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

REACTIVITY AND MECHANISM OF ANTITUMOR ACTION OF TRIAZENES

IV.* INTERACTION OF AROMATIC DIAZO-DERIVATIVES WITH L-AMINO ACIDS

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Earlier [3], we showed by biochemical experiments on Ca-ATPase that the interaction of aryldiazo-compounds, as one of the active ingredients in antitumor and antimetastatic 3,3-dimethyl-1-aryltriazenes, with the SH groups of the enzyme is irreversible. Since the biological activity and affinity of the drugs for the receptor in general are well correlated with their affinity for transport proteins (albumins), it can be assumed that the SH group of serum albumin does not participate in the transport of reactive aryldiazonium particles in the organism, which provides not only for their binding but also for their compulsory subsequent splitting out in the tumor.

From this standpoint the participation of free L-amino acids, as well as their fragments contained in natural proteins, as carriers of aryldiazo-compounds in the organism is the most realistic. However, the available literature data pertain to the study of reactions of diazo-compounds with amino acids and proteins only in alkaline media, and the experimental data obtained on the structure of the reaction products formed are contradictory [5, 8, 11, 13].

In this work, for a chemical demonstration of the existence of natural transport cryptodiazonium forms of aryldiazo-compounds, we synthesized a series of aryldiazo-compounds (I-III) and studied their interaction with certain derivatives of L-amino acids – glycine (IV), alanine (V), ornithine (VI), arginine (VII), valine (VIII), lysine (IX), leucine (X), isoleucine (XI), aspartic acid (XII), asparagine (XIII), glutamic acid (XIV), methionine (XV), phenylalanine (XVI), as well as the amide of N^{α}-tert-butoxycarbonyl-lysylglycine (XVII), which is a chemical model of the lysine target of proteins and peptides, permitting a simulation of the behavior of the N^{ε}-amino group of lysine in biological media. We studied the antitumor activity of the products formed from the investigated reaction – the corresponding diaryltriazenes (XXIV-XXVI).

EXPERIMENTAL CHEMICAL

The structure of the substances obtained was confirmed by the data of elementary analysis. The individuality of the compounds was monitored chromatographically on Silufol UV-254 plates in the system chloroform–ethanol 6:1, butanol–acetic acid–water 4:1:1, propanol–0.2 N ammonia 3:1.

The UV spectra were recorded on a Beckman model 26 Kinetic spectrometer (USA), the IR spectra on a UR-20 spectrometer in KBr tablets, and the PMR spectra on a Perkin Elmer R-12 spectrometer (60 MHz) with tetramethylsilane as the internal standard.

The diazo-compounds were produced according to the general method of [7], the amide of N^{α}-tert-butoxycarbonyllysylglycine XVII according to the scheme:

*For Communication III, see [3].

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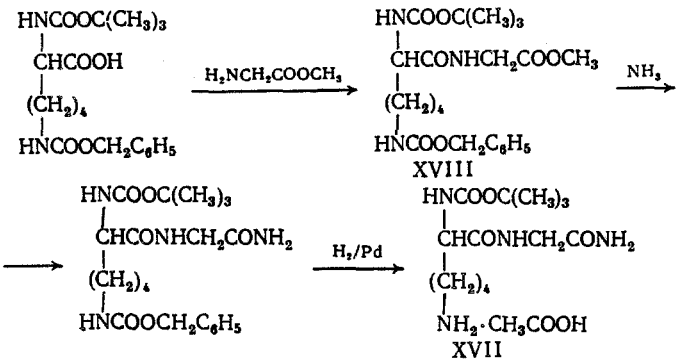
TABLE 1. Antitumor Activity of the Triazenes XXIV-XXVI, XXX-XXXII on Jensen Sarcoma in Rats

Compound	Dose, mg/kg	Inhibition of tumor growth, %	K_g, %
XXIV	50	98	—2
	100	86	—10
XXV	70	94	—1
XXVI	100	86	+4
XXX	75—100	69—75	From 1.5 to —7.3
XXXI	50—100	67—87	From +36 to +25
XXXII	50—100	92—81	From —4.4 to —13



Methyl Ester of N^{α} Tert-butyloxycarbonyl-N^{ε}-carbobenzyloxy-L-lysyl-glycine (XVIII). To a suspension of 3.8 g (0.01 mole) N^{α}-tert-butyloxycarbonyl-N^{ε}-carbobenzyloxy-L-lysine in 20 ml of chloroform, we added a solution of 1.25 g (0.01 mole) of the hydrochloride of the methyl ester of glycine [4] in 20 ml of chloroform at 0°C, then added a soluton of 2.2 g dicyclohexylcarbodiimide in 10 ml of chloroform. The reaction mass was mixed at room temperature for 4 h and left at the same temperature overnight. The dicyclohexylurea precipitate was filtered off, the filtrate washed with water, with a 10% solution of citric acid, a 2% solution $NaHCO_3$, and once again with water, and dried with sodium sulfate. The drying agent was filtered off, and the chloroform evaporated under vacuum. The residue was recrystallized from alcohol. Yield 3.15 g (70%). $C_{12}H_{33}N_3O_7$.

