Triazeno-*v*-triazole-4-carboxamides. Synthesis and Antitumor Evaluation[1,2]

Y. FULMER SHEALY AND C. ALLEN O'DELL

Kettering-Meyer Laboratory, Southern Research Institute, Birmingham, Alabama 35205

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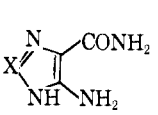
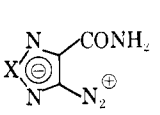
5-Diazo-*v*-triazole-4-carboxamide (IIa) cyclizes to 2,8-diazahypoxanthine slowly in acidic solution and rapidly at pH 7. It readily gives azo coupling products (IV) with N,N-dimethylaniline and with 2-naphthol and undergoes a replacement reaction with iodide. 5-(Substituted triazeno)-*v*-triazole-4-carboxamides (VI) were prepared from the diazotriazole and amines. A dialkyltriazeno derivative slowly gave rise to 2,8-diazahypoxanthine in solution in the presence of light. 5-(Butyltriazeno)-*v*-triazole-4-carboxamide, the only monoalkyltriazene prepared, is unstable both in the solid state and in aqueous solution. Rapid dissociation of the butyltriazeno derivative in solution to 5-amino-*v*-triazole-4-carboxamide was demonstrated. 5-(3,3-Dimethyl-1-triazeno)-*v*-triazole-4-carboxamide significantly increased the life span of leukemic (L1210) mice.

5-Amino-*v*-triazole-4-carboxamide (Ia) is a heterocyclic analog of 5(or 4)-aminoimidazole-4(or 5)-carboxamide (AIC, Ib), which, as its 1-β-D-ribofuranosyl phosphate derivative, is an intermediate in the biosynthetic pathway to purine ribonucleotides. Like AIC, the aminotriazole (Ia) gave an isolatable diazo derivative (IIa).[3] Since certain triazenes prepared from 5-diazoimidazole-4-carboxamide (IIb) have antineoplastic activity,[4,5] it was obviously desirable to prepare triazenes from the triazole analog IIa for antitumor evaluation. For this purpose, further knowledge of the properties and preparation of 5-diazo-*v*-triazole-4-carboxamide (IIa) was needed since study of this compound had previously been limited. The investigation described here included these studies together with the synthesis and antitumor evaluation of some triazeno derivatives.



Ia, X = N
b, X = CH



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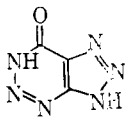
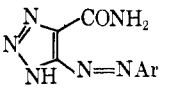
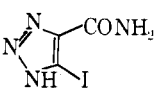


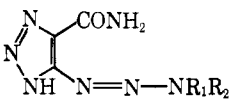
III



IVa, Ar = *p*-$(CH_3)_2NC_6H_4$
b, Ar = 2-hydroxy-1-naphthyl



V



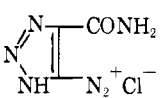
VI

	R_1	R_2
a	CH_3	CH_3
b	C_4H_9	C_4H_9
c	R_1R_2N =	
d	H	C_4H_9
e	CH_3	C_4H_9
f	CH_3	$(CH_3)_2CH$
g	CH_3	$C_6H_5CH_2$
h	CH_3	C_6H_5
i	H	*p*-BrC_6H_4

Although 5-diazo-*v*-triazole-4-carboxamide (IIa) cyclized in solution to 2,8-diazahypoxanthine[3] (*v*-triazolo-[4,5-*d*]-*v*-triazin-7(6H)-one, III), the fact that it was successfully recrystallized from water suggested that it might be more stable than the corresponding imidazole derivative. Observations of changes in the ultraviolet spectra of solutions of IIa (approximately 7×10^{-5} *M*) were performed by the methods previously described[3] for IIb. These studies showed that cyclization of IIa in 0.1 *N* hydrochloric acid in the dark at room temperature had occurred to only a slight extent after 6 hr and had almost reached completion after 3 days.[6] At pH 7 (phosphate buffer), the cyclization reached completion between 3 and 8 min. Thus, in comparison with the imidazole analog, IIa is more stable to cyclization in the 0.1 *N* acid and appears to be slightly less stable at pH 7. Cyclization of IIa in water (pH 5.5) is essentially complete in less than 4 hr and is estimated to occur at a rate similar to that of the imidazole derivative in water (at pH 5.9).

