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ANTITUMOUR ACTIVITY, BIOMIMETIC OXIDATION AND METABOLISM OF HETEROALICYCLIC TRIAZENES

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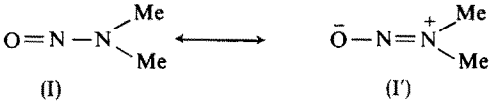
Abstract—Heteroalicyclic triazenes are, in general, inactive as antitumour agents. Chemical oxidation with potassium permanganate, biomimetic oxidation according to the Udenfriend process, or metabolic oxidation with rat liver fractions affords products oxidised in the α-, β- and γ-positions of the heteroalicyclic ring. α-Oxidation of the piperidine ring in 1-(4-chlorophenylazo)piperidine leads to ring-opening and the formation of an alkylating monoalkyltriazene which decomposes to 4-chloroaniline and valerolactone. The biotransformation pattern of the heteroalicyclic ring moeity in the triazenes is comparable with metabolic pathways so far elucidated for heteroalicyclic nitrosamines.

Dimethylnitrosamine (I) has the dubious distinction of being the most widely-studied carcinogen. The carcinogenic and antitumour triazene 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide (DTIC: II) and its aryldimethyltriazene counterparts (III) show similarities with dimethylnitrosamine in their chemical and biological properties. For example n.m.r. studies on dimethylnitrosamine [1] and X-ray crystallographic analysis of DTIC [2] indicate considerable double-bonded character about the N-NMe_2 linkage and imply that the dipolar forms (I′) and (II′) are important contributing forms in this type of compound. The effect of the electro-positive nitrogen renders the α-CH protons exchangeable by deuterium and probably influences their susceptibility to metabolism catalysed by mixed function oxygenases.

Dimethylnitrosamine is a pro-carcinogen [3]; similarly DTIC and the aryldimethyltriazenes are inactive as antitumour agents *in vitro* and only reveal their cytotoxic properties *in vivo* or when incubated with liver homogenates [4, 5]. We have recently devised a general synthesis of triazenoquinazolines (V) and (VI) and have shown that their chemical properties closely parallel those of DTIC [6]. The present study had two aims: (i) To examine if the triazenoquinazolines and certain of their derivatives possess tumour-inhibitory properties, and (ii) To elucidate the metabolic fate of triazenes bearing a heteroalicyclic ring.

In order to simplify the analytical identification of metabolites and degradation products, the chlorophenyltriazenes (IV) were chosen as model compounds in the biotransformation experiments.



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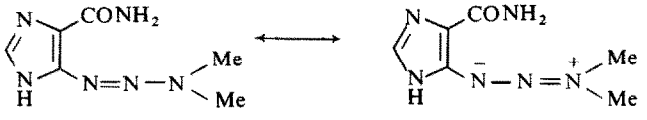

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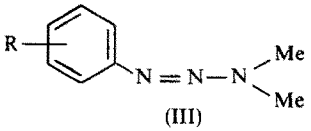

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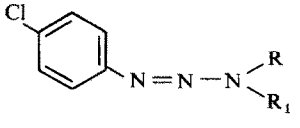

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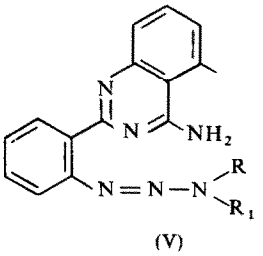

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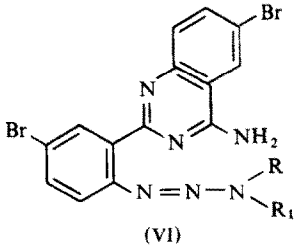

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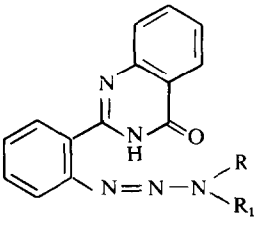

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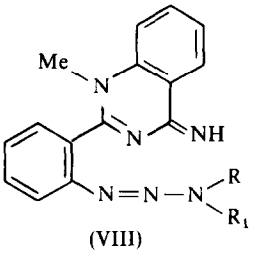
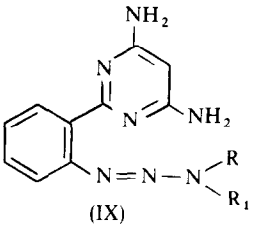


