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REACTIVITY AND MECHANISM OF THE ANTITUMOR ACTION OF TRIAZENES.

II. INTERACTION OF AROMATIC DIAZO-DERIVATIVES WITH A MODEL OF PHENOL-TYPE NUCLEOPHILIC SITES OF THE CELL MEMBRANES

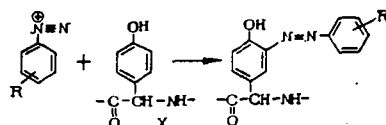
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Earlier [1] we showed that aromatic 3,3-dimethyltriazenes undergo protolysis extremely readily under conditions simulating their oral administration, forming the corresponding aromatic diazo-derivatives. The preservation of high antitumor activity of triazenes in the case of oral administration of the preparations thus cannot be due to the process of their microsomal oxidation, followed by generation of alkylating CH_3^+ cations but is associated with the action on the cell of reactive aryldiazonium cations formed during protolysis. On the basis of the literature data [3] it can be suggested that aromatic diazo-derivatives are capable of interacting with various nucleophilic sites of proteins, for example, with the residues of a number of amino acids contained in the active sites of the enzymes localized on the cell membranes.

One of these important nucleophilic sites, a potential biologically important target for reactive aromatic diazo-derivatives, is the L-tyrosine residues.

The possibility of the formation of azo-derivatives and the interaction of diazo-compounds with membrane proteins was postulated by Berg [3], who observed a staining of the cell membranes in a study of the action of p-diazobenzenesulfonate on erythrocytes.



R: p-OCH₃ (I), p-OC₂H₅ (II), p-CH₃ (III), m-CH₃ (IV), H (V), m-COOH (VI), p-COOH (VII), p-Br (VIII), p-NO₂ (IX).

X is a nucleophilic target.

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TABLE 1. Results of a Study of the Kinetics of the Azocoupling Reaction of Diazocompounds of the Benzene Series and 5-Diazoimidazole-4-carboxamide with p-Cresol at pH 7.4, 37°C, $\mu = 0.178$

Compound	Analytical wave-length, nm	Rate constant (K), min ⁻¹	$\Delta K, \text{min}^{-1}$	$\tau_{1/2}, \text{min}$
I	360	0,0053	0,0002	130,75
II	360	0,0090	0,0001	70,00
III	345	0,1117	0,0024	6,20
IV	350	0,3897	0,0060	1,78
V	350	0,3918	0,0044	1,77
VI	345	0,5460	0,0030	1,27
VII	345	1,3902	0,0018	0,50
VIII	—	7,2127*	—	0,096
IX	—	6845,08*	—	0,096
XI	320	1,158	0,0020	0,60

*The value of K of the reaction was found by an analytical method.

In this work we made a comparative study of the reactivity of a number of aryl diazocompounds (I-IX) and 5-diazoimidazole-4-carboxamide (XI) in the reaction of azocoupling with p-cresol — a close chemical model of the phenol-type nucleophilic sites of cell membranes — for a search for possible differences in the reactivity and mechanism of action of the diazocompounds formed in the decomposition of antitumor 3,3-dimethyl-1-aryltriazenes, and the preparation used clinically, dacarbazine [5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide (XII)].

EXPERIMENTAL (CHEMICAL)

Compounds XI and XII were synthesized according to the procedure described in [6]. An investigation of the kinetics of protolysis of XII was conducted at the temperature 37°C in a buffer solution with pH 1.2 and ionic strength $\mu = 0.178$, equal to the ionic strength of physiological saline solution.

The kinetics of azocoupling of the diazocompounds I-IX with a tenfold molar excess of p-cresol was studied at the temperature 37°C in phosphate buffer pH 7.4 with ionic strength $\mu = 0.178$. In both cases we used a spectrophotometric method of studying the reaction rate constants [1]. The measurements were performed on a Beckman model 26 Kinetic two-beam spectrophotometer (USA) with thermostatic control unit.

Determination of the Rate Constant of the Protolysis of the Triazene XIII. Compound XII (1.25 mmoles) was dissolved in 25 ml of ethanol, and 3 μ l of this solution was poured into a cuvette containing 3 ml of buffer solution, pH 1.2, heated to 37°C. The course of the reaction was followed according to the decrease in the optical density of the investigated triazene in solution. The reaction rate constant was determined according to the method described in [1].

Determination of the Rate Constant of the Reaction of Azocoupling of Diazocompounds of the Benzene Series with p-Cresol. For the experiment we prepared a solution of p-cresol with a concentration of $5 \cdot 10^{-4}$ M in phosphate buffer pH 7.4. A 3 μ l portion of a freshly prepared solution of the diazocompound with a concentration of $5 \cdot 10^{-2}$ M was poured into a cuvette with 3 ml of this solution, heated to 37°C; the dependence of the optical density of the investigated solution on the time was measured directly at a known rate of sliding of the spectrophotometer tape.