Amide of N^{α}-Tert-butyloxycarbonyl-L-lysylglycine (XVII). A 3.15-g (0.007 mole) portion of XVIII was dissolved in 50 ml of methanol, and ammonia was passed through for 2 h at 0°C. The reaction mass was left at room temperature overnight. The solvent was evaporated under vacuum. The oil obtained was dissolved in 30 ml of methanol, 1 g of palladium black [1] and several drops of acetic acid were added. Then hydrogen was passed through for 10 h with vigorous mixing. The catalyst was filtered off, the filtrate evaporated under vacuum, and the residue dried in a vacuum desiccator over P_2O_5. Yield of XVII 1.6 g (80%). $C_{13}H_{26}N_4O_4 \cdot CH_3COOH$.

The reaction of the aryldiazo-compounds I-III with amino acids IV-XVI and the dipeptide XVII was conducted according to the general procedure: to a suspension of 0.09 mole of the corresponding amino acid or dipeptide in 25 ml of phosphate buffer, pH 7.4 we added 5 ml of a freshly prepared solution of the corresponding diazo-compound in a concentration of 1 M at 37°C with vigorous mixing. The end of the reaction was determined chromatographically according to the disappearance of the original diazo-compound. The diaryltriazene precipitate was filtered off, washed with water, and dried in a vacuum desiccator. The filtrate was extracted with chloroform and the solvent evaporated under vacuum, isolating the aminobenzenes XXVI-XXIX.

In the interaction of p-nitrophenyldiazonium chloride I with the amino acids IV-XVI and the dipeptide XVII, we obtained 1,3-dinitrophenyltriazene XXIV with a yield of 1.7-2 g (39-46%), mp 240°C. $C_{12}H_9N_5O_4$. R_{f_1} 0.77; R_{f_2} 0.38. UV spectrum, λ_{max}(C_2H_5OH): 230, 265, 397 nm (log ε 4.08, 2.64, 4.52). IR spectrum, ν_{max}, cm^{-1}; 1330, 1500 (NO_2), 1580 (N=N), 3240 (NH). PMR spectrum (d_6-DMSO) ppm: 8.0 q (8H); as well as 4,5-dinitro-2-aminoazobenzene (XXVII) with a yield of 0.17-0.2 g (3.9-4%), mp 145°C (literature: mp 148°C [9]). R_{f_1} 0.7; R_{f_2} 0.5 UV spectrum, λ_{max} (C_2H_5OH):225, 375 nm (log ε 4.18, 4.54). IR spectrum, ν_{max}, cm^{-1}: 1480 (NO_2), 3500 (NH_2).

In the interaction of phenyldiazonium chloride (II) with lysine, arginine, and the dipeptide XVII, we obtained 1,3-diphenyltriazene XXV with a yield of 0.9-1 g (62-69%), mp 98-100°C. R_{f_1} 0.33; R_{f_2} 0.5. UV spectrum, λ_{max} (C_2H_5OH): 235, 290, 350 nm (log ε 4.55, 4.14, 4.59). IR spectrum, λ_{max}, cm^{-1}: 1590 (N=N), 3180 (NH), PMR spectrum (d_6-DMSO), δ, ppm: 7.4 s (10H), 12.4 s (1H); and 2-aminoazobenzene XXVIII with a yield of 0.1-0.15 g (4-6%), mp 58-59°C (literature: mp 59°C [9]). R_{f_1} 0.7; R_{f_2} 0.38. $C_{12}H_{11}N_3$. UV spectrum, λ_{max} (C_2H_5OH): 245, 380 nm (log ε 4.28, 4.68).

