diaminoacridine (17), was prepared according to the method of Albert and Gledhill.[11] The diazotization of 17 was effected by the same procedure given above for the preparation of 16: yield 440 mg (78%); homogenous by TLC (benzene–methanol, 5:2); IR (KBr) 2100 cm^{-1}. The fluorescence of 18 in aqueous solution is pale blue. When irradiated for 2 min with UV light, the fluorescence changed to bright yellow–green.

Biological Procedures. *Trypanosoma brucei* (EATRO 110) was kindly provided by Dr. M. Rifkin at Rockefeller University. The parasites were propagated in young adult male CD-1 Swiss mice (Charles River Co.) by intraperitoneal injections of 0.1 mL of whole blood containing about 1×10^7 parasites/mL. Larger quantities of parasites were obtained by injecting heavily parasitized mouse blood intraperitoneally into Long Evans rats followed by cardiac puncture in 3 days. Stocks of infected heparinized blood were stored at -70 °C in the presence of 12% glycerol. *T. brucei* was separated from blood cells by passage through a DEAE-cellulose (DE52 Whatman) column with Tris-buffered saline–glucose (TSG) (I = 0.129) as described by Lanham and Godfrey.[21] The parasites were pelleted by centrifugation at 1000g for 10 min and washed twice with TSG buffer. Survival following injection was scored for each group, and the mean of the number of days of survival was determined for each parasite dose.

Drug screening for trypanocidal activity was performed as described previously.[22] Parasites at a concentration of 5×10^6/mL in TSG and aliquots of drug diluted in TSG were added to obtain the appropriate final concentration. Each drug, whether photoreactive or not, was tested both with and without light activation. Light activation was carried out for 10 min in 9 × 50 mm plastic petri dishes (Falcon), using a GE 30W fluorescent lamp placed 4 cm above the surface of the sample. All nonphotoactivated (dark) experiments were carried out in the dark, with a red photographic safelight until after the mice were injected with parasites. Preirradiation conditions included a 1-h irradiation of the compound prior to its exposure to the parasites. The drug was mixed with parasites by gentle rotation for 10 min. Parasite viability was checked following all manipulations by monitoring motility. No differences in motility were noted between control and drug-treated parasites, and all preparations used for injection showed greater than 97% motility immediately prior to injection. The dose of parasites indicates the total number (motile plus nonmotile) of parasites injected. Mice were injected intraperitoneally with 0.1 mL containing 5×10^5 drug-treated parasites, and control groups of mice which received 5×10^5 untreated parasites were included for every parasite preparation. The numbers of surviving mice were scored each day over a period of 30 days. Trypanocidal activity is reported as the mean number of days of survival postinjection. The standard deviations are included, and the number of mice included in the determinations are given in parentheses (Tables II and III).

Acknowledgment. This investigation was supported by NIH Grant AI-17683 (L.W.Y.). We are indebted to Mary Burns for her help in preparing the manuscript.

Registry No. 1, 78276-17-2; 2, 17784-47-3; 3, 58658-11-0; 4, 89873-24-5; 4·HCl, 89873-25-6; 5, 20141-88-2; 6, 23043-62-1; 7, 78276-18-3; 8, 89873-26-7; 9, 78276-14-9; 10, 75586-70-8; 11, 78276-15-0; 12, 78276-10-5; 13, 21330-56-3; 14, 89873-27-8; 15, 75605-59-3; 16, 78276-12-7; 17, 951-80-4; 18, 78276-06-9; 9-chloroacridine, 1207-69-8; *p*-phenylenediamine, 106-50-3; 3-aminoacridine, 581-29-3; acriflavine, 86-40-8; proflavine, 92-62-6; 9-amino-1-nitroacridine, 21914-54-5; ledakrin, 6514-85-8; 9-amino-10-methylacridinium chloride, 5776-38-5; 1,9-diaminoacridine, 23043-60-9; berenil, 908-54-3; 3-amino-8-azido-5-ethyl-6-phenylphenanthridinium chloride, 65282-35-1; 3-amino-6-azidoacridine, 78276-16-1; 3,6-diazidoacridine, 57459-61-7.

Supplementary Material Available: Three tables containing the absorption characteristics and the ^1H NMR chemical shifts and coupling constants for the acridine compounds (6 pages). Ordering information is given on any current masthead page.

