Tetrahedron Letters No. 50, pp 5041 - 5044 0040-4039/78/1208-5041$02.00/0

α-HYDROXYLATED DERIVATIVES OF ANTITUMOUR DIMETHYLTRIAZENES

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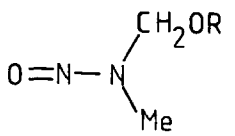
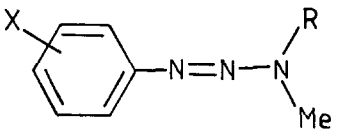
The prophetic suggestion[1] that metabolic α-hydroxylation could play a critical role in the carcinogenic activity of dimethylnitrosamine has been sustained by the recent development of synthetic routes to α-acetoxydimethylnitrosamine (1).[2] Unlike dimethylnitrosamine the acetoxy derivative is profoundly mutagenic without prior metabolic activation.[3-5] Chemical studies with a range of α-acetoxymethylnitrosamines corroborate the theory that such compounds are non-enzymatically deacetylated to unstable hydroxymethyl derivatives which decompose to afford alkylating species.[2,6,7]

A similar oxidative activation process has been proposed to explain the fact that 1-aryl-3,3-dimethyltriazenes (3) although inactive *in vitro* develop antitumour properties *in vivo* or after incubation with liver fractions.[8,9] In these cases alkylating monomethyltriazenes (4) are implicated as the cytotoxic species. Although the related α-hydroxymethyl intermediates (2) and (5) have hitherto been regarded as only transient species we have achieved a synthesis of the latter type simply by reacting aryldiazonium salts with a formaldehyde-methylamine mixture.



(1) R = Ac
(2) R = H



(3) R = Me
(4) R = H
(5) R = CH_2OH

In a typical synthesis methyl *p*-aminobenzoate (4.0 g), diazotised as normal in 1.5N-hydrochloric acid (60 ml) with sodium nitrite (1 mol. equiv.), was added (15 min.) to a pre-mixed solution of 40% aqueous formaldehyde (60 ml) and 25% aqueous methylamine (6 ml) at -5°. The white hydroxymethyltriazene (5a) was collected, washed with water, air-dried and crystallised from benzene. Physical characteristics of (5a) and related derivatives are listed in the Table.

The ^{1}H n.m.r. spectrum of (5a) recorded in $CDCl_3$ showed singlets at δ3.3 (N-CH_3) and 3.95 (O-CH_3), a doublet at δ5.3 (CH_2) which collapsed to a singlet with D_2O, and a typical aromatic AA'BB' pattern centred at δ7.8. The i.r. spectrum of (5a) showed significant peaks at 3440 (OH) and 1695 cm^{-1} (CO). More informative was the mass spectrum which showed a small molecular ion at m/e 223 (Relative Intensity ~ 1% at 70 eV) and significant peaks at m/e 193 (10%; M - CH_2O), m/e 163 (20%) and 151 (100%). The peak at 163 corresponds to the aryldiazonium ion fragment and that at 151 to the radical ion of methyl *p*-aminobenzoate. The appearance of abundant arylamine radical ion peaks in the mass spectra of all the hydroxymethyltriazenes is significant since only those triazenes which can fragment to monoalkyltriazenes normally afford such ions.[10] They are absent, for example, in the spectra of the dimethyltriazenes (3; X = *p*-CO_2Me or *p*-Ac) where the base peaks are the appropriate aryldiazonium ions, and feature to only a minor extent in the spectrum of the benzoate ester of (5a).

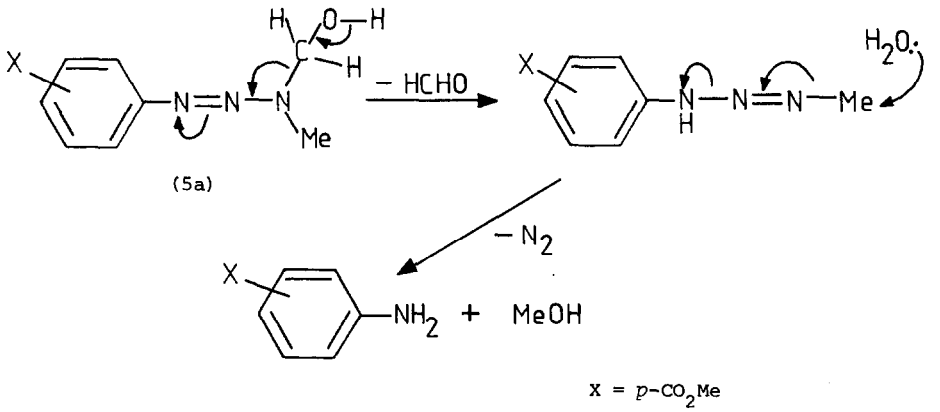
Table. α-Hydroxymethyltriazenes (5) from diazonium salts and formaldehyde-methylamine

	Compound	Yield (%)	M.p.°	Formula*	M^+
(5a)	X = *p*-CO_2Me	50	126-127	$C_{10}H_{13}N_3O_3$	223
	(benzoate ester)ø	73	88-89	$C_{17}H_{17}N_3O_4$	327
(5b)	X = *p*-CO_2Et	47	73-75	$C_{11}H_{15}N_3O_3$	237
(5c)	X = *p*-Ac	28	117-120	$C_{10}H_{13}N_3O_2$	207
(5d)	X = *p*-$CONH_2$	55	141-142	$C_9H_{12}N_4O_2$	208

* All compounds gave satisfactory CHN analyses.

