3. S. Fazekas de St. Groth, G. S. Watson, and A. F. Reid, J. Immunol., 80, 215 (1958).
4. F. Link, Methods of Virology, Vol. 4, New York (1968), p. 211.
5. V. I. Il'enko, Methods of Testing and Evaluating Antiviral Activity of Chemical Compounds against Influenza Virus [in Russian], Leningrad (1977).
6. C. E. Hoffmann, E. M. Neumayer, and R. F. Haff, J. Bact., 90, 623 (1965).

SYNTHESIS AND ANTITUMOR ACTIVITY OF SOME 4-OXO-1,2,3-TRIAZINO[5,6-b]INDOLES AND 1,1-DIALKYL-3-[INDOL-3-YL]TRIAZENES

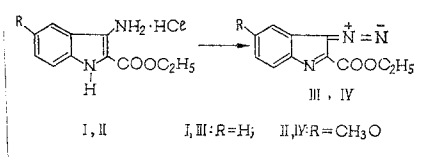
S. V. Simakov, V. S. Velezheva, T. A. Kozik, Yu. A. Ershova, V. A. Chernov, and N. N. Suvorov

UDC 615.277.3:547.336.2/.012.1

The diverse and high biological activity of certain heterocyclic compounds is related to the presence of a triazene or triazine group in their structure. Thus, the 1,1-dialkyl-3-aryltriazenes [1] and imidazolyltriazenes [2] exhibit antitumor activity. Many 1,2,4-triazino[5,6-b]indoles exhibit antiviral [3] and antiinflammatory [4] activity.

The present work was undertaken with the aim of working out a method of synthesis of the previously unknown 1,1-dialkyl-3-(indol-3-yl)triazenes and 1,2,3-triazino[5,6-b]indoles. The 2-carbethoxy-3-aminoindoles (I and II) were chosen as starting compounds.

The diazotization of the amines I and II with sodium nitrite in hydrochloric acid gave the diazoindoles (III and IV), which separated from the reaction mixture at pH 7.0-7.5.

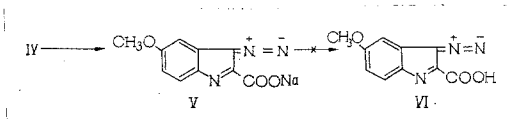


I, II I, III: R = H; II, IV: R = CH_3O

The formation of anhydrodiazo compounds of the type III and IV, in place of the expected diazo salts, has been observed, for example, in the diazotization of such π-electron deficient compounds as the 2-aryl-3-aminoindoles [5] and O-aminonaphthols [6].

In contrast to the literature report [7], we did not succeed in diazotizing 3-aminoindole and its 2-methyl derivative; use of either isoamyl nitrite and acetic acid in THF and dioxane, or sodium nitrite and hydrochloric acid as diazotizing agent led to the formation of a tar.

The diazoindoles III and IV have sharp melting points and crystallize well from organic solvents. Compounds III and IV are stable solids and they are also stable in alkaline solution. Alkaline hydrolysis of the diazoindole IV gives the stable salt V; the corresponding acid VI, however, could not be isolated.



V VI

The indolyltriazenes (VII-XI) were obtained in high yields (76-84%) by combining the diazoindoles III and IV with secondary amines; the reaction takes place on heating compounds III and IV with the amine in ethanol.

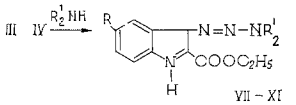
The NMR spectrum (acetone-d_6) of the indolyltriazene IX contains a singlet at 3.36 ppm from the protons of the dimethylamino group and a broad singlet from the indole NH at 10.26 ppm.

D. I. Mendeleev Moscow Chemical-Technological Institute, S. Ordzhonikidze All-Union Scientific-Research Institute of Pharmaceutical Chemistry, Moscow. Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 17, No. 10, pp. 1183-1188, October, 1983. Original article submitted December 30, 1982.

0091-150X/83/1710-0707$07.50 © 1984 Plenum Publishing Corporation

Both the indolyltriazene IX and the diazoindole IV combine with β-naphthol to give the azonaphthol (XII).

Alkaline hydrolysis of the indolyltriazene IX gives the salt V.



