CH_2CH_3), 79508-83-1; I [X = 3-$CH_2OC_6H_4$-3'-$CH(CH_3)_2$], 79508-84-2; I [X = 3-$CH_2OC_6H_4$-3'-$C(CH_3)_3$], 79508-85-3; I (X = 3-$CH_2OC_6H_4$-3'-C_6H_5), 87739-80-8; I (X = 3-$CH_2OC_6H_4$-3'-$NHCOCH_3$), 79508-86-4; I (X = 3-$CH_2OC_6H_4$-3'-$NHCSNH_2$), 79508-87-5; I (X = 3-$CH_2OC_6H_4$-3'-$NHCONH_2$), 70579-43-0; I [X = 3-$CH_2OC_6H_4$-4'-$(CH_2)_4CH_3$], 87739-81-9; I (X = 3-CH_2O-2-naphthyl), 87871-40-7; I (X = 3-CH_2O-1-naphthyl), 87871-41-8; I (X = 3-$CH_2SC_6H_5$), 80239-83-4; I (X = 3-$CH_2SC_6H_4$-3'-CH_3), 87739-85-3; I (X = 3-$CH_2SeC_6H_5$), 87739-79-5; I (X = 3-$CH_2C_6H_5$), 87739-83-1; I (X = 3-$SCH_2C_6H_4$-4'-Cl), 87739-84-2; I (X = 3-$CH_2OC_6H_2$-2',4',5'-Cl_3), 88253-89-8; I (X = 3,5-Cl_2), 2727-10-8; I [X = 3,5-$(CH_3)_2$], 88253-90-1; I (X = 4-SO_2NH_2), 90-08-4; I (X = 4-SO_2CH_3), 74798-28-0; I (X = 4-$CONH_2$), 87871-34-9; I (X = 4-$COCH_3$), 85304-88-7; I (X = 4-OH), 74798-26-8; I (X = NH_2), 87871-35-0; I (X = 4-$NHCOCH_3$), 74798-27-9; I (X = 4-CF_3), 47071-11-4; I (X = 4-F), 1542-59-2; I (X = 4-Cl), 516-21-2; I (X = Br), 3567-84-8; I (X = 4-I), 46781-41-3; I (X = 4-CN), 17711-68-1; I (X = 4-OCH_2CO-morpholine), 50574-87-3; I [X = 4-$O(CH_2)_2$-OC_6H_4-4'-NH_2], 87871-36-1; I (X = 4-CH_3), 15233-37-1; I [X = 4-$(CH_2)_3CH_3$], 4653-73-0; I [X = 4-$(CH_2)_5CH_3$], 87739-87-5; I [X = 4-$C(CH_3)_3$], 4653-75-2; I (X = 4-C≡CC_6H_5), 87871-37-2; I (X = 4-C≡CH), 87740-00-9; I [X = 4-C≡$CSi(CH_3)_3$], 87740-01-0; I (X = 4-OCH_3), 21316-30-3; I (X = 4-OCH_2CH_3), 46985-99-3; I (X = 4-OCH_2CH=CH_2), 88253-91-2; I [X = 4-$O(CH_2)_3CH_3$], 87739-96-6; I [X = 4-$O(CH_2)_5CH_3$], 4653-82-1; I (X = 4-$O(CH_2)_5CH_3$], 4653-85-4; I [X = 4-$O(CH_2)_7CH_3$], 4653-87-6; I [X = 4-$O(CH_2)_{10}CH_3$], 87739-97-7; I [X = 4-$O(CH_2)_{11}CH_3$], 79515-25-6; I (X = 4-$OCH_2C_6H_5$), 17944-10-4; I (X = 4-$OCH_2C_6H_3$-3',4'-Cl_2), 85304-89-8; I (X = $OCH_2C_6H_4$-4'-SO_2NH_2), 87739-88-6; I (X = $OCH_2C_6H_4$-4'-$CONH_2$), 87739-89-7; I (X = $OCH_2C_6H_4$-4'-CH_2OH), 87739-90-0; I (X = $CH_2SC_6H_5$), 87739-93-3; I (X = $CH_2SC_6H_4$-2'-CH_3), 87739-94-4; I (X = $CH_2SC_6H_4$-3'-CH_3), 87739-95-5; I (X = $SCH_2C_6H_5$), 87739-91-1; I (X = $SCH_2C_6H_4$-4'-Cl), 87739-92-2; methotrexate, 59-05-2; 2,4,5-trichlorophenol, 95-95-4; 3-nitrobenzyl chloride, 619-23-8; 3-nitrobenzyl 2,4,5-trichlorophenyl ether, 88253-92-3; *p*-nitrophenol, 100-02-7; ethyl bromide, 74-96-4; allyl bromide, 106-95-6; butyl bromide, 109-65-9; octyl bromide, 111-83-1; ethyl *p*-nitrophenyl ether, 100-29-8; allyl *p*-nitrophenyl ether, 1568-66-7; butyl *p*-nitrophenyl ether, 7244-78-2; *p*-nitrophenyl octyl ether, 49562-76-7; 4-ethoxyaniline, 6375-69-4; 4-butoxyaniline hydrochloride, 6927-73-7; 4-(octyloxy)aniline hydrochloride, 30402-00-7; 3-aminobenzyl 2,4,5-trichlorophenyl ether, 88253-93-4; 4-(allyloxy)aniline hydrochloride, 88271-75-4; dihydrofolate reductase, 9002-03-3; cyanoguanidine, 461-58-5.

