MOLECULAR BIOLOGICAL PROBLEMS OF THE CREATION OF DRUGS AND THE STUDY OF THE MECHANISM OF THEIR ACTION

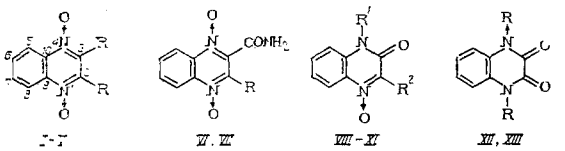
PHOTOCHEMICAL REACTIONS OF BIOLOGICALLY IMPORTANT QUINOXALINE N-OXIDES

G. G. Dvoryantseva, K. P. Tetenchuk,
V. I. Pol'shakov, and A. S. Elina

UDC 615.281:547.863.1].074:541.14

As is well known, heteroaromatic N-oxides undergo various types of photochemical reactions [7, 9, 14]. Some of these reactions are considered as promising methods of synthesizing a number of biologically important heterocyclic compounds, in particular, oxazepines and diazepines [7]. The ability of N-oxides of azines for photochemical conversions, together with the redox properties of this class of compounds, have recently been used successfully for the study of the mechanism of certain biochemical processes. Thus, the use of N-oxides of azines as enzyme-simulating photochemical oxidizing agents permits modeling of processes of biochemical oxidation in microsomes [6]. In connection with this, the study of the photochemical conversions of 1,4-di-N-oxides of quinoxaline derivatives, among which compounds with high biological activity have been detected, including the highly effective antibacterial preparations quinoxidine (I) and dioxidine (II), used in medical practice in the treatment of acute bacterial infections [4], is of special interest.

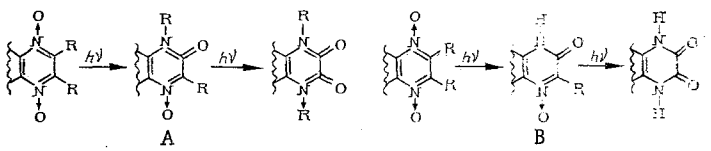
We were the first to study the photochemical reactions of I, II, and a number of related derivatives of quinoxaline 1,4-di-N-oxides, containing methyl, halomethyl, and carboxamide groups in the pyrazine ring (III-VII). The investigations were conducted during exposure of solutions of the starting materials I-VII in hydroxyl-containing and aprotic solvents under daylight conditions, as well as under irradiation by monochromatic light with a wavelength of 250 nm and a high-pressure mercury lamp at room temperature. Thin-layer chromatography, UV spectrophotometry, and NMR ^1H and ^{13}C spectroscopy were used as the main methods for monitoring the photolysis process and establishing the structure of the products VIII-XIII formed.



I: R = CH_2OCOCH_3; II: R = CH_2OH; III: R = CH_3; IV: R = CH_2Cl;
V: R = CH_2Br; VI: R = CH_2OH; VII: R = CH_3; VIII: $R^1 = R^2 = CH_2OCOCH_3$;
IX: $R^1 = R^2 = CH_3$; X: $R^1 = H$, $R^2 = CH_2OH$; XI: $R^1 = H$, $R^2 = CH_3$;
XII: R = CH_2OCOCH_3; XIII: R = H

RESULTS AND DISCUSSION

On the basis of our investigation it was established that two types of photochemical reactions are observed in the series of compounds discussed: photoisomerization with migration of a substituent to the nitrogen atom of the heterocycle (A), and photorearrangement with elimination of a substituent and the formation of the corresponding lactams (B).



The 1-N-oxide of 2,4-bis(acetoxymethyl)-3-oxo-3,4-dihydroquinoxaline (VIII) and 1,4-bis-(acetoxymethyl)-2,3-dioxo-1,2,3,4-tetrahydroquinoxaline (XII) were isolated as the main products of photolysis of I in hydroxyl-containing solvents (water, methanol, ethanol). The data of elementary analysis and the mass of the molecular ion in the mass spectra of I, VIII,

S. Ordzhonikidze All-Union Chemicopharmaceutical Scientific-Research Institute, Moscow.
Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 20, No. 4, pp. 399-408, April, 1986. Original article submitted July 22, 1985.