Determination of the Rate Constant of Cyclization of 5-diazoimidazole-4-carboxamide XI at pH 7.4 in the Presence of p-Cresol. Compound XI (1.25 mmoles) was dissolved in 25 ml of ethanol, and 3 μ l of this solution was poured into a cuvette containing 3 ml of buffer solution, pH 7.4, with p-cresol ($c = 5 \cdot 10^{-4}$ M). The change in the optical density of the solution at 37°C was monitored at λ 320 nm.

EXPERIMENTAL (BIOLOGICAL)

The biological activity of 1-(p-methylphenyl)-3,3-dimethyltriazene (XV), 1-phenyl-3-dimethyltriazene (XVI), and 1-(p-nitrophenyl)-3,3-dimethyltriazene (XVII) was studied earlier [1].

The antitumor activity of dacarbazine XII was studied on noninbred male white mice weighing 110-120 g with a continuous line of Jensen sarcoma. The test substance was

TABLE 2. Results of a Study of the
Protolysis of Triazenes of the Benzene
Series and Dacarbazine at pH 1.2, 37°C
 $\mu = 0.178$

Compound	Rate constant, $K \cdot 10^3$, sec ⁻¹	$\Delta K \cdot 10^3$, sec	$\tau_{1/2}$, sec
XV	4694,900	—	0,15
XVI	0,722	0,003	990
XII	0,070	0.0099	9830

TABLE 3. Influence of Triazenes on the
Growth of Jensen Sarcoma in Rats in the
Case of Oral Administration of the Preparations

Compound	Unit dose mg / kg (No. of administrations)	Ji, %	Kg, %
XII	150 (8)	88	11
XV	50 (8)	92	4,7
XVI	100 (8)	87	36
XVII	150 (8)	96	-7,3

administered orally in vegetable oil daily for a period of eight days, beginning with the third to fifth day after transplantation of the tumor. The antitumor activity of the preparation was judged according to the index of inhibition (J_i), calculated according to the formula:

$$J_i = \frac{B_e - B_c}{B_c} \times 100,$$

where B_c and B_e are the average values of the tumor mass in the control and experimental groups, respectively, in grams.

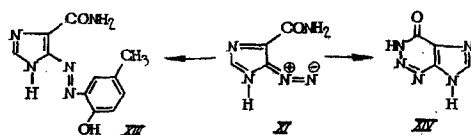
The toxic action of the preparation on animals was judged according to the coefficient of growth (K_g), which was calculated according to the formula:

$$K_g = \frac{(B_1 - A_1) \cdot C_2 \cdot 100}{(B_2 - A_2) \cdot C_1} - 100,$$

where C_1 and C_2 is the average body weight of the animals of the experimental and control groups at the beginning of the experiment, in grams; A_1 and A_2 is the average weight of the tumors in the experimental and control groups in grams; B_1 and B_2 is the average body weight of the animals in the experimental and control groups at the end of the experiment in grams. A positive value of K_g is evidence of a larger body weight gain of the animals in the experimental group and in the control.

RESULTS AND DISCUSSION

In a comparative study of the rates of the reactions of azocoupling of aryldiazocompounds I-IX and diazoimidazolecarboxamide XI with p-cresol, it was established that the diazocompound XI does not form the corresponding azo-derivative XIII under the conditions of the investigated reaction, but undergoes intramolecular cyclization to imidazo-[4,5-d]-1,2,3-triazinone-4 (XIV) at a high rate; according to the literature data, the product XIV does not possess biological activity [5].



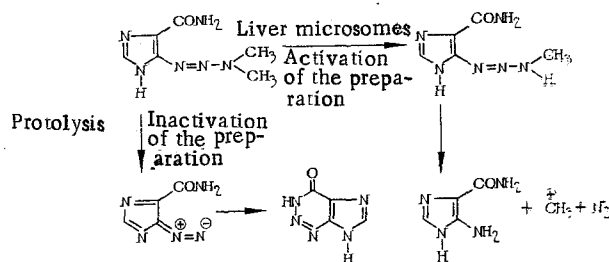
The calculated values of the rate constants of the diazocoupling of I-IX (Table 1) are well correlated with the values of the σ -constants of the substituents in the benzene ring ($\rho = 5.4528$, $n = 7$, $\gamma = 0.95$, $T = 6.8$, $\Delta S = 0.2665$, where ρ is the reaction constant, n the number of compounds, r the correlation coefficient, T the value of the Student criterion, and ΔS the standard deviation) (Table 2).

In connection with the differences found in the chemical behavior of diazocompounds I-IX, formed in the protolysis of the corresponding triazenes, we were interested in studying the antitumor activity of 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide XII under conditions of oral administration of the preparation which we had previously used in an investigation of the antitumor action of derivatives of 1-phenyl-3,3-dimethyltriazene [1, 2], as well as a study of the rate of protolysis of the substance of the dacarbazine preparation - the triazene (XIII).

As a result of our experiments it was established that XII possesses pronounced antitumor activity on a number of strains of animal tumors (Table 3), comparable with that of derivatives of 1-phenyl-3,3-dimethyltriazene. At the same time, as it follows from Table 2, the half-life of preparation XII under conditions simulating its oral administration, significantly exceeds $\tau_{1/2}$ of all the triazenes of the benzene series studied.

