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K-region oxides and imines derived from alkylated benz[*a*]anthracene congeners: synthesis, stability in aqueous media and mutagenicity

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The K-region oxides and imines of benz[*a*]anthracene, 1-methylbenz[*a*]anthracene, 7-methylbenz[*a*]anthracene, 7-ethylbenz[*a*]anthracene and 7,12-dimethylbenz[*a*]anthracene were synthesized and characterized (melting point, ^{1}H-NMR and electron impact mass spectra, elemental analysis, IR spectroscopy). All 10 compounds showed high mutagenic activity in *Salmonella typhimurium* (reversion of *his*$^-$ strains TA97, TA98, TA100 and TA104). The arene imines were more potent than the corresponding arene oxides. Alkyl substitutions strongly influenced the activities. Furthermore, all compounds were more active when exposure took place in the absence of inorganic ions than when KCl (125 mM) was present. The influence of the exposure medium was more pronounced with strain TA98 than with strain TA100. The half-lives of the test compounds were determined from mutagenicity experiments in which the compound was added to the exposure medium at varying times before the bacteria. In dilute sodium phosphate buffer (10 mM, pH 7.4), the half-lives of these chemicals (or their biological activity) varied from 0.5 to 110 min. Addition of KCl (150 mM) did not measurably affect the half-lives of some test compounds and appeared to slightly shorten those of others. Therefore, it is unlikely that the strong effect of KCl on mutagenicity and the dependence of this effect on the bacterial strain used can be explained by influences of KCl on the test compounds. Rather, it appears more likely that an effect of KCl on the bacteria may be an important factor. This study provides further examples of strong influences of unobtrusive media components on mutagenicity. It also demonstrates that small structural changes (alkyl substituents at diverse positions of the aromatic system) may play an important role in chemical reactivity and biological activity.

Introduction

K-region oxides are potent mutagens formed metabolically from various polycyclic aromatic compounds (Ames *et al.*, 1972; Glatt and Oesch, 1986; Thakker *et al.*, 1985). The corresponding arene imines exhibit even greater potency (Glatt *et al.*, 1985, 1986; Stark *et al.*, 1986; Roll *et al.*, 1990). We recently made two interesting observations: (i) the mutagenicity of the K-region oxide, 7-methylbenz[*a*]anthracene-5,6-oxide (7-MBA-O; structure **8** in Figure 1), in *Salmonella typhimurium* is strongly influenced by the ionic composition of the exposure medium (Glatt *et al.*, 1991) and (ii) 7,12-dimethylbenz[*a*]anthracene-5,6-imine (DMBA-I, **13**) is very unstable in aqueous media (data reported in this paper), in contrast to benz[*a*]anthracene-5,6-oxide (BA-O, **4**) and benz[*a*]anthracene-5,6-imine (BA-I, **5**). This led us to further explore alkyl substitution effects on the mutagenicity and solvolytic stability of this class of chemicals, and how these properties are influenced by the exposure medium. In the preceding study (Glatt *et al.*, 1991), the mutagenic activity of 7-MBA-O was the highest when the exposure took place in solute-free water and the lowest in the presence of K^+ ions (e.g. added as KCl). Furthermore, the influence was significantly stronger in strain TA98 than in strain TA100. The investigation of the new compounds therefore included the use of these exposure media and bacterial strains.

Alkyl substitution in the hindered 1 and 12 (bay region) positions of BA-O leads to a strong out of plane distortion of the aromatic system (Nashed *et al.*, 1991). It is likely that the same is true for the corresponding arene imines. It is expected that alkylation of the 7 position increases the reactivity of the oxirane and aziridine groups due to the electron-donating properties of the alkyl substituents. Both ethyl and methyl substituents exercise +I inductive and hyperconjugative effects, the hyperconjugative effect being more pronounced for the methyl group.

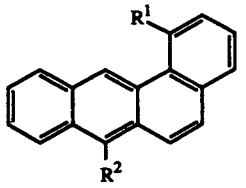
The parent hydrocarbons of the investigated arene oxides and imines widely vary in their carcinogenic acitvity (see Glatt *et al.*, 1981, 1989). 7,12-Dimethylbenz[*a*]anthracene is very potent, followed by 7-methylbenz[*a*]anthracene. Benz[*a*]anthracene and 7-ethylbenz[*a*]anthracene usually showed weak activities, whereas all data available for 1-methylbenz[*a*]anthracene are negative. 'Limited positive' carcinogenicity data are available for BA-O and 7-MBA-O (see Nesnow *et al.*, 1986), but it appears that other metabolites (e.g. bay region diol-epoxides) are much more important in the carcinogenicity of their hydrocarbons (Thakker *et al.*, 1985). Although no evidence is available for the metabolic formation of arene imines from polycyclic aromatic hydrocarbons, potential pathways have been presented (Glatt *et al.*, 1985).