(21) Lanham, S. M.; Godfrey, D. G. *Exp. Parasitol.* **1970**, *28*, 521.
(22) Cox, B. A.; Firth, W. J.; Hickman, S.; Klotz, F. B.; Yielding, L. W.; Yielding, K. L. *J. Parasitol.* **1981**, *67*, 410.

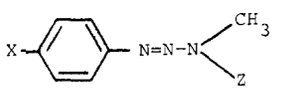
Tumor Inhibitory Triazenes.[1] 3. Dealkylation within an Homologous Series and Its Relation to Antitumor Activity

Derry E. V. Wilman,* Peter J. Cox,† Phyllis M. Goddard, Leigh I. Hart, Kanti Merai, and David R. Newell

Department of Biochemical Pharmacology, Institute of Cancer Research, Royal Cancer Hospital, Sutton, Surrey SM2 5PX, England. *Received October 5, 1983*

The in vivo antitumor activity and in vitro metabolic dealkylation have been measured for an homologous series of 3-alkyl-1-(4-carbamoylphenyl)-3-methyltriazenes and have been compared with their partition coefficients. This investigation has shown that the extent of oxidative metabolism in vitro and the antitumor activity in vivo of these compounds are dependent upon hydrophobicity. These findings provide confirmation for the relationship between metabolism and antitumor activity for aryldialkyltriazenes.

As part of our investigation of potential clinically less toxic second-generation analogues of the antitumor agent 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide (DTIC, Dacarbazine), we have recently reported[1] on an homologous series of 3-alkyl-1-(4-carboxyphenyl)-3-methyltriazenes (Ia). In this series there was a slight



Ia X = COOH

Ib X = $CONH_2$

improvement in antitumor activity against the TLX/5 lymphoma as the alkyl chain length increased from methyl to pentyl. At greater chain lengths, however, the activity rapidly diminished, the heptyl derivative being totally inactive. A number of possible reasons for this marked change in activity were suggested, and we now report on our investigations related to one of these.

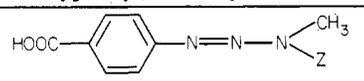
The antitumor activity of the dialkyltriazenes is thought to be dependent on oxidative metabolism.[2,3] We therefore set out to determine whether there is any alteration in the type or extent of metabolism that might account for the sudden change in activity referred to above. Unfortunately, from this point of view, the 1-(4-carboxyphenyl)-triazenes do not undergo measurable in vitro dealkylation,[4] an observation that is currently under investigation. We therefore turned our attention to a similar series of 3-al-

†Present address: Smith, Kline and French Research Ltd., The Frythe, Welwyn, Herts AL6 9AR, England.

(1) Previous paper in this series: D. E. V. Wilman and P. M. Goddard, *J. Med. Chem.*, **23**, 1052 (1980).
(2) T. A. Connors, P. M. Goddard, K. Merai, W. C. J. Ross, and D. E. V. Wilman, *Biochem. Pharmacol.*, **25**, 241 (1976).
(3) A. Gescher, J. A. Hickman, R. J. Simmonds, M. F. G. Stevens, and K. Vaughan, *Tetrahedron Lett.*, **50**, 5041 (1978).
(4) R. C. S. Audette, T. A. Connors, H. G. Mandel, K. Merai, and W. C. J. Ross, *Biochem. Pharmacol.*, **22**, 1855 (1973).

0022-2623/84/1827-0870$01.50/0

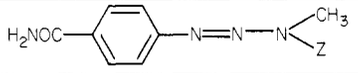
Table I. Physicochemical Data for the 3-Alkyl-1-(4-carboxyphenyl)-3-methyltriazenes



no.	Z	m^a	$R_m{}^b$	log P obsd^c	log P calcd^d
1	methyl	0	−0.094	−0.44	−0.29
2	ethyl	1	0.046	0.04	0.03
3	propyl	2	0.215	0.46	0.41
4	butyl	3	0.444	0.97	0.93
5	pentyl	4	0.672	1.49	1.44
6	hexyl	5	0.906	1.95	1.97
7	heptyl	6	1.084	2.58	2.37
8	octyl	7	1.347	2.77	2.96
9	dodecyl	11	2.147^e		4.77
10	octadecyl	17	3.403^e		7.60
11	isopropyl		0.222	0.34	0.42
12	2-methylbutyl		0.623	1.33	1.33
13	allyl		0.200	0.32	0.38
14	2-propynyl		−0.025	0.00	−0.13
15	benzyl		0.452		0.94

^{a} number of methylene groups in the alkyl chain: Z = $(CH_2)_mCH_3$. ^{b} Column conditions: 2.0 mL/min; 65% methanol/35% 0.37 M acetic acid (v/v). ^{c} From ref 1. ^{d} Calculated from eq 2. ^{e} Calculated from eq 1.

