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

S. V. Shorshnev, S. E. Esipov, and A. I. Chernyshev

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On the basis of an analysis of the changes in the chemical shifts of the signals in the ^1H 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 ^1H and ^{13}C NMR spectroscopy to investigate the behavior of pyrimidotriazinediones Ia, b and II in aqueous* and anhydrous acidic ($CDCl_3$–CF_3COOD) 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-azidofervenulin [10] and 3-azidoisofervenulin [11] to tetrazole derivatives and on the oxidation of 3-anilinofervenulin 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-triazine is the $N_{(1)}$ atom [15].

An analysis of the ^1H and ^{13}C NMR spectra of solutions of 7-azalumazines Ia, b in deuterochloroform with various amounts of trifluoroacetic acid (CF_3COOD) 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_2O–HCl, D_2O–DCl) was described in [6].

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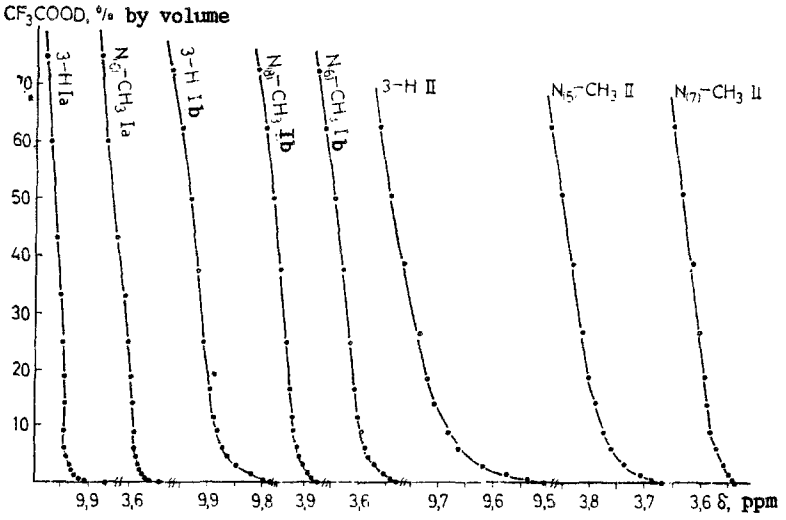
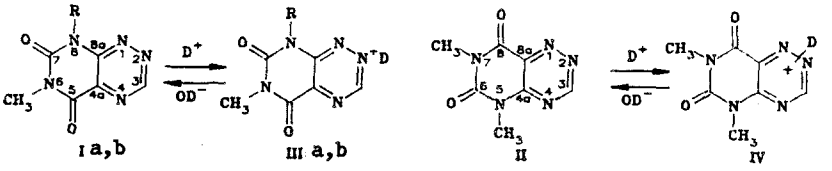


Fig. 1. Dependence of the chemical shifts of the protons in the ^1H NMR spectra of pyrimidotriazinediones Ia, b and II on the CF_3COOD concentration in $CDCl_3$.



Ia, IIIa R=H; b R=CH_3

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 methylidyne 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 methylidyne 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) (Δ_{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 methylidyne protons in the β position with respect to the center of cation formation than on the signals of the protons in the α 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 α position relative to the atoms that are the protonation centers; the signals of the β-carbon atoms are shifted to weak field and to a lesser extent [16, 19]. Measurements of the chemical shifts in the