Materials and methods

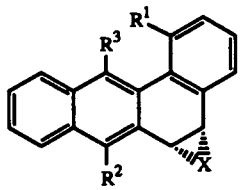
Chemicals

Benz[*a*]anthracene-5,6-oxide (BA-O, 1a,11b-dihydrobenz[3,4]anthra[1,2-*b*]-oxirene, **4**) (Newman and Blum, 1964), 7-methylbenz[*a*]anthracene-5,6-oxide (7-MBA-O, 1a,11b-dihydro-11-methylbenz[3,4]anthra[1,2-*b*]oxirene, **8**) (Newman and Blum, 1964), 7,12-dimethylbenz[*a*]anthracene-5,6-oxide (DMBA-O, 1a,11b-dihydro-6,11-dimethylbenz[3,4]anthra[1,2-*b*]oxirene, **12**) (Harvey *et al.*, 1975), benz[*a*]anthracene-5,6-imine (BA-I, 1a,11b-dihydrobenz[3,4]anthra[1,2-*b*]azirine, **5**) (Blum *et al.*, 1978) 7-methylbenz[*a*]anthracene-5,6-imine (7-MBA-I, 1a,11b-dihydro-11-methylbenz[3,4]anthra[1,2-*b*]azirine, **9**) (Blum *et al.*, 1978) and 7,12-dimethylbenz[*a*]anthracene-5,6-imine (DMBA-I, 1a,11b-dihydro-6,11-dimethylbenz[3,4]anthra[1,2-*b*]azirine, **13**) (Abu-Shqara and Blum, 1989) were prepared as described. The other compounds are new. Their ^{1}H-NMR and electron impact mass spectra are summarized in Tables I and II and their elemental analyses are given in Table III. No impurities were detected by high-pressure liquid chromatography. We therefore estimate that the purity of all investigated compounds was >98%.

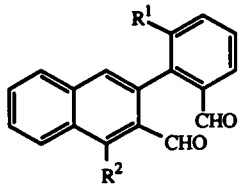
1-Methylbenz[a]anthracene-5,6-oxide (1-MBA-O, 1a,11b-dihydro-5-methylbenz-[3,4]anthra[1,2-b]oxirene, 6). A solution of 4.0 g (16.5 mmol) of 1-methylbenz-[*a*]anthracene (**1**) (Cook and Robinson, 1938) and 4.0 g (15.7 mmol) of osmium tetroxide in 40 ml of dry pyridine was stirred for 7 days under exclusion of light and air. A solution of 8 g of sodium bisulphite in 50 ml of water was added and the mixture was stirred for 3 h. Upon addition of excess water *cis*-5,6-



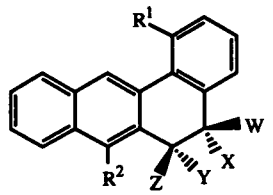
1 $R^1 = CH_3$, $R^2 = H$
2 $R^1 = H$, $R^2 = CH_3$
3 $R^1 = H$, $R^2 = C_2H_5$



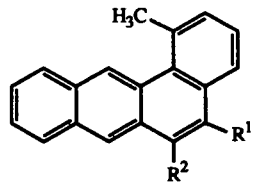
4 $R^1 = R^2 = R^3 = H$, X = O
5 $R^1 = R^2 = R^3 = H$, X = NH
6 $R^1 = CH_3$, $R^2 = R^3 = H$, X = O
7 $R^1 = CH_3$, $R^2 = R^3 = H$, X = NH
8 $R^1 = R^3 = H$, $R^2 = CH_3$, X = O
9 $R^1 = R^3 = H$, $R^2 = CH_3$, X = NH
10 $R^1 = R^3 = H$, $R^2 = C_2H_5$, X = O
11 $R^1 = R^3 = H$, $R^2 = C_2H_5$, X = NH
12 $R^1 = H$, $R^2 = R^3 = CH_3$, X = O
13 $R^1 = H$, $R^2 = R^3 = CH_3$, X = NH