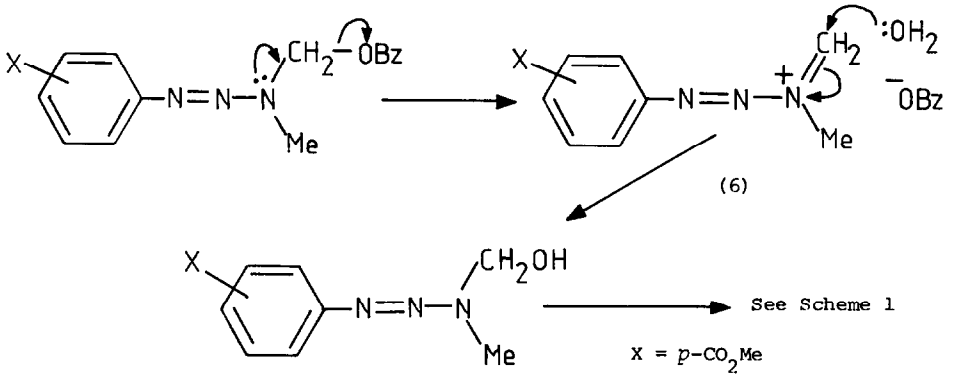
ø Prepared from (5a) with benzoyl chloride in pyridine at 25°.

The hydroxymethyltriazene (5a) afforded a crystalline benzoate ester but decomposed in acetic anhydride or acetyl chloride-pyridine. Although stable in non-polar solvents the hydroxymethyltriazene (5a) rapidly decomposed by a first order process ($t_{½}$ = 22 min.) in 0.5 M phosphate buffer (pH 7.5) at 25°. When the decomposition of (5a) was followed spectroscopically (λ max. 313 nm) the final spectrum was identical with that of methyl *p*-aminobenzoate and may be envisaged as proceeding by initial loss of formaldehyde according to Scheme 1.



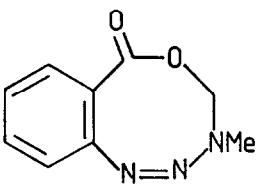
Scheme 1

However, decomposition of the benzoate ester of (5a) which also ultimately forms methyl *p*-aminobenzoate occurs at a rate faster ($t_{\frac{1}{2}}$ = 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 (Scheme 2) *via* an imminium intermediate (6) similar to that suggested to explain the anomalous stabilities of some esters of structurally related α-hydroxylated nitrosamines.[7]

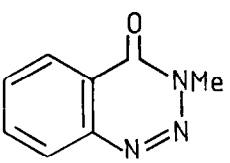


Scheme 2

Successful syntheses of hydroxymethyltriazenes (5) were only achieved from diazonium salts bearing - M substituents in the *p*-position of the aryl group; only unstable oils were formed from *p*-toluidine or *p*-chloroaniline. Attempted synthesis of a 'masked' hydroxymethyltriazene — the lactone (7) — from diazotised methyl anthranilate led to the formation of a high yield of the triazinone (8) presumably by cyclisation of a monomethyltriazene (4; X = *o*-CO_2Me) liberated by decomposition of the precursor hydroxymethyltriazene (5; X = *o*-CO_2Me).[11]



(7)



(8)

The hydroxymethyltriazene (5a) has pronounced antitumour properties giving a maximum percentage increase in survival time (IST) of 120% (5 daily doses of 5 mg/Kg) in mice innoculated with the TLX5 lymphoma. Comparable IST values for the related di- and mono-methyltriazenes (3 and 4; X = *p*-CO_2Me) are 58% (at 40 mg/Kg) and 87% (at 5 mg/Kg) respectively. Moreover, the ethyl ester (5b) is clearly an activated triazene since it inhibits the TLX5 lymphoma *in vitro* whereas its dimethyltriazene counterpart (3; X = *p*-CO_2Et) is inactive.

The close parallel between the chemical and biological properties of dimethylnitrosamine and aryldimethyltriazenes is further emphasised by the strong mutagenic activity of the hydroxymethyltriazenes (5a) and (5c) towards *Salmonella typhimurium* TA 100 and TA 1535; the corresponding dimethyltriazenes (3; X = *p*-CO_2Me or *p*-Ac) are non-mutagenic unless activated by liver fractions.

Acknowledgement: This work was supported by grants from the Cancer Research Campaign (to A.G. and R.J.S.).

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(Received in UK 2 October 1978)