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

## REACTIVITY AND MECHANISM OF THE ANTITUMOR ACTION OF TRIAZENES. V.* INTERACTION OF AROMATIC DIAZODERIVATIVES WITH THE METHYLAMIDE OF L-PROLINE

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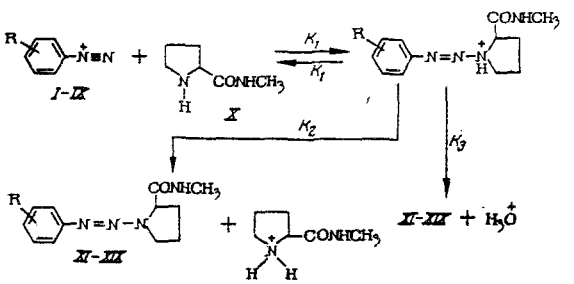
The L-proline residues contained in the structure of certain natural peptides [1], as well as the free amino acids L-proline and L-hydroxyproline of the blood, are potential nucleophilic targets for aryldiazo-compounds as one of the active ingredients in antitumor and antimetastic 3,3-dimethyl-1-aryltriazenes.

In the development of the investigations of [3], in this work we studied the reactivity of a series of aromatic diazoderivatives (I-IX) in reactions with a model of the proline target of peptides – the methylamide of L-proline (X). The latter is a good model for the behavior of the N-terminal proline residues of natural peptides, and the free amino acids L-proline and L-hydroxyproline in biological media.




R = H (IV, XIV), 4-OH (IX, XIX), 4-Cl (III, XIII), 3-$CH_3$ (V, XV), 4-$CH_3$ (VI, XVI), 4-$OCH_3$ (VII, XVII), 4-$OC_2H_5$ (VIII, XVIII), 4-$NO_2$ (I, XI), 4-$COOC_2H_5$ (II, XII).

## EXPERIMENTAL CHEMICAL

The reactions, composition of the reaction mixtures, and purity of the synthesized compounds were monitored by thin-layer chromatography on Silufol UV-254 plates in the systems chloroform–ethanol (6:1), propanol–0.2 N ammonia (3:1), and butanol–acetic acid–water (4:1:1). To demonstrate the structure of the compounds we measured their IR spectra on a UR-20 spectrometer in KBr tablets, as well as the PMR spectra on a Perkin-Elmer R-12 spectrometer (60 MHz) with tetramethylsilane in DMSO-$d_6$ as an internal standard.

N-Methylamide-L-proline Hydrochloride (X). A suspension of 10 g (0.09 mole) of L-proline in 100 ml of ethanol was saturated with gaseous HCl until the amino acid dissolved completely; the solvent was distilled off on a vacuum evaporator, and 100 ml of absolute ether and a 33% solution of NaOH were added to the residue at 0-5°C. After the addition of 10 g of potash, the ether layer was removed. The oily product was dissolved in 10 ml of ethanol

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TABLE 1. Properties of the Synthesized Compounds

| Compound | mp, °C | Found, % | | | Gross formula | Calculated, % | | | IR spectrum, ν, $cm^{-1}$ | PMR spectrum, δ, ppm |
|---|---|---|---|---|---|---|---|---|---|---|
| | | C | H | N | | C | H | N | | |
| X | 160—61 | 43,8 | 7,8 | 16,9 | $C_6H_{13}ClN_2O$ | 43,8 | 7,8 | 16,9 | 1690 (CO), 1520, 3240 (NH), | |
| XI | 125—6 | 52,0 | 5,4 | 25,3 | $C_{12}H_{15}N_5O_3$ | 52,1 | 5,6 | 25,5 | 1335, 1570 ($NO_2$), 1670 (CO), 3310 (NH) | 2,65 d (3H), 4,65 t (1H), 7, 86 q (4H), |
| XII | 135—6 | 59,2 | 6,6 | 18,5 | $C_{15}H_{20}N_4O_3$ | 59,2 | 6,6 | 18,4 | 1660, 1710 (CO), 3300 (NH) | 1,34 t (3H), 2,62 d (3H), 3,8 t (2H), 4,3 q (2H), 4,6 t (1H), 7,7 q (4H) |
| XIII | 110—11 | 53,8 | 5,5 | 20,9 | $C_{12}H_{15}N_4ClO$ | 54,0 | 5,6 | 21,0 | 840 (Cl), 1800 (CO), 3400 (NH) | 2,6 d (2H), 3,8 t (2H), 4,59 t (1H), 7, 35 s (4H), 8 q (1H) |
| XIV | | 61,7 | 6,8 | 23,7 | $C_{12}H_{16}N_4O$ | 62,0 | 6,9 | 24,1 | | |
| XVI | 105—6 | 63,8 | 7,2 | 22,6 | $C_{13}H_{18}N_4O$ | 63,4 | 7,3 | 22,8 | 1665 (CO), 3310 (NH) | 2,3 s (3H), 2,6 d (2H), 3,7 t (2H), 4,5 t (1H), 7,2 s (4H), 7,8 q (1H) |
| XVII | 120—21 | 59,1 | 6,9 | 21,4 | $C_{13}H_{18}N_4O_2$ | 59,5 | 6,9 | 21,4 | 1665 (CO), 3320 (NH) | 2,6 d (2H), 3,8 s (3H), 4,5 t (2H), 4,98 t (1H), 7,1 q (4H), 7,9 q (1H) |
| XVIII | 122—3 | 61,0 | 7,5 | 20,7 | $C_{14}H_{20}N_4O_2$ | 60,9 | 7,3 | 20,3 | | 2,3 s (3H), 2,6 d (2H), 3,7 t (2H), 4 q (2H), 7,1 q (4H), 7,9 q (1H) |

