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A STUDY OF THE ACID-BASE TRANSFORMATIONS OF OXODIHYDROTHIOCHROME

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The acid-base properties of oxodihydrothiochrome – the product of the redox disproportionation of the vitamin B_1 catabolite thiochrome (pH = 10.7, 4.5, and 0.5) – have been studied in the pH interval of 0-12 by PMR spectroscopy. It has been established that in acid medium (pH 0-1) the formation of a doubly charged ion is accompanied by a further structural transformation of the molecule of (I) with the formation of a vitamin B_1 analogue – oxothiamine (II). A possible mechanism of the action of (I) as an inhibitor of thiamine-dependent enzymes is discussed in the light of the results obtained. Details of the PMR spectra of (I) and (II) are given.

We have shown previously [1] that the natural catabolite of thiamine (vitamin B_1) thiochrome is converted as the result of redox disproportionation into oxodihydrothiochrome (I). The latter compound possesses a pronounced biological activity [2]; in particular, it inhibits transketolase – an enzyme catalysing the transfer of a glycolaldehyde fragment from a keto sugar to an aldo sugar. The coenzyme of this reaction is thiamine diphosphate. In view of the fact that the catalytic activity of thiamine and its analogues is a function of the pH of the medium, it appeared to us to be important to investigate the acid-base properties of oxodihydrothiochrome.

The study of the acid-base transformations was performed by PMR spectroscopy which not only enabled a quantitative estimate of them to be given but also revealed the corresponding sections of the molecule involved in these transformations. Analysis of the spectra (Fig. 1) showed that oxodihydrothiochrome undergoes three acid-base transformations in the pH range studied. As can be seen from Fig. 1, the first transition, in the alkaline region, has its greatest influence on the position of the 4-H signal ($\Delta\delta$ = 0.2 ppm), which indicates a nucleophilic attack of hydroxyl on the 5-C atom with the formation of the anion (II) (scheme). The upfield shift of this signal is due to a decrease in the screening effect of the hydrogen of the pyrimidine ring by the amide oxygen through a disturbance of the coplanarity between them and a change in the electric field as a consequence of the formation of a carboxylate ion.

The second transition in the weakly acid region is undoubtedly connected with the protonation of the cyclic secondary amino group. The formation of the ammonium ion (III) is reflected primarily in the position of the signal of the closest proton, 9a-H ($\Delta\delta$ = 0.13 ppm), and, to a smaller degree, in the position of the signals of the other proton-containing groupings directly connected with the pyrimidine and thiazole rings. The latter is due an increase in the "permeability" of the imine bridge for π-electrons of the two rings, which is clearly shown in the electronic spectra as a bathochromic shift of the long-wave absorption band ($\Delta\delta$ = 25 mm).

In more acidic media, the addition of a proton takes place at the most basic oxygen atom of the amide group with the formation of conjugate acids having the alternative structures (IV) and (V). The ambident nature of the cation (IV) finds its confirmation in the

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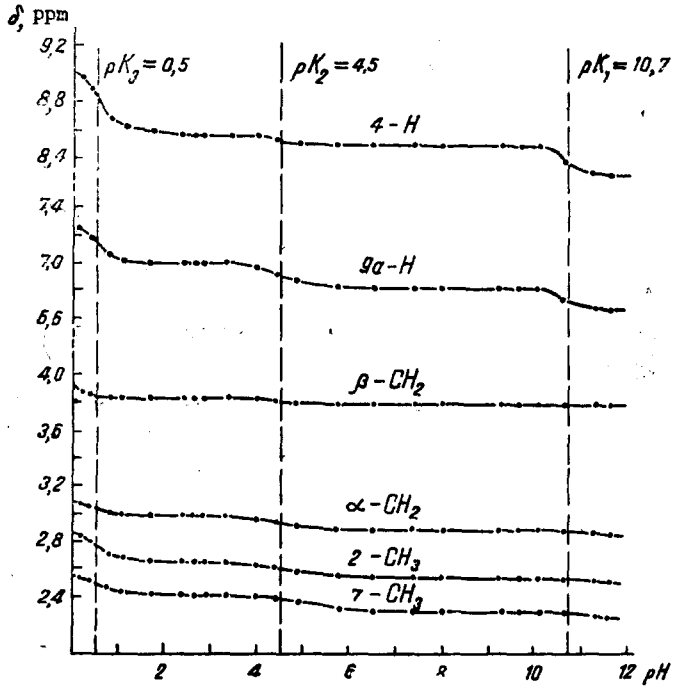
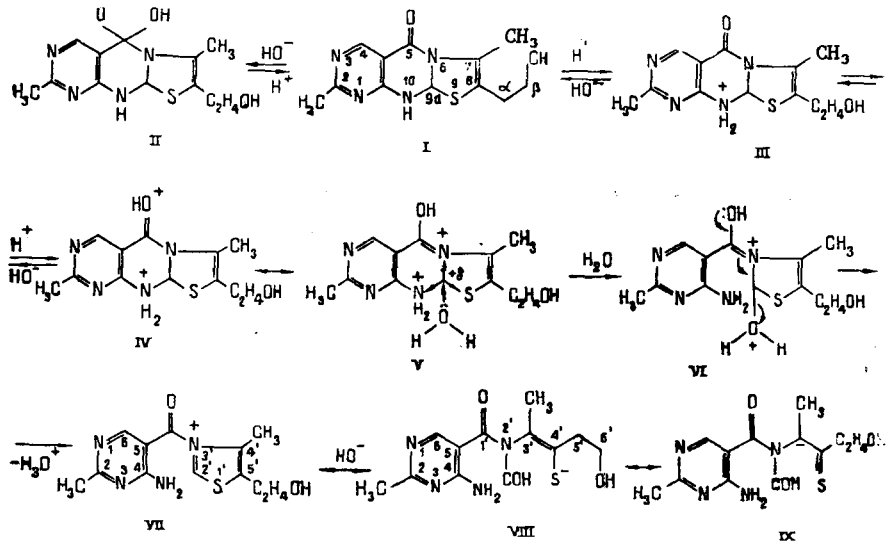