(VIII)

[in structures (IV)–(IX)]

	R	R_1
a:	$-(CH_2)_5-$	
b:	$-(CH_2)_4-$	
c:	$-(CH_2)_2\cdot O\cdot (CH_2)_2-$	
d:	$-CH(Me)\cdot (CH_2)_4-$	
e:	$-CH_2\cdot CH(Me)\cdot (CH_2)_3-$	
f:	$-(CH_2)_2\cdot CH(Me)\cdot (CH_2)_2-$	
g:	Me	Me
h:	Et	Et
i:	*n*-Pr	*n*-Pr



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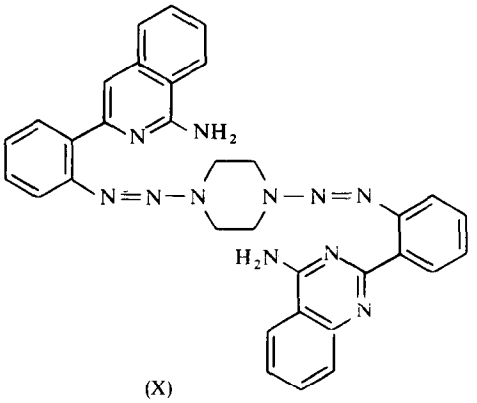
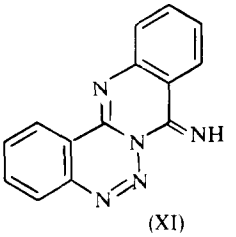


(X)



(XI)

MATERIALS AND METHODS

Chemicals. Synthesis of quinazolinotriazenes [6, 7] and 2,4-diamino-*s*-triazinotriazenes [8] was performed according to methods already published. Preparation of quinazolinotriazenes oxidized in the heteroalicyclic ring by potassium permanganate, and the chlorophenyltriazene series has been published elsewhere [9].

Antitumour screening. Triazenes were screened against L1210 lymphoid leukaemia, P388 lymphocytic leukaemia and B16 melanocarcinoma *in vivo*, and against human epidermoid carcinoma of the nasopharynx (H.Ep.-2) *in vitro* according to the protocols of the National Cancer Institute, Bethesda, Maryland [10].

The TLX5 lymphoma (approx. 10^5 cells) was implanted subcutaneously in female CBA/LAC mice. Compounds, suspended or dissolved in 10% dimethyl acetamide in arachis oil, were administered (i.p.) on days 3–7 after innoculation. Antitumour effectiveness was measured by determining the maximum increase in life-span of treated animals over control animals according to an established method [4].

Chemical oxidations. Oxidations of quinazolinotriazenes and chlorophenyltriazenes with potassium permanganate were accomplished by methods already published [9].

Udenfriend oxidations [11] were performed in a two-necked flask fitted with a condenser and a sintered glass oxygen inlet tube. The substrate (0.4 mM) was dissolved in 30% acetone in 0.1 M phosphate buffer (pH 7.4) containing ascorbic acid (9.6 mM), EDTA (4.2 mM) and $FeSO_4\cdot 7H_2O$ (0.85 mM). Blank reactions identical in all respects but omitting the triazene substrate were run in parallel. Oxygen was bubbled through the solutions for 24–30 hr and the temperature was maintained at 37°.

Metabolism experiments. Livers were obtained from male Wistar rats (200 g) which had been given sodium phenobarbital (0.5 g/litre) in their drinking water for the previous 5 days; 9000 *g* supernatant and microsomes were prepared from 25 per cent liver homogenates in 0.1 M Tris buffer (pH 7.4). Incubations were carried out at 37° for 50 min under air in 25 ml conical flasks. Each flask contained 9000 *g* supernatant (3 ml) or microsomal suspension (3 ml) equivalent to 1 g of liver, and 0.1 M Tris buffer (2 ml) with glucose-6-phosphate dehydrogenase type XV (Sigma, 3 units) and cofactors, giving final incubation mixtures containing NADP (0.55 mM), glucose-6-phosphate (11 mM) and $MgCl_2\cdot 6H_2O$ (10 mM). Incubations were initiated by adding substrate dissolved in acetone (0.2 ml) to a final concentration of substrate of 0.8 mM. After 50 min protein was precipitated with acetone (5 ml) and removed by centrifugation (15 min at 1200 *g*). All oxidations and incubations were performed in the dark.