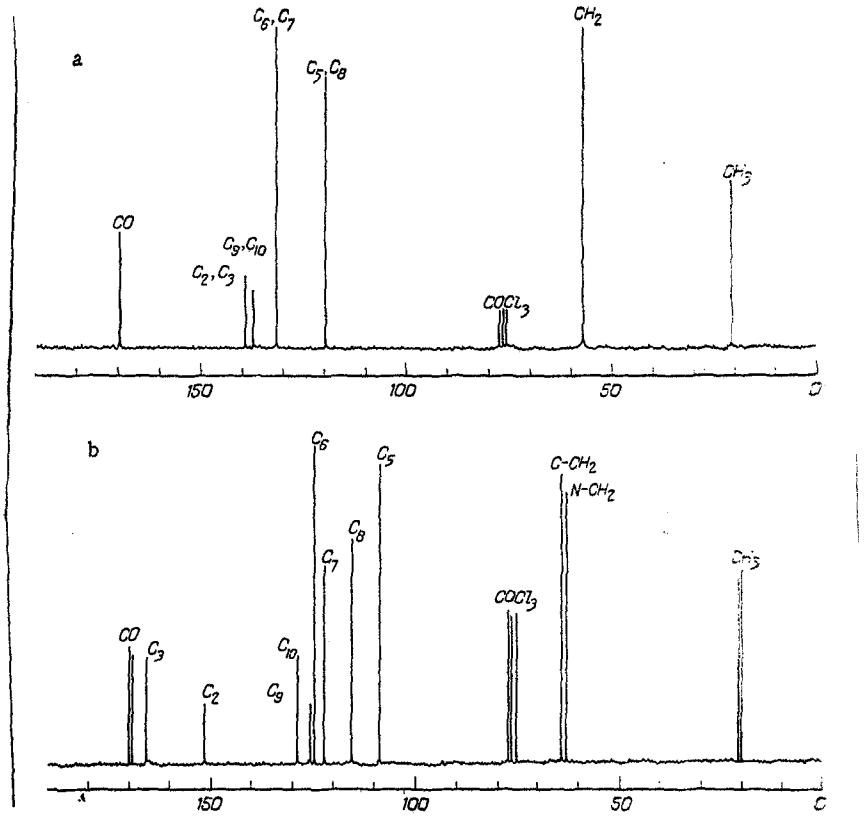


Fig. 1. NMR-^{13}C spectra in $CDCl_3$. a) Quinoxadine (I); b) 1-N-oxide of 2,4-bis(acetoxymethyl)-3-oxo-3,4-dihydroquinoxaline (VIII) (δ scale).

and XII (306.2) correspond to the same gross formula of all three compounds — $C_{14}H_{14}N_2O_6$. The structure of the photolysis products was demonstrated by the methods of NMR and IR spectroscopy.

A split doublet of H_8 (δ 8.16 ppm, $J_{8,7}$ 8 Hz) is situated in a weak field in the NMR-^{1}H spectrum of the mono-N-oxide VIII in $CDCl_3$. The multiplet with intensity three proton units (p.u.) in the region of 7.1-7.3 ppm was assigned to the protons H_5, H_6, and H_7. The signals of the methylene protons of the two acetoxymethyl groups, bonded to the N_4 and C_2 atoms, are observed in the form of singlets with intensity 2 p.u. at 5.90 and 5.37 ppm. The two corresponding signals of the methyl protons are situated in the strong field region (δ 2.25 and 2.11 ppm). The structure of VIII was confirmed by a comparison of the NMR-^{13}C spectra of this compound and I. In the spectrum of VIII, measured with complete suppression of spin-spin coupling (SSC) with protons (Fig. 1b), in contrast to the spectrum of the symmetrical molecule I (Fig. 1a), 14 signals are observed. The assignment of the signals (see Fig. 1; Table 1) was performed on the basis of the nature of the multiplicity of the spectral lines in the monoresonance ^{13}C spectra of the investigated compounds, the values of the SSC $^{13}C^1H$ constants, and a comparison of the spectral parameters (see Table 1) with the previously described spectral parameters of quinoxaline derivatives and their N-oxides [11, 12]. A characteristic of the spectrum of VIII is the weak field shift of the signal of C_3 into the region typical of the carbonyl carbon atoms (δ 166.9 ppm), as well as the doubling of the signals of the carbon atoms of the CH_2OCOCH_3 groups, corresponding to the different position of these groups in the pyrazine ring of the molecule. Thus, two triplets of the carbon atoms, possessing close values of the chemical shifts — 63.84 and 63.25 ppm — differ substantially in the values of the direct constants $^1J_{(C,H)}$, one of which (153.8 Hz) is close to the value of the corresponding constant for compound I (153.0 Hz), while the other (164.2 Hz) is significantly greater. According to this characteristic, the signal at 63.25 ppm with a larger value of the $^1J_{(C,H)}$ constant should be assigned to the N-CH_2 group.

A comparison of the spectra of I, VIII, and XII, measured in $CDCl_3$, unambiguously confirms the structure of compound XII. In the NMR-^{1}H spectrum of compound XII the signals in the region of 8-9 ppm, characteristic of quinoxaline N-oxides, are absent. The signals of

TABLE 1. Chemical Shifts of ^{13}C and Direct SSC $^{13}C^{1}H$ Constants of the Carbon Atoms of the Quinoxaline Bicycle

Compound	Solvent	δ, ppm								$^{1}J_{(C,H)}$, Hz			
		$C_{(2)}$	$C_{(3)}$	$C_{(5)}$	$C_{(6)}$	$C_{(7)}$	$C_{(8)}$	$C_{(9)}$	$C_{(10)}$	$C_{(5)}$	$C_{(6)}$	$C_{(7)}$	$C_{(8)}$
I	$CDCl_3$	138,9	138,9	120,2	131,9	131,9	120,2	137,4	137,4	172	167	167	172
VIII	$CDCl_3$	151,6	166,9	108,7	125,2	123,7	115,6	125,4	128,6	166	163	162	170
X	ДМСО-d_6	141,1	156,4	116,2	132,3	123,3	119,4	129,7	132,0	166	164	167	171,5
XIII	ДМСО-d_6	155,1	155,1	115,1	122,9	122,9	115,1	125,5	125,5	163	163	163	163

TABLE 2. Characteristic Absorption Bands in the UV Spectra of I and Products of Its Photoisomerization VIII and XII