Despite the competing intramolecular reaction, the diazotriazole readily coupled with N,N-dimethylaniline and with 2-naphthol when it was added slowly to aqueous solutions of these substrates. The azo compounds (IVa and IVb) were obtained in yields of 84 and 57%, respectively. Replacement of the diazo group by an iodo group in a potassium iodide–hydriodic acid solution afforded 5-iodo-*v*-triazole-4-carboxamide (V) and demonstrated a further similarity to the chemical behavior of benzenoid diazonium salts.[6]

Representative triazenes (VIa–i) (see Table I) were readily obtained from reactions of the diazotriazole



VII

(1) This investigation was supported by the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Contract No. SA-43-ph-1740 and PH-43-64-51, and by the C. F. Kettering Foundation.

(2) In structures I and IV–VII, the triazole-ring proton is arbitrarily placed at the position corresponding to the point of attachment of the ribofuranosyl group of 5-amino-1-(β-D-ribofuranosyl)imidazole-4-carboxamide 5'-phosphate. The tautomeric structure (III) used to represent 2,8-diazahypoxanthine is also arbitrarily chosen.

(3) Y. F. Shealy, R. F. Struck, L. B. Holum, and J. A. Montgomery, *J. Org. Chem.*, **26**, 2396 (1961).

(4) Y. F. Shealy, J. A. Montgomery, and W. R. Laster, Jr., *Biochem. Pharmacol.*, **11**, 674 (1962).

(5) (a) Y. F. Shealy and C. A. Krauth, *J. Med. Chem.*, **9**, 34 (1966); (b) *Nature*, in press.

(6) The slower rate of cyclization of IIa to III in 0.1 *N* acid may be due to protonation to a "normal" diazonium salt (VII). This species may also be present in the reaction medium used to prepare V.

TABLE I

5-(SUBSTITUTED TRIAZENO)-*v*-TRIAZOLE-4-CARBOXAMIDES (VI)

VI	R_1	R_2	Amine, ml g of IIa	Solvent,[a] ml g of IIa	Reaction time, hr[b]	Yield, %	Mp or approx dec pt,[c] °C	Formula	Calcd, % C	H	N	Found, % C	H	N
a	CH_3	CH_3	*d*	C, 43	1.5 + 2	94	165–170	$C_8H_{13}N_7O \cdot C_2H_7N$[e]	36.83	7.06	49.12	36.93	6.67	49.12
b	C_4H_9	C_4H_9	28.5	A	1.5 + 2.5	86	126	$C_{11}H_{21}N_7O$	49.41	7.92	36.68	49.11	7.86	36.84
c	R,R,N		10	B, 10	1.75 + 2	48[f]	255E	$C_7H_{11}N_7O$	40.48	5.30	46.88	40.38	5.45	46.79
d	H	C_4H_9	100	A	1 + 3	87[g]	118E	$C_7H_{13}N_7O \cdot C_4H_{11}N$[g]	46.46	8.51	30.41	46.45	8.51	35.40
e	CH_3	C_4H_9	8.5	B, 50	2 + 16	80	103–144, 244–246E	$C_9H_{15}N_7O$	42.65	6.71	43.53	42.97	6.45	43.38
f	CH_3	$(CH_3)_2CH$	4.4	B, 200	2.5 + 20	69	>160D, >210E	$C_7H_{13}N_7O$	39.80	6.21	46.43	39.82	5.95	46.25
g	CH_3	$C_6H_5CH_2$	11.5	B, 20	1.5 + 16	53	196D, >225E	$C_{11}H_{15}N_7O$	50.96	5.66	37.82	50.84	5.30	37.68
h	CH_3	C_6H_5	5	C, 20	1.5 + 2	93	265–267D	$C_{10}H_{13}N_7O$	48.97	4.53	39.98	48.71	4.76	39.63
i	H	*p*-BrC_6H_4	*h*	C, 50	2 + 16	95	270E	$C_9H_8BrN_7O$[i]	34.86	2.60	31.62	35.00	2.76	31.84

