DETERMINATION OF THE PRODUCTS FROM THE INACTIVATION OF d-CYCLOSERINE

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The stability of d-cycloserine in the solid state depends to a considerable degree on its purity. Extraneous compounds of a mineral and organic character and also products from the inactivation of d-cycloserine itself accelerate the decomposition process. In particular, it has been shown that the presence of 2,5-bis(aminohydroxymethyl)-3,6-diketopiperazine (DKP-1) and 2,5-dimethylene-3,6-diketopiperazine (DKP-2) in the d-cycloserine powder leads to a more rapid inactivation of the antibiotic [1]. In connection with this, specific limitations on the content of these compounds in commercial d-cycloserine have been introduced [2]. DKP-1 and DKP-2 are the products from the dimerization of d-cycloserine and can be formed during the isolation of the d-cycloserine or during its storage [3-6]. Methods which make it possible to determine quantitatively these compounds in d-cycloserine powder have been described in the literature [2, 7]. A spectrophotometric method based on the measurement of the optical density (D) of an alkaline solution of d-cycloserine at 285 nm has been the most widely used of them. This method makes it possible to determine the total diketopiperazine content. However, when carrying out a whole series of studies connected with the investigation of the mechanism of the inactivation of d-cycloserine, the necessity of determining the rate of formation of each of the diketopiperazines separately arises.

This paper is devoted to determining the DKP-1 and DKP-2 content in the presence of each other. We used the DKP-1 and DKP-2 which we isolated in the crystalline form* in thiswork. We used DKP-2 to construct the calibration curve. The measurements were carried out on an SF-4A spectrophotometer.

The separate determination of DKP-1 and DKP-2 in the presence of each other is based on the difference in the absorption spectra of these compounds. It is evident from Fig. 1 that the DKP-2 solutions in water have a characteristic absorption spectrum in the UV-region with a maximum at 270 nm. There is no absorption for DKP-1 under the same conditions. d-Cycloserine and β-aminohydroxyalanine [8] also have no absorption in this region. DKP-1 is irreversibly transformed into DKP-2 in a sodium hydroxide solution. It is evident from Fig. 2 that the absorption spectrum of DKP-1 becomes similar to the absorption spectrum of DKP-2 in an alkaline medium after maintaining it in the sodium hydroxide solution. Some shift in the spectra toward shorter wavelengths with a maximum at 270 nm takes place during the neutralization of the alkaline solutions. The rate of conversion of DKP-1 to DKP-2 is shown in Fig. 3, as is the stability of the latter in a 0.1 N sodium hydroxide solution at room temperature. It is evident from Fig. 3 that the magnitude of D remains unchanged for 45 min after having attained its maximum value within 5 min.

TABLE 1. Results of the Determination of the DKP-2 and DKP-1 Content in Synthetic Mixtures

Taken (in μg)		Found (in mg)		Relative error in (%)	
DKP-2	DKP-1	DKP-2	DKP-1	DKP-2	DKP-1
56	305	57	310	+1,8	+1,6
40	565	41	550	+2,5	—2,7
65	505	66	520	+1,5	+3,0
80	565	80	572	0	+1,2
96	606	94	612	—2,1	+1,0
120	690	119	700	—0,8	+1,5
120	565	120	570	0	+0,8
80	405	79	416	—1,2	+2,7
120	365	121	370	+0,8	+1,4

It is evident from that indicated above, that the amount of DKP-2 can be determined directly by measuring the D of a solution containing both diketopiperazines at 270 nm. It is necessary first to convert DKP-1

*The isolation of DKP-1 and DKP-2 was carried out by a method proposed by co-workers at the All-Union Scientific Research Institute of Antibiotics, V. B. Korchagin, A. M. Kan, and A. P. Kondrat'eva, to whom the authors express their deep appreciation.

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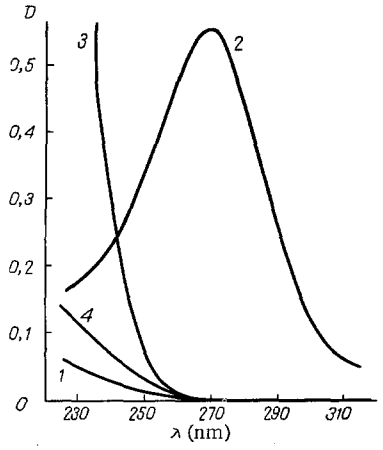

Fig. 1.