Compound	Solvent	λ_{max}, nm(lg ε)		
I	Water	242 (4,44);	260 (4,54);	375 (4,13)
	Ethanol	237 (4,44);	267 (4,47);	390 (4,12)
	Chloroform		269 (4,51);	395 (4,19)
VIII	Water	255 (4,70);	276 (3,64);	285 (3,60)
	Ethanol	252 (3,66);	277 (3,46);	286 (3,35)
	Choroform	256 (3,68);	277 (3,46);	288 (3,34)
XII	Water	229 (4,08);	260 (3,66);	308 (3,86)
	Ethanol	231 (4.08);	260 (3,58);	310 (3,86)
	Chloroform		260 (3,62);	310 (3,86)

the protons of the benzene ring $H_{(5)}$—$H_{(8)}$ are observed in the form of two overlapping multiplets at 7.1-7.3 ppm (summary intensity for p.u.). The singlet of the methylene protons (4 p.u.), according to the value of the chemical shifts (6.20 ppm), is close to the signal of the N-CH_2 group in VIII. The signal of the methyl protons (δ 2.04 ppm) represents a singlet with intensity 6 p.u.

In the IR spectra of compounds VIII and XII, together with the band of the stretching vibrations of the ester groups C=O (1730 and 1740 cm^{-1}), the characteristic band of the amide carbonyls at 1680 and 1670 cm^{-1} is detected.

An identification of structures VIII and XII permits us to conclude that in hydroxyl-containing solvents I undergoes photochemical isomerization with migration of the substituents to the nitrogen atom of the heterocycle (reaction of type A). The UV spectra of compounds I, VIII, and XII, measured in different media (Table 2), exhibit characteristic differences in position and intensity of the observed absorption bands, due to a change in the aromatic system of the chromophore in the process of photoisomerization of I. This permits the use of the method of UV spectrophotometry to monitor the process I → XII. Figure 2a presents a series of absorption curves of a solution of I in ethanol, measured for various moments of the reaction under daylight conditions. In time a gradual decrease in the longwave band at 390 nm, characteristic of the spectrum of I, is observed, down to complete disappearance of the corresponding maximum on the curve. The intensity of the band in the region 230-260 nm has a parallel decrease, and the intensity of the absorption band 310 nm, characteristic of XII, increases. The spectrum of the final state coincides with the spectrum of the isolated photolysis product XII. We should mention that as a result of the relatively low intensity of the absorption bands of compound VIII in the investigated region in comparison with the spectra of the original compound I and the final reaction product XII, the step of formation of the mono-N-oxide VIII could not be distinguished spectrophotometrically from the summary process I → XII. This problem was solved by thin-layer chromatography. The chromatograms presented in Fig. 3a, followed by UV detection of alcohol solutions of I stored in the light, characterize the change in the relative content of the starting materials and the substance formed over the passage of time. The data of thin-layer chromatography are distinct evidence of the successive occurrence of two steps of the reaction: the formation of the mono-N-oxide VIII and further conversion of VIII to the end product of the reaction XII. Consequently, photoisomerization of I proceeds in stages with excitation of the transition state, initially including one N → O group.

A similar type of photochemical reaction has been detected for the 1,4-di-N-oxide of 2,3-dimethylquinoxaline (III). The compound IX, which has been identified as the 1-N-oxide of 2,4-dimethyl-3-oxo-3,4-dihydroquinoxaline, has been isolated as the main photolysis product of III in aqueous and alcohol media. A split doublet of H_8 (δ 8.5 ppm, $J_{8,7}$ 8.5 Hz) and two

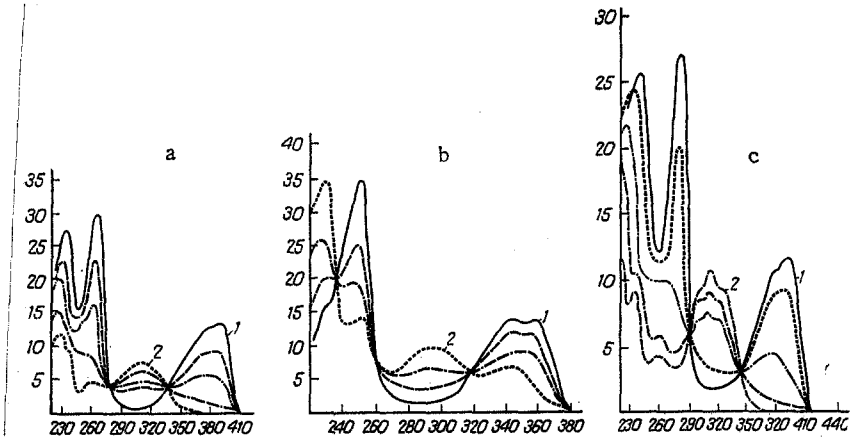
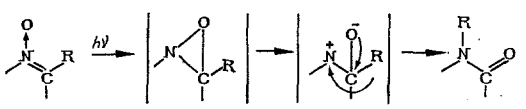


Fig. 2. Changes in the UV spectra of $4.0 \cdot 10^{-5}$ M solutions of compounds I, III, and V in the process of protolysis. a) Reaction I → XII in ethanol; b) reaction III → IX in water; c) reaction V → XIII in ethanol. 1) Initial state; 2) final state. Along x-axis, λ (in nm); along y-axis, $\varepsilon \cdot 10^{-3}$.