14 $R^1 = CH_3$, $R^2 = H$
15 $R^1 = H$, $R^2 = C_2H_5$



16 $R^1 = CH_3$, $R^2 = W = Z = H$, X = Y = OH
17 $R^2 = C_2H_5$, $R^1 = W = Z = H$, X = Y = OH
18 $R^1 = CH_3$, $R^2 = W = Y = H$, X = OH, $Z = N_3$
19 $R^1 = CH_3$, $R^2 = X = Z = H$, $W = N_3$, Y = OH
20 $R^1 = CH_3$, $R^2 = W = Y = H$, X = OH, $Z = NHP(O)(OC_2H_5)_2$
21 $R^1 = CH_3$, $R^2 = X = Z = H$, $W = NHP(O)(OC_2H_5)_2$, Y = OH
22 $R^1 = CH_3$, $R^2 = W = Y = H$, X = OH, $Z = N_3P(C_4H_9)_3$
23 $R^1 = CH_3$, $R^2 = X = Z = H$, $W = N_3P(C_4H_9)_3$, Y = OH
24 $R^1 = CH_3$, $R^2 = W = Y = H$, X = Cl, $Z = N_3$
25 $R^1 = CH_3$, $R^2 = X = Z = H$, $W = N_3$, Y = Cl
26 $R^2 = C_2H_5$, $R^1 = W = Y = H$, X = OH, $Z = N_3$
27 $R^2 = C_2H_5$, $R^1 = X = Z = H$, $W = N_3$, Y = OH



28 $R^1 = N_3$, $R^2 = H$
29 $R^1 = H$, $R^2 = N_3$

Fig. 1. Structure formulas of the investigated compounds (**4**–**13**) and intermediates in their syntheses (**1**–**3** and **14**–**29**).

dihydro-1-methylbenz[*a*]anthracene-5,6-diol (**16**) precipitated. Successive washing with water followed by drying under vacuum (5×10^{-2} mm Hg) afforded 3.66 g (84%) of colorless diol; melting point (m.p.) 170°C; IR (Nujol) 3451 cm^{-1} (OH).

A solution of 3.0 g (10.9 mmol) of **16** in 1 l of methanol was added to a solution of 13 g of sodium metaperiodate in 300 ml of water and 200 ml of methanol. The mixture was stirred for 24 h, the methanol was removed under reduced pressure and the residue was extracted with dichloromethane. The organic solution was dried (on magnesium sulfate) and the resulting oil was chromatographed on silica gel using hexane–ether mixtures (containing 10–70% ether) as eluent. We obtained 2.38 g (80%) of dialdehyde **14** as a yellow oil; IR (neat): 1682 cm^{-1} (C=O).

A solution of 2.0 g (7.30 mmol) of **14**, 1.4 ml (7.72 mmol) of tris(dimethylamino)phosphine and 25 ml of dry benzene was refluxed under nitrogen atmosphere for 3 h. The solvent was removed under reduced pressure and the residue triturated with a cold mixture of 5 ml of ether and 25 ml of hexane. The resulting colorless crystals were washed twice with the same mixture of solvents to give 1.73 g (93%) of **6**; m.p. 133–134°C (dec.); IR (Nujol): 1220 cm^{-1} (C–O).

trans-6-Azido-5,6-dihydro-1-methylbenz[a]anthracen-5-ol (18) and trans-5-azido-5,6-dihydro-1-methylbenz[a]anthracen-6-ol (19). A mixture of 1.0 g (3.87 mmol) of **6**, 10 g of sodium azide, 100 ml of acetone and 50 ml of water was stirred under nitrogen at room temperature for 48 h. The acetone was removed under reduced pressure and the resulting colorless precipitate was washed twice with water, and dried under vacuum (0.1 mm Hg) for 12 h. There was obtained 890 mg (77%) of a 2:3 mixture of **18** and **19**; m.p. (of mixture) 115–117°C (dec.); IR (Nujol) 3340 (OH), 2112 cm^{-2} (N_3).

Table I. ^{1}H-NMR spectra of the new compounds[a]