Table II. Physicochemical Data for the 3-Alkyl-1-(4-carbamoylphenyl)-3-methyltriazenes



no.	Z	m^a	$R_m{}^b$	log P obsd^c	log P calcd^d
16	methyl	0	−0.567	1.20	1.17
17	ethyl	1	−0.360	1.70	1.63
18	propyl	2	−0.147		2.09
19	butyl	3	0.083	2.46	2.60
20	pentyl	4	0.297		3.07
21	hexyl	5	0.534		3.59
22	heptyl	6	0.759		4.09
23	octyl	7	1.012	4.70	4.65
24	dodecyl	11	1.889^e		6.58
25	isopropyl		−0.182	2.00	2.02
26	*tert*-butyl		0.354		3.20
27	benzyl		0.527		3.58
28	2-hydroxyethyl		−0.979		0.26
29	hydroxy		0.062		2.55
30	methoxy		0.172		2.80
31	diethyl		0.404		3.31

^{a} Number of methylene groups in the alkyl chain: Z = $(CH_2)_mCH_3$. ^{b} Column conditions: 2.0 mL/min; 65% methanol/35% 0.05 M $HCOONH_4$, pH 6.3 (v/v). ^{c} From ref 11. ^{d} Calculated from eq 4. ^{e} Calculated from eq 3.

kyl-1-(4-carbamoylphenyl)-3-methyltriazenes (Ib), which are known to undergo dealkylation in vitro.[2] This series was tested for antitumor activity, and the relative extents of N-demethylation and other N-dealkylation were determined.

In a large number of structure–activity relationship studies, lipophilicity has been shown to be a major determinant of drug activity.[5,6] However, in view of the low aqueous solubility of the 1-(4-carbamoylphenyl)triazene derivatives, partition coefficients were not determined by the traditional method of direct measurement of the distribution of the solute between an octanol phase and an aqueous phase. Rather, they were calculated from retention data obtained on a reverse-phase high-performance liquid chromatography column (HPLC). In this system, hydrophobicity is measured by the value R_m. R_m was originally derived from reverse-phase paper and thin-layer chromatography, for which

$$R_m = \log\left[\frac{1}{R_f} - 1\right]$$

where R_f is the retention in the system.[7] It has since been applied to reverse-phase HPLC for several different compound types.[8-10] In HPLC, retention is described by the capacity factor k', such that

$$k' = \frac{V_R - V_0}{V_0}$$

where V_R is the elution volume of a retained compound, and V_0 is the elution volume of an unretained compound; thus, $R_m = \log k'$. The relationship between R_m and partition coefficient, P, is linear and of the form

$$\log P = aR_m + b$$

where a and b are constants.

Initially, we investigated the validity of this relationship in our series of 3-alkyl-1-(4-carboxyphenyl)-3-methyltriazenes (Ia), where the log P values were already known.[1] The partition coefficients for the 4-carbamoylphenyl series (Ib) were determined by HPLC with the values of log P published by Hatheway and co-workers[11] to form the initial relationship between log P and R_m. Finally, attempts were made to correlate the log P values obtained in this way with the in vivo antitumor activity and in vitro oxidative metabolism of the compounds.

Results and Discussion

Table I shows the values of R_m obtained for the 3-alkyl-1-(4-carboxyphenyl)-3-methyltriazenes (Ia). There is a good correlation between R_m and the number of methylene groups, m, in the alkyl chain, as shown in eq 1. In

$$R_m = 0.209\ (\pm\ 0.006)\ m - 0.155\ (\pm\ 0.025) \quad (1)$$
$$n = 8,\ r = 0.998,\ s = 0.039$$

view of this excellent correlation, the R_m values for compounds **9** and **10**, which were too hydrophobic to elute from the column under the conditions used, could be calculated (Table I).