overlapping multiplets at 7.6 and 7.3 ppm, with intensity ratio 1:2, belonging to the protons $H_{(6)}$, $H_{(5)}$, and $H_{(7)}$, are detected in the NMR-^{1}H spectrum of mono-N-oxide IX. The signals of the methyl protons of the N—CH_3 and C—CH_3 groups are observed in the form of singlets at 3.73 and 2.61 ppm, respectively. These data, as well as the results of a measurement of the UV and IR spectra, a determination of the mass of the molecular ion in the mass spectrum (190.0), and elementary analysis are in full agreement with the structure of III under daylight. The successive decrease in the intensity of the bands characteristic of the spectrum of III, with absorption maxima 250 and 360 nm, and a simultaneous increase in the absorption intensity at 230 and 295 nm, characteristic of the spectrum of IX, are evidence of the occurrence of the photoisomerization reaction III → IX. The presence of distinct isobestic points on a series of absorption curves measured at various moments of the reaction III → IX shows that in this process only two substnces are detected spectrophotometrically: the initial di-N-oxide III and the end product of the reaction IX. This result is confirmed by monitoring of the reaction by thin-layer chromatography. An analogous process occurs when a $4.0 \cdot 10^{-5}$ M aqueous solution of III is irradiated with monochromatic light with a wavelength 250 nm, at which the most intense $\pi \rightarrow \pi^*$ transition is observed in the spectrum of III. The high quantum yield of the reaction ($\varphi = 0.82$), close to the theoretical, when $\varphi = 1$, suggests that the photochemical conversion of the di-N-oxides I and III proceed as a monomolecular isomerization of the singlet $\pi \rightarrow \pi^*$ excited state.

In general, for photochemical rearrangements with displacement of the oxygen of the N → O group and migration of the substituent to the nitrogen atom of the heterocycle, a mechanism including the formation of metastable oxaziridine rings, followed by transition to a bipolar intermediate state, is proposed [7, 14]:



The formation of oxaziridine rings as the most probable structures arising from low-energy excited states agrees with the results of quantum chemical calculations [7, 10]. A transition state of this type has been considered, in particular, for the photoisomerization of certain derivatives of the 1,4-di-N-oxide of 2-benzylquinoxaline, with the formation of 1,4-disubstituted quinoxalinediones [9]. Moreover, it has been suggested that the most probable mechanism includes simultaneous excitation of both N → O functions in positions 1 and 4. However, the results that we obtained are distinct evidence of a step-wise mechanism of the photoisomerization of the investigated compounds. Moreover, a comparative study of the photochemical reactions of I and II in various media showed that for rearrangements with a displacement of oxygen and elimination of a fragment of the substituent (type B), excitation of a different structure of the transition state is the most probable.

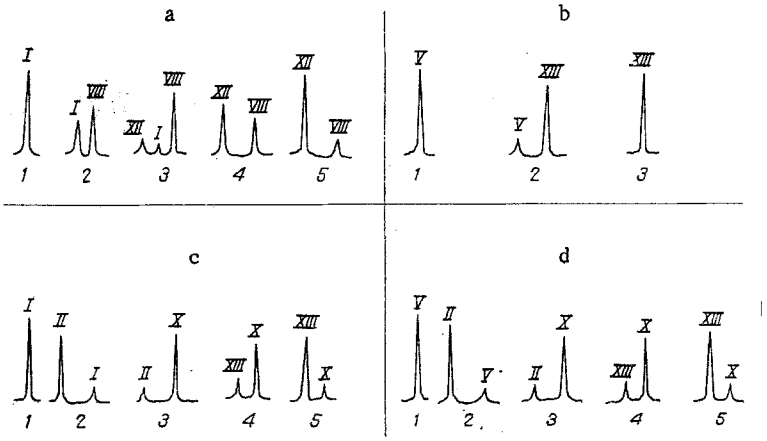
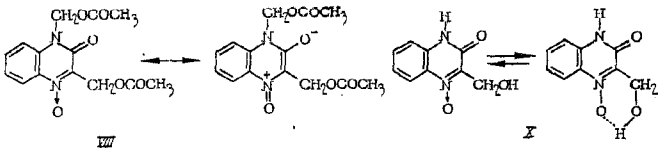


Fig. 3. Chromatograms followed by UV detection of 0.01% solutions of quinoxadine I and the 1,4-di-N-oxide of 2,3-bis(bromomethyl)quinoxaline V, stored in the light, in ethanol. a) Initial compound I, time (in days): 1) 0, 2) 1, 3) 2, 4) 3, 5) 4; b) initial compound V, time (in days): 1) 0, 2) 1, 3) 2; c, d) chromatograms of solutions of I (c) and V (d), prepared with heating. 1) Initial solution without heating; 2) initial solution after heating, time (in days): 3) 2, 4) 5, 5) 6.