For the urinary metabolism experiments, male Wistar rats (~200 g) were injected with triazene (150 mg/kg) dissolved in arachis oil at a concentration of 40 mg/

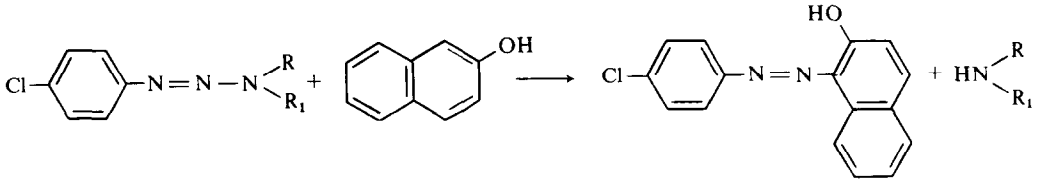


Fig. 1. Interaction of triazenes and 2-naphthol.

ml. Urine was collected for 24 hr after injection in ice-cooled vessels.

Chromatography. The bulk of the acetone in the oxidation mixtures and deproteinised incubates was evaporated under vacuum at 40°. The resultant solutions, or urine, were extracted with dichloromethane or ether. Extracts were dried over $MgSO_4$, concentrated by rotary evaporation and examined by t.l.c. or g.l.c.

Aliquots of the extracts of oxidation mixtures, homogenates and urine were examined on 0.25 mm layers of silica gel (Kieselgel GF 254; Merck). Plates were developed in benzene–ether (9:1) to give R_f values recorded in Table 2, or toluene/acetone/acetic acid (60:39:1). 4-Chloroaniline was visualised by spraying with Ehrlich's Diazo Test Reagent or with a saturated solution of *p*-dimethylaminobenzaldehyde in 10 N-hydrochloric acid. Triazenes were detected with a 3 per cent solution of 2-naphthol in acetic acid followed by heating at 100° for 10 min to give a red colour of 1-chloro-4-(2-hydroxy-1-naphthylazo)benzene (XII) (Fig. 1).

Preparative t.l.c. separations were achieved on 1 mm layers of silica gel. Products were recovered by removing the relevant bands and eluting with dichloromethane. Semiquantitative evaluation of preparative t.l.c. was possible with compounds (XIII), (XVI), (XVIII) and (XX) as their recovery from silica gel was > 75 per cent.

The percentage recovery of product/metabolite formation in the results refers to initial substrate concentration (*in vitro*) or injected dose (*in vivo*) as 100 per cent. Compounds (XVI) and (XX) were quantitatively determined by g.l.c., and derivatives (XIII) and (XVIII) estimated by u.v. spectroscopy.

Gas chromatograms were obtained with Pye Unicam 104 or GCV chromatographs fitted with flame ionisation detectors. Triazenes were separated on a 1.5 m column of 3% OV 17 on Chromosorb W.AW-DMCS, 80–120 mesh (Phase Separations Ltd.); nitrogen gas flow rate 50 ml min^{-1}; column oven temperature 178°. Retention times of products/metabolites are given in Table 2 relative to 4-chloroaniline. Quantitative analysis of 4-chloroaniline (XX) was performed on a 1.5 m column of 8% Carbowax 20 M and 2% KOH on Chromosorb W.AW-DCMS, 80–100 mesh; nitrogen gas flow rate 50 ml min^{-1}; column oven temperature 120°; 4-methoxyaniline as internal standard.

Spectra. Mass spectra were obtained on a low resolution magnetic sector V.G. Micromass 12B operating at 70 eV. For the g.c.–m.s. data the same instrument was linked to a Pye Unicam 104 gas chromatograph by a single stage glass jet separator with a helium flow rate of 45 ml $min.^{-1}$ I.r. spectra were recorded on a Perkin–Elmer 157G spectrometer, u.v. spectra on a Unicam SP 8000 instrument, and ^1H n.m.r. spectra on a Varian HA-100 spectrometer.

