Experiments utilizing this procedure in animals are in progress. Plasma flufenamic acid levels in two rats at various times after intravenous sublingual administration of 5 mg of I/kg in 0.2 *N* NaOH are shown in Table IV. Due to the high plasma I concentrations at the dose administered, only 50 μl of the plasma sample was taken for analysis. The assays described here may be used to measure therapeutic levels of I in patients.

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Antitumor Activity of Hydrazones and Adducts between Aromatic Aldehydes and *p*-(3,3-Dimethyl-1-triazeno)benzoic Acid Hydrazide

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Abstract □ Hydrazones and adducts between aromatic aldehydes and *p*-(3,3-dimethyl-1-triazeno)benzoic acid hydrazide were synthesized and tested for antitumor activity. Two adducts derived from 2,6-dinitro- and 4-cyanobenzaldehyde were active against TLX5 lymphoma in mice. The hydrazone of the latter aldehyde was significantly more active than the adducts.

Keyphrases □ *Antineoplastic agents, potential*—hydrazones and adducts between aromatic aldehydes and *p*-(3,3-dimethyl-1-triazeno)-benzoic acid hydrazide, structure–activity relationships □ *p*-(3,3-Dimethyl-1-triazeno)benzoic acid hydrazide—antineoplastic activity, hydrazones and adducts with aromatic aldehydes, structure–activity relationships

A series of 1-aryl-3,3-dimethyltriazene derivatives, characterized by the presence of a carbonyl group and a triazene function in the *para*-position on the aromatic nucleus, was previously synthesized and examined for antitumor activity against TLX5 lymphoma in mice (1). Among these compounds, the *o*-nitrophenylhydrazone of the *p*-(3,3-dimethyl-1-triazeno)benzoic acid hydrazide and the adduct between this hydrazide and *p*-nitrobenzaldehyde showed considerable activity. Therefore, a further group of adducts and related hydrazones carrying electron-withdrawing substituents was synthesized and tested for their antitumor activity. Their structures and activities are reported in Tables I and II.

EXPERIMENTAL[1]

Adducts I–IV—Adducts I, II, and IV were prepared by the addition of a solution of the aldehyde in hot ethanol to an equimolar solution of *p*-(3,3-dimethyl-1-triazeno)benzoic acid hydrazide (2) in the same solvent. After standing for a few minutes, the products precipitated and were washed with a few milliliters of cold ethanol. Methanol was used as a solvent for preparing adduct III, and this reaction mixture was heated gently for 15 min before allowing the precipitation.

The structures assigned to I–IV are in accordance with their elemental analyses and are supported by the following data:

1. When these substances were heated to 120°, they lost a molecule of water, yielding the corresponding hydrazone, except for III which decomposed.

2. TLC mobilities and UV spectra of these compounds were quite different from those of the relevant hydrazones, so they cannot be simply monohydrates of these substances. Furthermore, all of these compounds exhibited a strong characteristic band at 1040 cm^{-1} attributable to CO stretching, which disappeared after heating. In addition, the UV spectra of I–IV in ethanol corresponded to the sum of the spectra of the starting hydrazide and aldehydes. TLC on silica gel of these substances at very low concentrations showed that they are split, giving two spots corresponding to the starting reagents; the hydrazones, however, gave single spots. The very poor solubilities of these substances did not allow any NMR investigations of their structures.

Hydrazones V–VII—These hydrazones were prepared by heating the corresponding adducts for a few hours at 120°. The completion of the conversion was determined by TLC and by the disappearance of the strong characteristic band at ~1040 cm^{-1}; the yield was quantitative since no further recrystallization was required.

RESULTS AND DISCUSSION

Tests of the triazene derivatives against TLX5 lymphoma[2] in mice are reported in Table III. The activities observed for each compound were compared on the basis of the number of dose levels at which an ILS of ~50% was observed and by considering also the maximum ILS values obtained when >50%. The compounds carrying nitro groups as substit-

[1] Melting points were determined in open glass capillaries using a Büchi apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer model 225 spectrophotometer, and UV spectra were determined on a Hitachi Perkin-Elmer model 124. Kieselgel HF 254 + 366 (Merck) and methanol–ethyl acetate–ligroin (3:2:1) were used for TLC.