Compound	Chemical shift (δ p.p.m.): multiplicity, integration, coupling constants and assignment in parentheses
6	2.932 (s, 3, CH_3), 4.545 (d, 1, J = 4.2 Hz, H1a or H11b), 4.685 (d, 1, J = 4.2 Hz, H1a or H11b), 7.847–7.921 (m, 7, Ar*H*), 8.107 (s, 1, H11), 8.609 (s, 1, H6).
7	2.853 (s, 3, CH_3), 3.556 (d, 1, J = 5.2 Hz, H1a), 3.725 (d, 1, J = 5.2 Hz, H11b), 7.170–7.831 (m, 8H, Ar*H*), 7.972 (s, 1, H11), 8.518 (s, 1, H6).
10[c]	1.432 (t, 3, J = 7.6 Hz, CH_3), 3.408 (ABq, 1, J = 7.6 Hz, CH_2), 3.479 (ABq, 1, J = 7.6 Hz, CH_2), 3.506 (d, 1, $J_{1a,11b}$ = 4.2 Hz, H1a), 4.944 (d, 1, $J_{1a,11b}$ = 4.2 Hz, H11b), 7.34–7.55 (m, 4, Ar*H*), 7.643 (d, 1, $J_{7,8}$ = 7 Hz, H7), 7.890 (d, 1, $J_{9,10}$ = 9 Hz, H10), 8.102 (d, 1, $J_{2,3}$ = 8 Hz, H2), 8.253 (d, 1, $J_{4,5}$ = 8 Hz, H5), 8.436 (s, 1, H6).
11	1.456 (t, 3, J = 2 Hz, CH_3), 3.438 (q, 2, J = 2 Hz, CH_2), 3.588 (d, 1, $J_{1a,11b}$ = 5 Hz, H1a), 3.947 (d, 1, $J_{1a,11b}$ = 5 Hz, H11b), 7.307–7.578 (m, 4H, Ar*H*), 7.613 (dd, 1, $J_{7,8}$ = 2 Hz, H7), 7.897 (dd, 1, $J_{8,10}$ = 1.2 Hz, $J_{9,10}$ = 4.5 Hz, H10), 8.096 (d, 1, $J_{2,3}$ = 7.6 Hz, H2), 8.251 (d, 1, $J_{4,5}$ = 7.6 Hz, H5), 8.442 (s, 1, H6).
14	2.042 (s, 3, CH_3), 7.458–7.734 (m, 5, Ar*H*), 7.928 (t, 1, J = 7.3 Hz, H5 or H8), 8.085 (dd, 1, $J_{3',4'}$ = 7.2 Hz, $J_{3',5'}$ = 2.2 Hz, H3'), 8.585 (s, 1, H4), 9.685 (s, 1, *CHO*), 9.898 (s, 1, *CHO*).
15	1.452 (t, 3, J = 7.5 Hz, CH_3), 3.531 (q, 2, J = 7.5 Hz, CH_2), 7.354 (s, 1, H4), 7.383 (dd, 1, $J_{4',6'}$ = 1.2 Hz, $J_{5',6'}$ = 8.2 Hz, H6'), 7.536–7.863 (m, 5 Ar*H*), 8.053 (dd, 1, $J_{5,6}$ = 6 Hz, $J_{5,7}$ = 1.3 Hz, H5), 8.30–8.33 (m, 1, H7), 9.868 (s, 1, *CHO*), 10.236 (s, 1, *CHO*).
16	1.602 (br s, 2, O*H*), 2.744 (s, 3, CH_3), 4.745 (d, 1, J = 3.1 Hz, H5 or H6), 4.915 (d, 1, J = 3.1 Hz, H5 or H6), 7.135–7.880 (m, 7, Ar*H*), 8.069 (s, 1, H7), 8.091 (s, 1, H12).
17	1.347 (t, 3, J = 8 Hz, CH_3), 1.795 (br s, 1, O*H*, disappears by addition of D_2O), 2.863 (br s, 1, O*H*, disappears by addition of D_2O), 3.301 (q, 2, J = 8 Hz, CH_2), 4.910 (d, 1, $J_{5,6}$ = 3.5 Hz, affected by D_2O, H5 or H6), 5.235 (d, 1, $J_{5,6}$ = 3.5 Hz, affected by D_2O, H5 or H6), 7.40–7.88 (m, 7, ArH), 8.095 (d, 1, $J_{1,2}$ = 6.8 Hz, H1), 8.119 (s, 1, H12).