RESULTS

(i) *Antitumour properties of triazenoquinazolines.* The triazenoquinazolines (V) and (VI) and related derivatives were examined for tumour-inhibitory properties *in vivo* and *in vitro* but were found, in general, to be inactive: modifications to the quinazoline nucleus or the triazene side chain proved to have little effect. Compounds (V a–i), (VI a–f), (VII a), (IX a–c, h and i) and (X) were inactive against L 12 10 lymphoid leukaemia in mice in doses ranging from 200 to 400 mg/kg. Triazenes (V a, b and h) and (X) were inactive against P388 lymphocytic leukaemia and B 16 melanocarcinoma; in addition derivatives (V a, b, g and h) were inactive against the TLX5 lymphoma tumour. The dimethyltriazenoquinazolone (VII g) showed some activity against the latter tumour effecting a maximum increase in survival time of 32 per cent following five daily doses at 200 mg/kg. The inactive aminoquinazoline analogue (V g) proved to be toxic to mice at five daily doses of 50 mg/kg.

The aminoquinazoline series (V a–c, h and i) were the most toxic agents against human epidermoid carcinoma of the nasopharynx in cell culture with ED_{50} doses ranging from 1.2–2.3 μg/ml (Table 1). The related bistriazenopiperazine (X) was also active in this system. Substitution in the quinazoline nucleus by bromine atoms (VI f), or replacement of the aminoquinazoline by a 2,4-diamino-*s*-triazin-6-yl fragment had a dyschemotherapeutic effect.

(ii) *Biomimetic and metabolic oxidation of* 1-(4-*chlorophenylazo*) *piperidine* (IV a). This triazene when

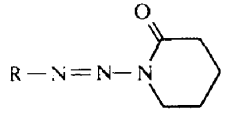
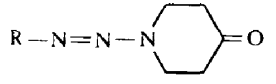
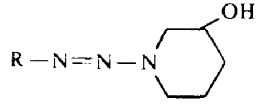
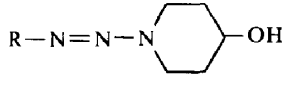
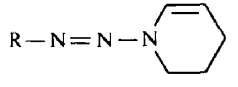
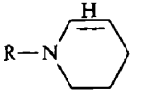
Table 1. Activity of triazenes against human epidermoid carcinoma of the nasopharynx (cell culture)

Compound	ED_{50} (μg/ml)*
V a (hydrate)	1.5†
V b	1.2†
V c	1.6†
V h (benzene solvate)	2.3†
V i	2.2†
VI f	100
VII a	64
VII g	30
VIII a (hydroidide salt)	28
IX a	75
IX b	120
IX c	100
IX h	40
IX i	43
X	3.3†

* Concentration that inhibits growth to 50 per cent of control growth.

† Average of three tests.

Table 2. Oxidation products and *in vitro* metabolites of 1-(4-chlorophenylazo)piperidine (IV a), their t.l.c. R_f values and g.l.c. retention times: R = 4-chlorophenyl

Product	Name	Structure	R_f value	G.l.c. retention time (Min)	Oxidation system $KMnO_4$/Udenfriend		Liver preparation 9000 *g*/microsomes	
XIII	1-(4-Chlorophenylazo)piperidin-2-one		0.16	—	+	+	—	(+)
XIV	1-(4-Chlorophenylazo)piperidin-4-one		0.35	9.4	(+)	+	—	(+)
XV	1-(4-Chlorophenylazo)piperidin-3-ol		0.20	10.7	(+)	+	—	(+)
XVI	1-(4-Chlorophenylazo)piperidin-4-ol		0.09	11.1 (8.2 for TMS ether)	+	+	+	+
XVII	1-(4-Chlorophenylazo)1,2,3,4-tetra-hydropyridine		0.36	9.6	+	+	—	+
XVIII	1,3-Bis-(4-chlorophenyl)triazene		0.57	—	+	+	+	+
XIX	1-(4-Chlorophenyl)-1,2,3,4-tetra-hydropyridine		0.38	3.6	+	-	—	+
XX	4-Chloroaniline		0.41	1	+	-	+	+

+ Products isolated and identified.
(+) Presence indicated by t.l.c. only.