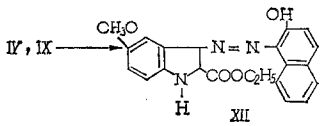
VII – XI

VII: R = H; R_2^1 = CH_3 X: R = CH_3O; R^1 = C_2H_5

VIII: R = H; R_2^1 = C_2H_5 XI: R = CH_3O; NR_2^1 = N⟨ ⟩;

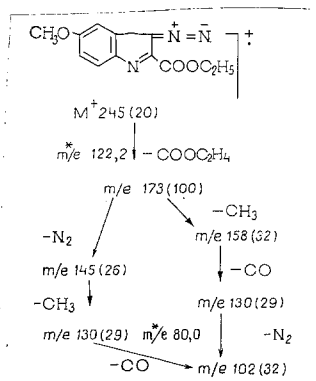
IX: R = CH_3O; R^1 = CH_3

In the IR, the diazo group absorbs at 2120-2140 cm^{-1}, while the absorption due to the indole NH is no longer seen.



XII

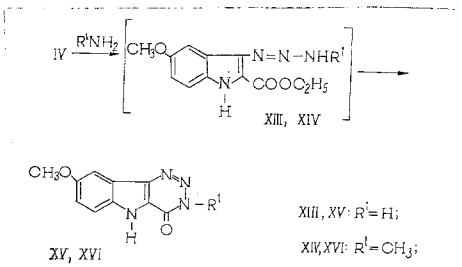
Mass-spectral data show that the way in which the diazoindole IV molecule splits is unusual for compounds capable of liberating nitrogen. The intensity of the molecular ion M^+ 245 is 20%. Only after the elimination of the $COCC_2H_4$ fragment, is a nitrogen molecule split off. At the same time, CH_3 and CO groups are produced from the methoxy group.



This diagram, showing the degradation of compound IV, confirms the presence of peaks due to metastable ions.

With ammonia and methylamine, the diazoindole IV does not give the indolyltriazene (XIII and XIV), but the 4-oxo-1,2,3-triazinoindole (XV and XVI); apparently, the reaction proceeds via the intramolecular cyclocondensation of the intermediately formed compounds XIII and XIV.

The triazinoindoles XV and XVI were obtained in yields of 23% and 76%, respectively.



XIII, XIV

XV, XVI

XIII, XV: R^1 = H;

XIV, XVI: R^1 = CH_3;

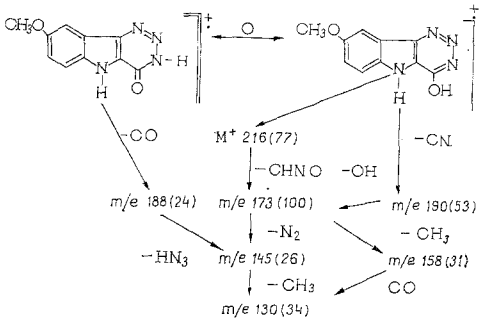
Compound XVI was obtained directly, but in lower yield, from the 3-aminoindole II, by-passing the separation of the diazoindole IV.

The presence of bands at 1600 cm^{-1} in the IR spectrum of the triazinoindole XVI confirms that in the solid state it exists in the oxo form.

The mass spectrum of the triazinoindole XVI shows the presence of ions with m/e 190(53) and 188(23), corresponding to the cleavage of CO and CN fragments, confirming that compound XVI is enolized by the ion-

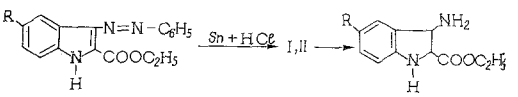
izing radiation. The ion fragment with m/e 190 can only be obtained from the hydroxyl form of the triazinoindole XVI. Comparison of the intensity of the peaks from the ion fragments with m/e 190 and 188 show that compound XVI is 75% enolized.

No variation in the ratio of the intensities of the peaks from the ions M^+/M^+-CO and M^+/M^+-CN is observed over the temperature range 125-300°C in the ionization chamber. The uniformity of these ratios confirms that no tautomeric change occurs in the 4-oxotriazinoindole XVI with change in temperature, since the mass spectrum selects the ratio of concentrations of keto and enol forms of compound XVI when the sample is vaporized in the system.



This scheme confirms the formation of metastable intermediates during decomposition.