Equation 2 was derived from the R_m and previously

$$\log P = 2.256\ (\pm\ 0.077)\ R_m - 0.076\ (\pm\ 0.050) \quad (2)$$
$$n = 12,\ r = 0.994,\ s = 0.117$$

determined log P values for the same series of compounds (Ia). Calculated values of log P from eq 2 are shown in Table I and demonstrate the reliability of this method for aryltriazenes.

Similar data for the series of 3-alkyl-1-(4-carbamoyl-

(5) C. Hansch in "Structure–Activity Relationships", Vol. 1, C. J. Cavallito, Ed., Pergamon Press, Oxford, 1973, p 75.
(6) A. K. Saxena and S. Ram, *Prog. Drug Res.*, **23**, 199 (1979).
(7) E. C. Bate-Smith and R. G. Westall, *Biochim. Biophys. Acta*, **4**, 427 (1950).
(8) J. M. McCall, *J. Med. Chem.*, **18**, 549 (1975).
(9) M. Takai, Y. Uehara, and J. M. Beisler, *J. Med. Chem.*, **22**, 1380 (1979).
(10) B. Rittich, M. Polster, and O. Kralik, *J. Chromatogr.*, **197**, 43 (1980).
(11) G. J. Hatheway, C. Hansch, K. H. Kim, S. R. Milstein, C. L. Schmidt, R. N. Smith, and F. R. Quinn, *J. Med. Chem.*, **21**, 563 (1978).
(12) W. J. Dixon and M. B. Brown, Eds., "BMDP Biomedical Computer Programs, P-series", University of California Press, Berkley, 1979, pp 399–417.

Table III. Metabolism of 3-Alkyl-1-(4-carbamoylphenyl)-3-methyltriazenes by Mouse Liver Microsomes in Vitro

compd	demethylation ± SD,[a] %	dealkylation ± SD,[a] %	metabolism ± SD,[a] %
16	55.5 ± 1.5	1.8 ± 1.8	57.3 ± 0.3
17	28.2 ± 0.9	33.7 ± 2.9	61.8 ± 3.7
18	25.3 ± 0.5	33.0 ± 2.9	58.3 ± 2.5
19	21.5 ± 2.0	33.5 ± 1.5	55.0 ± 3.5
20	18.0 ± 0.5	23.5 ± 0.0	41.5 ± 0.5
21	13.0	14.0	27.0
22	11.0	12.0	23.0
23	11.5 ± 1.5	8.0 ± 2.0	19.5 ± 3.5
Et_2 deriv	0.0	53.3 ± 1.3	53.3 ± 1.3

[a] Mean of at least two determinations. (The duplicates differed by no more than 10%.)

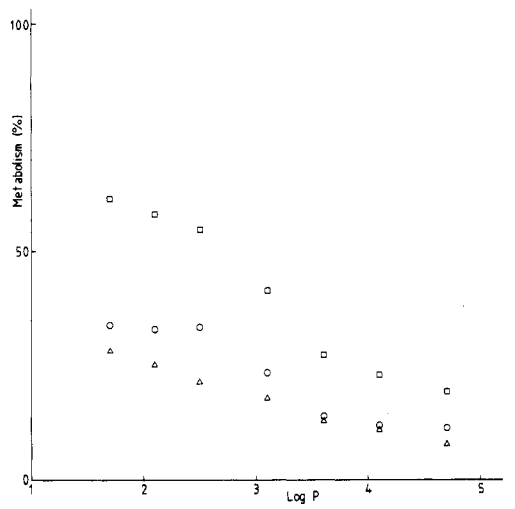


Figure 1. The relationship between log P and demethylation (△), dealkylation (○), and total metabolism (□) by mouse liver microsomes for a series of 3-alkyl-1-(4-carbamoylphenyl)-3-methyltriazenes.

phenyl)-3-methyltriazenes are contained in Table II. Equations 3 and 4 were derived from these data in a similar fashion to equations 1 and 2, respectively.

$$R_m = 0.225\ (\pm\ 0.010)\ m - 0.586\ (\pm\ 0.044) \quad (3)$$

$$n = 8,\ r = 1.000,\ s = 0.015$$

$$\log P = 2.203\ (\pm\ 0.081)\ R_m + 2.418\ (\pm\ 0.044) \quad (4)$$

$$n = 5,\ r = 0.998,\ s = 0.097$$

The calculated values of log P were used to investigate the existence of correlations between the partition coefficient of the 1-(4-carbamoylphenyl)triazenes and their in vitro metabolism and in vivo antitumor activity.