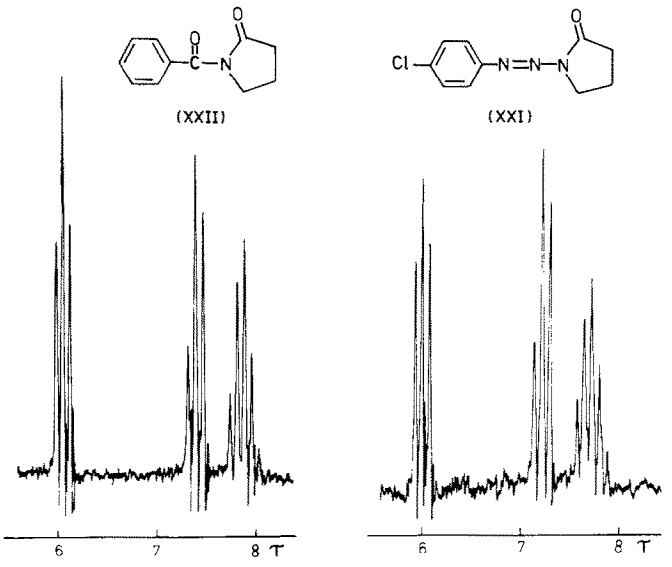


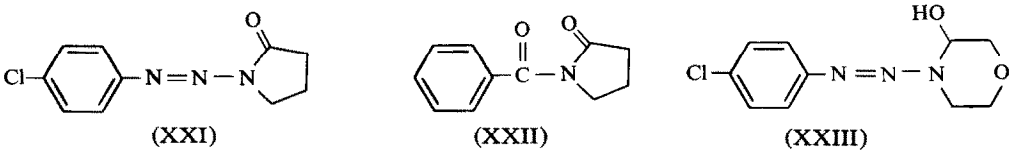
Fig. 2. 100 MHz ^{1}H n.m.r. spectra of 1-benzoylpyrrolidin-2-one(XXII) and 1-(4-chlorophenylazo)pyrrolidin-2-one (XXI) in deuterochloroform.

oxidized with potassium permanganate in aqueous acetone in the pH range 6–9 afforded eight oxidation products in addition to substantial amounts of starting material. Most of the oxidation products (Table 2) were identified by comparison with authentic reference samples prepared unambiguously by coupling 4-chlorophenyldiazonium fluoroborate with oxidised piperidines. Where reference compounds were unavailable the oxidation products were identified by g.l.c.–m.s. The chemical and spectroscopic characteristics of the oxidation products have been reported elsewhere [9]. A similar range of products was identified when triazene (IV a) was oxidized according to the Udenfriend process [11] or by incubation with rat liver homogenates or fortified liver microsomes. In a preparative permanganate oxidation the yield of 1-(4-chlorophenylazo)piperidin-2-one (XIII)—the major oxidation product—was 5 per cent [9]. U.v. analysis of the piperidin-2-one (XIII) and bis-chlorophenyltriazene (XVIII), which decomposed on the g.l.c. column, and g.l.c. assays of the piperidin-4-ol (XVI) and 4-chloroaniline (XX) from extracts of oxidation and incubation mixtures indicated the formation of about 0.7 per cent of (XIII), 0.5 per cent of (XVI), 0.5 per cent of (XVIII), and 1 per cent of (XX) in the case of Udenfriend oxidation; and 2 per cent of (XVI), 0.6 per cent (XVIII), and 1 per cent of 4-chloroaniline (XX) in the liver 9000 g incubation mixtures.

G.l.c. analysis of the purified residue from a dichloromethane extract of 24 hr urine samples of rats injected (i.p.) with 1-(4-chlorophenylazo)piperidine (IV a) revealed the presence of 4-chloroaniline (1.5%). T.l.c. examination of the extract showed traces of unchanged (IV a), the piperidin-2-one (XIII) and the bis-chlorophenyltriazene (XVIII).

