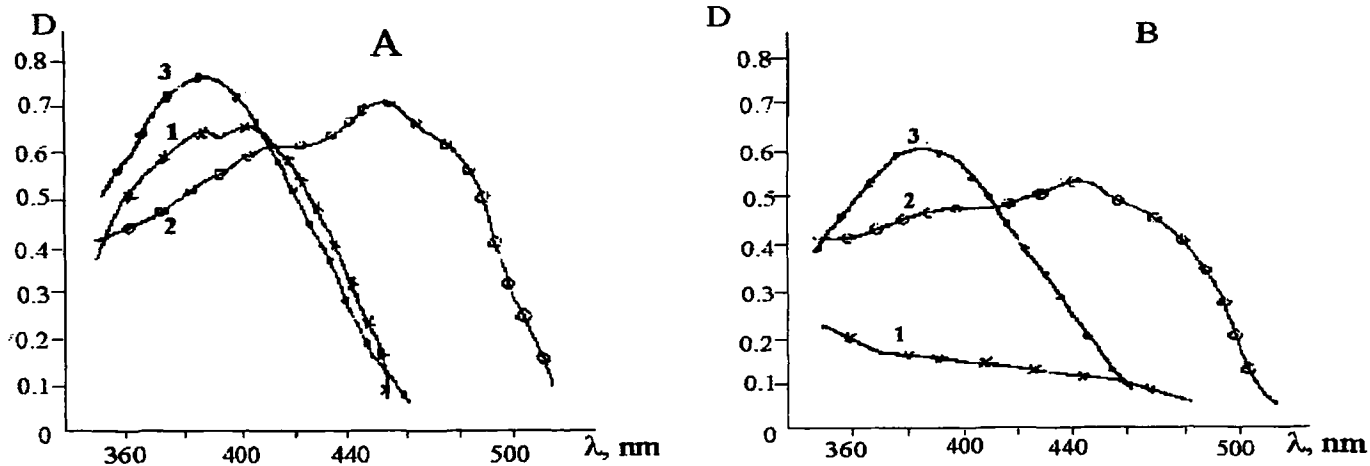


Fig. 1. UV spectra of solutions of megosin (1), ragosin (2), and gossypol (3) in methanol—water (1:10). Here and in Figs. 2—4: A) immediately after dissolution; B) after 120 h.

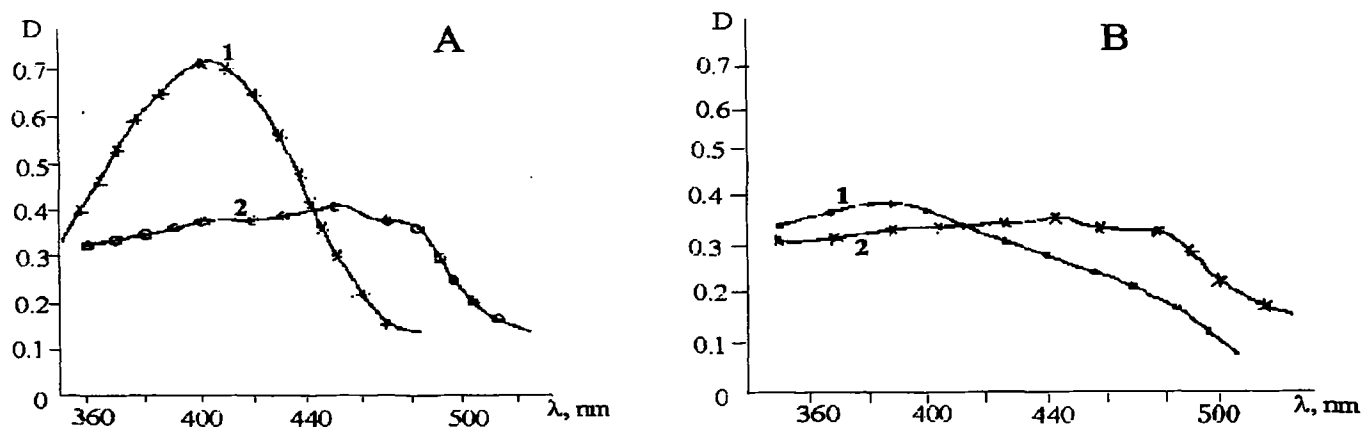


Fig. 2. UV spectra of solutions of megosin (1) and ragosin (2) in a buffer solution with pH 2.5.