[a] A = excess of the reactant amine (*cf.* column 4), B = ethyl acetate, C = methanol. [b] IIa was added during the period given by the first number. Stirring of the mixture was continued during the period given by the second number. [c] D = decomposition, E = explosive decomposition. Determined by sprinkling specimens along the gradiently heated bar of a Kofler Heizbank melting point apparatus. Some of the derivatives that melt without exploding will explode at a higher temperature. [d] Solvent was saturated with anhydrous dimethylamine. [e] Dimethylammonium salt of VIa. [f] Obtained in 92% yield when excess pyrrolidine was the solvent; see, however, footnote 7. [g] Butylammonium salt of VId. [h] 6.4 g of *p*-bromoaniline/g of IIa. [i] *Anal.* Calcd: Br, 25.8. Found: Br, 25.5.

with amines in organic solvents.[6a] Owing to instability[7] of monobutyltriazeno derivative VId, monoalkyltriazenes were not further investigated. The alkyltriazeno, but not the aryltriazeno, derivatives were initially obtained as salts of the reactant amines, and most of the salts were converted in slightly acid solutions to the un-ionized triazenes. The analogous triazenoimidazoles were not generally obtained as amine salts;[8] the greater acidity of the triazenotriazoles is consistent with the greater electron-withdrawing power of the triazole ring consequent to the introduction of an additional nitrogen atom.

The ultraviolet spectra of 5-(3,3-dibutyl-1-triazeno)-*v*-triazole-4-carboxamide (VIb) in phosphate buffer (pH 7) and of 5-(pyrrolidinylazo)-*v*-triazole-4-carboxamide (VIc) in water were unchanged after 24 hr in the dark. The absorbance values at 323 and 320 mμ for VIb in buffer and VIc in water, respectively, decreased very slowly after the solutions had been exposed to light. This process was far from complete after the solutions of VIb and VIc had stood for 6 days and for 7 days, respectively, unprotected from light during periods of daylight.[10] Since analogous disubstituted triazenoimidazoles give rise to 2-azahypoxanthine under similar conditions,[9] it was assumed that the changes in the ultraviolet spectra result from the formation of 2,8-diazahypoxanthine. Although the spectral changes during the period of observation did not proceed far enough to permit identification of the reaction product, the formation of 2,8-diazahypoxanthine from the pyrrolidinyl derivative (VIc) in a 1:1 water–ethanol solution, kept under similar conditions, was detected by thin layer chromatography.

The monobutyltriazeno derivative (VId) dissociated rapidly in phosphate buffer (pH 7) in the dark to 5-amino-*v*-triazole-4-carboxamide. This process was essentially complete when the first ultraviolet absorption curve was recorded; the recording was started 2 min after VId had been added to the buffer solution. The formation of the aminotriazole (Ia) was confirmed by isolation. The behavior of VId is, therefore, entirely analogous to that of its imidazole analog.[6a]

Antitumor Evaluation.[11,12]—Results of antitumor testing (at or below 500 mg/kg/day) in accordance with the protocols[13] of the Cancer Chemotherapy National Service Center are shown in Table II. Suspensions or solutions of the triazenes and the diazotriazole (IIa) in saline (0.85% NaCl in water) or in a carboxymethylcellulose vehicle (0.4% CMC in 0.85% saline) were injected intraperitoneally within 5 min of the time that the compounds came into contact with the aqueous solution. Toxic dose levels (not necessarily minimum toxic doses) for tumor-bearing animals were found for at least one of the tumor systems, and these data (mortality or difference in weight change between control and treated mice) were utilized in selecting doses for the other tumors.

(6a) NOTE ADDED IN PROOF.—Compound VIb and the analogous dipropyltriazene have recently been prepared and tested against the Ehrlich carcinoma: K. Hano, A. Akashi, I. Yamamoto, S. Narumi, Z. Horii, and I. Ninomiya, *Gann*, **56**, 417 (1965).