(iii) *Biomimetic and metabolic oxidation of* 1-(4-*chlorophenylazo*)*pyrrolidine* (*IVb*) *and* 4-(4-*chlorophenylazo*)*morpholine* (*IVc*). Oxidation of the pyrrolidinotriazene (IVb) with potassium permanganate in aqueous acetone, or incubation with rat liver homogenates and microsomes afforded three identifiable products in addition to starting material—these were 1-(4-chlorophenylazo)pyrrolidin-2-one (XXI), the bis-chlorophenyltriazene (XVIII) and 4-chloroaniline (XX). The pyrrolidinone was formed in 5 per cent yield in a preparative permanganate oxidation and was identified by its u.v., i.r. and mass spectroscopic characteristics [9]. In addition the ^{1}H n.m.r. spectrum of the aliphatic region of the spectrum of compound (XXI) was nearly identical (Fig. 2) to that of a model compound 1-benzoylpyrrolidin-2-one (XXII) conclusively showing that the pyrrolidine ring had suffered oxidation in the α-position.

Permanganate oxidation of the morpholinotriazene (IV c) afforded the bis-chlorophenyltriazene (XVIII), 4-chloroaniline (XX) and a compound (25 per cent; m.p. 120–122°) which had an intact triazene linkage (red colour with 2-naphthol/acetic acid) and analysed as a monohydroxy derivative of the starting material (Found: C, 50.0; H, 4.9; Cl, 15.0; N, 17.6. $C_{10}H_{12}ClN_3O_2$ requires C, 49.6; H, 5.0; Cl, 14.9; N, 17.4 per cent). The i.r. spectrum of this morpholinol derivative in carbon tetrachloride showed the presence of an intramolecularly bonded hydroxyl group (ν_{OH}



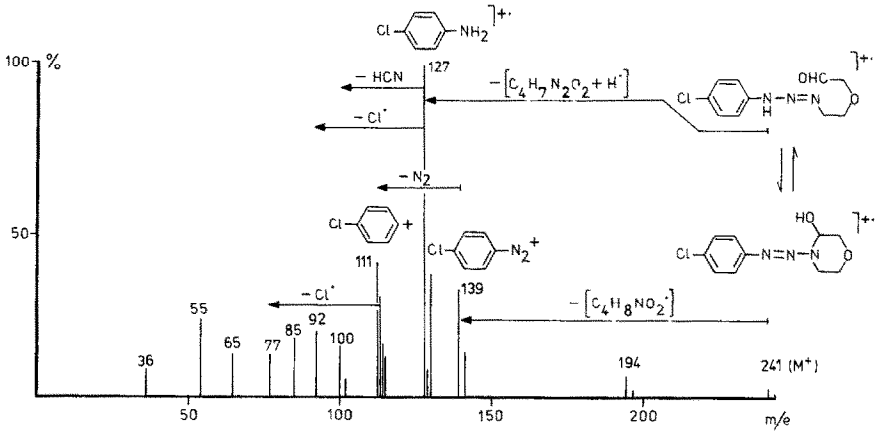


Fig. 3. Mass spectral fragmentation of 4-(4-chlorophenylazo)morpholin-3-ol(XXIII).

3410 cm^{-1}) which was unaffected by dilution. The mass spectrum showed a small molecular ion at m/e 241 (243), substantial peaks at 139 (141), 111 (113), 100 (102), 92 and a base peak at 127 (129): this fragmentation profile is consistent with the morpholin-3-ol structure (XXIII). The peaks at m/e 139 and 111 arise by cleavage of the N–N bond (Fig. 3) as expected for a heteroalicyclic triazene [7, 8]. The base peak at m/e 127 attributed to the 4-chloroaniline radical ion can only be formed from the ring-opened aldehyde tautomer which is, in effect, a monoalkyltriazene. Monoalkyltriazenes, in contrast to dialkyltriazenes, afford abundant radical ions of the appropriate arylamine [12]: as expected the peak at m/e 127 was not observed in the mass spectra of the heteroalicyclic triazenes (IV a–c) and the oxidised products (XIV–XVII).

Incubation of the morpholinotriazene (IV c) with liver homogenates and isolated microsomes also afforded the morpholinol (XXIII) which was detected by g.l.c. after silylation; although the bis-chlorophenyltriazene (XVIII) and 4-chloroaniline were identified in the urine of rats administered the morpholinotriazene, the morpholinol was not detected.