In a previous study [5] we established that II in hydroxyl-containing solvents undergoes a photochemical rearrangement to 2,3-dioxo-1,2,3,4-tetrahydroquinoxaline (XIII) with the formation of the 1-N-oxide of 2-hydroxymethyl-3-oxo-3,4-dihydroquinoxaline (X) as an intermediate product. At relatively low concentrations of the initial solutions of II (10^{-5}-10^{-3} M), both steps of the process II → X → XIII are clearly separated in time. When the initial concentration of II is increased to 10^{-2} M, the second step X → XIII is usually observed against a background of the continuing reaction II → X. Further investigations showed that the photolysis of II in ethanol and water is accompanied by a decrease in the pH of the solutions. After complete conversion of II to XIII in aqueous and alcohol solutions by the method of photometry [3], an amount of the formate ion equivalent to the elimination of two hydroxymethyl groups is detected. These data correspond to the elimination of a fragment of the hydroxymethyl group in the form of formaldehyde, which is then subjected to oxidation with the formation of formic acid. As is well known, many aldehydes are oxidized in air to the corresponding acids [2].

The polarity and solvating ability of the solvent have various effects on the ratio of the products of photochemical reactions I and II. In aprotic media with a low dielectric constant (benzene (ε 2.3) and chloroform (ε 4.7)), the photoisomerization of I is limited to the first step, i.e., the formation of the mono-N-oxide VIII. In the case of prolonged storage of solutions of VIII in the light, no further photochemical conversions are detected spectrophotometrically and chromatographically in these solvents. The photochemical stability is determined by the peculiarities of the electronic structure of this compound. A study of the NMR and UV spectra in various media showed that the structure of the mono-N-oxide VIII denotes a significant contribution of a structure with charge transfer from the N → O group to the carbonyl oxygen atom. Predominance of such a structure is indicated by the large hypsochromic shift of the longwave band in the UV spectrum of VIII (see Table 2) relative to the low energy transitions observed in the spectra of the mono-N-oxide X [5] (Δλ 70-80 nm) and 2,3-dioxo-1,2,3,4-tetrahydroquinoxalines XII and XIII (Δλ 20-40 nm). In the NMR-^{13}C spectrum of VIII (see Table 1), the signal of $C_{(3)}$=O is shifted in the weak field direction relative to the corresponding signals in the spectra of X and XIII (Δδ 10-12 ppm). This effect is evidence of a significant increase in the electron density on the carbonyl oxygen and a decrease in the order of the $C_{(3)}$=O bond. At the same time, no significant delocalization of electron density from the N → O group into the benzene ring is observed in the molecule of X: the signals of $C_{(5)}$-$C_{(10)}$ are situated in a stronger field relative to the corresponding signals in the spectrum of the mono-N-oxide X. Since the electronic effects of the substituents (CH_2OH and CH_2OCOCH_3) are rather similar, the observed differences in the electronic structure of VIII and X are evidently due to the ability of compound X to form intramolecular hydrogen bonds on account of the mobile hydroxyl protons of the hydroxymethyl group. The formation of hydrogen

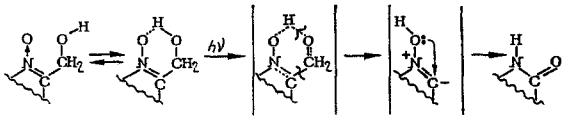
bonds stabilizes the structure with localization of the effective negative charge primarily on the oxygen atom of the N → O group.



VIII X

Intramolecular charge transfer from the N → O group to the carbonyl oxygen leads to an increase in the energy of excitation of the polar transition state in the absence of appreciable solvation effects. This evidently explains the photochemical stability of the mono-N-oxide VIII in aprotic media with a low dielectric constant. In polar hydroxyl-containing solvents (ethanol (ε 24.3) and water (ε 78.5)), there is a photoisomerization of VIII with the formation of XII. Aromatic N-oxides exhibit a substantial tendency to form hydrogen bonds with proton donor solvents [8]; moreover, the oxygen atom of the N → O group possesses greater proton acceptor capacity in comparison with the carbonyl oxygen. The effects of solvation stabilize the polar transition state, and the yield of photolysis products, as a rule, is appreciably increased with increasing polarity of the solvent [1, 7].

For the mono-N-oxide X, in contrast to VIII, a photochemical rearrangement of the type of B is observed, with the formation of the dioxoderivative XIII, both in hydroxyl-containing solvents (water, methanol, and ethanol) and in aprotic media with various dielectric constants (benzene, chloroform, and dimethylformamide (ε 36.7)). The detected differences in the influence of the polarity and solvating ability of the solvent on the relative photochemical stability of compounds VIII and X is evidence of a different mechanism of the two types (A and B) of photochemical reactions. The aggregate of data obtained suggests that the rearrangement of N-oxides of quinoxalines containing hydroxymethyl groups in the pyrazine ring proceeds according to a mechanism including the formation of six-membered rings on account of intramolecular hydrogen bonds with transfer of a proton to the oxygen atom of the N → O group in the excited state and elimination of a fragment of the substituent:



Such a mechanism permits the possibility of excitation of the transition state, including both N → O functions, in the absence of solvation effects. Actually, in benzene and chloroform the photochemical reaction II leads to the formation of XIII with a high yield, and in these media, in contrast to hydroxyl-containing solvents, the intermediate stage of formation of the mono-N-oxide X cannot be distinguished.