(7) The butylammonium salt of VId decomposed on standing in the solid state. One sample exploded and on one occasion a reaction mixture exploded when IIa was being added to butylamine. It should also be noted that the addition of finely divided IIa to undiluted pyrrolidine during one reaction (but not during two earlier preparations of VIc) was accompanied by flashes of light, formation of smoke, and some charring of the product.

(8) If salts were formed, they were dissociated either by washing with water, when this treatment was included in the purification process, or during drying under reduced pressure. However, the piperidine salt of 5(or 4)-(piperidinoazo)imidazole-4(or 5)-carboxamide[9] and the unstable cyclohexylamine salt of 5(or 4)-(cyclohexyltriazeno)imidazole-4(or 5)-carboxamide[6a] precipitated when these triazenes were prepared in the appropriate amine as solvent.

(9) Y. F. Shealy, C. A. Krauth, and J. A. Montgomery, *J. Org. Chem.*, **27**, 2150 (1962).

(10) These derivatives appear to be more stable to light in solution than similar disubstituted-triazenoimidazoles, but a comparison of their relative stabilities is not meaningful because the light wavelengths catalyzing decomposition are unknown and exposure to light might not have occurred under comparable conditions.

(11) Biological testing was performed by the Chemotherapy Department of Southern Research Institute under the auspices of the Cancer Chemotherapy National Service Center and under the supervision of Drs. F. M. Schabel, Jr., and W. R. Laster, Jr.

(12) L1210 = lymphoid leukemia L1210, Ca755 = Adenocarcinoma 755, S180 = Sarcoma 180, FVL = solid Friend virus leukemia.

(13) *Cancer Chemotherapy Rept.*, **No. 25**, 1 (1962); **No. 1**, 92 (1959). In order to pass stage 1 for one of the test systems mentioned here, the value of T/C in a single test at a nontoxic dose must be as follows: T/C ≧ 125% for L1210, T/C ≦ 53% for Ca755 and S180, T/C ≦ 63% for FVL. The maximum allowable difference in the average weight change of control and treated animals is 5 g for Ca755 and S180 and 4 g for FVL.

The dimethyltriazene VIa displayed activity against L1210 at three dose levels and, in addition, the product (1.7) of the T/C values at 250 mg/kg/day is above that required[13] (1.56) to pass stage II. The initial, limited data from the testing of the isopropylmethyltriazene VIf indicate that it is also inhibitory to L1210. None of the other triazenes displayed significant activity at the selected doses in L1210 tests. A compound that was inactive against L1210 at one dose was sometimes tested at a lower dose if there was reason to suspect that toxicity of the compound may have influenced the life span. For example, compound VIe was inactive at 50 mg/kg/day; however, at this dose in a Ca755 test it was toxic, and at the slightly higher dose of 62 mg/kg/day in S180 tests there was evidence of toxicity in the high weight-change differences. Compound VIe was, therefore, retested at 25 mg/kg/day.

Moderate inhibition of FVL by VIe and of S180 by III was observed in single tests of these compounds at 25 and at 500 mg/kg/day, respectively. Values of T/C below 53% were obtained in some of the tests (Table II) of certain triazenes *vs.* Ca755 or S180;[13] in these tests, however, either the weight-change differences were above or near the upper limit, or the apparent activity was not confirmed upon further testing. At best, these results may represent borderline activity, but considerable additional testing would be required to obtain definitive answers for a structure–activity correlation.

Experimental Section

Ultraviolet spectra were recorded with a Cary Model 14 recording spectrophotometer. Unless otherwise stated, melting points were determined on a Kofler Heizbank melting point apparatus and are corrected; "cap." means that the melting temperature was determined in a capillary tube.