(iv) *Semi-quantitative estimation of 4-chloroaniline from the oxidation of chlorophenyltriazenes with potassium permanganate.* Oxidation of the triazenes (5 mM) with potassium permanganate (10 mM) in aqueous acetone at 22° for 5 hr afforded the percentage yields of 4-chloroaniline recorded in Table 3. It should be emphasised that in a model experiment to determine the stability of 4-chloroaniline (1 mM) under identical oxidation conditions the recovery of amine was only 30 per cent.

Table 3. Formation of 4-chloroaniline by oxidation of triazenes with potassium permanganate

Starting triazenes	% Yield of 4-chloroaniline
IVa	1.3
IVb	0.6
IVc	0.3
IVe	3.7
IVg	1.7
1-(4-chlorophenylazo)-2,6-dimethyl-piperidine†	1.3
1-(4-chlorophenylazo)-2,2,6,6-tetramethylpiperidine†	<0.1
XIV	3.2
XVI	1.3

* Mean of three oxidations.
† For synthesis see [9].

DISCUSSION

An in-depth study on the structure–antitumour activity relationships in aryldialkyltriazenes [4, 5] revealed that the nature of the aryl substituent is relatively unimportant. Evidence was obtained which confirmed that only those aryltriazenes that can be metabolised *in vivo* to an aryl-N^3-monomethyltriazene have antitumour activity. Consequently the inactivity of the heteroalicyclic triazenes examined in the present work is not surprising. The inactivity of the dimethyltriazene (Vg) against the TLX5 lymphoma is however noteworthy since this tumour is normally exquisitely sensitive to dimethyltriazenes [4, 5]. Two possible reasons can be advanced to account for this inactivity. Like all the triazenes in series (V–IX) derivative (V g) is unstable particularly in acidic media [6] and readily cyclises to the tetracyclic triazine (XI). In this respect it resembles DTIC (II) which undergoes transformation in the light [13] and to a slight extent *in vitro* in the dark [14] to 2-azahypoxanthine. 2-Azahypoxanthine has no antitumour properties [15] and the tetracyclic triazene (XI) was inactive against all the test systems employed. Alternatively, the strongly basic character of the aminoquinazoline fragment of (V g) may militate against the transport of the compound to hepatic microsomal enzyme systems capable of bioactivating triazenes. Indeed the acidic dimethyltriazenoquinazolone (VII g) showed modest activity against the TLX5 lymphoma. Of the tumour systems on which the triazenes were

tested human epidermoid carcinoma of the nasopharynx (cell culture) was the only one to display appreciable sensitivity to the quinazolinotriazenes.

Metabolic α-hydroxylation is an activating step in the hepatic metabolism of antitumour dimethyltriazenes [4, 5] and the carcinogen dimethylnitrosamine [3]. α-Oxidised triazenes were detected amongst the products of oxidation of the heteroalicyclic triazenes (IV a–c). 1-(4-Chlorophenylazo)piperidin-2-one (XIII) and 1-4-(chlorophenylazo)-1,2,3,4-tetrahydropyridine (XVII) were formed both in the microsomal and in the biomimetic oxidations. Surprisingly, chemical oxidation with potassium permanganate or an ascorbic acid/$FeSO_4$/oxygen mixture yield all the products obtained by *in vitro* metabolic oxidation of (IV a) (Table 2). The unusual dehydropiperidine (XVII) could arise by dehydration of either the piperidin-2-ol (which was not detected) or the piperidin-3-ol (XV). The dehydropiperidine (XVII) was unstable and rapidly decomposed in polar media to 1-(4-chlorophenyl)-1,2,3,4-tetrahydropyridine (XIX) [9].

The pyrrolidin-2-one (XXI) was the major chemical and metabolic oxidation product of the pyrrolidinotriazene (IV b); the pyrrolidin-2-ol was not detected in any system. However, a stable morpholinol formed from the permanganate or metabolic oxidation of the morpholinotriazene (IV c) is apparently the α-hydroxymorpholine (XXIII), judging by its mass spectrum. Possibly the 3-ol arrangement is stabilised by intramolecular hydrogen bonding (Fig. 4), although the equatorial or axial disposition of the hydroxyl group is as yet unknown.

We have previously shown [9] that the piperidin-2-one (XIII) decomposes in aqueous alkali to afford 4-chloroaniline and valerolactone (XXV). We envisage that this α-oxidised triazene (XIII) is ring-opened by water to yield the corresponding monoalkyltriazene (XXIV) which can *alkylate* water (Fig. 5). Presumably it could also alkylate other biologically significant nucleophiles.