Studies of the Mode of Action of Antitumor Triazenes and Triazines. 6.† 1-Aryl-3-(hydroxymethyl)-3-methyltriazenes: Synthesis, Chemistry, and Antitumor Properties

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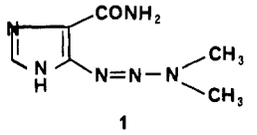
1-Aryl-3-(hydroxymethyl)-3-alkyltriazenes [ArN=NN(CH_3)CH_2OH] have been synthesized by diazonium coupling to the carbinolamine ($RNHCH_2OH$), generated in situ from the alkylamine and formaldehyde mixtures. The (hydroxymethyl)triazene structure has been confirmed by IR, NMR, and mass spectral analysis and also by the preparation of a crystalline benzoate derivative. The mass spectra of the (hydroxymethyl)triazenes suggest that they fragment by loss of formaldehyde to give the methyltriazene, which is also the product of hydrolysis in solution. The degradation of the (hydroxymethyl)triazenes in solution has been followed by UV spectroscopy and by HPLC analysis, and the half-lives were determined under a variety of conditions. The half-lives of the corresponding methyl- and (hydroxymethyl)triazenes are very similar. Both methyl- and (hydroxymethyl)triazenes decompose on silica plates during TLC analysis to give products consistent with known diazo-migration reactions. The (hydroxymethyl)triazenes have pronounced antitumor activity against the TLX5 tumor in vivo; in vivo–in vitro bioassay experiments suggest that the (hydroxymethyl)triazenes exert their in vivo antitumor activity via the degradation product, the alkyltriazene.

5-(3,3-Dimethyltriazen-1-yl)imidazole-4-carboxamide (1, DTIC, Dacarbazine, NSC 45388) has been used in the



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treatment of malignant melanoma, soft tissue sarcoma, and Hodgkin's disease.[1] The more accessible 1-aryl-3,3-dimethyltriazenes (ArN=$NNMe_2$) are also cytotoxic agents with a broad spectrum of activity against animal tumors.[2] It has been claimed that metabolic conversion of the dimethyltriazenes to methyltriazenes (ArN=NNHMe) is essential for antitumor activity.[3] However, recent studies[4] have suggested that dimethyltriazenes are metabolized to a mixture of selective and nonselective metabolites and that methyltriazenes are nonselective cytotoxic agents. An alternative candidate for the selectively cytotoxic species is the (hydroxymethyl)triazene [ArN=NN(CH_3)CH_2OH], since oxidative metabolic demethylation of dimethylamino compounds generally is considered to proceed via 1-hydroxymethyl intermediates.

Although the 1-(hydroxymethyl)triazenes have been regarded as only transient species, Kolar has adduced evidence that suggests that (hydroxymethyl)triazenes are relatively long-lived moieties in vivo. The hydroxymethyl metabolite of DTIC has been tentatively identified in the

† Part 5: Ross, D.; Langdon, A.; Gescher; Stevens, M. F. G. *Biochem. Pharmacol.*, in press.

‡ Saint Mary's University.

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urine of DTIC-treated rats,[5] and a urinary metabolite from rats treated with 1-(2,4,6-trichlorophenyl)-3,3-dimethyltriazene has been characterized as the *O*-glucuronide conjugate of the corresponding (hydroxymethyl)triazene.[6]

The search for a synthetic approach to (hydroxymethyl)triazenes has generated considerable controversy. In a preliminary report, we demonstrated that (hydroxymethyl)triazenes can be synthesized by simply reacting an arenediazonium salt with a formaldehyde–methylamine mixture:[7]

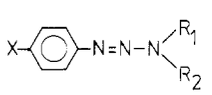
$$ArN_2^+ + MeNH_2/CH_2O \rightarrow ArN{=}NN(Me)CH_2OH$$

This method works well when the diazonium salt bears –M substituents in the aryl group. Subsequently, it was claimed that the use of the diazonium fluoroborate salt could extend the method to other substituents.[8] However, it has recently been demonstrated, by our group[9] and independently by Cheng et al.,[10] that the diazonium coupling reaction with formaldehyde–methylamine solutions is more complex and can afford mixtures of the (hydroxymethyl)triazenes and the previously unreported *N,N*-bis(triazenylmethyl)methylamines (**18**).

In this paper we report a full account of the synthesis and characterization of (hydroxymethyl)triazenes, with –M substituents in the aryl group, together with a study of the chemistry and pharmacology of these important molecules.

Results and Discussion

Synthesis and Mechanism. When an aqueous solution of an arenediazonium chloride, with a –M substituent in the para position, is treated with a mixture of aqueous methylamine and formaldehyde, the latter being in excess, a high yield of 1-aryl-3-(hydroxymethyl)-3-methyltriazenes (**4–8**) is obtained. These (hydroxymethyl)triazenes are



no.	X	R_1	R_2
2	Ac	Me	Me
3	CO_2Me	Me	Me
4	Ac	Me	CH_2OH
5	CO_2Me	Me	CH_2OH
6	CO_2Et	Me	CH_2OH
7	CN	Me	CH_2OH
8	$CONH_2$	Me	CH_2OH
9	CO_2Me	Me	CH_2OCOPh
10	CO_2Me	Et	CH_2OH
11	CN	Et	CH_2OH
12	Ac	Me	H
13	CO_2Me	Me	H
14	CN	Me	H
15	Ac	Et	H
16	CO_2Me	Et	H
17	CN	Et	H

stable, crystalline substances, which recrystallize unchanged from organic solvents and have sharp melting points. The optimum molar ratio of formaldehyde–methylamine for the reaction with these diazonium salts is