5-Diazo-*v*-triazole-4-carboxamide[3] (**IIa**) was prepared by an improved procedure. Redistilled isoamyl nitrite (41.2 g) was added dropwise to a mixture of 30.0 g of 5-amino-*v*-triazole-4-carboxamide[14] (Ia), 810 ml of water, and 90 ml of glacial acetic acid. The mixture was stirred for 4 hr at room temperature, filtered to remove a small amount of insoluble material, and concentrated *in vacuo* at 40–45° to about 200 ml. The crystalline product (IIa) was removed by filtration, washed thoroughly with water, and dried *in vacuo* at 55°; yield 21 g (65%), mp *ca.* 178° with explosive decomposition.

2,8-Diazahypoxanthine (**III**) was more easily prepared by modifying previous procedures.[3] A mixture consisting of 5.5 g of IIa, 100 ml of water, and a sufficient amount of 10% NaOH to raise the pH to 8 was stirred at room temperature for 2 hr. The mixture was treated with activated carbon, filtered, acidified to pH 5 with acetic acid, chilled, and filtered again to remove the white crystalline precipitate. The first crop of 2,8-diazahypoxanthine dihydrate, after it had been washed with water and dried *in vacuo* at 56°, amounted to 4.5 g (65%); explosive decomposition, 270–275°. A second crop of 1 g (total yield, 79%) was isolated from the filtrate.

5-Iodo-*v*-triazole-4-carboxamide[15] (**V**).—To a solution of 8.36 g (50.4 mmoles) of KI, 6.39 g (25.2 mmoles) of iodine, 160 ml of water, and sufficient HI to lower the pH to 0.6 was added 3.5 g (25.2 mmoles) of IIa in small portions during 1 hr. Hydriodic acid was added, as needed, to maintain the pH of the reaction mixture at 0.6–1.0. Nitrogen was evolved. The mixture was stirred at room temperature for an additional 3 hr, chilled to 5° and filtered to remove 1.1 g of a gray crystalline precipitate (mp 206–210°). A second crop of 1.78 g of gray crystals (mp 208–210°), obtained from the cold filtrate, was combined with the first crop; and the combined material, upon recrystallization from a mixture of ethyl acetate and hexane, afforded 2.0 g of white crystals; mp 208–210°.

Anal. Calcd for $C_3H_3IN_4O$: C, 15.14; H, 1.27; N, 23.54; I, 53.33. Found: C, 15.29; H, 1.45; N, 23.42; I, 53.29.

5-(*p*-Dimethylaminophenylazo)-*v*-triazole-4-carboxamide (**IVa**).—A mixture of 1.32 g of N,N-dimethylaniline and 15 ml of water was acidified to pH 4 with 1 *N* HCl and then diluted with 20 ml of ethanol to give a homogeneous mixture. Small amounts of a 300-mg portion of IIa were added during 1.75 hr, and 1 *N* HCl was added, as needed, to maintain the acidity at pH 4. Stirring was continued for 0.5 hr. The red precipitate was removed by filtration, washed with water, and dried *in vacuo* at 55° (P_2O_5); yield 470 mg (84%); mp 240–241° dec (cap.). A specimen for analysis was precipitated from dimethylformamide by the addition of water; mp 240–242° dec (cap.).

Anal. Calcd for $C_{11}H_{13}N_7O$: C, 50.95; H, 5.05; N, 37.82. Found: C, 50.70; H, 4.94; N, 37.72.

5-(2-Hydroxy-1-naphthylazo)-*v*-triazole-4-carboxamide (**IVb**).—A solution of 15.68 g (109 mmoles) of 2-naphthol and 400 ml of 50% ethanol was made alkaline with 0.1 *N* NaOH until the observed pH reached 7.9. The mixture was stirred and protected from light while 3.0 g (21.7 mmoles) of IIa was added in small portions during 1.5 hr, and 0.1 *N* NaOH was introduced, as needed, to maintain the pH at 7.6–7.9. Stirring was continued for 1 hr, and the orange product was isolated in the same way as was IVa; yield 3.5 g (57%), mp 250° dec (cap.). A specimen recrystallized twice from a dimethylformamide–water mixture melted with decomposition at the same temperature.

Anal. Calcd for $C_{13}H_{10}N_6O_2$: C, 55.33; H, 3.57; N, 29.78. Found: C, 55.36; H, 3.53; N, 29.90.