Within the framework of the mechanism that we are proposing, the ability of N-oxides for photochemical rearrangement of the type of B is determined chiefly by the presence of a substituent in the α-position to the N → O function, capable of forming intramolecular hydrogen bonds with transfer of a proton at relatively low energies of excitation. Of substantial interest from this standpoint was a study of the photochemical reactions of N-oxides of 3-carboxamidoquinoxaline derivatives. We established that 1,4-di-N-oxides of 2-hydroxymethyl- and 2-methyl-3-carboxamidoquinoxaline (VI and VII) also undergo photochemical rearrangement with elimination of the substituent containing labile protons. In the photolysis of VI in water, ethanol, and DMFA, compound XIII is formed with a high yield. The 1-N-oxide of 2-methyl-3-oxo-3,4-dihydroquinoxaline XI was isolated as the main product of the photochemical reaction of VII in aqueous and alcohol media. The structure of XI was demonstrated on the basis of a comparison of the NMR-^{1}H spectrum of the product isolated with the spectra of the mono-N-oxides IX and X and was confirmed by the data of other physicochemical methods of investigation.

We should note that in the process of photochemical reactions of compounds II, VI, VII, and X in various solvents, including those under conditions excluding the possibility of hydrolysis, no formation of photoisomerization products with migration of the hydroxymethyl or carboxamide group to the nitrogen atom of the heterocycle was detected.

The photolysis of 1,4-di-N-oxides of halomethyl derivatives of quinoxaline IV and V in aqueous and alcohol solutions with the formation of XIII (see Figs. 2c and 3b) is probably

associated with hydrolysis processes. The feasibility of hydrolyzing compounds IV and V to II in these media was confirmed in our experiments. The rate of the dark reaction of hydrolysis increases sharply with increasing temperature, whereas the rate of photochemical reactions does not depend on the temperature [1]. Therefore, the successive steps of the summary process — the reaction of hydrolysis V → II (or IV → II) and further photochemical conversions of dioxidine II → X → XIII — are detected chromatographically after preparation of a solution of the initial compound with heating (Fig. 3d). In an analogous experiment a similar process is also observed for I. In a 0.01% alcohol solution of I, prepared with heating, a predominant content of the product of hydrolysis I → II is detected (Fig. 3c). Subsequent chromatograms of this solution with exposure to daylight are evidence of the occurrence of a photochemical rearrangement, characteristic of II. However, in the process of photochemical conversions of I in aqueous and alcohol solutions, prepared at room temperature, the formation of compounds II, X, and XIII was not observed (see Fig. 3a). Consequently, under the usual conditions, photoisomerization of I is not accompanied by processes of hydrolysis both of the initial compound and of the reaction products VIII and XII formed.

Thus, as a result of our investigation it was established that the antibacterial preparations I and II exhibit an ability for two different types of photochemical rearrangements with displacement of the oxygen of the N → O group, proceeding according to different mechanisms; moreover, each preparation is characterized by one definite photochemical reaction. The high selectivity of the photochemical conversions of I and II reflects the specifics of their molecular structure. The presence of hydroxymethyl groups containing labile hydroxyl protons in the α-positions to the N → O functions is the main structural factor determining the peculiarities of the mechanism of the rearrangement of II.

EXPERIMENTAL

The UV spectra of the investigated compounds were recorded on a Specord M-40 spectrophotometer (German Democratic Republic). The IR spectra of crystalline samples were measured on a UR-20 spectrometer (German Democratic Republic) in tablets with KBr. The NMR-^{1}H spectra were recorded on a Varian XL-200 spectrometer (Switzerland) with working frequency 25.2 MHz, internal standard TMS. The mass spectra were measured on a Varian MAT-112 chromato-mass spectrometer (Federal Republic of Germany). Thin-layer chromatography was conducted on Silufol UV-254 plates, using the solvent system chloroform–heptane–ethanol 50:30:30 and carbon tetrachloride–chloroform–ethanol 70:20:15 (detection in UV light). The relative content of the substances in the chromatographic spots was determined with an Opton recording spectrophotometer (Federal Republic of Germany).

The photolysis of compound III in the case of irradiation with monochromatic light (λ 250 nm) was conducted in a $4.0 \cdot 10^{-5}$ M aqueous solution, placed in a thermostatically controlled quartz cuvette, using a DDS-30 radiation source and a prism monochromator. For a calculation of the quantum yield of the reaction III → IX, the light intensity was measured with a ferrioxalate actinometric solution according to the procedure described in [1].

1-N-Oxide of 2,4-Bis(acetoxymethyl)-3-oxo-3,4-dihydroquinoxaline (VIII). A. A solution of 1 g I in 100 ml chloroform was exposed to light for approximately one week. The degree of photochemical conversion was monitored by the method of thin-layer chromatography. After disappearance of the starting material, the solvent was removed and the precipitate recrystallized from ethanol. Yield: 0.9 g (90%) VIII, mp 150-151°C. Found, %: C 54.73; H 4.54; N 9.14. $C_{14}H_{14}N_2O_6$. Calculated, %: C 54.88; H 4.60; N 9.14. Mass spectrum, m/e: 306.2 (M^+). IR spectrum, cm^{-1}: 1730 (C=O ester), 1680 (C=O amide). UV spectrum: see Table 2 and Fig. 2a. NMR-^{13}C spectra: see Table 1 and Fig. 1b; NMR-^{1}H spectrum: see explanation in text.