18 + 19[b]	2.685 [s, 1.2, CH_3 (**18**)], 2.705 [s, 1.8, CH_3 (**19**)], 4.545 [d, 0.6, J = 8.6 Hz, affected by D_2O, H6 (**19**)], 4.675 [br s, 1, H5 (**19**), H6 (**18**)], 4.792 [d, 0.6, J = 8.6 Hz, H5 (**19**)], 6.258 (br s, 1 disappears by addition of D_2O, O*H*), 7.297–8.000 (m, 7, Ar*H*), 8.058 (s, 1, H7), 8.213 [s, 0.4, H12 (**18**)], 8.231 [s, 0.6, H12 (**19**)].
20 + 21	1.265 (two overlapping t, 6, 6.9 Hz, CH_2CH_3), 2.723 (s, 3, CH_3), 4.122 (q, 4, J = 6.9 Hz, CH_2CH_3), 4.254 [d, 0.4, J = 8.7 Hz, H5 (**20**)], 4.306 [d, 0.6, J = 9.7 Hz, H5 (**21**)], 4.524 [d, 0.6, J = 9.7 Hz, H6 (**21**)], 4.662 (d, 0.4, J = 8.7 Hz, H6 (**20**)], 7.276–7.572 (m, Ar*H*), 8.060 (s, 1, H7), 8.147 (s, 1, H12).
22 + 23	0.895 (t, 9, J = 7.7 Hz, CH_2CH_3), 1.176–1.668 (m, 12, CH_2), 1.753–1.936 (m, 6, PCH_2), 2.746 [s, 1.2, CH_3 (**22**)], 2.766 [s, 1.8, CH_3 (**23**)], 4.590 (two overlapping d, 1, J = 10 Hz, *CH*OH), 5.211 (two overlapping d, 1, J = 10 Hz, *CH*N_3), 7.152–8.100 (m, 7, Ar*H*), 8.144 (s, 1, H7), 8.215 (s, 1, H12).
24 + 25	2.799 [s, 1.2, CH_3 (**24**)], 2.818 [s, 1.8, CH_3 (**25**)], 4.818 [d, 0.6, J = 3.1 Hz, H5 (**25**)], 4.915 [d, 0.4, J = 3 Hz, H6 (**24**)], 5.145 [d, 0.4, J = 3 Hz, H5 (**24**)], 5.259 [d, 0.6, J = 3.1 Hz, H6 (**25**)], 7.258–8.017 (m, 8, Ar*H*), 8.263 [s, 0.6, H12 (**25**)], 8.283 [s, 0.4, H12 (**24**)].
26 + 27[c]	1.428 (two overlapping t, 3, J = 7 Hz, CH_3), 3.295 (two overlapping q, 2, J = 7 Hz, CH_3), 4.827 (d, 0.3, $J_{5,6}$ = 3 Hz, H5 (**27**)], 4.867 [d, 0.7, $J_{5,6}$ = 3 Hz, affected by D_2O, H5 (**26**)], 5.206 [d, 0.7, $J_{5,6}$ = 3 Hz, H6 (**26**)], 5.287 [d, 0.3, $J_{5,6}$ = 3 Hz, affected by D_2O, H6 (**27**)], 7.259–7.591 (m, 8, Ar*H*), 7.895–8.140 (m, 4, Ar*H*), 8.211 [s, 0.3, H12 (**27**)], 8.229 [s, 0.7, H12 (**26**)].
28 + 29	3.112 [s, 1.2, CH_3 (**29**)], 3.124 [s, 1.8, CH_3 (**28**)], 7.172–7.606 (m, 8H, Ar*H*), 8.159 [s, 0.6, H7 (**28**)], 8.605 [s, 0.4, H7 (**29**)], 9.41 [s, 0.6, H12 (**28**)], 9.203 [s, 0.4, H12 (**29**)].