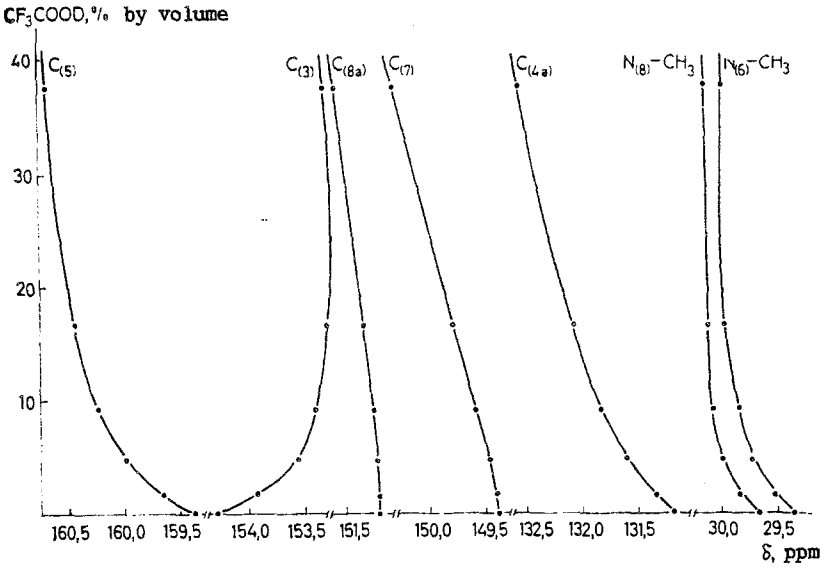


Fig. 2. Dependence of the chemical shifts of the carbon atoms in the ^{13}C NMR spectra of fervenulin (Ib) on the CF_3COOD concentration in $CDCl_3$.

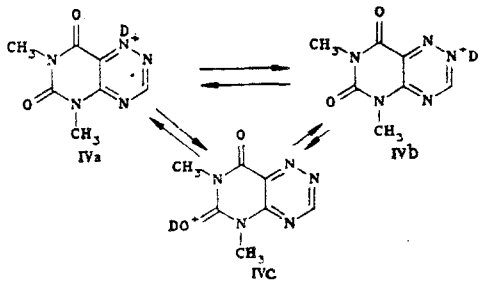
TABLE 1. ^1H NMR Spectra of Pyrimidotriazinediones Ia, b, II, IIIa, b, and IV

Compound	Solvent	Chemical shifts, δ, ppm		
		3-H	$N_{(6)}CH_3$*	$N_{(8)}CH_3$†
Ia	$CDCl_3$	9,87	3,55	—
IIIa	60% $CF_3COOD/CDCl_3$‡	9,97	3,64	—
$\Delta_{obs} = \delta_{IIIa} - \delta_{Ia}$		0,10	0,09	—
Ib	$CDCl_3$	9,79	3,53	3,88
IIIb	60% $CF_3COOD/CDCl_3$	9,95	3,66	3,97
$\Delta_{obs} = \delta_{IIIb} - \delta_{Ib}$		0,16	0,13	0,09
II	$CDCl_3$	9,50	3,54	3,67
IV	60% $CF_3COOD/CDCl_3$	9,81	3,65	3,87
$\Delta_{obs} = \delta_{IV} - \delta_{II}$		0,31	0,11	0,20
II	D_2O	9,50	3,39	3,55
IV	24% DCl/D_2O	9,89	3,39	3,65
$\Delta_{obs} = \delta_{IV} - \delta_{II}$		0,39	0,00	0,10

*$N_{(7)}CH_3$ for II and IV.
†$N_{(5)}CH_3$ for II and IV.
‡Here and subsequently, the percent sign denotes the percent of acid by volume.

^{13}C NMR spectra of solutions of fervenulin Ib showed that only the signal of the $C_{(3)}$ atom is shifted to strong field with an increase in the acid concentration (Fig. 2). This indicates unequivocally that the protonation center in the fervenulin molecule is the $N_{(2)}$ atom. Protonation at the $N_{(4)}$ atom should have led to a strong-field shift of the signals of the methylidyne $C_{(3)}$ atom and the nodal $C_{(4a)}$ atom.

The titration curves of isofervenulin (II) constructed from ^{13}C NMR data have more complex character (Fig. 3). The signals of the $C_{(3)}$, $C_{(6)}$, and $C_{(8a)}$ carbon atoms are shifted to strong field; the maximum shift is observed for the $C_{(8a)}$ signal (Δ_{obs} = 2.1 ppm), while the other two are shifted to a lesser extent [Δ_{obs} = 1.5 and 0.9 ppm for $C_{(3)}$ and $C_{(6)}$, respectively]. The fact of the strong-field shift of the signals of the $C_{(8a)}$, $C_{(3)}$, and $C_{(6)}$ atoms makes it possible to conclude that an equilibrium with the participation of three prototropic tautomeric cations IVa-c, the protonation centers in which are the $N_{(1)}$, $N_{(2)}$, and $O_{(6)}$ atoms, exists in solutions of isofervenulin and trifluoric acid in chloroform (see scheme on following page).