The 2-carbethoxy-3-aminoindoles I and II were obtained in yields of 58 and 63%, respectively, by reduction of the 3-phenylazoindoles (XVII and XVIII) with tin in hydrochloric acid [8] without further purification. Higher yields of the hydrochlorides I and II were achieved only by the use of finely divided tin foil; very little reaction occurs when using granulated tin. Aniline hydrochloride, a by-product of the reaction, is readily eliminated by washing with water. The 3-aminoindole hydrochlorides I and II are converted to the free bases Ia and IIa with sodium carbonate.



XVII, XVIII Ia, IIa

XVIII, I, Ia: R = H, XVIII, II, IIa: R = CH_3O;

The antitumor activity of the compounds was studied in tests on rats and mice with transplanted tumors (Jensen's sarcoma 45 and 180, and Ehrlich's carcinoma) by the standard methods [9]. Only the diazoindole III, on administration of a single daily injection of 50 mg/kg for 5-6 days, exhibited a moderate (up to 52%) antitumor action on Jensen's sarcoma. The results are given in Table 1.

EXPERIMENTAL

Infrared spectra of the compounds in mineral oil were taken on a UR-20 (GDR) instrument, UV spectra were taken in ethanol on a Specord-UV, and NMR spectra on a Varian CFT-20 (80 MHz) with tetramethylsilane as internal standard (δ scale). Mass spectra were recorded on an MX-1303 instrument with the direct introduction of the sample into the ion source; ionizing voltage 50 eV, cathode emission current 1.25 mA. The course of the reaction and the purity of the products were checked by TLC using Silufol UV-254 (ChSSR) plates.

2-Carbethoxy-3H-diazo-5-methoxyindole (IV). To a suspension of 2.7 g (10 mmoles) of the 3-aminoindole hydrochloride II in 50 ml of water was added 1.8 ml (20 mmoles) of hydrochloric acid (d 1.17 g/cm^3), the solution cooled to 0-5°C and a solution of 0.76 g (11 mmoles) of sodium nitrite in 10 ml of water added. The reaction mixture was mixed for 30 min at 5-10°C and neutralized with 20% aqueous sodium carbonate to pH 7.0-7.5. The precipitated material was filtered off and washed with water to give 2.1 g (84%) of the diazoindole IV with mp 182-183°C (from isopropyl alcohol). IR spectrum, ν, cm^{-1}: 2125 $(\overset{\oplus}{N}{=}\overset{\ominus}{N})$, 1730 (C = O). UV spectrum, λ_{max}, nm (log ε): 213 (4.51), 260 (4.18), 298 (4.25), 322 (390). NMR spectrum (acetone-d_6), ppm: 1.39 and 4.39 (t and q, C_2H_5), 3.85 (s, CH_3O), 6.94 (dd, J_{6-4} 8.8 Hz, J_{6-7} 2.3 Hz, 6-H), 7.32 (d, J_{4-6} 2.3 Hz, 4-H), 7.67 (d, J_{7-6} 8.8 Hz, 7-H). Found, %: C 59.1; H 4.8; N 17.3. M^+245. $C_{12}H_{11}N_3O_3$. Calculated, %: C 58.8; H 4.5; N 17.1%. M 245.

TABLE 1. Antitumor Activity of Compounds III, IV, and IX

Transplanted tumor	Compound								
	IX			IV			III		
	LD_{100}, mg/kg	dose, mg/kg	index of retardation of growth, %	LD_{100}, mg/kg	dose, mg/kg	index of retardation of growth, %	LD_{10} mg/kg	dose, mg/kg	index of retardation of growth, %
Jensen's sarcoma	500	50	21					50	52
		30	24	500	50	0	300	30	24
		20	15					10	0
Sarcoma 45	500	50	0	500	50	0	300	10	24
Sarcoma 180	500	12,5	20	500	12,5	19	300	10	17
Ehrlich's carcinoma	500	12,5	13	500	12,5	0	300	10	14

Note. The LD_{100} was determined from a single injection of the preparation.