5-(Substituted triazeno)-*v*-triazole-4-carboxamides (**VIa–i**) were prepared by the same general procedure, which is illustrated by the procedure for the dibutyltriazene (VIb).

A 3.5-g specimen of 5-diazo-*v*-triazole-4-carboxamide was added in small portions during a period of 1.5 hr to 100 ml of di-*n*-butylamine contained in a flask wrapped with aluminum foil to exclude light. The mixture was stirred an additional 2.5 hr at room temperature. A white solid was removed from the reaction mixture with a filter funnel wrapped with aluminum foil, washed thoroughly with benzene, and dried *in vacuo* at 60°; yield 9.5 g (95% as the dibutylammonium salt), mp 112–114°. A mixture of 6.5 g of the dibutylammonium salt and 600 ml of water was stirred in the darkness at room temperature while 1 *N* HCl was added until the pH of the mixture stabilized at 5.7. The crystalline precipitate was removed by filtration, washed well with water, and dried *in vacuo* at 60°; yield 3.96 g (91%), mp 126°.

Data on the preparation and characterization of the individual triazenes are recorded in Table I. The dimethyltriazene VIa was characterized and tested biologically as the dimethylammonium salt, which was obtained as a chromatographically homogeneous,[16] yellowish white solid by evaporation of methanol and dimethylamine from the reaction mixture. The other triazenes precipitated from the reaction mixtures. The aryl derivatives (VIh and VIi) precipitated as the un-ionized triazenes; the alkyl derivatives separated as salts of the reactant amines and were obtained, with the exception of VId and VIf, as un-ionized triazenes by the method described above. Compound VIf was freed from its salt by passing an ethanol solution of the salt through a column of a weakly acidic ion-exchange resin (Amberlite CG-50). The monobutyl derivative[7] (VId) was characterized as the (white) butylammonium salt because of its instability. All of the un-ionized triazenes were white solids except for VIh and VIi, which were dull orange solids.

2,8-Diazahypoxanthine from VIb and VIc.—The ultraviolet spectrum of VIc in water, determined within 5 min after the compound had been added to the solvent, showed maximum absorption at 320 mμ (ε 15,700). The spectrum was essentially unchanged after the solution had stood for 24 hr in the dark. The solution was then allowed to stand in a Pyrex flask under labora-

(14) L. L. Bennett, Jr., and H. T. Baker, *J. Org. Chem.*, **22**, 707 (1957); J. S. Webb and A. S. Tomcufcik, U. S. Patent 2,714,110 (July 26, 1955); *cf.* J. R. E. Hoover and A. R. Day, *J. Am. Chem. Soc.*, **78**, 5832 (1956); S. Yamada, T. Mizoguchi, and A. Ayata, *Yakugaku Zasshi*, **77**, 455 (1957); see *Chem. Abstr.*, **51**, 14698c (1957).

(15) The first specimen was prepared by Mr. C. A. Krauth.

(16) Thin layer chromatography on silica gel in 3:1 chloroform–methanol; detection by ultraviolet light after spraying with Ultraphor WT (Highly Concentrated, BASF Colors and Chemicals, Inc., Charlotte, N. C.).

TABLE II

EVALUATION OF 5-(SUBSTITUTED TRIAZENO)-*v*-TRIAZOLE-4-CARBOXAMIDES AND RELATED COMPOUNDS AGAINST TRANSPLANTABLE MOUSE TUMORS[a]