The low solubility of rheumycin (Ia) in chloroform made it possible to investigate the protonation of this compound by ^{13}C NMR spectroscopy. However, considering the similar character of the titration curves of rheumycin (Ia) and fervenulin (Ib) obtained from ^{1}H NMR data (Fig. 1) and the close shifts of the signals of the methylidyne and N-methyl protons due to acidification [Δ_{obs} (3-H) = 0.10 and 0.16 ppm, and Δ_{obs} [$N_{(6)}CH_3$) = 0.09 and 0.13 ppm, respectively] it may be assumed that the protonation of the molecules of these compounds takes place at the same center, viz., the $N_{(2)}$ atom.

For the quantitative evaluation of the basicities of Ia, b and II the equilibrium constants of the protonation processes were measured from the dependences of the shifts of the signals in the ^{1}H NMR spectra of these substances on the trifluoroacetic acid and pyrimidotriazinedione concentrations.

The equation that links the observed shifts of the ^{1}H NMR signals with the initial concentrations of the base and acid [20]

$$K=\frac{\Delta_{obs}}{(\Delta_{max}-\Delta_{obs})\left(A_0-B_0\frac{\Delta_{obs}}{\Delta_{max}}\right)},$$

can be represented in the form

$$A_0+B_0=-\frac{1}{K}+A_0\frac{\Delta_{max}}{\Delta_{obs}}+B_0\frac{\Delta_{obs}}{\Delta_{max}}, \quad (1)$$

where A_0 and B_0 are the initial concentrations of the acid and base, respectively, K is the equilibrium constant of the process A + B $\rightleftarrows$ AB, Δ_{obs} is the observed shift of the signal (the difference in the chemical shifts of the pyrimidotriazinediones in the presence and absence of acid), and Δ_{max} is the maximum shift of the signal (the difference in the chemical shifts in the adduct and base: $\Delta_{max} = \delta_{AB} - \delta_B$). The legitimacy of the use of Eq. (1) to evaluate the equilibrium constant of the protonation of isofervenulin (II) is due to the

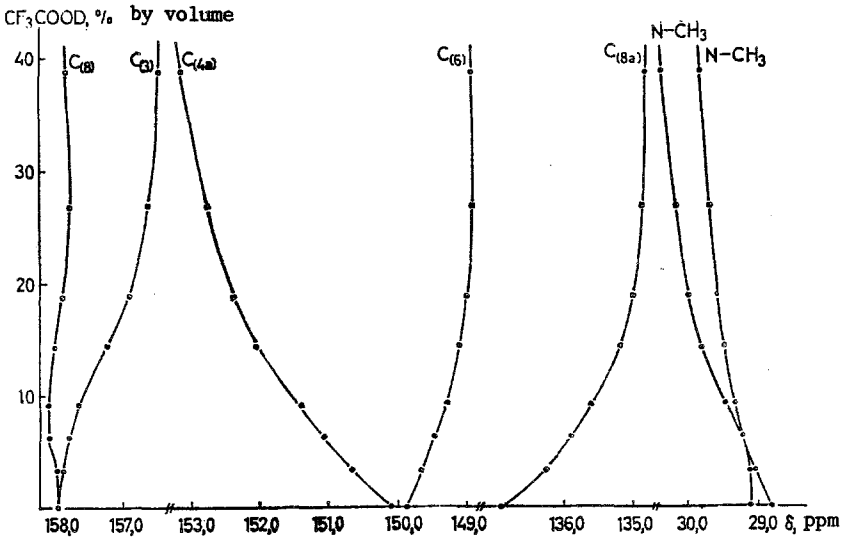


Fig. 3. Dependence of the chemical shifts of the carbon atoms in the ^{13}C NMR spectra of isofervenulin (II) on the CF_3COOD concentration in $CDCl_3$.

