PROTONATION OF PYRIMIDOTRIAZINEDIONES BY $^1$H AND $^{13}$C NMR SPECTROSCOPY

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On the basis of an analysis of the changes in the chemical shifts of the signals in the $^1$H and $^{13}$C NMR spectra on the pyrimidotriazinedione and trifluoric acid concentrations in CDCl$_3$ it was established that the protonation of rheumycin and fervenulin takes place at the N(2) atom, whereas the protonation of isofervenulin takes place competitively at the N(1), N(2), and O(6) atoms. The equilibrium constants of the investigated protonation processes were measured.

Natural pyrimido[5,4-e]-1,2,4-triazinedione antibiotics (7-azalumazines) rheumycin (Ia) and fervenulin (Ib) have a broad spectrum of antitumorigenic activity [1, 2]. Isofervenulin (II) (dimethyl-6-azalumazine), which is obtained by chemical means, has antiviral activity [3]. To ascertain the molecular mechanisms of their biological activity one must study chemical models of the behavior of these substances in a living organism. In a study of the peculiarities of the behavior of 7-azalumazines in a living cell [4] it was shown that these antibiotics are capable of being reduced by oxidizing cytoplasmatic nicotinamide-adenine dinucleotide (NADH). It has been assumed that dihydro derivatives of Ia, b participate in these processes [5]. In this connection it seems of interest to study the possibility of the formation and the structures of the protonated and hydrated forms of antibiotics Ia, b and their isomeric analog II as possible intermediates in reversible electron-transfer reactions.

For this we used $^1$H and $^{13}$C NMR spectroscopy to investigate the behavior of pyrimidotriazinediones Ia, b and II in aqueous* and anhydrous acidic (CDCl$_3$–CF$_3$COOD) media.

Azalumazines Ia, b and II have five potentially possible protonation centers: the three nitrogen atoms of the triazine fragment and the two oxygen atoms of the uracil fragment. The amide nitrogen atoms of the uracil ring are excluded from the discussion, since their basicities should be substantially lower than the basicities of the nitrogen atoms of the triazine ring.

An analysis of the literature data on the reactivities of azalumazines and related compounds does not make it possible to unequivocally determine the preferred protonation center of these molecules. Thus, the protonation of lumazine occurs successively at the oxygen atom of the carbamido group of the uracil ring and at one of the nitrogen atoms of the pyrazine ring [7-9]. Data on the cyclization of 3-azidoisofervenulin [10] and 3-azidoisofervenulin [11] to tetrazole derivatives and on the oxidation of 3-aminofervenulin to the 2-N-oxide [12] constitute indirect evidence for the greater basicity of the N(2) atom as compared with the N(4) atom. The quaternization of 5,6-substituted 1,2,4-triazines, which is related to protonation, also takes place at the N(2) atom [13, 14], while the quaternization center of 3-morpholino- and 3-pyrrolidino-1,2,4-triazines is the N(1) atom [15].

An analysis of the $^1$H and $^{13}$C NMR spectra of solutions of 7-azalumazines Ia, b in deuterochloroform with various amounts of trifluoroacetic acid (CF$_3$COOD) showed that the protonation of rheumycin and fervenulin takes place primarily at the N(2) atom of the triazine ring. In the case of isofervenulin (II) the N(1) and N(2) atoms of the triazine ring and the O(6) atom of the uracil fragment are competitive protonation centers.

* A study of the chemical peculiarities of the behavior of Ia, b in aqueous acidic media (H$_2$O–HCl, D$_2$O–DCI) was described in [6].
The addition of trifluoroacetic acid to solutions of Ia, b and II in chloroform leads to a shift of the signals of the N-methyl and methyldyne protons in the $^1H$ NMR spectra to weak field. The smooth trend of the titration curves and the presence of only one inflection point (Fig. 1) indicate that the formation of only monocations of the pyrimidotriazinediones occurs over the investigated range of substrate and acid concentrations. A comparison of the shifts of the signals of the methyldyne 3-H proton in the spectra of azalumazines Ia, b and II (Table 1) shows that the greatest deshielding effect on acidification is observed in the case of isofervenulin (II) ($\Delta_{obs} = 0.31$ ppm). For rheumycin (Ia) and fervenulin (Ib) the protonation effects are only 0.10 and 0.16 ppm, respectively. This is evidently associated with the protonation of isofervenulin (II) and 7-azalumazines Ia, b at different centers. It is known [16, 17] that the protonation of azines has a greater effect on the shifts of the signals of the methyldyne protons in the $\beta$ position with respect to the center of cation formation than on the signals of the protons in the $\alpha$ position. Thus, the substantial difference in the shifts of the signals of the 3-H protons of II from Ia, b indicates that the protonation of isofervenulin takes place at the $N(1)$ atom, whereas the protonation of rheumycin and fervenulin takes place at the $N(2)$ or $N(1)$ atom.

The $^{13}C$ NMR spectra provide more detailed information regarding the structures of the cations of pyrimidotriazinediones Ib and II. In connection with the fact that the maximum protonation effects in the $^1H$ NMR spectra are observed with a change in the acid concentration from 0 to 15% (by volume) (Fig. 1), the investigation of the protonation of pyrimidotriazinediones by $^{13}C$ NMR spectroscopy was restricted to the range of acid concentrations up to 40%. The assignment of the signals to the individual carbon atoms of the azalumazine molecules was made taking into account the multiplicities and spin-spin coupling constants (SSCC) measured from the spectra recorded without suppression of the spin-spin coupling of the $^{13}C$ nuclei with the protons, as well as by comparing the chemical shifts of the signals with the data previously obtained for fervenulin in $d_6$-DMSO [18]. The principal diagnostic feature in the determination of the protonation centers from the $^{13}C$ NMR spectra is the shift to strong field of the signals of the carbon atoms in the $\alpha$ position relative to the atoms that are the protonation centers; the signals of the $\beta$-carbon atoms are shifted to weak field and to a lesser extent [16, 19]. Measurements of the chemical shifts in the