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GOSSYPOL AND ITS DERIVATIVES AND THEIR COMPLEXES IN SOLUTIONS

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The results are given of a study of gossypol, its physiologically active derivatives, and their complexes with N-polyvinylpyrrolidone (M.m. 8000) in various media. It has been shown that the production of complexes permits the creation not only of water-soluble forms but also of more stable forms of the compounds.

One of the methods for imparting to drugs a capacity for dissolving in water is the production of their complexes with polymers for medical use and, in particular, N-polyvinylpyrrolidone (PVP). In the development of standardizing and technical documentation for such drugs it is necessary to determine the stability of their aqueous solutions and their behavior in media modeling physiological liquids (for example, gastric and intestinal juices).

The present paper gives the results of a chromatographic and UV-spectrophotometric study of the behavior of gossypol (GP) and megosin and batriden and their 1:9 complexes with PVP — pogosin, rometin, and mebavin, respectively — in aqueous solutions and in buffer mixtures in the pH interval of 2.5-10.5.

The instability of GP itself in aqueous solutions and even in weak alkalis is a well known fact [1]. Appreciable changes take place even in the process of dissolving GP, and the intensity of transformation increases on storage. The UV absorption spectra of GP in 0.5% KOH and Na_2CO_3 solutions taken after these solutions had stood at 20°C for 3 h were identical with the UV absorption spectra of the substances obtained in the alkali refining of cottonseed oil and the heat treatment of the seeds. The substances isolated from these solutions do not give the characteristic reactions for gossypol. The hypothesis has been expressed that under the given conditions a far-reaching transformation of GP takes place, an important part in which is assigned to oxidation processes [1, 2].

We have studied the UV spectra of solutions of GP in buffer solutions at pH 2.5-10.5. In all cases the spectra showed three absorption maxima, at 240-245, 290, and 385-390 nm — i.e., with practically no differences from the UV spectrum of GP taken in a freshly prepared chloroform solution. The stability of these solutions for a considerable time (up to 8-10 h), like the stability of GP solutions in a buffer, is apparently due to the formation of complexes with the boric and phosphoric acids present as components of buffer solutions.

In the UV spectrum of an aqueous solution of pogosin an absorption maximum was observed at 380 nm the position and intensity of which scarcely change in the course of 42 h, which showed a fairly high stability of the complex of GP with PVP. Solutions of pogosin in a buffer mixture at pH 2.5 proved to be fairly stable. PG was detected chromatographically in ether extracts from such a solution that had been stored for more than 72 h. This fact, and also the appearance in these solutions of a precipitate — obviously PVP — can be explained by a partial decomposition of the complex. GP was not detected chromatographically in buffer solutions at pH 7.0-10.5, although precipitation of PVP was observed and so was a change in the color of the solution to dark yellow, which may indicate an oxidation of GP under these conditions.

The UV spectrum of megosin in water was represented by a maximum at 245 nm and a broad absorption band with two maxima, at 385 and 405 nm. By analogy with the UV spectra of alkylimines of *ortho*-hydroxyaldehydes, which exist in the quinoid form, the latter may be assigned to an electronic $\pi-\pi^*$ transition. A study of the dependence of the optical density

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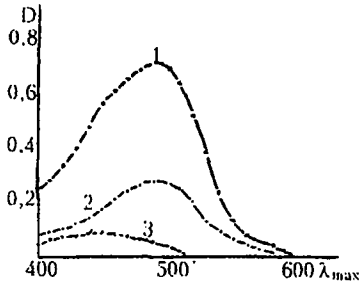

Fig. 1. UV spectra of solutions of batriden in DMSO: *1*) immediately after preparation; *2*) after 72 h; *3*) after 168 h.

of an aqueous solution of megosin on the time of storage showed that a considerable fall in the optical density had taken place after only an hour [3].

We have previously observed a considerable instability of the product of the condensation of GP with *para*-nitroaniline, in a methanolic solution of which GP was detected after only 30 min.

Megosin is likewise very unstable in a buffer mixture with pH 2.5 and the optical density almost halved in the course of a day. Megosin is fairly stable at pH 7.0, and even when its solution was stored for 120 h the optical density had scarcely changed and GP was not detected chromatographically; hydrolysis to GP took place when the solution was heated. When megosin was dissolved in a mixture with pH 10.5, hydrolysis to GP was observed after 24 h and GP was detected in the solution chromatographically.