The in vitro metabolism of the 1-(4-carbamoylphenyl)triazenes in the presence of mouse liver microsomes produced the following three sets of data: the extent of demethylation, the extent of other dealkylation, and the total extent of metabolism as the sum of these (Table III). The assumption is made that the alkyltriazene so formed is not metabolized further, as was demonstrated by Connors and co-workers.[2] That there is little interference between the demethylation and dealkylation determinations is demonstrated by the results obtained for the dimethyl derivative **16** and the diethyl derivative.

In all cases, metabolism was inversely related to lipophilicity (Figure 1), although the extent of dealkylation of the compounds with shorter alkyl chains (**17–19**) did not differ significantly. The mechanism by which increased hydrophobicity reduces metabolism is unclear. However, it could be argued that the extremely hydrophobic nature

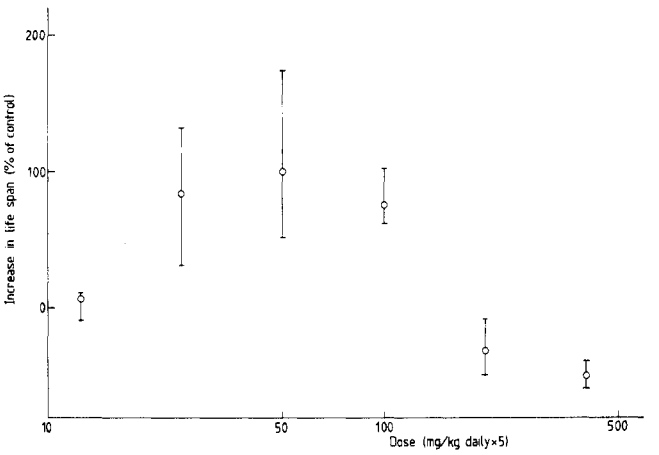


Figure 2. Activity of a typical triazene, 1-(4-carbamoylphenyl)-3-ethyl-3-methyltriazene (**17**), against the TLX/5 lymphoma.

of these molecules, along with their increased bulk as the chain increases, limits their access to the active site of the cytochrome P450–enzyme complex.

It is of interest that compounds **17–23** displayed an equal propensity to demethylate (46.7 ± 6.2%) and to dealkylate (53.4 ± 6.3%) despite wide variation in overall metabolism (61.8–19.5%) (Table III). This is in contrast to previous data, which demonstrated that N-dealkylation occurred preferentially in the shorter alkyl chain of *N*-ethyl-*N*-methylaniline[13] and propanolol.[14]

Equation 5 correlates log P with the extent of demethylation for compounds **17–23**. Compound **16** has been excluded from this set of data, since it has two possible sites for demethylation, whereas the remainder have only one. Similarly, eq 6 relates to the dealkylation of compounds **17–23**, and eq 7 relates to the total extent of metabolism of compounds **16–23**.

$$\text{extent of demethylation} = 37.5\ (\pm 2.2)\ \log P - 6.18\ (\pm 0.67) \quad (5)$$

$$n = 7,\ r = 0.972,\ s = 1.77$$

$$\text{extent of dealkylation} = 39.2\ (\pm 2.6) - 1.68\ (\pm 0.22)\ (\log P)^2 \quad (6)$$

$$n = 7,\ r = 0.957,\ s = 3.56$$

$$\text{extent of metabolism} = 65.2\ (\pm 3.3) - 2.35\ (\pm 0.28)\ (\log P)^2 \quad (7)$$

$$n = 8,\ r = 0.959,\ s = 5.37$$

In all the above correlations we have considered only log P, since it is the physicochemical parameter that is varied by the addition of methylene groups to an alkyl chain. Although the molar refractivity also varies, this directly parallels the alteration in log P for such an homologous series.