B. A solution of 0.5 g I in 100 ml of chloroform was irradiated with a high-pressure mercury lamp for 3 h. Yield 0.40-0.45 g (80-90%) VIII.

1-N-Oxide of 2,4-Dimethyl-3-oxo-3,4-dihydroquinoxaline (IX). A. An aqueous solution of III (1 g in 400 ml) was exposed to light until the initial compound disappeared (∿2 weeks), concentrated under vacuum (10 mm Hg, 25°C) to 50 ml, and extracted with chloroform; the extract was concentrated to 25 ml, 10-15 ml, and extracted with chloroform; and the precipitate was filtered. Yield: 0.81 g (81.6%) IX, mp 188-191°C. Found, %: C 63.10; H 5.27; N 14.70. $C_{10}H_{10}N_2O_2$. Calculated, %: C 63.15; H 5.30; N 14.72. Mass spectrum, m/e: 190.0 (M^+). IR spectrum, cm^{-1}: see explanation in text.

B. A solution of 0.25 g III in 50 ml of water was irradiated with a high-pressure mercury lamp for 3 h. Yield: 0.2 g (80%) IX.

1-N-Oxide of 2-Methyl-3-oxo-3,4-dihydroquinoxaline (XI). A. An ethanol (aqueous) solution of VII (0.05 g in 500 ml) was left in the light until the initial compound disappeared (∿3 days), concentrated to 25 ml under vacuum, and the precipitate filtered off. Yield: 0.036 g (90%) XI, mp 195-197°C. Found, %: C 61.05, H 4.55; N 15.80. $C_9H_8N_2O_2$ Calculated, %: C 61.35; H 4.59; N 15.89. Mass spectrum, m/e: 176.2 (M^+). IR spectrum, cm^{-1}: 1650 (C=O amide). UV spectrum (ethanol), λ_{max}, nm (log ε): 252 (3.71), 275 (3.57), 285 (3.50). NMR-^{1}H spectrum (DMSO-d_6), δ, ppm: 11.43 br. s. (N-H), 8.05 d, $H_{(8)}$, 7.24 t, $H_{(6)}$, 7.14 t, $H_{(7)}$, 7.08 d, $H_{(5)}$, 2.61 s (C-CH_3).

B. A solution of 0.05 g VII in 500 ml of ethanol was irradiated with a high-pressure mercury lamp for 1 h. Yield 0.032-0.036 g (80-90%) XI.

1,3-Bis(acetoxymethyl)-2,3-dioxo-1,2,3,4-tetrahydroquinoxaline (XII). A solution of 0.1 g I in 200 ml of ethanol was left in the light until the initial compound disappeared (∿5 days). The content of XII in solution was determined spectrophotometrically. Yield: 0.067 g (67%) XII. The solution was concentrated under vacuum to 25 ml, 50 ml of diethyl ether was added, the mixture exposed for 3-4 h, and the precipitate filtered off. Yield: 0.012 g (12%) XII, mp 160-163°C. Found, %: C 54.73; H 4.82; N 9.12. $C_{14}H_{14}N_2O_6$. Calculated, %: C 54.88; H 4.60; N 9.14. Mass spectrum, m/e: 306.0 (M^+). IR spectrum, cm^{-1}: 1470 (C=O ester), 1670 (C=O amide). UV spectrum: see Table 2. NMR-^{1}H spectrum: see explanation in text.

2,3-Dioxo-1,2,3,4-tetrahydroquinoxaline (XIII). A. Production of XIII from II in Benzene. A solution of 0.1 g II in 400 ml of benzene was exposed in the light until the initial compound disappeared (∿3 days), concentrated in a rotary evaporator (10 mm Hg, 25°C) to 10 ml, and the precipitate filtered off. Yield: 0.065 g (90%) XIII, identical with the 2,3-dioxo-1,2,3,4-tetrahydroquinoxaline produced by the well-known method in [13].

B. Production of XIII from VI in Ethanol and Water. An ethanol (aqueous) solution of VI (0.25 g in 500 ml) was exposed to light until the initial compound disappeared (∿5 days). Further treatment of the solution was performed analogously to A. Yield: 0.16 g (93.3%) XIII.

C. Production of XIII from IV and V in Ethanol and Water. An ethanol (aqueous) solution of IV or V (0.25 g in 500 ml) was exposed to light until the initial compound disappeared (2-3 weeks). The solvent was distilled off under vacuum at room temperature. Yield: 0.14 g (89.7%) XIII from IV and 0.10 g (84.7%) XIII from V.

Quantitative Determination of the Formate Ion. A 0.02% aqueous solution of II was left in the light until compounds II and X disappeared (pH 5.7, monitored by the method of thin-layer chromatography). Then 5 ml of the solution was placed in a 25-ml volumetric flask, 2 ml of Griss reagent was added, the mixture exposed for 10 min, the volume of the solution brought up to the mark with ethanol, and the optical density measured at 480 nm. Calculation was performed with the aid of the specific index of absorption, obtained on a standard solution of formic acid, and adjusted to pH 5.7 with alkali. The yield of formate ion found was 99.8% of the theoretical amount.