The ease of hydrolysis of the products of the condensation of GP with amines is due not only to the influence of electron-accepting substituents in the amine component but also to the fact that, as we have established previously, they are enamines for which the ketoimine form of existence in solutions and in crystals has been clearly shown [4, 5]. The basicity of enamines is lower than that of azomethines and they readily undergo acid hydrolysis. The hydrolysis of such compounds in an alkaline medium is usually hindered, but the "*ortho*-effect" connected with the possibility of the formation of a hydrogen bond between the imine group and a substituent present in the *ortho*-position to it somewhat distorts the usual pattern of the dependence of hydrolysis on the basicity of the compound formed, which makes hydrolysis under alkaline conditions possible [6].

With respect to the positions of the maxima, the UV spectrum of an aqueous solution of rometin was analogous to that of an aqueous solution of megosin. But rometin itself in aqueous solution was more stable than megosin: the intensity of the absorption bands at 240 and 385 nm did not change for almost 40 h. The optical density of a solution of rometin in a buffer mixture at pH 2.5 did not change for 20 h. After 96 h, GP was detected in the solution chromatographically and the deposition of a precipitate, obviously PVP, was observed, which showed a breakdown of the complex and hydrolysis of the megosin to GP. In buffer solutions in the pH interval from 7.0 to 10.5 the complex decomposed partially after storage for 96 h.

In taking UV spectra of batriden, one must bear in mind the fact that, in hydroxyl-containing solvents and aqueous–organic mixtures, with various polarities, arylidenebarbituric acids, with which batriden may be classed, decompose rapidly with the cleavage of the exocyclic multiple bond, forming aryl(2,4,6-trioxohexahydropyrimidin-5-yl)carbinols, which break down further into barbituric acid and aldehydes. The half-period of decomposition ranges from a few seconds to several hours, depending on the nature of the substituent in the aldehyde ring, on the nature of the solvent, and on the pH of the medium [7]. In anhydrous aprotic solvents the arylidenebarbituric acids are more stable and exist in the tricarbinol form (pyrimidine ring [8]).

In the UV spectrum of batriden in DMSO taken immediately after the dissolution of the substance two absorption maxima were observed: in the 320 nm region, corresponding to electronic transitions in the pyrimidine ring, and at 430-530 nm — a long-wave absorption maximum the appearance of which is connected with $\pi-\pi^*$ transitions in the branched chain of conjugation GP–CH=C^5–(C^6–O)–C^4=O. The optical density of the long-wave absorption maximum at 495 nm decreased during storage, and after 168 h the absorption maximum had disappeared (Fig. 1). The same situation was observed when batriden was dissolved in DMSO–ethanol (1:9), but here the fall in the intensity of the absorption maximum takes place faster.

According to the literature, this fact can be explained by the addition of water or other elements of the solvent to the exocyclic bond with the formation of a carbinol and possible subsequent solvolysis to GP and barbituric acid or a mixture of

them [7, 8]. And, in actual fact, spots of batriden and GP and an additional spot were observed on a chromatogram of solutions of batriden in DMSO and in DMSO–ethanol (1:9) after storage for 120 h. The presence of barbituric acid in these solutions was established with the aid of a qualitative reaction [9].

The weak-field region of the PMR spectrum of a freshly prepared specimen of batriden in DMSO solution contained the signals of the C^5=CH methine proton at 10.40 ppm and of an aromatic proton at 7.81 ppm, and a set of one-proton singlets corresponding to hydroxy and amine groups. The parameters of the spectrum showed that under the given conditions batriden was present in the form characteristic for arylidenebarbituric acids. When the spectrum of the same specimen was taken after 24 h it showed a singlet at 6.38 ppm, the intensity of the signal at 10.40 had diminished, and a redistribution of the signals of the proton-exchanging groups was observed. On continued storage of the solution, the intensity of the signal at 6.38 ppm continued to rise, with a simultaneous decrease in the intensity of the signal at 10.40 ppm going as far as its complete disappearance. The nature of the change in the spectrum with time showed that the structure of the batriden molecule had clearly undergone changes. On further storage of this solution (for about 6 days) the signals of GP appeared in the spectrum. When this solution was chromatographed, as on the chromatography of stored solutions of batriden in DMSO and DMSO–ethanol (1:9), spots of batriden and GP and one additional spot were observed, and barbituric acid was detected qualitatively.

The UV spectra of batriden in buffer mixtures with pH 2.5 and 7.0 were each represented by a maximum at 260-265 nm, an inflection in the 340-350 nm region, and a weak maximum at 495 nm. The dissolution of batriden in a buffer solution with pH 10.5 was accompanied by a change in the color of the solution from red to blue, the appearance of an absorption maximum at 235-240 nm and of an inflection in the 310-340 nm region, and a plateau in the interval from 360 to 390 mn; the long-wave maximum disappeared, i.e., there were considerable changes in the structure of the batriden molecule, which is in harmony with the known fact of the instability of arylidene compounds in alkaline media [9, 10].