Compd (R_1, R_2 of VI)	Tumor[b]	Dose, mg/kg/day	Mortality	Av wt[c] change, T/C, g	Tumor data T/C[d]	T/C ratio, %
VIa[e]	L1210	500	0/6	−0.9/+0.4	13.5/8.3	162
(CH_3, CH_3)		375	0/6	−2.5/+0.2	13.3/9.0	147
		250	0/6	−1.3/+0.3	11.8/8.3	142
		250	0/6	−0.6/+2.1	10.0/8.3	120
		168	0/6	−1.7/+0.3	9.7/8.3	116
		100	0/6	−0.4/+0.3	8.8/8.3	106
		66	0/6	+0.7/+0.3	9.3/8.3	112
	Ca755	500	0/10	−2.5/+3.3[f]	302/1690	17
		375	0/10	−1.5/+1.6	681/1174	58
		250	0/10	+2.8/+3.1	855/1436	59
	S180	500	1/6	−4.0/−0.9	342/1318	25
		250	0/6	−3.9/−0.4	461/1544	29
		250	0/6	−0.3/+1.1	803/1355	59
		250	0/6	−2.8/+1.0	822/1006	81
		125	0/6	+0.5/+0.8	703/1608	43
		62	0/6	−1.0/+0.8	1354/1608	84
	FVL	375	0/10	−0.8/+3.3[f]	757/1875	40
		250	0/10	+1.0/+3.3	1415/1875	75
VIb	L1210	500	6/6			Toxic
(C_4H_9, C_4H_9)		250	0/6	−0.5/+0.7	7.7/7.9	97
	Ca755	375	7/10			Toxic
		187	3/10	−0.7/+0.3	756/1025	73
	S180	280	2/6	−2.1/+1.0	898/1307	68
VIc	L1210	300	0/6	−0.9/+0.5	7.5/8.3	90
	Ca755	350	4/10	−2.3/+0.5	372/757	Toxic
		175	0/10	+1.7/+1.9	1199/1245	95
	S180	500	2/6	−3.3/−0.3	825/1199	68
		250	0/6	+0.4/+0.3	1243/1139	109
VIe	L1210	50	0/6	−2.3/+1.2	10.3/9.1	113
(CH_3, C_4H_9)		25	0/6	−0.1/+1.2	10.8/9.1	118
	Ca755	50	8/10			Toxic
		25	0/10	+0.4/+0.6	866/921	94
	S180	162	3/6	−4.8/+0.1	433/1168	Toxic
		62	1/6	−4.4/+0.1	453/1168	38
		62	0/6	−3.5/+1.0	638/1307	48
		62	0/6	−5.1/+1.9[f]	1260/1847	56
		31	1/6	−5.0/+0.1[f]	994/1168	85
		16	0/6	−0.6/+0.1	1090/1168	93
	FVL	50	2/10	−3.9/+1.9[f]	192/844	22
		25	0/10	−0.2/+1.9	400/844	47
VIf	L1210	200	0/6	−3.5/+2.4[g]	9.2/8.8	104
(CH_3, $CH(CH_3)_2$)		100	0/6	−2.1/+2.4	12.0/8.8	136
	Ca755	100	0/10	−0.2/+2.7	1276/1260	101
VIg	L1210	250	0/6	−2.3/−1.5	9.3/8.4	110
(CH_3, $CH_2C_6H_5$)		175	0/6	−1.6/+1.3	9.3/8.0	116
	Ca755	250	2/10	−2.4/+3.6[f]	551/1816	30
		200	0/10	−2.4/+2.3	464/817	56
		100	0/10	+0.7/+2.3	744/817	91
	S180	375	1/6	−3.2/+1.3	648/928	69
VIh	L1210	500	0/6	−2.1/−1.3	8.5/9.2	92
(CH_3, C_6H_5)		375	0/5	−0.6/+1.0	9.2/9.7	94
	Ca755	500	5/10	−3.5/+2.2[f]	156/704	Toxic
		375	1/10	−0.9/+1.6	381/1174	32
		375	1/10	−0.1/+2.9	714/954	74
		375	4/10	−0.6/+3.6	940/1816	Toxic
	S180	500	2/6	−4.0/−0.9	676/1318	51
		375	0/6	−1.9/+1.1	944/1355	69
		250	0/6	−1.5/−0.4	706/1544	45
		125	0/6	−0.1/+0.8	1369/1948	70
VIi	L1210	200	0/6	−1.4/+1.1	8.3/8.1	102
(H, C_6H_4Br-*p*)		100	0/6	−0.4/+0.7	7.8/8.1	96
	Ca755	250	4/9	−0.1/+2.1	896/1491	Toxic
		125	0/9	+0.7/+3.1	1023/1250	81
	S180	200	3/6	−1.5/+0.1	635/1034	Toxic
		100	0/6	+1.2/+2.4	938/736	127