The result of a typical TLX/5 tumor inhibition test is shown in Figure 2. It will be noted that the dose–response curve is not sigmoidal. This is due to to the nature of the end point of the assay, i.e., increase in life span (ILS). Thus, antitumor activity, as measured by ILS, increases

(13) J. W. Gorrod, D. J. Temple, and A. H. Beckett, *Xenobiotica*, **5**, 453 (1975).
(14) O. M. Bakke, D. S. Davies, L. Davies, and C. T. Dollery, *Life Sci.*, **13**, 1665 (1973).
(15) R. Carubelli and G. F. Kolar, *Proc. Int. Cancer Congr., 12th*, 1, 171 (1978).

Table IV. Antitumor Activity of an Homologous Series of 3-Alkyl-1-(4-carbamoylphenyl)-3-methyltriazenes (IG)

		% increase in life span of TLX/5 tumor bearing animals at the following dose levels, daily × 5						max % ILS μmol^{-1} kg^{-1}	
no.	Z	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg	(C_{max})	log $1/C_{max}$
16	methyl	94	47	65	51	−7	−26	1.447	−0.161
17	ethyl	7	84	100	76	−31	−49	0.693	0.159
18	propyl	7	113	60	41	20	−45	0.996	0.002
19	butyl	18	23	91	83	−12	−51	0.426	0.371
20	pentyl	3	7	79	71	−28	−51	0.392	0.407
21	hexyl	4	9	11	83	56	30	0.218	0.662
22	heptyl	2	6	15	65	15	−20	0.180	0.745
23	octyl	−2	4	4	19	112	56	0.163	0.788
24	dodecyl	1	3	3	−1	13	9	0.023	1.638
25	isopropyl	21	52	45	41	−10	−60	0.458	0.339
26	*tert*-butyl	4	−4	−2	0	0	NT[a]		
27	benzyl[b]	−3	−1	3	9	86	−36	0.145	0.839
28	2-hydroxyethyl	1	0	5	7	42	38	0.047	1.328
29	hydroxy	NT	NT	19	19	37	55	0.036	1.444
30	methoxy	−3	−3	−5	−3	−7	−3		

[a] NT = not tested at this dose level. [b] Dose levels used for this compound were 10, 20, 40, 80, 160, and 320 mg/kg.

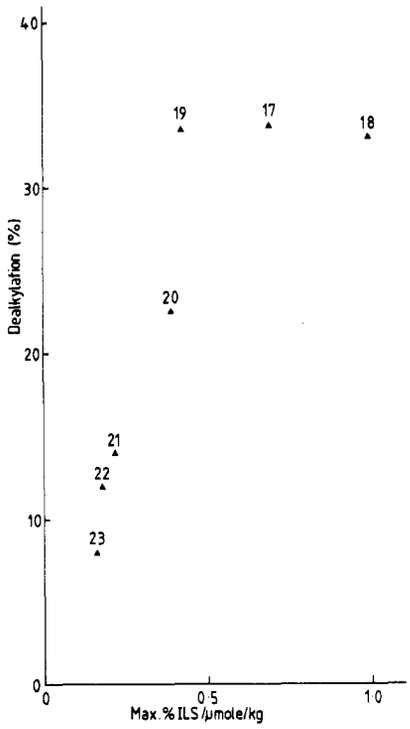


Figure 3. Relationship between in vitro dealkylation and in vivo antitumor activity.

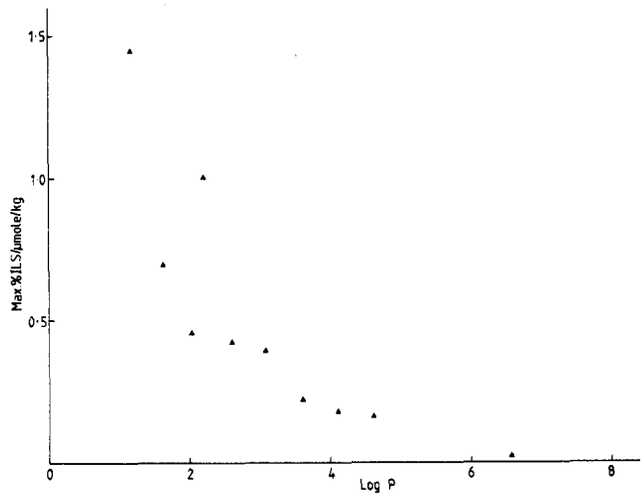


Figure 4. Relationship between antitumor activity [maximum percent increase in life span/micromole/kilogram and lipophilicity (log P).

with increasing dose until toxicity is encountered. Thereafter ILS decreases with increasing dose as the severity of the toxicity becomes progressively greater. Quantification of antitumor activity has been achieved in this case by conversion of the observed ILS values to percent ILS/micromole/kilogram of drug administered at each dose level. The maximum percentage increase in life span per micromole/kilogram for each triazene (Table IV) was then used to study correlations between antitumor activity, in vitro dealkylation, and lipophilicity.