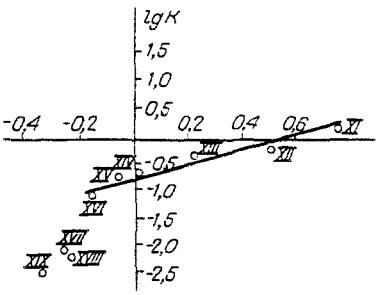


Fig. 1. Dependence of $K_{obs}$ on the Jaffe σ constants of the reaction of aryldiazo-compounds I-IX with the methylamide of L-proline. Along x-axis: σ; along y-axis: log K.

TABLE 2. Results of a Study of the Kinetics of the Proteolytic Cleavage of Triazenes XVI-XVIII

| Compound | Rate constant K, min | Half-conversion time $\tau_{1/2}$, min |
|---|---|---|
| XVI | 0,0073 | 94,9 |
| XVII | 0,0139 | 49,9 |
| XVIII | 0,0104 | 96,2 |

Note. pH 7.4, T = 37°C, μ 0.178, analytical wavelength 315 nm.

and treated with gaseous methylamine at 0°C analogously to the procedure of [5]. The product was isolated in the form of the hydrochloride. Yield 4.2 g (29.4%). The data of the IR and PMR spectra and the melting points are presented in Table 1.

1-(4-Nitrophenylazo)pyrrolidine-2-methylcarboxamide (XI). To a solution of 0.003 mole X in 10 ml of water, a freshly prepared solution of the diazo-compound I, produced according to the procedure of [6], was added with mixing and cooling to 0°C, maintaining pH 7.0-8.0 in the process by adding a saturated solution of soda. The crystalline precipitate was filtered off, washed with water, and dried in a vacuum desiccator. Yield 1 g (48.5%).

Analogously to the procedure described for the triazene XI, we produced 1-(4-carbethoxyphenylazo)-pyrrolidine-2-methylcarboxamide (XII) with a yield of 65.8%, 1-(4-chlorophenylazo)-pyrrolidine-2-methylcarboxamide (XIII) with a yield of 53.5%, 1-(phenylazo)pyrrolidine-2-methylcarboxamide (XIV) with a yield of 57.8%, 1-(4-methylphenylazo)pyrrolidine-2-methylcarboxamide (XVI) with a yield of 68.8%, 1-(4-methoxyphenylazo)pyrrolidine-2-methylcarboxamide (XVII) with a yield of 67.8%, and 1-(4-ethoxyphenylazo)pyrrolidine-2-methylcarboxamide (XVIII) with a yield of 77.5%.

The rate constants of the reaction were determined spectrophotometrically on a Beckman Model 26 Kinetic 26 two-beam recording spectrophotometer with thermostatic control unit (USA). The experimental data were treated according to the procedure of [2].

Determination of the Rate Constant of the Reaction of Diazo-Compounds I-IX with the Methylamide of L-Proline. For the experiment we prepared a solution of X with a concentration of $5 \cdot 10^{-4}$ M in phosphate buffer pH 7.4. Into a cuvette with 3 ml of this solution, heated to 37°C, we poured 0.03 ml of a freshly prepared solution of the diazo-compound [4] with a concentration of $5 \cdot 10^{-3}$ M. The course of the reaction was followed according to the change in the optical density of the solution at the analytical wave-length.