TABLE II (*Continued*)

Compd	Tumor[b]	Dose, mg/kg/day	Mortality	Av wt[c] change, T/C, g	Tumor data T/C[d]	Tumor data T/C ratio, %
IIa	L1210	25	0/6	−0.3/+0.3	9.3/7.4	125
		12.5	0/6	+0.3/+0.3	7.0/7.4	94
	Ca755	25	6/10			Toxic
		12.5	0/10	+1.6/+2.7	903/1411	63
	S180	25	0/6	−0.6/+0.2	433/583	74
III	L1210	375	0/6	−1.4/−0.6	7.0/6.6	106
	Ca755	500	0/10	+4.1/+3.7	1392/1181	117
	S180	500	1/6	−3.0/+0.2	465/941	49
		250	2/6	0/−2	1256/1395	90
IVa	L1210	300	0/6	−0.5/+1.1	8.3/8.3	100
	Ca755	500	4/10	−1.2/+1.1	923/1003	Toxic
		250	2/10	−0.6/+2.2	1133/1141	99
	S180	500	1/6	−2.1/−0.7	607/753	80
IVb	Ca755	200	0/10	+0.6/+2.2	663/992	66
	S180	250	3/6	−0.2/−0.3	1010/1381	Toxic
		125	1/6	−0.6/−1.3	704/869	81
V	S180	500	0/6	−1.3/−1.0	1662/1474	112

[a] T = treated animals, C = control animals. [b] See footnote 12. [c] Average weight change of host animals during the duration of the S180, Ca755, and FVL tests and during the first five days of the L1210 tests. [d] Average tumor weights in milligrams for S180, Ca755, and FVL; average survival time in days for L1210. [e] Tested as the dimethylammonium salt. [f] Average weight-change difference (C − T) above the acceptable levels (5 g for S180 and Ca755 and 4 g for FVL). [g] The high weight-change difference suggests toxicity at this dose.

tory conditions unprotected from incandescent lighting and indirect sunlight. Absorption at 320 mμ slowly decreased in intensity during 7 days of observation. A slight shoulder appeared to be forming in the 270–300-mμ region after 7 days, but the spectral changes were not sufficiently pronounced to permit identification of 2,8-diazahypoxanthine [λ_{max} 278 mμ (ε 5100) at pH 7].[3]

A solution of 50 mg of VIc in 12.5 ml of 50% aqueous ethanol was then stirred under similar conditions of light exposure. Aliquots were withdrawn and subjected to thin layer chromatography on silica gel with water or 1:1 chloroform–methanol as developing solvents and with VIc and III as reference compounds. 2,8-Diazahypoxanthine (III) was detected within 27 hr, and the amount slowly increased. After 2 weeks, the concentration of III was judged to be somewhat less than that of VIc.

The ultraviolet spectrum of VIb in phosphate buffer (pH 7), determined within 20 min, was also unchanged after 24 hr in the dark; λ_{max} 323 mμ (ε 14,500). Exposure to light in the manner described for VIc resulted in a slow decrease in absorption at 323 mμ and the appearance, within 6 days, of a slight shoulder in the 270–300-mμ region, suggestive of III.

5-Amino-*v*-triazole-4-carboxamide from VId.—A mixture of 200 mg of the butylammonium salt of 5-(butyltriazeno)-*v*-triazole-4-carboxamide and 8 ml of water was stirred at room temperature until the solid dissolved. A gas was evolved. The pH of the solution was lowered from 8.9 to 5.0, and water was evaporated *in vacuo;* the residual white solid was suspended in 1 ml of water, separated by filtration, washed twice with 0.5-ml portions of water, and dried *in vacuo* at 50°; 72 mg (80%). The melting point (228°), infrared spectrum, and R_f values (in three paper chromatographic solvent systems) showed that this material was 5-amino-*v*-triazole-4-carboxamide.[14]

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