Figure 3 shows the relationship observed between antitumor activity and in vitro dealkylation, the metabolic process that yields the cytotoxic methyltriazene. For compounds **19–23** (butyl to octyl), antitumor activity increases linearly with in vitro metabolism, as shown by eq 8. However, compounds **17** (Ib, Z = ethyl) and **18** (Ib, Z = propyl) show greater antitumor activity than would be predicted from their in vitro oxidative metabolism. This serves to emphasize the point that in vivo antitumor activity is also determined by pharmacokinetic parameters other than metabolism, a number of which are dependent on lipophilicity.

$$\log 1/C_{max} = 0.261\ (\pm 0.041) \log P - 0.362\ (\pm 0.134) \quad (8)$$

$$n = 7,\ r = 0.944,\ s = 0.109$$

The relationship of lipophilicity (log P) to antitumor activity for compounds **16–25** is shown in Figure 4. Equation 9 correlates these data for compounds **16–25** and

$$\log 1/C_{max} = 0.447\ (\pm 0.098) + 0.305\ (\pm 0.028) \log P \quad (9)$$

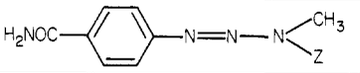
$$n = 11,\ r = 0.965,\ s = 0.137$$

27. The remaining compounds were omitted because of the absence of a proton on the α-carbon atom of the side chain or the presence of an hydroxyl group.

These findings alone do not distinguish between the relative merits of the two suggested mechanisms for the antitumor activity of the aryldialkyltriazenes.[2,3] Further synthetic work is in progress, in conjunction with the pharmacokinetic studies, in an attempt to resolve this problem.

Of the triazenes examined in this study, compound **18** (Ib, Z = propyl) displayed the greatest activity against the

Table V. Some New 3-Alkyl-1-(4-carbamoylphenyl)-3-methyltriazenes



no.	Z	mp, °C	solvent[a]	anal.[b]	UV spectra[c] λ_{max}, nm	UV spectra[c] ϵ
18	propyl	115–116	A–C	C, N; H[d]	322	20 500
21	hexyl	103–104	B–C	C, H, N	322	21 500
22	heptyl	111–112	B–C	C, H, N	322	22 100
24	dodecyl	97–99	B–C	C, H, N	322	21 300
30	methoxy	110–114	A–C	C, H, N	298	15 200

[a] A = ethyl acetate; B = benzene; C = petroleum ether, bp 60–80 °C. [b] Elements cited were all within ±0.3% of the theoretical value. [c] UV spectra were determined in ethanol solution. [d] H: calcd, 7.3; found, 6.8.

TLX/5 lymphoma. This photostable analogue of DTIC thus warrants further preclinical study as a potential second-generation antitumor triazene.

Experimental Section

Melting points were determined on a Reichert micro hot stage apparatus and are uncorrected. Ultraviolet spectra were recorded in ethanol solution on a Pye SP8-150 spectrometer. Elemental analyses were obtained from Butterworth Laboratories Ltd., Teddington, England, or from Dr. F. B. Strauss, Oxford, England.

Triazene Synthesis. All the amines required were obtained from commercial sources and used without further purification. The physicochemical properties of the new compounds are described in Table V. The ultraviolet absorbance in the region of 320 nm is characteristic for the triazene structure. The preparation of compound 18 is typical.