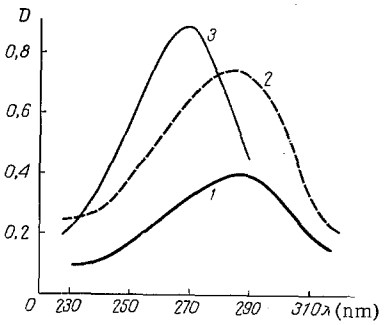

Fig. 2.

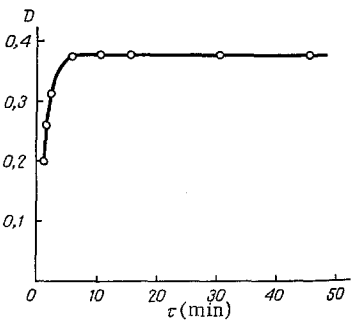

Fig. 3.

Fig. 1. Absorption spectra of the following aqueous solutions: 1) DKP-1; 2) DKP-2; 3) d-cycloserine; and 4) β-aminohydroxyalanine.

Fig. 2. Absorption spectra of DKP-2 (curve 1 – in 0.1 N sodium hydroxide); and DKP-1 (curve 2 – in sodium hydroxide; curve 3 – after neutralizing the solution).

Fig. 3. Rate of conversion of DKP-1 to DKP-2 in a 0.1 N sodium hydroxide solution.

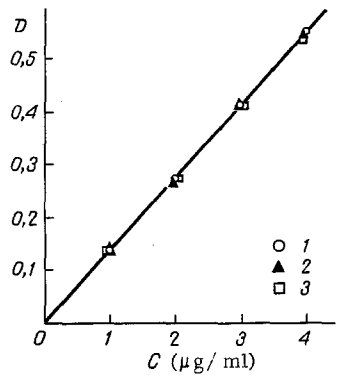

Fig. 4. Calibration curve for DKP-2. 1) In water; 2) in 0.1 N sulfuric acid; 3) in 0.1 N sodium sulfate.

into DKP-2 in order to determine it quantitatively. Since the determination of the DKP-1 content includes the obligatory neutralization of the alkaline solution with sulfuric acid, we checked how the acidity of the solution and also the presence of sodium sulfate in it affect the experimental results. Solutions of DKP-2 in water, 0.1 N sulfuric acid, and 0.1 N sodium sulfate were prepared for this purpose. It follows from Fig. 4 that the same values of D for the appropriate DKP-2 concentrations were obtained in all cases. The fact that the change in acidity of the solution to 0.1 N does not change the magnitude of D makes it possible to add an excess of acid during the neutralization of the alkaline solutions and to conduct the measurements of D in an acid medium.

METHOD OF ANALYSIS

An aqueous solution containing DKP-1 and DKP-2 in concentrations of 8-12 and 2-3 μg/ml respectively was measured at 270 nm (1-cm cells) and D_1 was determined. Then 5 ml of the original solution was placed into a 25-ml volumetric flask, 5 ml of a 0.2 N sodium hydroxide solution was added, the solution was maintained at room temperature for 15 min, the solution in the flask was brought to the mark with 0.1 N sulfuric acid, and the D was measured at 270 nm (D_2). The D_1 value characterizes the amount of DKP-2 in the solution, and D_2 the total DKP-1 and DKP-2. The diketopiperazine concentrations were calculated from the calibration (see Fig. 4) using the values of D measured. The amount of DKP-1 was calculated from the difference between the results of the two determinations by multipyling the value obtained by the ratio of the molecular weights of the diketopiperazines: 204/138.

The results of the determination of the DKP-2 and DKP-1 content in synthetic mixtures by the method we developed are given in Table 1; ratios between DKP-2 and DKP-1 of 1 : 3 to 1 : 4 were used. The relative error does not exceed ± 3%. The sensitivity of the method equals 1 μg/ml. d-Cycloserine at a concentration of 25 μg/ml and β-aminohydroxyalanine at a concentration of 100 μg/ml do not interfere with the determination.

We used this method to investigate the decomposition mechanism of d-cycloserine in the solid state. It was shown with its aid that DKP-1 is preferentially formed during the inactivation of crystalline d-cycloserine.

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