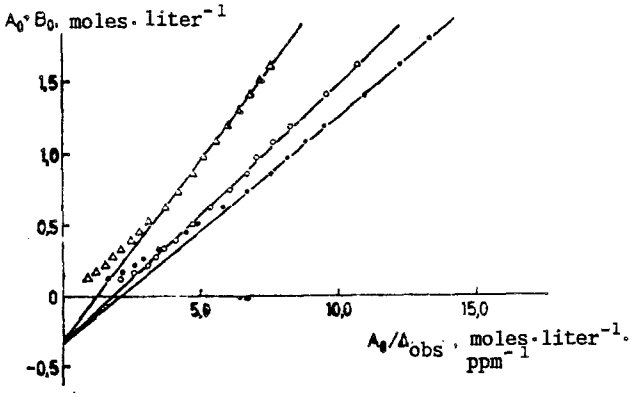


Fig. 4. Dependence of the sum of the starting concentrations of the base and trifluoroacetic acid $A_0 + B$ on A_0/Δ_{obs}: •) rheumycin (Ia); o) fervenulin (Ib); Δ) isofervenulin (II).

TABLE 2. Equilibrium Constants for the Protonation of Pyrimidotriazinediones Ia, b and II in $CDCl_3$ and Maximum shifts of the 3-H Signal in the ^1H NMR Spectra

Compound	Protonation equilibrium constant, K liter/mole	Maximum shift λ_{max}, ppm	Correlation coefficient r
Ia	2,92	0,16	0,999
Ib	2,91	0,18	0,996
II	3,21	0,25	0,999

fact that the shift of the resonance signals of the 3-H proton for each of the IVa-c cations as compared with the 3-H signal of base II should occur in the same direction, viz., to weak field. Thus, a weak-field shift of all of the signals of the ring protons upon protonation was previously shown in a series of azines [16], including a 1,2,4-triazine derivative [21]. In this connection the contributions of the individual IVa-c cations to the observed shift of the signal of the 3-H proton upon protonation of isofervenulin (II) should be summed up but should not compensate one another. On the basis of this, Eq. (1) can be used to determine the equilibrium constant of the protonation of isofervenulin.

An analysis of Eq. (1) shows that in the case of excess acid ($A_0 \gg B_0$), taking into account the postulate $\Delta_{max} > \Delta_{obs}$, the last term in this equation can be disregarded. Under these conditions the relationship between the sum of the starting concentrations $A_0 + B_0$ and the A_0/Δ_{obs} value has linear character. The equilibrium constant of the protonation process can be determined from the magnitude of the segment cut out on the axis of ordinates (= −1/K), and the maximum shift of the signal can be determined from the slope of the line ($b = \tan\alpha = \Delta_{max}$). The experimental results for Ia, b and II are presented in Fig. 4. It follows from the graphs that distinct linearity is realized at a trifluoroacetic acid concentration above 0.6 M (correlation coefficients greater than 0.996). The equilibrium constants and maximum shifts of the signals for Ia, b and II at 40°C calculated by the method of least squares are presented in Table 2. The equilibrium constants of protonation for rheumycin (Ia) and fervenulin (Ib) are virtually equal (2.92 and 2.91 liters/mole, respectively); this constitutes evidence for the identical basicities of these compounds. The constant is somewhat greater in the case of isofervenulin (II) (3.21 liter/mole); this makes it possible to assume that it is a somewhat stronger base than 7-azalumazines Ia, b. This is evidently associated with the greater thermodynamic stabilities of isofervenulinium cations IVa-c as a consequence of their stabilization due to delocalization of the positive charge over three centers – the $N_{(1)}$, $N_{(2)}$, and $O_{(6)}$ atoms.