Table I. 1-Aryl-3-(hydroxymethyl)-3-alkyltriazenes[a]

no.	yield, %	mp, °C	solvent	IR ν_{max},[b] cm^{-1}	^1H NMR chem shift, ppm: solvent	arom AA'BB	CH_2[c]	OH[c]	NR	X
4	28	117–120	benzene	3342 (OH), 1650 (C=O)	$(CD_3)_2SO$	7.47–7.98	5.16 (d)	6.40 (t)	3.18 (s)	2.56 (s, MeC=O)
5	85	112–114	benzene	3440 (OH), 1690 (C=O)	$(CD_3)_2SO$	7.5–8.0	5.15 (d)	6.39 (t)	3.17 (s)	3.84 (s, OMe)
					$CDCl_3$	7.35–8.1	5.20 (d)	3.0 (br)	3.28 (s)	3.88 (s, OMe)
6	91	73–75	hexane	3450 (OH), 1690 (C=O)	$(CD_3)_2SO$	7.6–8.1	5.20 (d)	6.40 (t)	3.20 (s)	1.3 (t, 3 H), 4.33 (q, 2 H)
					$CDCl_3$	7.4–8.2	5.30 (d)		3.35 (s)	1.4 (t, 3 H), 4.4 (q, 2 H)
7	100	114	ether/MeOH	3420 (OH), 2230 (C≡N)	$(CD_3)_2SO$	7.4–8.0	5.17 (d)	6.43 (t)	3.20 (s)	
8	90	141–142	ethanol	3445 (OH), 3300, 3180 (NH_2), 1640 (C=O)	$(CD_3)_2SO$	7.3–8.0	5.16 (d)	6.35 (t)	3.16 (s)	7.3 (br s, NH_2)
10	56	101–103	benzene/pet. ether	3400 (OH), 1690 (C=O)	$CDCl_3$	7.3–8.15	5.20 (d)		1.3 (t, 3 H),[d] 3.85 (q, 2 H)[d]	3.88 (s, OMe)
11	70	71–73	hexane	3445 (OH), 2240 (C≡N)	$CDCl_3$	7.5–7.6	5.20 (d)		1.33 (t, 3 H),[d] 3.90 (q, 2 H)[d]	

[a] Analytical results were within ±0.040% of theoretical values for all elements (C, H, and N). [b] As Nujol mulls. [c] J = 6.5 Hz. [d] J = 7.0 Hz.

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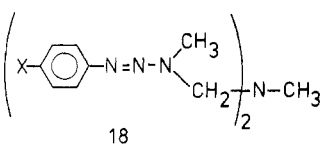
10:1; the yield is also enhanced when the formaldehyde–methylamine mixture is in excess over the diazonium salt such that the methylamine–diazonium salt ratio is 3:1. Maximum precipitation of the (hydroxymethyl)triazene is achieved by neutralization of the diazonium salt–formaldehyde–methylamine mixture with saturated sodium bicarbonate solution. The synthesis is extendable to the 3-ethyl homologues (**10** and **11**); the yields and physical data of these triazenes are listed in Table I.

Application of this synthesis to (hydroxymethyl)triazenes with other than –M groups (e.g., CH_3 or halogen) in the aryl moiety is more complicated. Reaction of the *p*-chloro- and *p*-bromobenzenediazonium salts with a 10:1 formaldehyde–methylamine mixture affords mainly the *N,N*-bis(triazenylmethyl)methylamine (**18**, X = Cl or Br); the (hydroxymethyl)triazene [*p*-Br-C_6H_4-N=NN(CH_3)-CH_2OH] has been obtained from the diazonium coupling reaction with a 60:1 formaldehyde–methylamine mixture. A preliminary account of the latter results has been reported;[9] the formation of the bis(triazene) (**18**) has been observed in an independent study by Cheng et al.[10]



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The formation of the (hydroxymethyl)triazene and bis(triazene) from the same reactants suggests a degree of complexity of the equilibria present in formaldehyde–methylamine mixtures not previously encountered. Nucleophilic addition of the amine to the carbonyl group affords zwitterions, which readily tautomerize to the "carbinolamines". The latter are not often stable enough to be isolated and dehydrate readily:

$$RNH_2 + CH_2O \rightleftharpoons [RN^+H_2CH_2O^-] \rightleftharpoons RNHCH_2OH \rightleftharpoons RN{=}CH_2 + H_2O$$

The most likely pathway for (hydroxymethyl)triazene formation is diazo coupling to the carbinolamine:

$$ArN_2^+ + RNHCH_2OH \rightarrow ArN{=}NN(R)CH_2OH + H^+$$

Further reaction of the Schiff base ($RN{=}CH_2$) with alkylamine results in the formation of a *gem*-diamine, and a continuation of this "polymerization" leads to the tris(alkylamino)bis(methylene) compound:

$$RN{=}CH_2 + RNH_2 \rightarrow RNHCH_2NHR$$

$$RNHCH_2NHR + CH_2{=}NR \rightarrow RNHCH_2N(R)CH_2NHR$$

Diazo coupling to the latter species is a plausible route to the bis(triazene) (**18**). These complex reactions have now been studied in more detail.[11]

Characterization. A characteristic feature of the (hydroxymethyl)triazenes is the intense hydroxyl group absorption at ca. 3400–3450 $cm.^{-1}$ in the IR spectra; the OH bands are significantly broadened as expected of a hydrogen-bonded group. The IR spectra also exhibit the predicted bands for the functional groups in the X moiety, e.g., cyano or carbonyl (Table I).

Unequivocal corroboration of the (hydroxymethyl)triazene structure is evident in the ^1H NMR spectra of these products (Table I). Spectra recorded in deuteriochloroform solution show the signals of the aromatic AA′BB′ system, the *N*-alkyl group, and the protons (if any) in the X group. The *N*-methylene signal from the CH_2OH group is frequently (but not always) seen as a doublet, presumably due to vicinal coupling to the OH proton; however, the OH signal is either extremely broad or not seen at all in the $CDCl_3$ spectra. This problem was not encountered when Me_2SO-d_6 was used as the solvent; the methylene doublet (5.2 ppm, J = 6.5 Hz) and the hydroxyl triplet (6.4 ppm, J = 6.5 Hz) are always clearly evident. Addition of D_2O to the Me_2SO-d_6 solution causes the disappearance of the OH triplet, the collapse of the methylene doublet to a singlet, and the appearance of the HOD signal.