LITERATURE CITED

1. G. O. Bekker, ed., Introduction to the Photochemistry of Organic Compounds [Russian translation], Leningrad (1976), pp. 89-124.
2. R. Shriner, R. Fieson, J. Curtin, and P. Morrill, Identification of Organic Compounds [Russian translation], Moscow (1983), p. 188.
3. I. M. Korenman, Photochemical Analysis: Methods of Determination of Organic Compounds [in Russian], Second Edition, Moscow (1975), p. 67.
4. E. N. Padeiskaya, in: New Chemotherapeutic Preparations for the Treatment of Patients with Infectious Diseases [in Russian], Moscow (1976), pp. 89-103.
5. K. P. Tetenchuk, G. G. Dvoryantseva, I. S. Musatova, and A. S. Elina, Khim.-Farm. Zh., No. 12, 1493-1499 (1984).
6. M. N. Akhtar, D. R. Boyd, J. D. Neill, and D. M. Jerina, J. Chem. Soc. Perkin Trans. I, 1693-1699 (1980).
7. A. Albini and M. Alpegiani, Chem. Rev., 84, 43-71 (1984).
8. Z. Dega-Szafran, M. Szafran, and M. Kreglewski, J. Chem. Soc. Perkin Trans. II, 1516-1521 (1980).
9. A. A. Jarrar and Z. A. Fataftan, Tetrahedron, 33, 2127-2129 (1977).
10. C. Kaneko, S. Yamada, and I. Yokoe, Tetrahedron Lett., 2333-2336 (1970).
11. A. F. Kluge, M. L. Maddox, and G. S. Lewis, J. Org. Chem., 45, 1090-1914 (1980).
12. H. McNab, J. Chem. Soc. Perkin Trans. I, 357-363 (1982).

13. M. A. Phillips, J. Chem. Soc., 2392-2399 (1928).
14. G. G. Spence, E. C. Taylor, and O. Buchardt, Chem. Rev., 70, 231-265 (1970).

PHARMACOKINETIC STUDY OF POLYMERIC DRUGS.

RADIOISOTOPIC METHOD FOR PHARMACOKINETIC INVESTIGATIONS

G. N. Pimenova, V. A. Matveev, and V. N. Kulakov

UDC 615.014.474:678].033.073.916

The absence of standard procedures for pharmacokinetic analysis inhibits the introduction into practice of preparations based on polymeric compounds. The radioisotopic procedure (RP) of [3] for checking using gamma emitters is most informative and effective in the pharmacokinetic study of high molecular drugs. The distribution of labeled compounds in the organism is recorded in the majority of cases in experimental investigations by direct radiometry of the organs and tissues of laboratory animals after their death [5]. A large number of animals is used for obtaining statistically significant data which complicates these investigations and hinders their use for operative pharmacokinetic checking. The error with which the pharmacokinetic parameters of a studied polymer are determined may serve as a criterion of the usefulness of the RP. Only at sufficiently small errors and in the presence of a good correlation with the chosen pharmacokinetic model (PM) can the studied substance be characterized with the aid of the values of the indicated parameters and a comparative study of a series of compounds be carried out.

The possibilities of the RP are illustrated in the present work using as example a study of the pharmacokinetics of δ-chymotrypsin (I) labeled with ^{125}I, (^{125}I-I).

MATERIALS AND METHODS

Labeled ^{125}I-(I) was obtained by the electrochemical iodination of (I) at constant potential as in [4] when no appreciable change in the enzymic activity of the preparation occurred. The solution of ^{125}I-(I) has the following characteristics: enzyme concentration 1 mg/ml, pH 7.0 (0.1 M phosphate buffer, prepared from physiological solution), volume radioactivity 22 MBq/ml, and content of radioactive iodide up to 2%. Random bred white rats of weight 150-180 g were used in the work, and ^{125}I-(I) solution (0.2 ml) was injected into the tail vein. Weighing of the syringe was carried out on an analytical balance with a precision of 0.0001 g. Animals were killed by decapitation. Organs and tissues (blood, liver, kidneys, heart, lungs, stomach, large and small intestine, spleen, thyroid, brain) were subjected to direct radiometry after 1, 3, 5, 15, 30, 60, 180, 300, and 1440 min. Measurement of radioactivity was carried out with a Gamma apparatus (Hungary). The results of radiometry were expressed as the percentage of the injected radioactivity relative to the weight of organs and tissues.

Mathematical processing of data for calculating pharmacokinetic parameters was carried out by first-order regression analysis. Coefficients of the regression equation were determined, as also were their standard deviations [1, 2], the confidence intervals at a significance level P = 0.05, $\Delta = S \cdot t^P_{n-2}$, where t^P_{n-2} is the Student coefficient and n the number of experimental points. Calculations were carried out with an Elektronika MK-56 programmable microcalculator.

The experiment was carried out in two stages. Stage I was a preliminary assessment of the time of removal (accumulation) of the preparation from 3-4 time points; stage II was an investigation of a selected time range. It was usually adequate to select 6-8 time points.

To check the extent of the destruction of ^{125}I-(I), blood serum and urine of rats was subjected to analysis by electrophoresis on a cellulose acetate film using ^{125}I-(I) as reference.

Scientific-Research Institute for the Biological Testing of Chemical Compounds, Moscow Province. Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 20, No. 4, pp. 408-412, April, 1986. Original article submitted March 11, 1985.

0091-150X/86/2004-0235$2.50