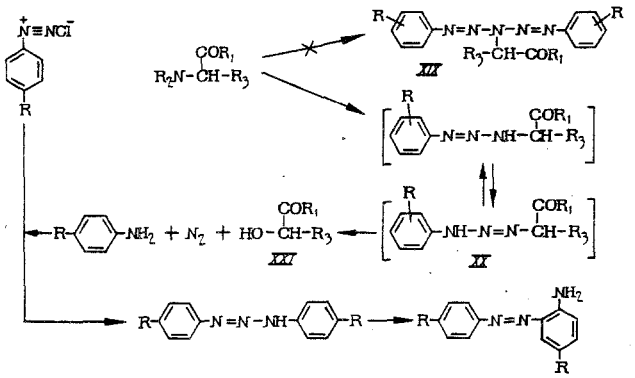
In the interaction of p-tolydiazonium chloride III with L-lysine, L-arginine, and the dipeptide XVII, we obtained 1,3-dimethylphenyltriazene (XXVI) with a yield of 0.72-0.8 g (55-61%), mp 116-117°C. R_{f_1} 0.86; R_{f_2} 0.33. $C_{14}H_{15}N_3$. UV spectrum, λ_{max} (C_2H_5OH): 240, 290, 360 nm (log ε 4.49; 4.3; 4.55). PMR spectrum (d_6-DMSO), δ, ppm: 2.3 s (6H), 7.25 q (8H), 12.4 s (1H), as well as 4,5-dimethyl-2-aminoazobenzene XXIX with a yield of 0.08-0.09 g (6-7%), mp 100°C (literature: mp 102°C [9]). R_{f_1} 0.83; R_{f_2} 0.68. UV spectrum, λ_{max} (C_2H_5OH): 247, 330 nm (log ε 4.19, 4.43).

EXPERIMENTAL BIOLOGICAL

The antitumor activity of the diaryltriazenes XXIV-XXVI was studied on noninbred male white rats weighing 110-120 g with transplanted Jensen sarcoma. The test substances were administered orally in vegetable oil for a period of eight days beginning with the third to fifth day after transplantation of the tumor. The index of inhibition of tumor growth (I_i) and the coefficient of growth (K_g) were determined according to the customary formulas [3].

RESULTS AND DISCUSSION

A study of the reaction of aryldiazonium compounds I-III with natural amino acids IV-XVI and the dipeptide XVII at biological values of the pH, temperature, and ionic strength of the solution (pH 7.4; 37°C; μ 0.178) showed that regardless of the nature of the substituent in the benzene ring of the diazo-compound and the amino acid component, 1,3-diaryltriazenes XXIV-XXVI are formed as the main product according to the Zann–Wollemann–Waschka scheme [13] and partially isomerize to the azo-compounds XXVII-XXIX.



We should note specially that under these conditions the formation of the corresponding pentazenes – bis(aryldiazoamino)amines XIX is not observed under these conditions in the reactions of diazo-compounds with glycine IV and with basic amino acids VI, VII, and IX, as it is in alkaline medium [2, 4].

Since the dipeptide XVII, containing basic N^{ε}-amino group of L-lysine, also forms diaryltriazenes XXIV-XXVI in the reaction with diazo-compounds I-III, it can be assumed that in the case of L-lysine IX and ornithine VI, both amino groups of these amino acids take part in the reactions.

The results obtained from model experiments thus permit us to conclude that free amino acids, as well as the terminal basic amino groups of natural proteins and peptides, are capable of participating in the formation of unstable disubstituted triazines with aryldiazo-compounds under conditions of biological values of the pH, temperature, and ionic strength of the solution; the products undergo further rapid transformation into 1,3-diaryltriazenes, which, according to the literature data [8, 13], is accompanied by a simultaneous deamination of the amino acids or protein amino acid fragments. The conclusions drawn are in good agree-

ment with the data of a number of researchers, showing that symmetrical 1,3-diaryltriazenes are actually products of the metabolism of 3,3-dimethyl- and 3-monoalkyl-1-aryltriazenes [6, 10, 12].

The results of a study of the antitumor activity of the triazenes XXIV-XXVI obtained, cited in Table 1, are evidence that these compounds possess high antitumor activity, commensurate with the activity of the corresponding 3,3-dimethyl-1-aryltriazenes [13].

In view of this, 1,3-diaryltriazenes, possessing cryptodiazonium properties and capable of readily undergoing hydrolysis to form aryldiazo-compounds [4], can evidently play the role of a transport depot form of diazo-compunds as the active ingredient in triazenes under the conditons of the organism.

A kinetic study of the conversion of diazo-compounds according to the Zann–Wollemann–Waschka scheme, which we were the first to observe in neutral medium, will be the subject of our next investigations.

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INFLUENCE OF EXTRACT OF *Eleutherococcus* AND ELEUTHEROSIDES A, B, C, D + E ON RELEASE AND METABOLISM OF ARACHIDONIC ACID

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Often the search for the active ingredient of a drug leads to the conclusion that the action of a mixture of substances is more effective than the action of the individual compounds, as, for example, in the case of ginseng and *Eleutherococcus* [2], or the active substances have a very broad spectrum of action, as is the case for eleutheroside B or cucurbitacins of bryony [5].

Earlier [3, 5] we suggested that the biological activity of certain natural compounds may be a consequence of their influence on the system of hormone-like bioregulators, present in the organs and tissues of the organism – the system of eicosanoids. These autocoids are modulators in the chain of events from stimulus to response and give the most varied pharmacological effects [8]. Precisely such a system of bioregulators can evidently play a vital role in the mechanisms of the action of adaptogens, as, for example, in the case of the

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