Table II. Mass Spectroscopic Data of 1-Aryl-3-(hydroxymethyl)triazenes

no.	mass spectrum, m/e (relative intensity)
5	223 (M^+, 2), 193 (M – CH_2O, 8), 165 (15), 163 (MeO_2C-C_6H_4-N_2^+, 23), 151 (100), 135 (84), 120 (100)
10	237 (M^+, 2), 207 (M – CH_2O, 11), 179 (20), 164 (52), 163 (MeO_2C-C_6H_4-N_2^+, 15), 151 (MeO_2C-C_6H_4-$NH_2^{+\cdot}$, 100), 135 (42), 120 (100)
8	208 (M^+, 3), 178 (M – CH_2O, 60), 150 (96), 148 (NH_2CO-C_6H_4-N_2^+, 99), 136 (H_2NCO-C_6H_4-$NH_2^{+\cdot}$, 100), 134 (100)
4	207 (M^+, 3), 177 (M – CH_2O, 10), 149 (14), 147 (Ac-C_6H_4-N_2^+, 29), 135 (Ac-C_6H_4-$NH_2^{+\cdot}$, 100), 134 (36), 120 (100)

The structure of the 1-[*p*-(methoxycarbonyl)phenyl]-3-(hydroxymethyl)triazene (**5**) was confirmed by the ^{13}C NMR spectrum. The carbon chemical shifts and respective assignments are as follows: 162.33 (C=O), 150.3, 126.6, 122.7, and 116.8 (aromatics), 74.57 (OCH_3), 48.24 (CH_2), 29.41 (NCH_3) ppm. Further proof of the structure of **5** was evident from the formation of a crystalline benzoate ester (**9**) by reaction with benzoyl chloride in pyridine; **9** was characterized by the absence of OH absorption and an ester carbonyl band at 1710 cm^{-1} in the IR spectrum. The NMR and mass spectra confirmed the structure of **9**.

The mass spectra of the (hydroxymethyl)triazenes (**4**, **5**, **8**, and **10**) (Table II) are consistent with the assigned structures. All show weak molecular ions and significant fragments at M – 30, indicating loss of formaldehyde, and M–$RNCH_2OH$, corresponding to the aryldiazonium fragment. The appearance of abundant arylamine radical ion peaks in the mass spectra of the (hydroxymethyl)triazenes is significant, since only those triazenes that can fragment to monoalkyltriazenes normally afford such ions.[12] Indeed, the mass spectrum of **5** from m/e 193 and below is remarkably similar to that of the analogous monomethyltriazene (**13**). The mass spectrum of the benzoate **9** is quite different from its precursor (hydroxymethyl)triazene (**5**); **9** behaves like a dialkyltriazene and undergoes a similar fragmentation to the dimethyltriazene (**3**). A principle fragmentation of both compounds is at the N2–N3 bond to give the diazonium ion, m/e 163, whereas the fragment at m/e 151 (the radical ion $MeO_2CC_6H_4NH_2^+\cdot$) is relatively weak in the spectrum of **9** and absent in the spectrum of the dimethyltriazene.

Degradation and Chemical Stability. The correlation of structure and activity between the monoalkyltriazenes and the (hydroxymethyl)alkyltriazenes (see later) led us to examine and compare the stability of these molecules in solution (Table III). Half-lives were measured either by the disappearance of substrate by HPLC or by meas-

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Table III. Half-life Determinations of Arylalkyltriazenes

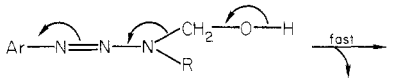
no.	method	UV λ_{max}, nm	buffer[a]	pH	temp, °C	$t_{1/2}$, min
2	UV	337	P	2.5	37	1.23
	UV	337	P	3.5	37	11.8
	UV	337	P	4.5	37	107.7
	UV	337	P	5.2	37	>24 h
	UV	337	P	7.4	37	>24 h
3	UV	328	P	7.5	25	>24 h
12	UV	325	P	7.4	37	13.8
	HPLC	325	P	7.4	37	15.1
	HPLC	325	tris	7.4	37	36.2
	HPLC	325	Earl's	7.4	37	66.6
15	HPLC	325	Earl's	7.4	37	15.1
4	UV	325	P	7.4	37	14.5
13	UV	313	P	7.4	37	12.1
	UV	313	P	7.5	25	35.0
16	UV	312	P	7.5	25	9.0
5	UV	313	P	7.4	37	12.60
	UV	313	P	7.5	25	22
10	UV	315	P	7.5	25	7
14	HPLC	300	Earl's	7.4	37	99.0
17	HPLC	300	Earl's	7.4	37	18.3
9	UV	302	P	7.5	25	26.5

[a] P = phosphate buffer.

uring the change of absorbance in the UV spectrum with time.

Arylmethyltriazenes have been shown to decompose in aqueous solutions at physiological pH in a way that can be described by first-order kinetics.[13] The half-life of a methyltriazene (**12**) in phosphate buffer at pH 7.4 was similar whether determined by UV spectroscopy (13.81 min) or HPLC (15.10 min), but it was very different in buffers of different composition but identical pH (7.4) (15.10 min in phosphate, 36.20 min in Tris, and 66.60 min in Earl's buffer) as determined by HPLC. The decomposition of arylmethyltriazenes has been previously shown to exhibit a marked dependence on the composition of the medium, at least in aprotic solvents.[14] The stability of a methyltriazene (**13**) in phosphate buffer was predictably found to be temperature dependent (12.1 min at 37 °C compared with 35 min at 25 °C). The half-lives of all the derivatives studied here are significantly longer than the half-life of 1-phenyl-3-methyltriazene (PhN=NNHMe), which was found to be 3.5 min at 37 °C in pH 7.4 buffer.[15]