[a]Unless stated otherwise the spectra were recorded at 200 MHz in $CDCl_3$.
[b]In DMSO-d_6.
[c]Recorded at 300 MHz.

Table II. The 70 eV electron impact mass spectra of the new compounds

Compound	Ionization temperature (°C)	Masses of main fragments (*m/z*): assignment and relative intensity in parentheses
6	140	258 ($M^{\cdot+}$, 44), 240 ($C_{19}H_{12}^{\cdot+}$, 6), 229 ($C_{18}H_{13}^+$, 47), 215 ($C_{17}H_{11}^+$, 100), 202 ($C_{16}H_{10}^{\cdot+}$, 18), 115 ($C_8H_3O^+$, 9).
7	150	257 ($M^{\cdot+}$, 42), 243 ($C_{19}H_{15}^+$, 14), 241 $C_{19}H_{13}^+$, 14), 227 ($C_{18}H_{13}^+$, 20), 215 ($C_{17}H_{11}^+$, 93), 202 ($C_{16}H_{10}^{\cdot+}$, 37), 152 ($C_{12}H_8^{\cdot+}$, 22), 128 ($C_{10}H_6^{\cdot+}$, 100).
10	115	272 ($M^{\cdot+}$, 100), 275 ($C_{19}H_{13}^+$, 82), 228 ($C_{18}H_{12}^{\cdot+}$, 42), 215 ($C_{17}H_{11}^+$, 33), 202 ($C_{16}H_{10}^{\cdot+}$, 11), 181 ($C_{14}H_{13}^+$, 12), 114 ($C_9H_6^{\cdot+}$, 9).
11	90	271 ($M^{\cdot+}$, 79), 256 ($C_{20}H_{16}^{\cdot+}$, 100), 228 ($C_{18}H_{12}^{\cdot+}$, 7).
14	130	274 ($M^{\cdot+}$, 84), 259 ($C_{18}H_{11}O_2^+$, 8), 245 ($C_{18}H_{13}O^+$, 100), 231 ($C_{17}H_{11}O^+$, 48), 202 ($C_{16}H_{10}^{\cdot+}$, 48), 126 ($C_{10}H_6^{\cdot+}$, 7).
15	80	288 ($M^{\cdot+}$, 18), 270 $C_{20}H_{14}O^{\cdot+}$, 14), 260 ($C_{19}H_{16}O^{\cdot+}$, 28), 259 ($C_{19}H_{15}O^+$, 100), 231 ($C_{17}H_{11}O^+$, 36), 215 ($C_{17}H_{11}^+$, 50), 202 ($C_{16}H_{10}^{\cdot+}$, 36).
17	150	290 ($M^{\cdot+}$, 52), 272 ($C_{20}H_{16}O^{\cdot+}$, 27), 257 ($C_{19}H_{13}O^+$, 36), 243 ($C_{18}H_{11}O^+$ or $C_{19}H_{15}^+$, 25), 231 ($C_{17}H_{11}O^+$, 21), 229 ($C_{18}H_{13}^+$, 32), 228 ($C_{18}H_{12}^{\cdot+}$, 37), 215 ($C_{17}H_{11}^+$, 100), 203 ($C_{16}H_{11}^+$, 13), 202 ($C_{16}H_{10}^{\cdot+}$, 30).
18 + 19	110	301 ($M^{\cdot+}$, 40), 273 ($C_{19}H_{15}NO^{\cdot+}$, 21), 258 ($C_{19}H_{14}O^{\cdot+}$, 51), 245 ($C_{18}H_{13}O^+$, 65), 244 ($C_{18}H_{12}O^{\cdot+}$, 100), 230 ($C_{18}H_{14}^{\cdot+}$, 26), 228 ($C_{18}H_{12}^{\cdot+}$, 18), 215 ($C_{17}H_{11}^+$, 48), 202 ($C_{16}H_{10}^{\cdot+}$, 36).
20 + 21	80	410 ($M^{\cdot+}$, 2), 293 ($C_{23}H_{24}NO_3P^{\cdot+}$, 6), 320 ($C_{19}H_{15}NO_2P^+$, 22), 295 ($C_{19}H_{15}O^+$, 22), 258 ($C_{19}H_{14}O^{\cdot+}$, 100), 257 ($C_{19}H_{15}N^{\cdot+}$, 22), 242 ($C_{19}H_{14}^{\cdot+}$, 9), 240 ($C_{19}H_{12}^{\cdot+}$, 5), 229 ($C_{18}H_{13}^+$, 48), 215 ($C_{17}H_{11}^+$, 10).
22 + 23	100	301 [(M-$PC_{12}H_{27}$)$^+$, 7], 273 ($C_{19}H_{15}NO^{\cdot+}$, 92), 244 ($C_{18}H_{12}O^{\cdot+}$, 54), 218 ($C_{16}H_{10}O^{\cdot+}$, 83), 189 ($C_{15}H_9^+$, 100).
24 + 25	90	321, 319 ($M^{\cdot+}$, 9, 27), 278 ($C_{19}H_{13}{}^{37}Cl^{\cdot+}$, 3), 276 ($C_{19}H_{13}{}^{35}Cl^{\cdot+}$, 9), 256 ($C_{19}H_{14}N^+$, 87), 254 ($C_{19}H_{12}N^+$, 14), 241 ($C_{19}H_{13}^+$, 38), 229 ($C_{18}H_{13}^+$, 100), 227 ($C_{18}H_{11}^+$, 35).
26 + 27	100	315 ($M^{\cdot+}$, 41), 258 ($C_{19}H_{14}O^{\cdot+}$, 100), 256 ($C_{19}H_{12}O^{\cdot+}$, 17), 244 ($C_{18}H_{12}O^{\cdot+}$, 19), 243 ($C_{18}H_{11}O^+$, 25), 241 ($C_{18}H_{13}^+$, 19), 215 ($C_{17}H_{11}^+$, 28).