Determination of the Rate Constant of the Reaction of Protolytic Cleavage of Triazenes XVI-XVII at pH 7.4. Into a cuvette with 3 ml of buffer solution, pH 7.4, heated to 37°C, we poured 0.03 ml of a solution of the investigated substance in ethanol, with a concentration of $5 \cdot 10^{-3}$ M.

## RESULTS AND DISCUSSION

A study of the kinetics of the reaction of aryldiazo-compounds I-IX with the model of the proline target X showed that the values found for the rate constants (see Fig. 1) are well-correlated with the values of the σ constants of the substituents in the benzene ring ($\rho$ = 1.1304, n = 6, T = 9.8, r = 0.98, $\Delta S \approx 0.077$, where $\rho$ is the reaction constant; n is the number of compounds; r is the correlation coefficient; T is the value of the Student criterion; $\Delta S$ is the standard deviation).

However, the dependence of K on the σ constants of the substituents is linear only for the diazo-compounds I-VI, possessing acceptor and weak donor substituents (see Fig. 1). Further increasing the donor capacity of the substituent R leads to a deviation of the experimental points corresponding to the diazo-compounds VII-IX from the dependence found.

In search for the cause of the indicated deviations, we studied the stability of triazenes XVI-XVIII, containing donor substituents in the ring, in phosphate buffer with ionic strength $\mu$ = 0.178 at pH 7.4 and 37°C (Table 2).

The data cited in Table 2 are evidence that the triazenes XVII-XVIII undergo appreciable protolytic cleavage, the rate of which increases with increasing donor capacity of the substituent R. It is known [4] that the rate of the diazocoupling reaction of the diazo-compounds with dialkylamines is limited by the step of formation of the diazoammonium cation, while the observable reaction constant ($K_{obs}$) can be represented in the form

$$K_{obs} = \frac{K_1 (K_2 [B] + K_3 [Sol])}{K_{-1} + (K_2 [B] + K_3 [Sol])},$$

where $K_1$ and $K_{-1}$ are the rate constants of the formation and decomposition of a diazoammonium cation; $K_2$ and $K_3$ are the rate constants of the interaction of the diazoammonium cation with the base and solvent, respectively; Sol is the solvent; B is any base.

In view of this, for the triazenes XI-XV, stable under the experimental conditions ($K_{-1} = 0$), $K_{obs} = K_1$. Increasing the rate constant of the reverse reaction ($K_{-1}$) of the triazenes XVII and XVIII thus leads to a regular decrease in $K_{obs}$ and a significant deviation of the experimental points from a linear relationship (see Fig. 1) for the diazo-compounds VII-IX. An experimental point on the graph, corresponding to the diazo-derivative VI, still fits onto the straight line as a result of the small influence of the rate of the reverse reaction of protolytic cleavage of the triazene XVI on $K_{obs}$.

Attempts to isolate the triazene XIX, produced by the interaction of the diazo-derivative IX with the methylamide of L-proline, did not lead to the desired result on account of its instability. This is in good agreement with the results of the kinetic observations (see Fig. 1).

An analysis of the results the kinetics of model experiments, simulating the behavior of aryldiazo-derivatives in the organism with respect to the proline target of natural peptides and the free amino acids L-proline and L-hydroxyproline, does not participate in the transport of diazo-compounds containing acceptor substituents ($\sigma_R > 0$), since the triazene derivatives formed are rather stable to protolytic decomposition and are incapable of further generating diazo-cations.

On the contrary, diazo-derivatives containing donor substituents ($\sigma_R < 0$) form products of interaction with the proline target at a lower rate; however, these products are capable of exhibiting cryptodiazonium properties at biological values of the pH, temperature, and ionic strength of the solution, which increase with increasing donor capacity of the substituent R in the benzene ring, which is subsequently accompanied by a further slow isolation of the diazo-compound from the transport arylazopyrrolidine depo-form.

Considering the literature data [7], it can be assumed that the enzymatic C-hydroxylation of diazenes containing acceptor substituents in the benzene ring substantially lower their stability in biological media as a result of the equalization of the influence of acceptors by the introduction of OH, capable of ionization, into the ring of the donor group. This in turn should lead to an increase in the role of the proline target in the biological transport of diazo-compounds, as the active ingredient of antitumor and antimetastatic triazenes, containing not only donor but also acceptor substituents in the aromatic ring.

We should consider, however, that a decrease in the rate of interaction of diazo-compounds with the proline ring may lead to competitive side reactions of diazo-derivatives with other amino acid nucleophilic centers of proteins and peptides (for example, tyrosine residues), to a loss of the ability of diazo-compounds for transport. A study of this important question will be the subject of further investigations.

## LITERATURE CITED


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