It has been shown previously that when Gp is dissolved in an aqueous solution of boric acid a stable complex with an absorption maximum at 390 nm is formed. Apparently, Gp, to which megosin is hydrolyzed on dissolution in the buffer mixture with pH 2.5, forms just such a complex with the boric and phosphoric acids that are components of the buffer solution.

When megosin was dissolved in a buffer solution with pH 7.0, the formation of this complex was observed immediately, as was shown by the appearance of an intense absorption maximum at 390 nm the optical density of which scarcely changed during 120 h, and there was no maximum in the 400—405 nm region (Fig. 3).

When ragosin was stored for 24 h its optical density in the 400—450 nm interval changed only slightly, but after 48 h the broad band in the 400—450 nm region disappeared and an absorption maximum appeared at 380—390 nm. This fact shows that, although ragosin has proved to be more stable than megosin under these conditions, it is also hydrolyzed to Gp with the subsequent formation of a complex.

The difference in the behaviors of megosin and ragosin on hydrolysis can be explained by the influence of the nature of the amine component. It is known that the rate of acid hydrolysis of azomethines is in direct dependence on the degree of basicity of the initial amine: the higher the basicity the greater the rate of hydrolysis [3]. Starting from the known fact of the greater basicity of aliphatic amines than of aromatic and heterocyclic amines, together with the influence of the electron-donating groups in 4-aminoantipyrine and the presence of the NaOSO<sub>3</sub> group in the molecule of β-aminoethyl sulfate, it is possible to explain the difference in the rates of hydrolysis of megosin and ragosin by the lower basicity of the amine component in the latter [4].

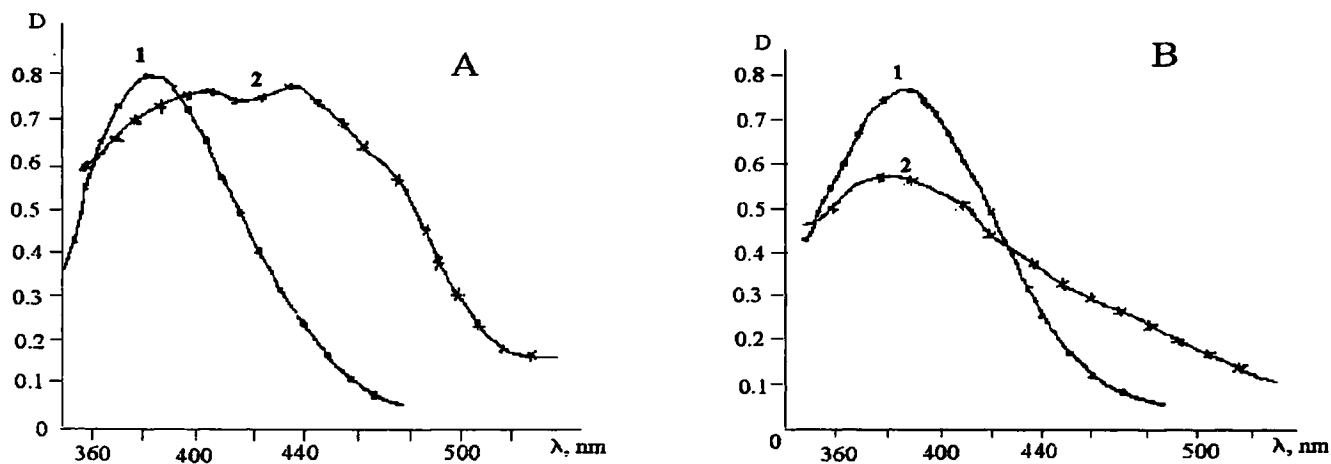


Fig. 3. UV spectra of solutions of megosin (1) and ragosin (2) in a buffer solution with pH 7.0.