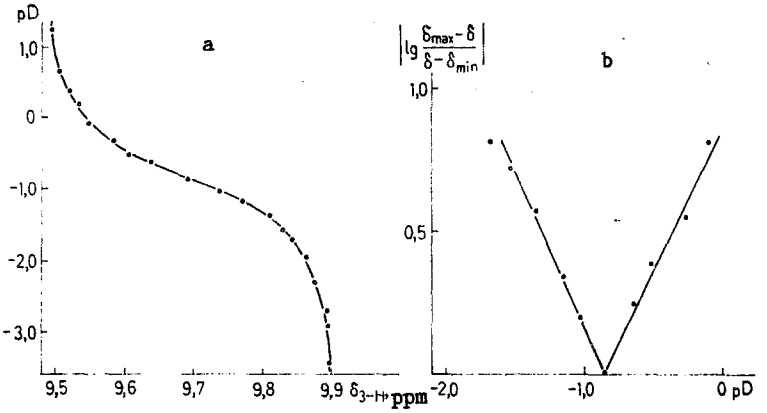


Fig. 5. Dependence of the chemical shift of the 3-H proton of isofervenulin (II) on the acidity of the medium: a) titration curve; b) logarithmic anamorphosis of the titration curve.

The protonation of isofervenulin also occurs in an aqueous medium. The dependence of the chemical shift of the methylidyne 3-H proton on the pD value is presented in Fig. 5a. The pK_a value of the isofervenulinium ion conjugate acid in aqueous (D_2O) solution indicated from the graph in the Henderson–Hasselbach coordinates [22] (Fig. 5b) is −0.86 (correlation coefficient 0.993). The behavior of 7-azalumazines Ia, b in aqueous acidic media differs fundamentally from the protonation of these compounds and isofervenulin in an anhydrous medium that was described in the present paper. Hydration of these compounds with the formation of covalent adducts involving the $N_{(4)}$–$C_{(4a)}$ bond occurs in the case of rheumycin (Ia) and fervenulin (Ib) [6].

EXPERIMENTAL

The ^1H and ^{13}C NMR spectra of solutions of the investigated compounds in $CDCl_3$ or D_2O were recorded at 40°C with a Bruker WH-90 spectrometer (West Germany) at 90 (^1H) and 22.62 MHz (^{13}C). The chemical shifts on the δ scale were measured for solutions in $CDCl_3$ relative to the signal of the residual protons of the solvent at δ 7.26 ppm (^1H) and of carbon at δ 77.0 ppm (^{13}C); in the case of aqueous solutions the chemical shifts were measured relative to the signal of dioxane as the internal standard at δ 3.65 ppm (^1H). The accuracies in the measurement of the chemical shifts determined by digital resolution were 0.001 ppm (for ^1H) and 0.02 ppm (for ^{13}C). In the measurement of the equilibrium constants from the ^1H NMR spectra the initial concentrations of bases Ia, b and II (B_0) were 0.102 to 0.014 M, and the initial trifluoroacetic acid concentration (A_0) ranged from 0 to 1.79 M. The acid (CF_3COOD) was dispensed with a microsyringe. The acidity of the solutions of isofervenulin (II) in D_2O was varied by the addition of hydrochloric acid (DCl/D_2O). The positive pD values were determined with a Knick-643 digital pH meter (West Germany); the H_0 scale [23] was used in the case of negative acidities.

LITERATURE CITED

1. S. M. Navashin, I. P. Fomina, V. G. Koroleva, T. G. Terent'eva, and L. A. Shtegel'man, Antibiotiki, No. 10, 892 (1967).
2. S. M. Navashin, L. P. Ivanitskaya, T. G. Terent'eva, and L. V. Egorov, Farmatsiya, No. 3, 55 (1981).
3. C. Kuechler, W. Kuechler, and L. Heinisch, Arzneim.-Forsch., 16, 1122 (1966).
4. N. P. Golovchenko, Author's Master's Dissertation Abstract, Institute of the Biochemistry and Physiology of Microorganisms, Academy of Sciences of the USSR, Pushchino (1977).
5. S. E. Esipov, N. A. Klyuev, L. A. Saburova, and V. M. Adanin, Khim. Prikl. Spektrosk., No. 1, 85 (1981).
6. S. V. Shorshnev, G. G. Aleksandrov, S. E. Esipov, N. A. Klyuev, and A. I. Chernyshev, Khim. Geterotsikl. Soedin. (1988, in press).
7. U. Ewers, H. Günther, and L. Jaenicke, Chem. Ber., 107, 3275 (1974).
8. E. Uhlmann and W. Pfleiderer, Heterocycles, 15, 437 (1981).
9. G. Müller and W. von Philipsborn, Helv Chim. Acta, 56, 2680 (1973).
10. S. Nishigaki, M. Ishiba, and K. Senga, J. Org. Chem., 48, 1628 (1983).