[2] TLX5 lymphoma cells (10^5) were injected subcutaneously in the inguinal region of CBA/LAC female mice, 20–25 g, bred in the Chester Beatty Research Institute. The drugs were used as a solution freshly prepared by sonication in acetone–arachis oil (10:90 v/v); the treatment was performed by daily intraperitoneal administration from Day 3 to 7 after tumor inoculation. For each substance, 10 control mice and groups of five mice for each dose level were used. ILS is the percent increase of the mean survival time of each treated group to that of the relevant controls.

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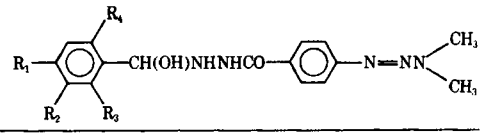

Table I—Physical Constants of the Adducts

Compound	R_1	R_2	R_3	R_4	Melting Point	Yield, %	Formula	Analysis, %	Calc.	Found
I	H	NO_2	H	H	210° dec.	31	$C_{16}H_{18}N_6O_4$	C	53.62	53.58
								H	5.06	5.08
								N	23.45	23.50
II	NO_2	H	NO_2	H	238° dec.	76	$C_{16}H_{17}N_7O_6$	C	47.64	47.55
								H	4.25	4.20
								N	24.31	24.38
III	H	H	NO_2	NO_2	116–118° dec.	59	$C_{16}H_{17}N_7O_6$	C	47.64	47.50
								H	4.25	4.12
								N	24.31	24.17
IV	CN	H	H	H	254–256° dec.	61	$C_{17}H_{18}N_6O_2$	C	60.34	60.40
								H	5.36	5.40
								N	24.84	24.90

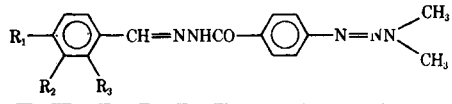

Table II—Physical Constants of the Hydrazones

Compound	R_1	R_2	R_3	Melting Point	Yield, %	Formula	Analysis, %	Calc.	Found
V	H	NO_2	H	210° dec.	100	$C_{16}H_{16}N_6O_3$	C	56.46	56.40
							H	4.74	4.80
							N	24.69	24.71
VI	NO_2	H	NO_2	243° dec.	100	$C_{16}H_{15}N_7O_5$	C	49.87	49.93
							H	3.92	3.95
							N	25.44	25.25
VII	CN	H	H	254–256° dec.	100	$C_{17}H_{16}N_6O$	C	63.74	63.80
							H	5.03	5.03
							N	26.23	26.21

Table III—Antitumor Activity of Compounds III, IV, and VII against TLX5 Lymphoma in Mice

Compound	Dose, mg/kg/day	Average Day of Death ± *SE*	ILS, %
III	—	10.1 ± 0.1	—
	12.5	14.0 ± 0.8	38.6
	25	16.6 ± 0.8	64.3
	50	16.2 ± 0.6	60.8
	100	16.0 ± 0.5	58.4
	200	4.0 ± 0.0	−60.4
IV	—	9.9 ± 0.2	—
	25	11.4 ± 0.2	15.1
	50	14.4 ± 0.5	45.4
	100	15.0 ± 0.3	51.5
	200	15.4 ± 0.7	55.5
	400	7.0 ± 0.9	−29.3
VII	—	10.2 ± 0.2	—
	12.5	11.0 ± 0.3	7.8
	25	16.2 ± 1.6	58.8
	50	19.8 ± 0.4	94.1
	100	20.2 ± 1.7	98.0
	200	17.6 ± 0.8	72.5
	400	17.4 ± 0.2	70.5

uents (I, II, V, and VI) were inactive over a dosage range that included maximum tolerated doses, with the exception of III which was effective at three dose levels. The adduct of *p*-cyanobenzaldehyde (IV) also was active at three dose levels, whereas the corresponding hydrazone (VII) was effective at the five highest dosages employed. An analysis of variance (3) indicated that the ILS values obtained for this compound at 50 and 100 mg/kg/day were significantly higher than those observed for the other compounds tested ($F = 17.2$, $p < 0.0002$).

These results, together with those already reported for a related series of compounds (1), indicate a marked antileukemic activity for derivatives characterized by single electron-withdrawing substituents in either the *ortho*- or *para*-position. High activity does not appear to be peculiar for hydrazones or adducts. In this series, the hydrazone derived from *p*-cyanobenzaldehyde (VII) was significantly more active than the corresponding adduct (IV). For the previously examined *p*-nitro-substituted derivatives, the hydrazone proved completely inactive and the adduct showed remarkable activity. The effects of these compounds on other experimental tumors is being examined.

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