Table III. Analytical data for the new polycyclic compounds

Compound	Formula	Found (%)				Calculated (%)			
		C	H	N	X	C	H	N	X
6	$C_{19}H_{14}O$	88.36	5.65			88.34	5.46		
7	$C_{19}H_{15}N$	88.49	5.70	5.64		88.68	5.88	5.44	
11	$C_{20}H_{17}N$	88.28	6.01	4.89		88.52	6.31	5.16	
15	$C_{20}H_{16}O_2$	82.95	5.83			83.31	5.59		
17	$C_{20}H_{18}O_2$	82.46	6.43			82.73	6.25		
18 + 19	$C_{19}H_{15}N_3O$	75.49	5.04	13.56		75.73	5.02	13.94	
20 + 21	$C_{23}H_{25}NO_4P$	67.31	6.09	3.41	7.57[a]	67.30	6.14	3.41	7.55
24 + 25	$C_{19}H_{14}ClN_3$	71.08	4.41	13.02	11.09[b]	71.36	4.41	13.14	11.09[b]
26 + 27	$C_{20}H_{17}N_3O$	76.22	5.65			76.17	5.43		

[a]X = P.
[b]X = Cl.

Diethyl trans-(5,6-dihydro-1-methyl-6-benz[a]anthracen-5-ol)amidophosphate (20) and diethyl trans-(5,6-dihydro-1-methyl-5-benz[a]anthracen-6-ol)amidophoshate (21). A solution of 100 mg (0.33 mmol) of the mixture of azido-alcohols **18** and **19** and 60 μl (0.35 mmol) of triethyl phosphite in 5 ml of dichloromethane was refluxed under nitrogen atmosphere for 24 h. The solvent was evaporated under reduced pressure and the residue triturated with a mixture of 0.5 ml of ether and 0.5 ml of hexane. Filtration and washing of the crystals (twice) with ether and hexane (1:1) gave 80.4 mg (59%) of a 2:3 mixture of **20** and **21**; m.p. 95–100°C (dec.); IR (Nujol): 3625 (NH), 3382 (OH), 1700 cm^{-1} (P=O).

Reaction of azido-alcohols 18 and 19 with thionyl chloride. To a solution of 200 mg (0.66 mmol) of the mixture of isomeric azido-alcohols **18** and **19** in 10 ml of dry benzene was added dropwise at 7°C under nitrogen atmosphere 1 ml of thionyl chloride. The solution was stirred at this temperature for 3 h and then poured onto ice and ether. The organic phase was washed successively with water, 5% aqueous sodium bicarbonate and again with water. The solution was dried, concentrated and chromatographed on silica gel with hexane containing from 0 to 10% of ether as eluent. The first fraction consisted of 34 mg (19%) of a 3:2 mixture of 5- and 6-azido-1-methylbenz[*a*]anthracene (**28** and **29**, respectively); yellow semi-solid; IR (Nujol): 2015 cm^{-1} (N_3). The second fraction of 112 mg was composed of a 2:3 mixture of *trans*-6-azido-5-chloro- and *trans*-5-azido-6-chloro-5,6-dihydro-1-methylbenz[*a*]anthracene (**24** and **25** respectively). Yield 63%; m.p. 100–105°C (dec.); IR (Nujol): 2100 cm^{-1} (N_3).

1a,11b-Dihydro-5-methyl-1H-benz[3,4]anthra[1,2-b]azirine (1-MBA-I, 7). (i) A solution of 50 mg (15.6 μmol) of the mixture of chloro-azides **24** and **25** in 4 ml of absolute ether was added dropwise under nitrogen at 0°C to a suspension of 40 mg (80 μmol) of lithium aluminum hydride in 5 ml of the same solvent. The mixture was stirred at room temperature for 3 h and the excessive hydride was decomposed with ethyl acetate. The precipitate of lithium and aluminum compounds was filtered off and washed several times with benzene. The organic solvents were removed under reduced pressure and the residue dried under vacuum (0.01 mm Hg) to yield 34 mg (85%) of **7** as pale yellow crystals; m.p. 100–101°C (dec.): IR (Nujol) 3550 cm^{-1} (NH).

(ii) A solution of 50 mg (0.166 mmol) of the mixture of **18** and **19** and 50 μl of tri-*n*-butylphosphine in 3 ml of degassed anhydrous ether was stirred under nitrogen at room temperature for 45 min. The colorless precipitate that separated was filtered and washed with cold anhydrous ether to give 65.4 mg (81%) of a mixture of the isomeric 'Staudinger adducts' *trans*-6-[tributylphosphor-(azido)]-5,6-dihydro-1-methyl-5-benz[*a*]anthracenol (**22**) and *trans*-5-[tributyl-phosphor(azido)]-5,6-dihydro-1-methyl-6-benz[*a*]anthracenol (**23**) in the ratio 2:3; m.p. 100–101°C (dec.).

