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REACTIVITY AND MECHANISM OF THE ANTITUMOR EFFECT OF TRIAZINES

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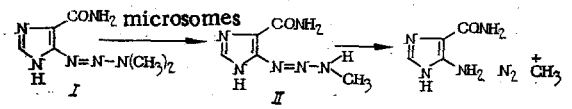
UDC 615.277.3:547.236.2].015.4

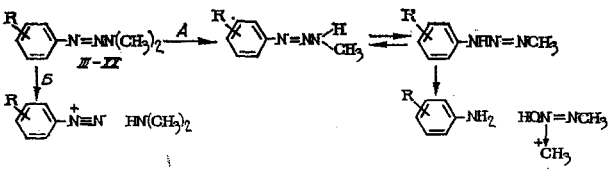
5-(3,3-Dimethyl-1-triazeno)imidazole-4-carboxamide (dicarbazine) (I), synthesized by J. Shealy et al. in 1961 [12], has now found clinical use in the treatment of melanoma. However, the therapeutic effectiveness of the preparation in monochemotherapy, as well as in combination with antitumor agents, is limited by the short lifetime of the enzymatically formed active metabolite 5-(3-methyl-1-triazeno)imidazole-4-carboxamide (II), which is a donor of reactive methyl cations, which are alkylate nucleic acids [11].



Attempts to create long-action drugs have stimulated a search for biologically active compounds in the series of 3,3-dimethyl-1-aryltriazenes, since the corresponding monomethyl derivatives, in contrast to 3-methyl-1-triazenes of the imidazole series, are substantially more stable compounds.

In experiments on animals, the aryltriazines synthesized, in comparison with dicarbazine, showed not only lower toxicity but also a different spectrum of antitumor action [5, 7], which prompted a more thorough and comprehensive study of the mechanism of their antitumor effect.

After the publication of the work of F. Kruger et al. [8], who detected incorporation of radioactivity into nucleic acids after the introduction of 3,3-dimethyl-1-phenyltriazene, labeled on the 3,3-dimethyl group with ^{14}C, it might seem that aryltriazines, like dicarbazine, are characterized by the same mechanism of antitumor action, including N-demethylation as a necessary step (pathway A).



R = p-CH_3 (III), m-CH_3 (IV), H (V), p-Cl (VI), p-Br (VII),

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 0091-150X/85/1809-0592$08.50

n-COOH (VIII), m-COOH (IX), p-$COOC_2H_5$ (X), p-NO_2 (XI), p-$COOCH(CH_3)_2$ (XII), p-$CONHCH(CH_3)_2$ (XIII), p-OC_2H_5 (XIV), p-OCH_3 (XV), p-$COOCH_3$ (XVI), p-$CONH_2$ (XVII), p-$CONHCH_3$ (XVIII), p-$CON(CH_3)_2$ (XIX), p-CO—morpholino (XX).

However, recently a number of experimental facts have emerged, which do not fit into the framework of the traditional concepts. Thus, for example, it has been shown [3, 6] that 1-aryl-3,3-dimethyltriazines, containing donor substituents in the ring, evidently, by forming the corresponding diazo compounds in the organism in spontaneous hydrolysis, strongly inhibit processes of microsomal N-demethylation. Moreover, just as we should have expected, there is no correlation between the rate of N-demethylation and the antileukemic activity of the triazenes. On the contrary, such a correlation is present between the antitumor effect and the half-conversion time of the triazenes to the corresponding diazo compounds in neutral medium [10].

The second alternative mechanism of the antitumor effect of aryltriazenes (pathway B) is also supported by [4] and our data [1, 2], showing the aryltriazenes exhibit high antitumor activity even in the case of oral administration of the preparations.

The present work is devoted to a study of the antitumor activity and the reactivity of a series of aryltriazenes under conditions simulating the oral mode of administration of the preparations, to establish the interrelationship of their reactivity and the possibile mechanism of their antitumor effect.

EXPERIMENTAL CHEMISTRY

The structure of the substances obtained was confirmed by the data of elementary analysis. The individuality of the compounds and the course of the reactions were monitored chromatographically on Silufol UV-254 plates in the systems: n-propanol—ammonia 0.2 N (3:1) (Rf_1), chloroform—ethanol (3:1) (Rf_2), and n-butanol—acetic acid—water (4:1:1) (Rf_3).

The triazenes III-XIV were produced according to the general procedure of [9].