There is very little difference between the half-lives of corresponding methyl and (hydroxymethyl)triazenes (e.g.: **12**, 13.8 min; **4**, 14.5 min; **13**, 12.1 min; **5**, 12.6 min); also, the λ_{max} of corresponding derivatives was identical. Thus, it appears that, under these conditions, the initial step in the decomposition of a (hydroxymethyl)triazene is rapid loss of formaldehyde and that the rate-determining step is the decomposition of the alkyltriazene, as follows:

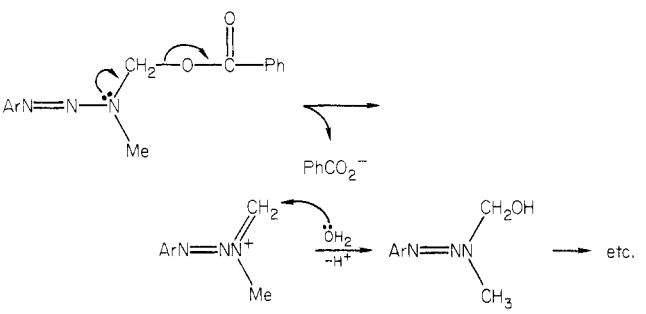


$$ArHNN{=}NR \xrightarrow[H_2O,\ -N_2]{\text{slow}} ArNH_2 + ROH$$

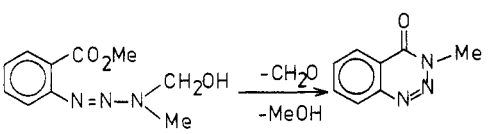
The product of decomposition of these methyl- and (hydroxymethyl)triazenes was, in each case, shown to be the corresponding arylamine, i.e., *p*-aminoacetophenone or methyl *p*-aminobenzoate, by TLC analysis.

A further structural correlation is seen when the half-lives of corresponding methyl- and ethyltriazenes are compared. Methyltriazenes have half-lives 4–5 times as long as the corresponding ethyltriazenes whether measured in phosphate or Earl's buffer. Similarly, the 3-(hydroxymethyl)-3-methyltriazene (**5**) has a half-life of 22 min (phosphate, pH 7.5, 25 °C) compared to 7 min for the 3-(hydroxymethyl)-3-ethyltriazene (**10**).

Decomposition of the benzoate ester (**9**), which also ultimately forms methyl *p*-aminobenzoate, occurs at a rate faster ($t_{1/2}$ = 26.5 min) than can be explained simply by invoking an initial hydrolysis of the benzoate group. In this case we propose an alternative route via an iminium intermediate similar to that suggested to explain the anomalous stability of some esters of structurally related (acetoxymethyl)nitrosamines.[16]



The tendency for the (hydroxymethyl)triazene toward spontaneous loss of formaldehyde seems to be enhanced by location of the X substituent in the ortho position. Attempted synthesis of the ortho isomer (**19**) of **5** from



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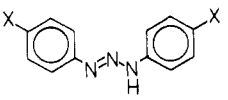
diazotized methyl anthranilate led only to the formation of the triazinone (**20**), presumably by cyclization of a methyltriazene liberated by decomposition of the precursor (hydroxymethyl)triazene.[7]

The development of a TLC system for analysis of methyl- and (hydroxymethyl)triazenes was thwarted by the instability of these compounds. Samples of the methyltriazene (**12**) decomposed on silica plates to give three spots on the chromatogram: *p*-aminoacetophenone, the diaryltriazene (**21**, X = Ac), and another spot, which is



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probably the methyltriazene itself. The sample of the methyltriazene used in this experiment gave only one peak on HPLC. Decomposition of the same methyltriazene in phosphate buffer yielded only the arylamine, with no evidence of formation of the diaryltriazene, which is proposed, therefore, to be the product of a diazo-migration reaction,[17] catalyzed by the slightly acidic silica gel. The

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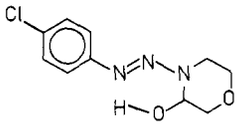
(hydroxymethyl)triazenes are always seen as a streak on the TLC plate, indicating breakdown during the development of the chromatogram. Julliard et al.[8] have claimed to obtain discrete spots with their TLC system to check the purity of (hydroxymethyl)triazenes; however, this suggestion is clearly erroneous, sine their products have now been shown to be bis(triazene)s (18) and not (hydroxymethyl)triazenes.[9,10] Similar decomposition problems have been encountered with HPLC assays of (hydroxymethyl)triazenes, even using reverse-phase columns; in this case, the polarity of the solvent system may be the cause of decomposition. In one case, the 3-(hydroxymethyl)-3-ethyltriazene [p-EtO_2C-C_6H_4-N=NN(C_2H_5)CH_2OH] decomposed in the solid state on prolonged storage and also gave the diazo-migration product (21, X = CO_2Et).

Molecular Structure. An important question concerning (hydroxymethyl)triazenes is their molecular structure, in particular, the geometry around the N=N bond and the juxtaposition of the hydroxymethyl group. Intramolecular H bonding may be responsible for stabilization of the 2-hydroxymorpholinotriazene (22), which



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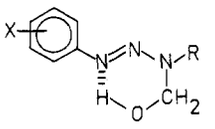

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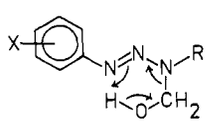
is formed from the morpholinotriazene either by permanganate oxidation or by a metabolic process after intraperitoneal (ip) injection in mice.[18] Similar H bonding in N-(hydroxymethyl)triazenes (23) could inhibit the facile elimination of formaldehyde characteristic of some unstable carbinolamines.[19] Alternatively, one might argue that the lone pair of sp^2 electrons on the terminal azo nitrogen could initiate the elimination by a neighboring group effect (24). The balance between stabilization and elimination



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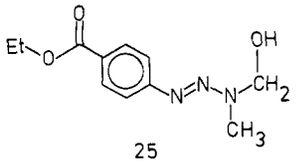

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should then be finely tunable by modification of the aryl substituent X: electron-donating substituents should then encourage the elimination process, whereas electron-withdrawing substituents should stabilize the carbinolamine. Perhaps significantly, the moxt active aryldimethyltriazenes (against the TLX5 lymphoma) seem to be those derivatives with –M substituents in the aryl group,[3,4] and these are the very ones that form the most stable N-hydroxymethyl derivatives.

