STUDY OF THE EPIMERIZATION OF TETRACYCLINE BY THE NMR METHOD

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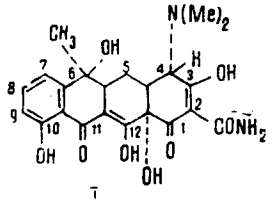
UDC 615.779.931

It is known that natural tetracyclines, and also many of their derivatives, readily epimerize at the C_4 asymmetric center [1, 2]. This reaction is reversible, and both epimers can be isolated.

The study of the process of epimerization is of interest from the point of view of determining the mechanism of the reaction, which, apparently, is closely connected with the tautomerism of the diketo amide grouping of ring A. In addition to this, it is important to investigate the factors accelerating and inhibiting epimerization, particularly in organic solvents, in view of the numerous studies on the production of synthetic tetracycline derivatives [3, 4] (see formula below)



On investigating the transformations of tetracycline (I) under various conditions, we found that in pyridine solution tetracycline hydrochloride undergoes rapid epimerization. We used the NMR method to study this process.

At 32°C, the equilibrium TC $\rightleftharpoons$ ETC* in pyridine is established 3 h after the dissolution of TC, and the equilibrium mixture contains 48.4% of epitetracycline (Fig. 1). The achievement of equilibrium was judged from the absence of further changes in the ratio of the epimers with time. Figure 2 shows the NMR spectra of tetracycline hydrochloride taken soon after dissolution (a) and in the state of equilibrium (b). It can be seen from the spectra of Fig. 2 that the epimers have different chemical shifts (CSs) not only for the protons at C_4 [5] but also for the C-methyl and N-methyl protons. The assignments that we made are given below.

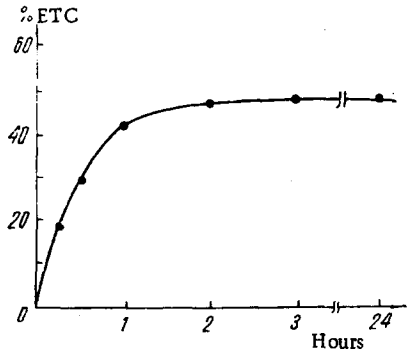


Fig. 1. Process of formation of ETC in a solution of TC in pyridine.

Chemical Shifts of Some Protons of Tetracycline and Epitetracycline Hydrochlorides in ppm in Pyridine-d_5

Protons	TC	ETC
C_4–H	4.13	5.12
C_6–CH_3	1.65	1.75
$N(CH_3)_2$	2.86	3.14

*Here and below, tetracycline hydrochloride is denoted by TC and epitetracycline hydrochloride by ETC.

All-Union Scientific-Research Institute of Antibiotics. All-Union Scientific-Research Institute of Medicinal Plants. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 67-72, January-February, 1974. Original article submitted October 25, 1972.

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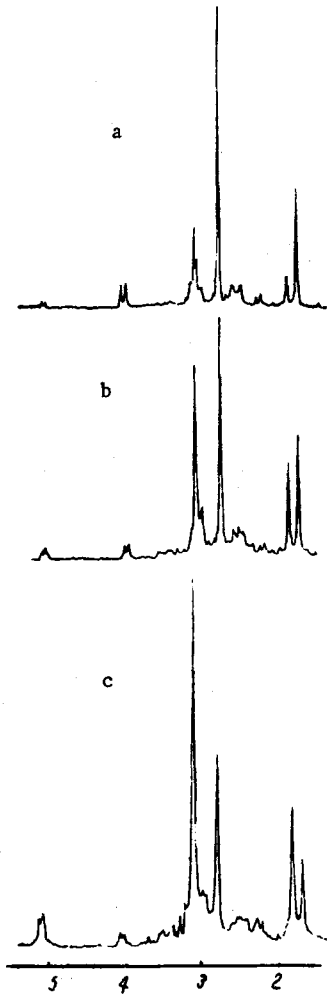


Fig. 2

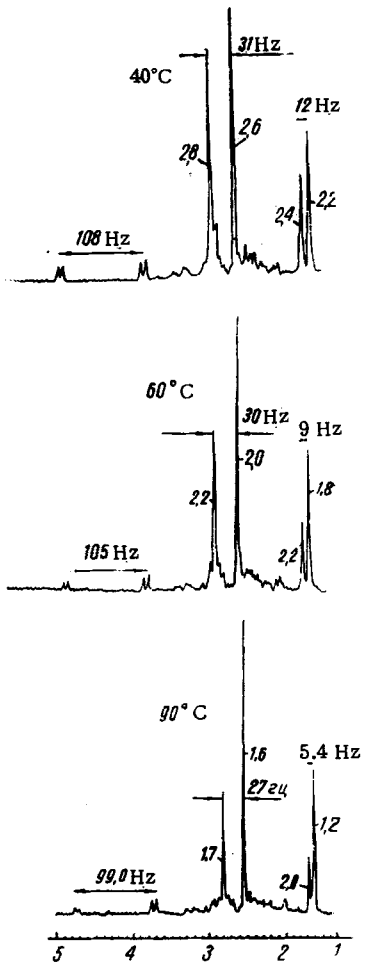


Fig. 3

Fig. 2. NMR spectra in pyridine-d_5 immediately after the dissolution of TC (a), the equilibrium mixture TC ⇌ ETC (b), and a mixture of TC and ETC in a ratio of 1 : 3 (c).