Fig. 1. Dependence of the PMR signals (δ, ppm) of proton-containing groupings in oxodihydrothiochrome on the pH.



considerable shift not only of the signal of the protons of the pyrimidine ring (Δδ = 0.5 ppm), but also that of the proton in the dihydrothiazole ring (Δδ = 0.3 ppm) (Fig. 1).

The constants of the acid-base transitions of oxodihydrothiochrome considered above amount to 10.7, 4.5, and 0.5 in the pK scale. It must be mentioned that the formation in an acid medium of the doubly charged ion (IV) is accompanied not only by a downfield shift of the signals of the proton-containing groupings but also by the appearance in the spectrum of a second set of monotypical signals of magnetically nonequivalent protons. The latter indicates a further structural transformation of the oxydihydrothiochrome molecule in an acid medium.

The most probable mechanism of this transformation, in our view, is the above (scheme). Protonation of the lactam (III) raises the electrophilicity of the 9a-C atom of the thiazole ring with a contribution of the resonance structure (V) and promotes nucleophilic attack of a water molecule at the reaction center formed. The cleavage of the 9a-C–

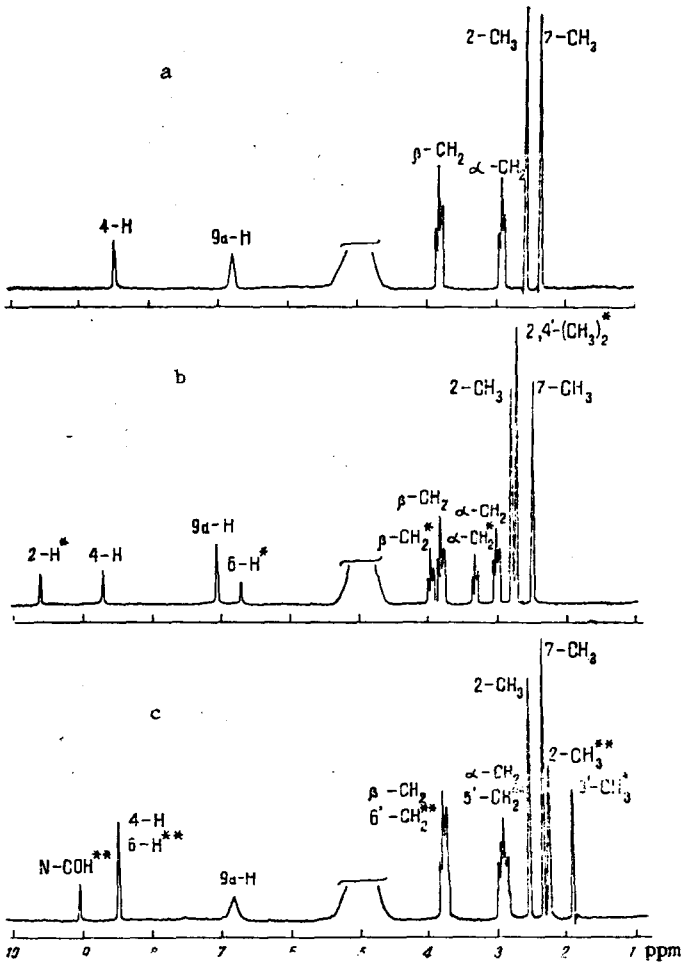


Fig. 2. PMR spectra of oxodihydrothiochrome (I) at pH 9.65 (a) and 0.76 (b), and after the return from pH 1.1 to pH 9.7 (c). The signals of the protons of form (VII) are marked with one asterisk, and those of (VIII) with two asterisks.