Surprisingly, a crystal structure determination[20] of the 3-(hydroxymethyl)-3-methyltriazene (6) shows that it exists as the rotamer (25) with the N-hydroxymethyl group in a trans arrangement with respect to the azo linkage.

Table IV. Antitumor Activity of the 1-Aryl-3-(hydroxymethyl)triazenes and Analogues against the TLX5 Lymphoma in Vivo

no.	tumor	% ILS (T/C)	opt dose, mg kg^{-1} (5 times daily)
2	TLX5S	65	20
2	TLX5R	0	20
3[a]	TLX5S	58	40
3[a]	TLX5R	0	40
4	TLX5S	72	20
5	TLX5S	120	5
5	TLX5R	0	5
7	TLX5S	54	160
8	TLX5S	62	20
9	TLX5S	31	20
11	TLX5S	0	160
13[a]	TLX5S	87	5
13[a]	TLX5R	4	20

[a] See ref 4.

Within the unit cell, the hydroxy group is intermolecularly H bonded to the ester carbonyl group, and there are strong stacking attractions between the benzene rings.

Antitumor Activity. (Hydroxymethyl)triazenes have pronounced activity against the TLX5(S) lymphoma in vivo (Table IV); the activity of the (hydroxymethyl)triazenes (4, 5, 7, and 8) is at least comparable to the dimethyltriazenes (2 and 3), which are active on the TLX5S tumor but not the TLX5R. The most active (hydroxymethyl)triazene of this series is 5, which gives a maximum increase in survival time of 120% for five daily doses of 5 mg/kg in mice inoculated with 10^5 TLX5 lymphoma cells and is superior to the analogous methyl- (13) and dimethyltriazene (3) (87 and 58%, respectively). The antitumor activity of the 3-(hydroxymethyl)-3-methyltriazenes (4, 5, 7, and 8) is not shared by the 3-ethyl-3-(hydroxymethyl)triazene (11). These results suggest that the (hydroxymethyl)triazenes may exert their in vivo antitumor effect via the decomposition product, the methyltriazene; the 3-ethyl derivative is inactive, presumably because it decomposes to a ethyltriazene. Previous studies had suggested that if a group was removed from the 3-position by metabolism (or, as would be the case here, by chemical decomposition) to yield a species other than a methyltriazene, then antineoplastic activity was lost.[3]

The in vivo activity of the (hydroxymethyl)triazene (5) against the sensitive line of the TLX5 lymphoma but not against the resistant line (Table IV) is a result similar to that obtained with the methyltriazene (13).[4] The result is suggestive evidence that 5 exerts its activity via 13, and, indeed, the results of in vitro–in vivo bioassays also suggest this. Thus, in a comparison of 5 with 13 against the resistant TLX5 in a bioassy, equivalent toxicity was observed (results not shown). If the (hydroxymethyl)triazene was a selective antitumor species, it would be expected that the TLX5 resistant line would show a degree of resistance to it.

Although the role of the (hydroxymethyl)triazenes as the selective species responsible for the antitumor activity of dimethyltriazenes has been called into question by these results, it is still possible that these compounds or conjugates of them may be circulating metabolites of the dimethyl compounds. Attempts to quantify circulating (hydroxymethyl)triazenes in the plasma of mice given dimethyltriazenes have so far been unsuccessful.

The benzoate derivative (9) of 5 has low antitumor activity (% ILS = 31 at 20 mg/kg). Evidently, the benzoate is not an effective transport form or prodrug modification of the (hydroxymethyl)triazene. At best it appears to be

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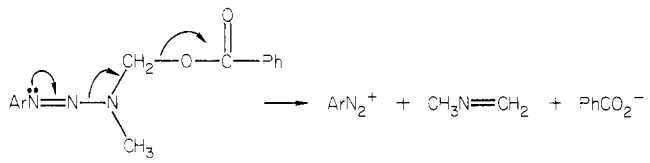
a slightly active dialkyltriazene that may be undergoing preferential demethylation to give the inactive alkyltriazene ($ArN{=}NNHCH_2OCOPh$). An alternative explanation for the relative inactivity of **9** could be a facility to fragment to the diazonium ion, the driving force being the formation of the benzoate ion:



Whatever the explanation is, the inactivity of the benzoate (**9**) is paralleled by the inactivity of the glucuronide conjugate of the urinary metabolite of 1-(2,4,6-trichlorophenyl)-3,3-dimethyltriazene.[6,22]

The results of in vitro experiments clearly show that the (hydroxymethyl)triazenes are "activated" triazenes that inhibit tumor cell growth without metabolic activation unlike the dimethyltriazene counterparts; the latter show no in vitro activity. This behavior is also shown in a study of the inhibition of M21 melanoma cell growth by triazenes. The (hydroxymethyl)- and methyltriazenes inhibit cell growth in culture, whereas the dimethyltriazenes are inactive in this assay.[23]

A similar oxidative activation process has been proposed[24] to explain the carcinogenic and mutagenic activity of dimethylnitrosamines, which are nonmutagenic without prior metabolic activation. The (hydroxymethyl)triazene is a structural analogue of the (hydroxymethyl)nitrosamine, which can be isolated only as the (acetoxymethyl)nitrosamine derivative.[25] The close parallel between the chemical and biological properties of dimethylnitrosamine and aryldimethyltriazenes is further emphasized by the strong mutagenic activity of the (hydroxymethyl)triazenes **4** and **5** towards *Salmonella typhimurium* TA 100 and TA 1535;[26] the corresponding dimethyltriazenes are nonmutagenic unless activated by liver fractions.