The optical density of an aqueous solution of mebavin at 260 nm did not change in the course of 24 h. On further storage a precipitate — obviously PVP — deposited and the solution was shown by chromatography to contain batriden (R_f 0.8), GP (R_f 0.55), a substance giving a spot with R_f 0.7, and barbituric acid (qualitatively). Obviously, there had been not only breakdown of the complex but also partial solvolysis of the batriden to GP, batriden, and a possible intermediate — gossypolidene(2,4,6-trioxotetrahydropyrimidin-5-yl)carbinol [7].

An analogous pattern was observed in solutions of mebavin with pH 2.5 and 7.0. A difference is that under these conditions, judging from the decrease in intensity of the absorption maximum at 490 nm, the breakdown of the complex proceeded faster than in aqueous solution. The sharpest decrease took place with mebavin on its dissolution in a buffer mixture with pH 10.5. As also in the case of batriden, the solution immediately became blue. The UV spectrum shows an absorption maximum at 255 nm and a plateau in the 360-390 nm region; the long-wave maximum had disappeared.

A comparative consideration of the results obtained has enabled us to trace the nature of the behavior of GP, megosin, and batriden and of their complexes with PVP in solutions with various pH values in dependence on the nature of the substituents introduced through the aldehyde groups. One of the conclusions is that the formation of complexes of GP derivatives with PVP has enabled the production of drug forms that are not only soluble in water but are also more stable on storage. The results obtained can be used to establish the storage lives of aqueous solutions of the complexes if freeze-dried powders are proposed as their medicinal forms. Moreover, the study of the behavior of these compounds in buffer mixtures at pH 2.5-10.5, modeling physiological liquids, will permit the selection of the most effective schemes of application of the medicaments.

EXPERIMENTAL

Batriden and megosin were obtained as in [11], and pogosin, mebavin, and rometin as in [12]. TLC was conducted on Silufol UV-254 plates. UV spectra were taken on a SF-26 spectrophotometer; in the cases of GP, batriden, and megosin at C = 0.002% and in the cases of rogosin, mebavin, and rometin at C = 0.01%.

The buffer solutions were prepared according to [13]. Qualitative reactions for batriden and megosin were performed as prescribed in PS [Pharmacopeial Standard] 42-30-64-94 and PS 42-30-68-94, respectively, and qualitative reactions for barbituric acid as given in [9].

REFERENCES

1. V. P. Rzhekhin, in: The Use of Spectroscopic Methods in the Provisions Industry and in Agriculture [in Russian], LGU (1955), p. 110.
2. A. I. Ismailov, Z. Sh. Shukurov, S. A. Talipov, F. G. Kamaev, R. G. Mardanov, and B. T. Ibragimov, Khim. Prir. Soedin., 50 (1994).
3. L. Biktimirov, Kh. L. Ziyaev, N. I. Baram, and A. I. Ismailov, in: Questions of Transplantology and Immunosuppression [in Russian], Tashkent (1983), p. 3.
4. P. Zuman, Chem. Listy, **46**, 668 (1962).
5. N. I. Baram, F. G. Kamaev, Kh. L. Ziyaev, L. Biktimirov, A. I. Ismailov, G. B. Nazarov, and B. T. Ibragimov, Khim. Prir. Soedin., 650 (1989).
6. B. A. Porai-Koshits, É. M. Poznanskaya, V. S. Shevchenko, and L. A. Pavlova, Zh. Obshch. Khim., **17**, No. 10, 1774 (1947).
7. A. I. D'yachkov, B. A. Ivin, N. A. Smorygo, and E. G. Sochilin, Zh. Org. Khim., **12**, No. 5, 1115 (1976).
8. B. A. Ivin, A. I. D'yachkov, I. M. Vishnyakov, N. A. Smorygo, and E. G. Sochilin, Zh. Org. Khim., **11**, No. 6, 1337 (1975).
9. Chemical Reagents and Preparations [in Russian], Goskhimizdat, Leningrad (1953), p. 229.
10. A. P. Grishchuk and S. N. Baranov, Zh. Obshch. Khim., No. 4, 896 (1958).
11. L. Biktimirov, Kh. L. Ziyaev, B. Khodzaniyazov, D. Ziyamov, N. I. Baram, and A. I. Ismailov, Khim. Prir. Soedin., 198 (1996).
12. G. N. Naumchik and V. A. Vainshtein, Inventors' Certificate 232,718 (1967); Byull. Izobret., No. 35, 36 (1969).
13. Yu. Yu. Lur'e, Handbook of Analytical Chemistry [in Russian], Khimiya, Moscow (1971), p. 238.