An analysis of the results obtained from biological testing, the kinetics of the protolysis of triazenes in acid medium, and the reaction of azocoupling of the corresponding diazoproducts with the nucleophilic target X permits us to conclude that the antitumor action of dacarbazine, in contrast to triazenes of the benzene series, is not associated with its ability to be digested forming a diazoproduct XI, capable of interacting with portions of the cell membranes containing the L-tyrosine residue.

Probably this is associated primarily with the fact that even under rigorous conditions of oral administration of preparation XII, the rate of its protolysis is significantly lower than the rate of protolysis of aryltriazenes, and the diazo-derivative XI formed in small amounts is irreversibly and noncompetitively cyclized to the biologically inactive triazinone XIV. In view of this, the observed antitumor effect of dacarbazine in the case of its oral administration, as well as in the case of intravenous administration [4], is a consequence only of the process of microsomal oxidation of the CH_3 group by the triazene chain, stable to protolysis, followed by generation of a methyl cation that alkylates the nucleic acids of the tumor. The negligible formation of 5-diazoimidazole-4-carboxamide XI that occurs is evidently the result of inactivation of the preparation.



On the contrary, as the experiments show, for aryltriazenes the interaction of the diazo-compounds formed in the case of oral administration with the nucleophilic sites of proteins that contain L-tyrosine residues in neutral medium is one of the most probable pathways of the action of these substances on biologically important targets, leading to their irreversible transformation.

Unquestionably there is not yet any sufficient basis for asserting that interactions of this kind can play a deciding role in the manifestation of antitumor activity of aryltriazenes. We are currently conducting a detailed study of this aspect of the problem; however, the data obtained confirm our opinion that there are substantial differences in the mechanism of the chemical and biological effects of aryltriazenes and dacarbazine on the cell; that they are profound and closely associated with the peculiarities of the reactivity and structure of the aromatic fragment of the molecules of biologically active triazene derivatives.

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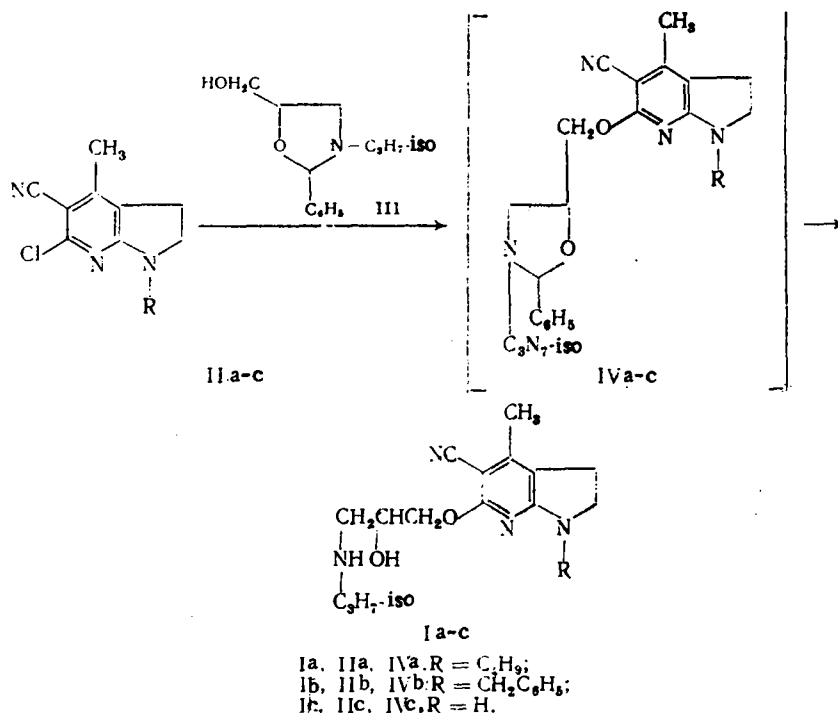
AZAINDOLES. LXVIII.* 7-AZAINDOLES AS β -ADRENOBLOCKING AGENTS

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Recent reports of enhanced β -blocking activity in conjunction with vasodilatory and hypotensive properties following the introduction of cyano-substituents into benzene, pyridine, and indole in addition to the 3-isopropylamino-2-hydroxypropoxy group [7, 8, 11] are of considerable interest in selecting the best routes in the search for cardiovascular drugs. We have previously [3] shown that the introduction of these groups into the 6- and 7-positions of 5-azaindoles has a similar effect, but such compounds are readily converted into 6-oxo-7-cyano-5-azaindoles as a result of the energy advantage of the 6-oxo-5-azaindole system [6]. In contrast to 5-azaindoles, 7-azaindoles (or 7-azaindoles) are less prone to form lactams [10], and should be more stable.

We have obtained 1-benzyl- and 1-butyl-4-methyl-5-cyano-6-(3'-isopropylamino-2'-hydroxypropoxy)-7-azaindoles (Ia, b) as follows:



4-Methyl-5-cyano-6-chloro-7-azaindoles [5] were reacted as described in [9] using the modifications reported in [3] with 2-phenyl-3-isopropyl-5-hydroxymethyloxazolidine (III)

*For communication LXVII, see [5].

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