1-(p-Isopropylaminocarbonylphenyl)-3,3-dimethyltriazene (XIII). To 1 g (4.7 mmoles) of the isopropylamide of p-aminobenzoic acid, dissolved in 6.6 ml of dilute (1:7) hydrochloric acid, a solution of 0.4 g (5.8 mmoles) sodium nitrate in 2.3 ml of water was added dropwise with mixing and cooling in an ice bath. The temperature of the reaction mass should not exceed 5-10°C. A solution of dimethylamine is slowly added to the solution of the diazo compound obtained, to pH 8.0-9.0. The precipitate formed is filtered off, recrystallized from ethanol, and 0.8 g (87%) of compound XIII is obtained, with mp 171-173°C. Rf_1 0.89; Rf_2 = 0.69; Rf_3 = 0.85. Found %: C 60.7; H 8.0; N 24.6. $C_{12}H_{18}N_4O$. Calculated %: C 61.3; H 7.7; N 24.9.

A study of the kinetics of the acid cleavage of triazenes was conducted at a constant temperature of 37°C in buffer solution with pH 1.2 and ionic strength I = 0.178 mole/kg, equal to the ionic strength of physiological saline solution, by a spectrophotometric method on a Beckman Model 26 Kinetic spectrophotometer with thermostatic control unit (USA).

Determination of the Rate Constants of the Decomposition of Triazenes. A 1.25-mmole portion of the triazene is dissolved in 25 ml of ethanol, and 3 ml of this solution is added to a cuvette containing 3 ml of buffer solution, pH 1.2, heated to 37°C. The course of the reaction is followed according to the decrease in the optical density of the investigated substance in solution at the analytical wavelength. The following formula was used to determine the rate constants of the reaction:

$$\ln \frac{D_0 - D_\infty}{D_\tau - D_\infty} = K\tau,$$

where D_0 is the optical density of the reaction solution at the analytic wavelength at the zero moment of time; D_∞ is the optical density of the reaction solution at the analytical wavelength at the moment of time $\tau = \infty$; D_τ is the optical density of the reaction solution at the analytical wavelength at the moment of time τ; K is the rate constant of the reaction (in sec^{-1}); τ is the time (in sec).

Kinetic curves of the investigated pseudo-first-order reaction were constructed according to the experimental data, then treated according to the method of least squares. The half-conversion time of triazenes ($\tau_{1/2}$) was determined according to the formula

TABLE 1. Influence of 3,3-Dimethyl-1-aryltriazines III-XX on the Growth of Jensen's Sarcoma in Rats after Oral Administration

Compound	Single dose, mg/kg (No. of administration)	J_T, %	K_g, %
III	100 (8)	+81	—13
	50 (8)	+92	—4,7
V	100 (8)	+87	+36
	50 (8)	+67	+25
VIII	100 (8)	+85	+9,3
	50 (8)	+39	+15
XI	150 (8)	+96	—7,3
	100 (8)	+69	+13
	75 (8)	+75	+1,5
XII	200 (7)	+73	+4
	200 (5)	+82	+5
	150 (7)	+84	+5
	50 (5)	+34	+3
XIII	150 (5)	+81	+18
	100 (7)	+50	+3
	50 (7)	+41	+6
XIV	200 (5)	+43	—9
	100 (5)	+51	—3
	50 (5)	+44	+1
XV	100 (5)	+38	—5
	50 (5)	+35	0
XVI	200 (5)	+89	—10
	100 (6)	+30	—18
XVII	100 (6)	+76	+1
XVIII	200 (5)	+83	—2
	50 (7)	0	—7
XIX	200 (5)	+76	—13
	100 (7)	+64	—8
	100 (6)	+55	+2
	50 (7)	+86	—7
XX	200 (5)	+70	+1

$$\tau_{1/2} = \frac{0.693}{K},$$

where K is the rate constant of the reaction (in sec^{-1}).

EXPERIMENTAL BIOLOGY

The antitumor activity of the compounds obtained was studied on noninbred male white rats with a body weight of 110-120 g with transplanted Jensen's sarcoma. The tested substances were administered orally in vegetable oil daily for eight days beginning with the third to fifth day after the transplantation of the tumor. The antitumor activity of the preparations was judged according to the index of inhibition (J_i), calculated according to the formula

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

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

The value of the toxic effect of the preparations on animals was judged according to the value of the growth coefficient (K_g), which was calculated according to the formula

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

where C_1 and C_2 are the average body weights of the animals of the experimental and control groups at the beginning of the experiment (in g); A_1 and A_2 are the average weights of the

TABLE 2. Results of a Study of the Kinetics of Protolysis of 3,3-Dimethyl-1-aryltriazenes III-XIV at pH 1.2, 37°C, μ = 0.178

Compound	Analytical wavelength, nm	Rate constant (K), sec^{-1}	ΔK, sec^{-1}	$\tau_{1/2}$, sec
III	—	4,6949*	—	0,15
IV	—	2,2990*	—	0,31
V	303	0,7463	0,0002	1,0
VI	312	0,1255	0,0017	5,5
VII	320	0,0219	0,0013	31,6
VIII	320	0,0152	0,0022	46,8
IX	315	0,0469	0,0032	14,8
X	325	0,0130	0,0002	53,3
XI	325	0,0007	0,00003	990,0
XII	320	0,0148	0,0002	46,8
XIII	300	0,0316	0,0016	21,9
XIV	—	8,4600*	—	0,08

*The value of the rate constant of the protolysis reaction was found by an analytical method.

tumors in the experimental and control groups (in g); B_1 and B_2 are the average body weights of the animals in the experimental and control groups at the end of the experiment (in g). A value of K_g with a "plus" sign is evidence of a larger increase in the body weight of the animals in the experimental group in comparison with the control.