Experimental Section

Infrared spectra were recorded on a Perkin-Elmer 299 spectrophotometer with Nujol mulls. NMR spectra were obtained on a Varian EM360 spectrometer with Me_4Si as internal standard, and ultraviolet spectra were recorded on Unicam SP-8005 and Cary 219 spectrophotometers. Mass spectra were recorded on a VG Micromass 12B spectrometer. Microanalyses were performed by the Canadian Microanalytical Laboratory, Vancouver, British Columbia, and by the Butterworth Microanalytical Consultancy, Teddington, United Kingdom. Melting points were recorded on a Koffler hot stage apparatus calibrated with Fisher Thermetric standard samples.

1-Aryl-3-(hydroxymethyl)-3-methyltriazenes (4–8). General Procedure. A solution of the arylamine (0.033 mol) in concentrated hydrochloric acid (10 mL), diluted with water (65 mL), was diazotized at 0 °C with sodium nitrite (2.5 g) in water (20 mL) over a period of 1 h or until a clear diazonium salt solution was obtained. A mixture of 40% aqueous methylamine (7.5 mL) and 40% aqueous formaldehyde (75 mL) was prepared with cooling at 0 °C, and this mixture added slowly to the diazonium salt solution. The mixture was then neutralized carefully with saturated sodium bicarbonate solution and stirred until precipitation appeared complete (ca. 1 h). The product was filtered, dried under suction, and recrystallized from an appropriate solvent to afford the 1-aryl-3-(hydroxymethyl)-3-methyltriazene. Yields and physical data are given in Table I.

1-Aryl-3-(hydroxymethyl)-3-ethyltriazenes (10 and 11). The 1-aryl-3-(hydroxymethyl)-3-ethyltriazenes were prepared with an equimolar amount of 70% aqueous ethylamine instead of 40% aqueous methylamine (Table I).

[1-[*p*-(Methoxycarbonyl)phenyl]-3-methyltriazen-3-yl]-methyl Benzoate (9). 1-[*p*-(Methoxycarbonyl)phenyl]-3-(hydroxymethyl)-3-methyltriazene (**5**; 3.0 g) was dissolved in redistilled pyridine (12.0 mL) at room temperature. The solution was stirred vigorously and cooled in ice, while freshly distilled benzoyl chloride (2.0 g) was added dropwise over 15 min. The resulting mixture was filtered, and the filtrate was poured over an ice–water mixture (25 g). The product appeared at this stage as a white precipitate, which was separated by suction filtration, dried in a dessicator, and recrystallized from petroleum ether to afford the benzoate (**9**): yield 3.2 g (73%); mp 88–89 °C (petroleum ether); IR ν_{max} (Nujol) 1710 and 1695 (C=O) cm^{-1}; NMR ($CDCl_3$) δ 3.91 (s, 3 H, O-Me), 3.34 (s, 3 H, N-Me), 6.09 (s, 2 H, CH_2), 7.4–8.2 (m, 9 H, aromatic); mass spectrum, *m/e* 327 (8) (M^+), 296 (5) (M-OMe), 268 (2) (M-CO_2Me), 224 (2), 193 (1), 163 (54) (MeO_2C-C_6H_4-N_2^+), 151 (15), 135 (96), 122 (100), 120 (38), 105 (100), 92 (22).

3-Methyl-1,2,3-benzotriazin-4(3*H*)-one (20). Diazotization of methyl anthranilate, followed by reaction with aqueous methylamine–formaldehyde mixture at 0 °C and then basification to pH 9 with saturated $KHCO_3$, afforded a yellow oil, which was isolated by ether extraction. After standing for 3 weeks, a crystalline product grew in the oil; the product (32% yield) was separated by filtration and was identical (mp and IR) spectrum) with an authentic sample of 3-methyl-1,2,3-benzotriazin-4(3*H*)-one (**20**).[27]

Half-life Determinations. (a) Spectroscopic scale decomposition of samples kept in the dark in the spectrometer thermostated at 37 °C or at ambient temperature (25 °C) was monitored by scans taken at time intervals determined by a Unicam SP 8005 program controller operating in the repeat scan mode. The decrease in absorbance with time was measured at the λ_{max} of the compound under study, and the half-life was calculated from the slope of a logarithmic plot of the extent of decomposition on the ordinate against time on the abscissa.

(b) For half-life measurement by HPLC, the triazenes were incubated at a concentration of 100 $\mu g\ mL^{-1}$ in closed Universal containers at 37 °C. Samples were taken at intervals and added to an equal volume of ice-cold acetone to prevent further decomposition. The decrease in triazene concentration with time as assayed by HPLC analysis on a Cyano 10-μm radialpak liquid chromatography cartridge (Waters). Chromatography was performed with an Altex 100A pump in conjunction with a Pye-Unicam variable-wavelength LC–UV detector; the mobile phase was 30% aqueous acetonitrile with 0.1% diethylamine added, and the flow rate was 2 mL/min. The UV detector was set at the maximum absorption wavelength of the triazene being studied (see Table III).

Buffer Solutions. (a) Phosphate (Sorensen's) Buffer. Potassium dihydrogen phosphate (9.073 g L^{-1}) and disodium hydrogen phosphate dihydrate (11.87 g L^{-1}) solutions were mixed in the appropriate proportions to give the desired pH.

(b) Earl's Buffer: Sodium chloride (6.80 g), sodium bicarbonate (2.20 g), glucose (1.00 g), potassium chloride (0.40 g), sodium dihydrogen phosphate dihydrate (0.14 g), and distilled water (to 1 L). The buffer was adjusted to pH 7.4 by the addition of hydrochloric acid.