Fig. 3. NMR spectra of equilibrium mixtures of TC ⇌ ETC in pyridine-d_5 at 40, 60, and 90°C (the distances between some of the peaks and the half-widths of these peaks are expressed in Hz).

Figure 2c, shows the spectrum of a mixture of TC and ETC. This spectrum confirms the fact that the signals at 5.12, 1.75, and 3.14 ppm are due to the protons of the epi form. Two hours after dissolution of the mixture, the solution had a spectrum identical with that of the equilibrium state (Fig. 2b). It must be mentioned that in the spectra of mixtures of the epimers obtained in heavy water and deuteromethanol, the CSs of the protons of the C-methyl group were identical.

The results of a study of the temperature dependence of the equilibrium constant of the epimerization reaction in pyridine showed that with a rise in the temperature the equilibrium is shifted in the direction of the starting material (Fig. 3). The amount of epitetracycline in the equilibrium mixture at 90°C is 37.7%. The ratios of the epimers at corresponding temperatures during the raising and lowering of the temperature coincided within the limits of experimental error. Consequently, the epimerization reaction is reversible over the whole range of temperatures investigated.

Table 1 gives the thermodynamic characteristics of the process of epimerization in pyridine obtained on the basis of the temperature dependence (Fig. 4). As can be seen from the table the free energy of the transition TC → ETC is small, and with a rise in the temperature ΔF increases somewhat. The enthalpy of the reaction, ΔH, is negative, i.e., the reaction is exothermic. The entropy decreases during the epimerization process ($T\Delta S < 0$). Since in the transition TC → ETC the number and nature of the bonds in the molecule do

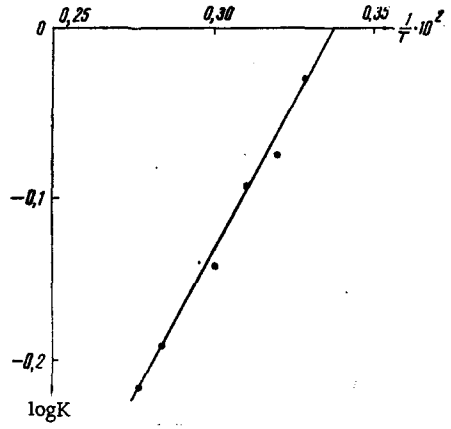


Fig. 4. Constant of the equilibrium TC $\rightleftarrows$ ETC as a function of the temperature.

TABLE 1. Thermodynamic Characteristics of the Epimerization Reaction of Tetracycline Hydrochloride in Pyridine-d_5

T, °C	TC, %	$\lg K_c^*$	ΔF	ΔH	$T\Delta S$
			cal · mole^{-1}		
32	51,6	−0,027	38		−1645
40	54,3	−0,075	107		−1714
50	65,4	−0,094	130	−1607	−1733
60	58,1	−0,143	218		−1825
70	59,2	−0,161	252		−1859
80	61,1	−0,195	314		−1921
90	62,3	−0,218	362		−1969

* $K_c = \frac{[ETC]}{[TC]}$.

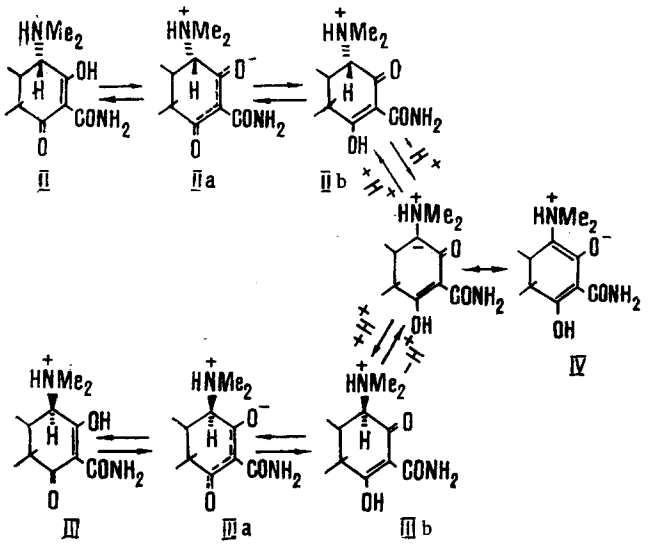
not change, and no apparent advantage for the formation of intramolecular hydrogen bonds is created, the exothermicity of the reaction is apparently connected with the greater solvation energy of epitetracycline than of tetracycline. In this case, the decrease in entropy on passing to the epi form also becomes understandable: a greater degree of solvation leads to a smaller mobility of the solvent molecules. Although the thermodynamics of the equilibrium TC $\rightleftarrows$ ETC has not been studied previously and has not been discussed in papers on the epimerization [2, 6, 7], the experimental results published in them have enabled us to calculate the thermodynamic characteristics of the equilibrium mixtures under various conditions. The results of the calculations showed that in all the cases described the epimerization reaction is exothermic. The value of ΔH is little affected by the pH of the solution and the concentration of buffer ions. As calculated for the conditions described in preceding investigations [2, 7] it varies from −2.5 to −0.75 kcal/mole. In all cases, the entropy decreases in the transition TC → ETC. The free energy of the transition ΔF rises with an increase in the pH of the solution and falls with a rise in the concentration of buffer ions.