RESULTS AND DISCUSSION

Data on the effectiveness of the antitumor action of the investigated preparations are cited in Table 1. For the triazenes XII, XIV-XX the values of the indices K_g and J_i were taken from [1].

As can be seen from Table 1, all the 3,3-dimethyl-1-aryltriazenes studied possess pronounced antitumor activity on Jensen's sarcoma in rats in the case of an oral mode of administration of the preparations. In this case a characteristic feature of the substances is their low toxicity and good tolerability by the experimental animals (the absence of side effects or any sharp inhibition of weight gain).

Data on the kinetics of the decomposition of a series of aryltriazenes III-XIV, cited in Table 2, show that all the 3,3-dimethyl-1-aryltriazenes studied undergo an extremely rapid cleavage under conditions simulating the oral mode of administration of the preparations, forming the corresponding aromatic diazocompounds, which thus represent the only reactive particle formed, responsible for the manifestation of the antitumor effect of the triazines.

An analysis of the values found for the rate constants of the protolysis of triazenes shows that they depend greatly on the electronic nature of the substituents in the aromatic ring of the molecule ($\rho = -3.2031$). In this case a good correlation of the values of the rate constants of the reaction and the Hammett σ constant, found for the investigated series of substances ($n = 9$, $r = 0.95$, $T = 6.5247$, $\Delta S = 0.2813$, where n is the number of compounds, r is the correlation coefficient, T is the value of the Student criterion, and ΔS is the standard deviation), is observed.

It should be noted that the values of K of the protolysis of aryltriazines III, IV, and XIV were obtained by an analytical method, since they could not be registered experimentally by the method used in the work as a result of the high rate of the process.

Thus, on the basis of the data obtained it can be concluded that in the oral mode of administration of 3,3-dimethyl-1-aryltriazenes, the possibility of activation of the preparations by their microsomal N-demethylation, followed by decomposition to alkylating methyl cations or radicals, is excluded.

It can be asserted that the antitumor effect of aryltriazenes under the experimental conditions is realized on account of the rapid cleavage of the latter to the corresponding

aromatic diazo derivatives, representing the active particle of the investigated antitumor preparations.

The data obtained permit a realistic estimation of the effectiveness of the antitumor action of triazenes, observed under conditions ensuring the predominance of one of the two possible mechanisms of their decomposition, and open up new prospects for the directed synthesis of antitumor preparations of the triazene series.

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ACID HYDROLYSIS OF 6-FLUORO DERIVATIVES OF CERTAIN ANALOGS OF THE NUCLEIC BASES OF THE PYRIMIDINE SERIES

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UDC 615.31:547.853.3].015.4:542.938

6-Fluoro-containing analogs of certain nucleic acid bases — 6-fluorouracil (I), 6-fluorothymine (II), and 6-fluoroisocytosine (III) — the synthesis of which was described in [2, 5], may be of significance as antimetabolites. Nucleosides of 6-fluorouracil possess antiviral activity [6]; the absence of antitumor activity is attributed to their possible hydrolysis *in vivo* [7]. In our experiments, conducted on mice and rats, in certain cases we observed the appearance of antitumor activity with low toxicity, but sometimes the effect was the reverse: High toxicity and low antitumor activity were observed. In view of this, the hydrolysis of the C_6—F bond in systems simulating the pH, salt composition, and temperature of certain biological media is of interest.

The hydrolysis of compounds I-III does not occur appreciably in a system simulating the salt composition of blood, as well as in buffer systems with pH 8.0-8.4 (intestinal juice). In view of the low solubility of the indicated compounds in aqueous systems and in media close to neutral, the most convenient mode of administration of our preparations into the organism is oral. In view of this, to evaluate the possibility of the hydrolysis in the case of oral administration, it was necessary to investigate the conversion of preparations I-III in acid systems, simulating gastric juice.

Experiments conducted in dilute hydrochloric acid at pH 1.0 and 2.5 and a temperature of 37°C showed that in both the systems there is a hydrolysis of substances I-III, leading to the formation of the corresponding barbituric acid derivatives. The rate of hydrolysis

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Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 18, No. 9, pp. 1041-1044, September, 1984. Original article submitted December 20, 1984.

0091-150X/84/1809-0596$08.50