TABLE 2. Yields and Melting Points of the Indolyltriazenes VII-XI and Starting Materials for Their Synthesis

Compound	Starting diazoindole	Starting amine	Quantity of amine, mmoles	Yield, %	mp, °C
VII	III	HN $(CH_3)^*_2$	40	76	154—6
VIII	III	HN $(C_2H_5)_2$	30	78	151—2
IX	IV	HN $(CH_3)^*_2$	40	84	183—4
X	IV	HN $(C_2H_5)_2$	30	79	185—7
XI	IV	HN C_5H_{10}	30	86	182—3

*33% aqueous solution.

TABLE 3. Elemental Analysis and IR Spectra of the Indolyltriazenes VII-XI

Compound	Found, %			Empirical formula	Calculated, %			IR spectra, ν, cm^{-1}		
	C	H	N		C	H	N	(indoles NH)	C=O	C=N
IV	60,0	6,2	21,6	$C_{13}H_{16}N_4O_2$	60,0	6,1	21,5	3310	1665	1535
VIII	62,9	7,1	19,5	$C_{15}H_{20}N_4O_2$	62,5	6,9	19,4	3325	1670	1530
IX	58,1	6,2	19,3	$C_{14}H_{18}N_4O_3$	57,9	6,2	19,3	3330	1675	1530
X	60,5	7,1	17,7	$C_{16}H_{22}N_4O_3$	60,4	6,9	17,6	3320	1670	1535
XI	62,0	6,9	16,9	$C_{17}H_{22}N_4O_3$	61,8	6,7	17,0	3315	1670	1530

2-Carbethoxy-3H-diazoindole (III). Using the same method used to prepare compound IV, 2.4 g (10 mmoles) of the 3-aminoindole I gave 1.6 g (73%) of the diazoindole III, mp 102-121°C (from isopropanol). IR spectrum, ν, cm^{-1}: 2120 $(\overset{\oplus}{N}{=}\overset{\ominus}{N})$, 1725 (C = O). UV spectrum, λ_{max}, nm (log ε): 214 (4.37), 268 (4.26), 306 (4.12). Found, %: C 61.6; H 4.4; N 19.6%. $C_{11}H_{19}N_3O_2$. Calculated, %: C 61.4; H 4.2; N 19.5%.

General Method of Preparation of the 1,1-Dialkyl-3-(indol-3-yl)triazenes (VII-XI). To a solution of 10 mmoles of the diazoindole III or IV in 50 ml of ethanol at 65-70°C was added an excess of the secondary amine. The mixture was heated to boiling, allowed to stand for 2-3 h at 20-25°C, and poured into ice water. The precipitate was filtered off, washed with water and a small amount of cold ethanol, and recrystallized from isopropanol.

Reagents, yields, and melting points for compounds VII-XI are given in Table 2; elemental analysis data and IR spectral data in Table 3.

1,1-Dimethyl-3-(2-carbethoxy-5-methoxyindol-3-yl)triazene (IX). UV spectrum, λ_{max}, nm(log ε): 208 (4.38), 255 (4.27), 299 (4.39), 370 (4.24). NMR spectrum (acetone-d_6), ppm: 1.36 and 4.32 (t and q, C_2H_5), 3.36

TABLE 4. Physical Properties of the 2-Carbethoxy-3-aminoindoles Ia and IIa, and Their Hydrochlorides I and II

Compound	Yield, %	mp, °C	Found, %				Empirical formula	Calculated, %				IR spectrum, ν, cm^{-1}		
			C	H	N	Cl		C	H	N	Cl	(indole NH)	C=O	NH_2
I	58	215–6	55,1	5,1	11,8	15,3	$C_{11}H_{12}N_2O_2HCl$	54,9	5,4	11,6	14,8	3140	1690	3300 3360 3510
Ia	84	151–2	61,5	6,3	14,0	—	$C_{11}H_{12}N_2O_2$	64,7	5,9	13,7	—	3140	1665	3310 3370 3310
II	63	234–35	53,6	5,7	10,6	13,6	$C_{12}H_{14}N_2O_3HCl$	53,2	5,5	10,4	13,1	3150	1700	3360 3530 3390
IIa	87	175–6	61,4	6,1	11,8	—	$C_{12}H_{14}N_2O_3$	61,5	6,0	12,0	—	3150	1670	3320

[s, $N(CH_3)_2$], 3.78 (s, CH_3O), 6.90 (dd, J_{6-4} 2.6 Hz, J_{6-7} 9 Hz, 6-H), 7.34 (d, J_{4-6} 2.6 Hz, 4-H), 7.65 (d, J_{7-6} 9 Hz, 7-H), M^+ 290.