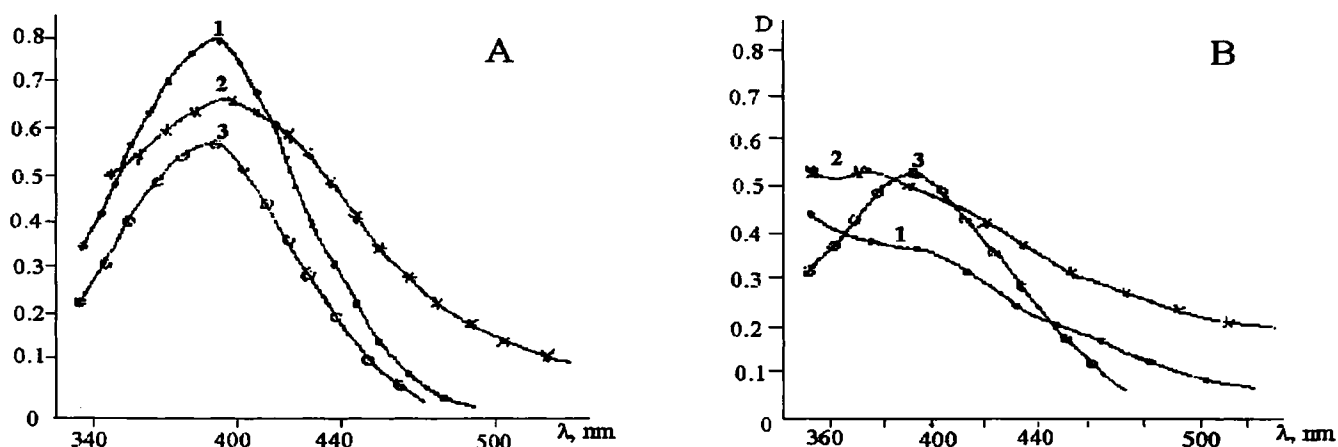


Fig. 4. UV spectra of solutions of megosin (1), ragosin (2), and gossypol (3) in a buffer solution with pH 10.5.

The results of a comparative consideration of the spectra of Gp, megosin, and ragosin taken immediately after their dissolution in a buffer mixture at pH 10.5 proved to be interesting (Fig. 4). In all three spectra an intense absorption maximum was observed in the 390–400 nm region. When the solutions were stored, the pattern changed: in the case of Gp, the optical density at 390 nm fell (from 0.58 to 0.35) but the form of the spectrum did not change. In the case of megosin, in 48 h there was also a fall in the optical density of the maximum at 390 nm (from 0.80 to 0.48), and then the absorption band at 390 nm disappeared. In the case of ragosin, no maximum at 390 nm was observed after only 24 h; i.e., ragosin was more subject to alkaline hydrolysis than megosin. The absence of Gp, established chromatographically, permits the assumption that it undergoes further transformation in an alkaline medium [5].

Thus, in the case of amine derivatives of Gp we observe alkaline hydrolysis of the imines, which, as is known from the literature is less characteristic for enamines and is sometimes observed on heating with alkalis [6–8]. Alkaline hydrolysis is due to the capacity of imines for adding an OH group; i.e., it depends on the acidity of the product formed. And if in the molecule of the azomethine formed there are substituents having such an acidifying action that the acid properties in it predominate over the basic properties, alkaline hydrolysis becomes possible. Cases are known where azomethines did not undergo acid hydrolysis at all but were unstable to the action of alkali [8]. The fact that ragosin proved to be more stable at pH 10.5 than megosin also shows the influence of the nature of the substituent (lower basicity) on hydrolysis, which agrees with information on the alkaline hydrolysis of azomethines [8].

Moreover, the general pattern of the dependence of the hydrolysis of azomethines is somewhat distorted by the *ortho*-

effect — the presence of a hydroxy group in the *o*- position with respect to the azomethine bond, which favors the formation of an intramolecular hydrogen bond, as has been confirmed by NMR spectroscopy in the case of megosin and ragosin [9].

Thus, the results of a study of the hydrolysis of gossypol enamines at various pH values have shown the greater stability of ragosin than of megosin in an acid medium. At the same time, the rate of hydrolysis of ragosin in an alkaline medium is higher than in an acid medium.

## EXPERIMENTAL

Megosin and ragosin were obtained as in [9]. TLC was conducted on Silufol plates in the acetone—diethyl ether (5:2) system. UV spectra were taken on a SF-26 spectrometer ( $C = 0.002\%$ ). Buffer solutions were prepared as described in [10].

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