Although it is conceivable that the metabolite 4-chloroaniline could arise by bio-reduction of the triazene linkage this is impossible in the potassium permanganate *oxidations*. The finding that all the heteroalicyclic triazenes, with the exception of 1-(4-chlorophenylazo)-2,2,6,6,-tetramethylpiperidine which has no α-CH groups, yield significant amounts of 4-chloroaniline by permanganate oxidation (Table 3) reinforces the view that oxidative attack at the α-

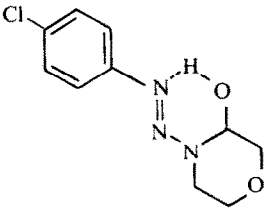


Fig. 4. Intramolecular H-bonding in 4-(4-chlorophenylazo)morpholin-3-ol.

position is crucial for subsequent ring-opening and the development of alkylating character. In the case of the morpholin-3-ol (XXIII) the ring opened aldehyde tautomer could serve as an alkylating agent. The formation of 1,3-bis-(4-chlorophenyl)triazene (XVIII) in the chemical and metabolic degradations of triazenes (IV a–c) is simply explained in terms of a "diazo-migration" reaction [16] involving monoalkyltriazenes [e.g. (XXIV)]; therefore (XVIII) is better described as a metabonate rather than a metabolite.

In conclusion, the heteroalicyclic triazenes are seen to be metabolised in similar fashion to their nitroso counterparts. For example, Krüger and Bertram [17] identified nitrosopyrrolidin-3-ol as a metabolite of nitrosopyrrolidine in the rat; nitrosopiperidin-4-ol and nitrosopiperidin-4-one are an *in vitro* metabolite and Udenfriend oxidation product, respectively, of nitrosopiperidine [18]. α-Oxidation also occurs in the metabolism of nitrosopiperidine as evidenced by the presence of 5-hydroxypentanal in incubation mixtures containing rat liver microsomes [19]; similarly ε-aminocaprolactam and ε-aminocaproic acid are urinary metabolites of nitrosohexamethyleneimine in the rat [20]. Significantly, blockage of the α-CH groups by methyl in nitrosopiperidine reduces or eliminates mutagenic and carcinogenic activity [21].

Thus although α-oxidation bioactivates the carcinogenic heteroalicyclic nitrosamines, similar processes do not convert inactive heteroalicyclic triazenes to tumour-inhibitory agents; 1-(4-chlorophenylazo)piperidin-2-one (XIII) is totally inactive against the TLX5 lymphoma. However, preliminary studies indicate that the α-hydroxylated triazene 4-(4-chlorophenylazo)morpholin-3-ol (XXIII) is a powerful direct-acting mutagen towards *Salmonella typhimurium* TA 100 and TA 1535. These strains detect base-pair substitutions and

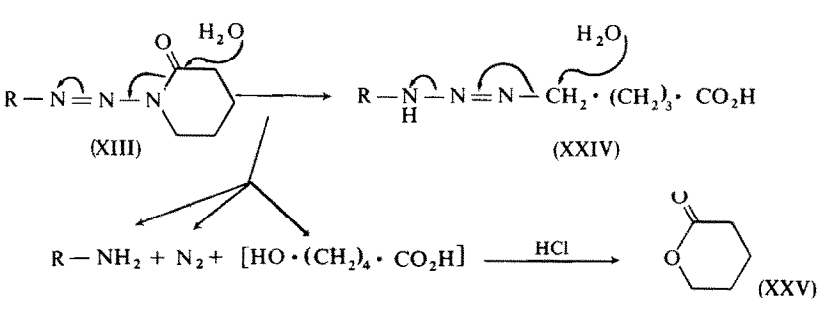


Fig. 5. Decomposition of 1-(4-chlorophenylazo)piperidin-2-one in 0.1N-potassium hydroxide at 25° in the dark.

are sensitive to alkylating agents [22]. The morpholinol is not mutagenic towards the TA 1537 strain which is reverted by frameshift mutagens [23]. The parent morpholinotriazene (IVc) is not a direct-acting mutagen of either type.

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