Thus, the results of the thermodynamic calculations show that the suggested selective solvation of the epi form is not specific for the reaction in pyridine but is characteristic of epimerization under all the conditions described. The predominant solvation of epitetracycline is probably a consequence of the different configurations [8] of the normal and epi forms. If solvation ensures the stabilization of the epi form, the role of buffer ions probably consists of lowering the energy barrier of the transition. In water, the potential barrier of this transition is fairly high, since the rate of epimerization in water at pH 4.0 is 70 times smaller than under the same conditions in 1.0 M phosphate buffer [6]. The activation energy decreases with an increase in the strength of the buffer solution [7]. In our case, pyridine plays the role both of a solvating solvent and of a catalyst. The rapid establishment of the equilibrium TC $\rightleftarrows$ ETC in pyridine shows a low activation energy under these conditions. With a rise in the temperature, of course, the stability of the solvates falls, which leads to a nonproportional rise in the rate of the reverse reaction and, consequently, to a displacement of the equilibrium in the direction of the normal form.

The mechanism of the epimerization reaction of the tetracyclines has not been studied; nevertheless, a number of hypothetical schemes of this process have been put forward [9]. It is assumed that epimerization is connected with the formation of an enolic tautomer with a double bond at C_3-C_4.

With a rise in the temperature there is not only a change in the ratio of the intensities of the corresponding signals of the epimers but also a contraction of the peaks of the C-methyl and N-methyl protons and some approach of the signals of the epimers (see Fig. 3). The fact that in the epimerization process the epimers have different CSs and not an averaged signal shows that one of the stages of the epimerization process is fairly slow. At the same time, the contraction of the signals with a rise in the temperature shows their averaged nature; each of the epimers exists as two or more forms, transitions between which take place fairly fast and are still more accelerated with a rise in the temperature.

On the basis of ideas on tautomeric transformations, the mechanism of the epimerization of tetracycline can be represented by the following scheme:



As is well known, the slow stage in transformations of this type is the splitting off of the proton [10], i.e., the transitions (IIb) → (IV) and (IIIb) → (IV). We showed the formation of the tautomer (IV) in the following way. To the equilibrium mixture TC ⇌ ETC in pyridine we added a few drops of deuteromethanol. This led to a decrease in the intensity of the signal of the C_4 proton after 3 h from one proton unit to 0.35 proton unit, while there were no changes in the remainder of the spectrum. Consequently, after this time 75% of the C_4 protons had been replaced by deuterium. If it is borne in mind that in the deuteration of tetracycline with deuteromethanol the C_4 proton is not replaced by deuterium, it becomes clear that deuteration at C_4 takes place in the conversion of the enolic tautomer (IV) into (IIb) and into (IIIb).

EXPERIMENTAL

The NMR spectra were taken on a Varian HA-100D spectrometer; the solvents used, apart from deuteropyridine, were heavy water and methanol-d_4. Solutions (3%) of tetracycline hydrochloride of 99% purity were used, and the signal of HMDS was taken as 0. The relative concentrations of the epimers were calculated from the spectra recorded with a field sweep of 250 Hz as the ratio of the products of the half-width of the signals and their heights.

The mixture of TC and ETC hydrochlorides was obtained from the ammonium salt of epitetracycline [2] by the method adopted in tetracycline chemistry for obtaining hydrochlorides. The mixture contained 76% of epitetracycline (determined spectrophotometrically [6] from the ratio of the intensities of absorption at 254 and 267 nm).

CONCLUSIONS

1. By means of the NMR method it has been shown that tetracycline hydrochloride undergoes rapid epimerization in pyridine solution.

2. The temperature dependence of the constant of the equilibrium TC ⇌ ETC has been studied and the thermodynamic characteristics of this equilibrium have been calculated.

3. The scheme of the transformations of tetracycline hydrochloride leading to the formation of the epimer has been put forward.

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