11. Yu. A. Azev, I. Ya. Postovskii, E. L. Pidemskii, and A. F. Goleneva, Khim.-Farm. Zh., No. 4, 39 (1980).
12. S. Nishigaki, H. Kanazawa, Y. Kanamori, M. Ichiba, and K. Senga, J. Heterocycl. Chem., 19, 1309 (1982).
13. C. M. Atkinson and H. D. Cossey, J. Chem. Soc., No. 3, 1628 (1963).
14. G. Doleschall and K. Lempert, Acta Chim. Acad. Sci. Hung., 77, 345 (1973).
15. S. G. Alekseev, V. N. Charushin, O. N. Chupakhin, S. V. Shorshnev, A. I. Chernyshev, and N. A. Klyuev, Khim. Geterotsikl. Soedin., No. 11, 1535 (1986).
16. R. J. Pugmire and D. M. Grant, J. Am. Chem. Soc., 90, 697 (1968).
17. G. G. Dvoryantseva, T. Ya. Filipenko, I. S. Musatova, A. S. Elina, P. V. Petrovskii, E. V. Arshavskaya, and É. I. Fedin, Izv. Akad. Nauk SSSR, Ser. Khim., No. 5, 1060 (1977).
18. S. Ya. Mel'nik, A. A. Bakhmedova, Yu. Yu. Volodin, M. N. Preobrazhenskaya, A. I. Chernyshev, S. E. Esipov, and S. M. Navashin, Bioorg. Khim., No. 11, 1723 (1981).
19. R. J. Pugmire and D. M. Grant, J. Am. Chem. Soc., 93, 1880 (1971).
20. R. Drago, Physical Methods in Chemistry [Russian translation], Vol. 1, Mir, Moscow (1981), p. 333.
21. S. G. Alekseev, P. A. Torgashev, M. A. Fedotov, A. I. Rezvukhin, S. V. Shorshnev, A. V. Belik, V. N. Charushin, and O. N. Chupakhin, Khim. Geterotsikl. Soedin., No. 4, 525 (1988).
22. E. Breitmaier and W. Voelter, ^{13}C NMR Spectroscopy, Verlag Chemie, Weinheim–New York (1978), p. 82.
23. A. Gordon and R. Ford, The Chemist's Guide [Russian translation], Mir, Moscow (1976), p. 79.

MASS-SPECTROMETRIC STUDY OF THE PRODUCTS OF INTRAMOLECULAR CYCLIZATION OF 1,3-AMIDO ALCOHOLS. 5-6-DIHYDRO-4H-1,3-OXAZINES AND 2-OXAZOLINES

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The mass spectra of substituted 5,6-dihydro-4H-1,3-oxazines and 2-oxazolines were studied for the identification of the products of intramolecular cyclization of 1,3-amido alcohols. The fragmentation of the molecular ions of 1,3-oxazines under the influence of electron impact proceeds via both fragmentation of the retrodiene type and with the formation of rearrangement ions, the relative intensities of the peaks of which are determined by the nature and position of the substituents in the heteroring. The molecular ions of 2-oxazolines undergo fragmentation chiefly with the loss of a molecule of a ketone.

One of the most widely used methods for the synthesis of 5,6-dihydro-4H-1,3-oxazines is the intramolecular cyclization of 1,3-amido alcohols under the influence of concentrated sulfuric acid [1]. However, it was recently established that the formation of both 5,6-dihydro-4H-1,3-oxazines and 2-oxazolines or mixtures of both isomeric cyclic imino esters is possible during this reaction, depending on the structure of the starting 1,3-amido alcohol [2, 3]. The closeness of the ^{1}H and ^{13}C NMR spectra of these compounds often makes it possible to draw an unambiguous conclusion regarding their structures. The analysis of the parameters of the NMR spectra of mixtures of both possible cyclic imino esters is fraught with

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