1-(4-Carbamoylphenyl)-3-methyl-3-propyltriazene (18). A solution of 3.4 g (0.025 mol) of 4-aminobenzamide in 70 mL (0.07 mol) of hydrochloric acid was diazotized at 0 °C, by the addition of 1.75 g (0.025 mol) of sodium nitrite in 10 mL of water. After 0.5 h, excess nitrite was destroyed with sulfamic acid. The mixture was then made alkaline with 8.4 g (0.1 mol) of sodium bicarbonate, and 1.9 g (0.025 mol) of *N*-methylpropylamine in 10 mL of water added. After a further 0.5 h, the mixture was extracted with 4 vol of ethyl acetate. The combined, dried (Na_2SO_4) organic phase was concentrated to small volume and applied to the top of a column of alumina. Elution with ethyl acetate and evaporation of the solvent gave a white solid, which on crystallization from ethyl acetate–petroleum ether (bp 60–80 °C) (1:1) yielded 4.4 g of 18 (80%): mp 115–116 °C. Anal. Calcd for $C_{11}H_{16}N_4O$: C, 60.0: H, 7.3; N, 25.4. Found: C, 60.2; H, 6.8; N, 25.1.

Antitumor Activity. The TLX/5 lymphoma was transplanted subcutaneously in female CBA/LAC mice as previously reported.[4] The triazenes were administered intraperitoneally in 10% *N,N*-dimethylacetamide in arachis oil for 5 consecutive days, commencing 3 days after tumor transplantation, by using our usual system.[1] The previously unpublished results are shown in Table IV.

Determination of Partition Coefficient by HPLC. The HPLC was undertaken on a Model ALC/GPC 204 chromatograph (Waters Associates, Milford, MA). Compounds were dissolved at a concentration of 0.1 mg/mL in methanol and 5-μl samples were applied to the column by a WISP automatic sample injector (Waters Associates). Chromatography was accomplished on a 30 cm × 5.6 mm μBondapak C18 column, and compounds were detected in the eluate by virtue of their absorbance at 254 nm. The 1-(4-carboxyphenyl)triazenes were eluted isocratically with 65% methanol–35% 0.37 M acetic acid (v/v) at a rate of 2 mL/min. The 1-(4-carbamoylphenyl)triazenes were eluted similarly with 65% methanol–35% 0.05 M ammonium formate, pH 6.3 (v/v). The void volume of the column (V_0) was determined with uracil.

Data Processing. Equations 1–9 were obtained with the aid of a stepwise multiple linear regression computer program (BMDP2R).[12] In these equations, the figures in parentheses are the standard errors of the regression coefficients. For a given equation, n is the number of compounds, r is the multiple correlation coefficient, and s is the standard error of estimate.

Extent of Metabolism. The extent of metabolism of the 1-(4-carbamoylphenyl)triazenes by liver microsomes from phenobarbitone-treated mice in 60 min at 37 °C was determined by the method of Cox et al.,[16] in terms of demethylation,[17] other dealkylation, and total metabolism as the sum of these two.[18] These results, calculated as a percentage of the total possible metabolism, are shown in Table IV.

Acknowledgment. This work was supported by grants to the Institute of Cancer Research, Royal Cancer Hospital, from the Cancer Research Campaign and Medical Research Council. We are grateful to Professor W. C. J. Ross and Dr. K. R. Harrap for their interest.

Registry No. 1, 7203-91-0; 2, 74109-20-9; 3, 74109-21-0; 4, 74109-22-1; 5, 74109-23-2; 6, 74109-24-3; 7, 74109-25-4; 8, 66974-67-2; 9, 74109-26-5; 10, 74109-27-6; 11, 74109-28-7; 12, 85514-39-2; 13, 74109-30-1; 14, 74109-31-2; 15, 65587-38-4; 16, 33330-91-5; 17, 59708-19-9; 18, 89529-99-7; 19, 59708-21-3; 20, 59708-22-4; 21, 89530-00-7; 22, 89530-01-8; 23, 66521-49-1; 24, 89530-02-9; 25, 59708-23-5; 26, 59708-25-7; 27, 59708-24-6; 28, 59708-20-2; 29, 42548-73-2; 30, 66974-76-3; 4-aminobenzamide, 2835-68-9; *N*-methylpropylamine, 627-35-0; *N*-methylhexylamine, 35161-70-7; *N*-methylheptylamine, 36343-05-2; *N*-methyldodecylamine, 7311-30-0; *N*-methylmethoxamine, 1117-97-1.

(16) P. J. Cox, K. Merai, and D. E. V. Wilman, in preparation.
(17) T. Nash, *Biochem. J.*, **55**, 416 (1953).
(18) E. Sawicki, T. R. Hauser, T. W. Stanley, and W. Elbert, *Anal. Chem.*, **33**, 93 (1961).