10-N bond accompanying this and the subsequent stabilization of the intermediate oxonium ion (VI) leads to a thiamine analogue – oxothiamine (VII).

The proposed scheme of the formation of oxothiamine (VII) is in harmony with the PMR spectral results. As an illustration, Fig. 2b shows the spectrum of oxodihydrothiochrome at pH 0.76 recorded 30 min after the preparation of the solution. The increase in the aromaticity of the thiazole ring in oxothiamine (VII) finds its reflection in a downfield shift of the signal of the 4'-CH_3 protons by 0.22 ppm and of the signals of the protons on the α- and β-carbon atoms in the 5'-hydroxyethyl radicals by 0.28 and 0.11 ppm, respectively, as compared with the initial oxodihydrothiochrome. The SSCCs of the methylene protons did not change under these conditions. A weighty piece of evidence in favor of the formation of a structure in which the nitrogen atom of the thiazole component is quaternary is the appearance in the spectrum of a weak-field signal of a proton at 9.63 ppm belonging to the 2'-H atom of thiazole, which agrees well with literature information for thiamine [3].

It is known [4] that thiamine and its analogues undergo transformations connected with the opening of the thiazole ring in an alkaline medium. The consequence of such a structural rearrangement is the appearance of a N-formyl grouping the signal of the aldehyde proton of which resonates in the form of a characteristic singlet in the ∿9 ppm region [5]. In view of this, to confirm the structure of oxothiamine (VII) we performed an additional experiment: A solution containing a mixture of compounds (IV) and (VII) (pH 1.1) was rapidly made alkaline, to pH 9.7. In the PMR spectrum obtained at this pH value (Fig. 1c), in addition to the signals of the untransformed oxodihydrothiochrome (I) (Fig. 1a), the signals of a new compound appeared. The absence in the spectrum of the new structure of the signal

of the proton of a thiazole ring and the appearance of a weak-field singlet at 9.06 ppm typical for a N=COH grouping [5] indicated the opening of the thiazole ring of the oxothiamine (VII) in the alkaline medium with the formation of the thiol form (VIII). The latter was also characterized by a substantial downfield shift of the signal of the 3'-methyl protons (δ = 1.92 ppm), because of a considerable contribution of structure (IX) to the localized negative charge on the 3'-C atom.

Thus, the totality of the results obtained agrees completely with the structure proposed for oxothiamine (VII).

The results of the present work permit a possible mechanism of the action of oxodihydrothiochrome (I) as an inhibitor of transketolase to be put forward. Probably, in the animal organism under certain conditions (sharp jumps in the concentration of H^+ ions in the mitochondria, strongly acid medium in the gastric juice), oxodihydrothiochrome is partially converted into oxothiamine (VII), which is then phosphorylated with the formation of the anticoenzyme form of thiamine diphosphate – oxothiamine diphosphate. It is important to note that the conversion of oxydihydrothiochrome into oxothiamine at low pH values reflects the specific nature of its chemical structure. No similar transformations have been observed for thiochrome, which is probably the reason why it lacks similar biological activity.

EXPERIMENTAL

The PMR spectra (in D_2O) were obtained on a Varian SC-300 spectrometer with a working frequency of 300 MHz in the pulsed regime with Fourier transformation on a computer of the 620/L-100 type. The chemical shifts of the signals were measured relative to the internal standard sodium 3-(trimethylsilyl)tetradeuteropropionate (δ 0.015 ppm) with an accuracy of ±0.005 ppm. The titrants used were 0.05 N DCl and KOD, and pH values being determined on a pH-meter of the Orion Research-601 type with an Ingold 405 M 3 combined electrode. The conditions for isolating oxodihydrothiochrome have been described previously [1].

SUMMARY

In the pH range of 0-12, oxodihydrothiochrome is characterized by three acid-base transitions with pK 10.7, 4.5, and 0.5.

In an acid medium (pH 0-1), the formation of a doubly-charged ion is associated with a further structural transformation of the molecule of oxodihydrothiocvhrome, leading to an analogue of vitamin B_1 – oxothiamine.

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