(c) Tris Buffer (0.01 M): Trizma base (1.21 g) and distilled water (to 1 L). The buffer was adjusted to pH 7.4 by the addition of hydrochloric acid.

Tumor Passage. The TLX5S lymphoma was passaged at 7-day intervals by intraperitoneal injection of approximately 2 × 10^5 cells into 20-g male CBA/LAC mice. The TLX5R lym-

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(27) LeBlanc, R. J.; Vaughan, K. *Can. J. Chem.* **1972**, *50*, 2544.

phoma was passaged in the same way; previously, this tumor had been made resistant to ethyl 5-(3,3-dimethyltriazen-1-yl)-2-phenylimidazole-4-carboxylate and was found to be resistant to the optimum antitumor dose of the dimethyltriazenes used in subsequent studies.[4] Cells were counted with a Model ZBI Coulter counter.

Antitumor Test. Approximately 2×10^5 lymphoma cells were injected subcutaneously in the inguinal region of 20-g female CBA/LAC mice. After 2 days, drugs were administered daily for 5 days by intraperitoneal injection of 0.1 mL of a solution or suspension of drug made by sonication in either 10% acetone–arachis oil or 10% dimethyl sulfoxide–arachis oil. The day of death of the animals, which were in groups of five, was recorded, and the survival time of treated animals was compared with untreated controls. Survival time was shown to be proportional to the number of cells injected.[21]

Bioassay. In vitro–in vivo bioassays were performed as described previously.[21]

Acknowledgment. The authors are grateful to the following agencies for financial support. The Cancer Research Campaign (United Kingdom), The Natural Sciences and Engineering Research Council of Canada, and the Saint Mary's University Senate Research Comittee. The carbon-13 spectrum was provided by the Atlantic Regional Nuclear Magnetic Resonance Centre at Dalhousie University, Halifax, Nova Scotia.

Registry No. **2**, 52416-18-9; **3**, 29168-87-4; **4**, 70346-60-0; **5**, 69467-93-2; **6**, 70303-37-6; **7**, 73779-16-5; **8**, 70346-61-1; **9**, 70346-62-2; **10**, 88392-04-5; **11**, 82647-12-9; **12**, 51029-21-1; **13**, 40643-38-7; **14**, 51029-20-0; **15**, 82647-08-3; **16**, 79868-88-5; **17**, 82647-11-8; **20**, 22305-44-8; methyl anthranilate, 134-20-3; 4-acetylanaline, 99-92-3; methyl 4-aminobenzoate, 619-45-4; ethyl 4-aminobenzoate, 94-09-7; 4-cyanoanaline, 873-74-5; 4-aminobenzamide, 2835-68-9.

Potential Antitumor Agents. 40. Orally Active 4,5-Disubstituted Derivatives of Amsacrine

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The DNA-intercalating agent amsacrine is an effective drug for the treatment of human leukemias and lymphomas but has minimal solid tumor activity. As a first step in identifying analogues with a wider spectrum of activity, a comparison was made of the in vivo antileukemic (P-388) activity of amsacrine analogues given by oral (po) and intraperitoneal (ip) routes. A series of 4-substituted and 4,5-disubstituted derivatives all showed high activity when administered ip against ip-implanted P-388, but activity varied widely when the compounds were given orally. 4-Methoxy and 4-carbamoyl derivatives proved essentially inactive, whereas 4-methyl and 4-methylcarbamoyl derivatives retained activity. Exceptional oral activity was shown by the 4-methyl-5-methylcarbamoyl derivative, making this amsacrine derivative worthy of further testing.

The DNA-intercalating agent amsacrine [*m*-AMSA, 4′-(9-acridinylamino)methanesulfon-*m*-anisidide, **1**] has proved an effective clinical drug for the treatment of various disseminated tumors, especially acute leukemia,[1,2] and certain lymphomas.[3] However, many clinical trials have shown it to be ineffective or only marginally effective against a wide range of solid tumors in man.[4,5]

Amsacrine is a member of the 9-anilinoacridine series of drugs. A very large number of these now exist,[6] and efficient and flexible synthetic routes have been devised[7,8] that make a much larger number of potential structures easily accessible. Thus, an important goal of current research is to identify analogues of amsacrine that possess enhanced activity against solid tumors. A severe limitation to the effective drug treatment of solid tumors, and one of the principal reasons why many cationic drugs of the DNA-intercalating type are relatively inactive against such tumors notwithstanding high activity against leukemia, is the difficulty of ensuring adequate drug distribution into the solid tumor structure. One approach to selective derivatives of amsacrine with better distribution properties, therefore, is to determine the structural features associated with these properties in biological systems. In earlier work addressing this question for analogues of amsacrine, we employed the L1210 leukemia in vivo, comparing the antitumor effectiveness of compounds when given intraperitoneally (ip) against ip, sc (subcutaneous), and ic (intracerebral) implanted tumors.[9] This system proved to be a severe test for amsacrine derivatives, with many of the ip active compounds proving inactive against sc tumor and only a very few showing activity against ic tumor. This study showed that drugs active against the remotely implanted tumors were more lipophilic than the optimum value for ip activity. A useful structural feature was found to be a 4-CH_3 or 4-OCH_3 group, which greatly enhanced drug distribution to remote sites. In particular, the 4-CH_3 and 4,5-$(CH_3)_2$ derivatives (**2** and **6**; Table I) showed a significant activity against both sc and ic tumors.[9]

A further indication of favorable drug distribution properties is the ability to show activity following oral (po) administration. In addition, antitumor drugs that are effective when given orally are much simpler to administer, so that such a property represents a real clinical advantage. While many uncharged antitumor drugs of the antimetabolite (e.g., 6-mercaptopurine) and alkylating agent (e.g., cyclophosphamide and melphalan) classes are effective when given orally, the DNA-binding agents (usually cationic drugs) are not. In order to have activity when given orally an antitumor drug must be capable of being effi-

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