Sodium Salt of 3H-Diazo-5-methoxyindol-2-carboxylic Acid (V). A. To a solution of 2.4 g (10 mmoles) of the diazoindole IV in 40 ml of ethanol was added 2.4 g (60 mmoles) of NaOH. The reaction mixture was refluxed for 30 min, cooled, and diluted with diethyl ether (100 ml). The precipitated material was filtered off and washed with ethanol to give 2.1 g (87%) of the salt V.

B. Using the same method, 0.98 g (81%) of the salt V, mp 275-276°C (with decomp.) was prepared from 1.45 g (5 mmoles) of the indolyltriazene IX and 1.45 g (34 mmoles) of NaOH in 40 ml of ethanol. IR spectrum, ν, cm^{-1}: 2140 $(\overset{\oplus}{N}{=}\overset{\ominus}{N})$, 1620 (C=O). Found, %: C 50.6; H 2.8; N 17.8%. $C_{10}H_6N_3O_3Na$. Calculated, %: C 50.2; H 2.5; N 17.6%.

1-(2-Carbethoxy-5-methoxyindole-3-yl)azonaphthol-2 (XII). A. A solution of 1.22 g (5 mmoles) of the diazoindole IV and 1.44 g (10 mmoles) of β-naphthol in 50 ml of ethanol was refluxed for 4 h. The reaction mixture was cooled to 45-50°C, the precipitated material filtered off and washed with hot ethanol. Yield of azonaphthol XII 1.26 g (65%).

B. 1.45 g (5 mmoles) of the indolyltriazene IX and 1.44 g (10 mmoles) of β-naphthol in 60 ml of ethanol gave 1 g (54%) of compound XII with mp 299-300°C (from a 5:1 mixture of DMFA and water). IR spectrum, ν, cm^{-1}: 1630 (N=N), 1690 (C=O), 3300 (broad OH, indole NH). Found, %: C 68.1; H 5.1; N 11.1%. $C_{22}H_{19}N_3O_4$. Calculated, %: C 67.9; H 4.9; N 10.8%.

5H-4-Oxo-3H-8-methoxy-1,2,3-triazino[5,6-b]indole (XV). To a solution of 1.32 g of the diazoindole IV in 50 ml of ethanol at 60°C was added 10 ml of 25% aqueous ammonia. The mixture was heated to boiling, left for 1 h at 25°C and treated with 0.01 N hydrochloric acid to pH 7.0-7.5. The precipitate was filtered off, and washed with water and hot ethanol to give 0.3 g (28%) of compound XV with mp 241-242°C (from DMFA). IR spectrum, ν, cm^{-1}: 1660 (C=O), 3150 (indole NH), 3270 (NH). Found, %: C 56.1; H 4.0; N 26.2%. M^+ 216. $C_{10}H_8N_4O_2$. Calculated, %: C 55.6; H 3.7; N 26.0%. M 216.

5H-4-Oxo-3-methyl-8-methoxy-1,2,3-triazino[5,6-b]indole (XVI). A. By the method used for the synthesis of XV, 0.7 g (76%) of the triazoindole XVI, mp 257-258°C (from DMFA) was obtained from 1 g of the diazoindole IV and 10 ml of 25% aqueous methylamine.

B. An aqueous solution of the diazoindole IV, obtained from 2.7 g (10 mmoles) of the 3-aminoindole II was added with vigorous stirring to a mixture of 50 ml of 33% aqueous methylamine, 3.2 g (30 mmoles) of sodium carbonate, and 20 g of ice. The reaction mixture was stirred for 30 min at 5-10°C, the precipitated material filtered off, and washed with water and hot ethanol to give 0.73 g (31%) of compound XVI. IR spectrum, ν, cm^{-1}: 1680 (C=O), 3150 (indole NH). NMR spectrum (DMSO-d_6), ppm: 3.86 (s, CH_3O), 3.98 (s, CH_3), 7.16 (dd, J_{6-4} 2.7 Hz, J_{6-7} 8.8 Hz, 6-H), 7.51 (d, J_{7-6} 8.8 Hz, 7-H), 7.58 (d, J_{4-6} 2.7 Hz, 4-H). Found, %: C 57.5; H 4.7; N 24.24%. M^+ 230. $C_{11}H_{10}N_4O_2$. Calculated, %: C 57.4; H 4.4; N 24.4%. M 230.

2-Carbethoxy-3-phenylazo-5-methoxyindole (XVIII). A solution of 10.2 g (110 mmoles) of aniline in 40 ml (250 mmoles) of 20% hydrochloric acid was diazotized with a solution of 7.6 g (110 mmoles) of sodium nitrite in 30 ml of water at 3-5°C. The diazo solution was added to a cooled (0-2°C) solution of 21.9 g (100 mmoles) of 2-carbethoxy-5-methoxyindole in 400 ml of DMFA, brought to pH 7.5-8.0 by the addition of 15% aqueous sodium

carbonate with the temperature kept below 5°C. After the addition of all the diazo solution, mixing was continued for a further hour, the precipitate filtered off and washed with water to give 22.3 g (69%) of compound XVIII, mp 209-211°C (from isopropyl alcohol). IR spectrum, ν, cm^{-1}: 1680 (C=O), 1535 (N=N), 3300 (indole NH). Found, %: C 67.1; H 5.5; N 12.9%. $C_{18}H_{17}N_3O_3$. Calculated, %: C 66.9; H 5.5; N 13.0%.

2-Carbethoxy-3-phenylazoindole (XVII). Using the same method given for XVIII, 16.7 g (63%) of the 3-phenylazoindole XVII, mp 193-194°C (from isopropyl alcohol) was obtained from 18.9 g (100 mmoles) of 2-carbethoxyindole and phenyldiazonium chloride [prepared from 10.2 g (110 mmoles) of aniline in 400 ml of DMFA]. Literature value for mp of XVII, 191-193°C [8].

Hydrochlorides of 2-carbethoxy-3-aminoindole (I) and 2-carbethoxy-3-amino-5-methoxyindole (II). To a solution of 10 mmoles of the 3-phenylazoindole (XVII or XVIII) in 30 ml of isopropyl alcohol at 60°C was added 6.1 g (50 mmoles) of finely divided tin. With vigorous mixing was then added dropwise 8.5 ml (100 mmoles) of hydrochloric acid (d 1.15 g/cm^3). The mixture was refluxed for 2 h, cooled to 0-5°C, the precipitate filtered off and washed with ice-water (50 ml).

Yields, melting points, elemental analysis, and IR spectral data are given in Table 4.

2-Carbethoxy-3-aminoindole (Ia) and 2-carbethoxy-3-amino-5-methoxyindole (IIa). The hydrochlorides I and II (10 mmoles) were dissolved in 100 ml of water (70-80°C), the solutions filtered and 15% aqueous sodium carbonate added to the filtrate to pH 8.0. The precipitate was filtered off, washed with water, dried, and recrystallized from isopropyl alcohol.

Yields, melting points, elemental analysis, and IR spectral data are given in Table 4.

LITERATURE CITED

1. S. Cristian and J. R. Rondestved, J. Org. Chem., 22, 200-203 (1957).
2. R. Osika, S. Seeber, U. Bruntsch, et al., Dtsch. Med. Wshchr., 99, 2468 (1974).
3. G. N. Pershin and N. S. Bogdanova, Chemotherapy of Viral Infections [in Russian], Moscow (1973), p. 76.
4. A. B. Tomchin, A. N. Poskalenko, and M. M. Ponomareva, Chemistry, Biochemistry, and Pharmacology of Indole Derivatives [in Russian], Tbilisi (1981), p. 112.
5. L. S. Éfros and M. V. Gorelik, Chemistry and Technology of Intermediates and Dyes [in Russian], Moscow (1978), pp. 418-420.
6. A. Gonzales and C. Galvez, Synthesis, 9, 741-742 (1981).
7. S. P. Hiremath, A. G. Bruke, and J. L. Nelson, Indian J. Chem., 198, 767-769 (1980).
8. G. N. Kurilo, O. N. Boyarintseva, and A. N. Grinev, Khim. Geterotsikl. Soedin., No. 5, 664-665 (1975).
9. N. N. Suvorov, V. A. Chernov, V. S. Velezheva, et al., Khim.-farm. Zh., No. 9, 27-34 (1981).