When the suspension of the mixture of **22** and **23** was refluxed in 5 ml of either degassed dichloromethane or *n*-hexane for 24 h the resulting product proved by ^{1}H-NMR analysis to be a mixture of 40% of the expected imine **7** and 60% of unreacted **23**. The mixture did not contain any **22**. Compounds **7** and **23** could be separated by fractional crystallization from ether-hexane mixtures only with substantial losses.

cis-7-Ethyl-5,6-dihydrobenz[a]anthracene-5,6-diol (17). A solution of 4.2 g (16.4 mmol) of 7-ethylbenz[*a*]anthracene (**3**) (Fieser and Hershberg, 1937) and 4.0 g (16.32 mmol) of osmium tetroxide in 25 ml of pyridine was stirred at 20°C under exclusion of air and light for 8 days. Treatment of the resulting precipitate with a solution of 7.3 g of sodium bisulphite in 100 ml of water for 3 h and dilution with further 300 ml of water afforded 3.45 g (76%) of analytically pure **17**; m.p. 170–171°C; IR (Nujol): 3200–3450 cm^{-1} (OH).

1-Ethyl-3-(2-phenylformyl-2-naphthalenecarboxaldehyde (15). A mixture of 1.754 g (6.048 mmol) of **17**, 4 g of sodium metaperiodate, 350 ml of methanol and 100 ml water was stirred at room temperature for 48 h. The methanol was removed under reduced pressure and the residue extracted with dichloromethane. Chromatography on silica gel with a 2:3 ether–hexane mixture as eluent yielded 0.955 g (56%) of **15** as a yellow oil; IR (neat): 1680 cm^{-1} (C=O).

11-Ethyl-1a,11b-dihydrobenz[3,4]anthra[1,2-b]oxirene (7-EBA-O, 10). In analogy to the preparation of **6**, a mixture of 745 mg (2.586 mmol) of **15**, 512 mg (3.14 mmol) of tris(dimethylamino)phosphine and 20 ml of benzene was refluxed under nitrogen for 3 h. After the usual workup there was obtained 612 mg (87%) of **10** as colorless crystals; m.p. 144–145°C (from ethyl acetate). A single crystal was subjected to X-ray diffraction analysis. The compound proved to belong to space group *Pbca* with a = 7.906(2), b = 22.297(4), c = 15.672(3) Å; V = 2762.7(8) Å^3; Z = 8; R = 0.057 and R_w = 0.064 for 1767 reflections with $I > 2\sigma_I$, and to be a down epoxide. Full crystallographic data which include experimental details, tables of positional and thermal parameters, bond lengths, bond angles, observed and calculated structure fractures, ORTEP and stereoscopic drawings are available from the authors upon request.

trans-6-Azido-7-ethyl-5,6-dihydrobenz[a]anthracen-5-ol (26) and trans-5-azido-7-ethyl-5,6-dihydrobenz[a]anthracen-6-ol (27). In the manner described for the preparation of **18** and **19**, 809 mg of **10** was reacted for 48 h with 13.5 g of sodium azide. The usual workup and chromatography of the product on silica gel (using a 3:7 mixture of hexane–ether as eluent) afforded 598 mg (64%) of a 7:3 mixture of **26** and **27**; m.p. 57–58°C; IR (Nujol): 3300 (OH), 2100 cm^{-1} (N_3).

11-Ethyl-1a,11b-dihydrobenz[3,4]anthra[1,2-b]azirine (7-EBA-I, 11). To a solution of 234 mg (724 mmol) of the mixture of azido-alcohols **26** and **27** in 15 ml of dichloromethane was added under nitrogen atmosphere 124 mg (0.816 mmol) of triethyl phosphite. The mixture was refluxed for 3 h. The solvent was removed under reduced pressure and the residue was triturated with cold ether. The crystals were washed successively with pentane and dried at 0.01 mm Hg. Yield of **11** 158.8 mg (79%); m.p. 142–143°C; IR (Nujol): 3360 cm^{-1} (NH); UV (CH_2Cl_2): λ_{max} (log ε) 210 (3.88), 224 (3.91), 256 (4.31), 260 (4.29), 276 (4.43), 292 (3.78), 312 (3.86), 323 (3.76), 255 (2.69).

Mutagenicity investigations

Bacteria. The *his*$^-$ *S.typhimurium* strains TA97, TA98, TA100 and TA104 were kindly provided by Professor B.N.Ames (Berkeley, CA). Stock cultures were stored at −70°C. Bacteria were grown overnight in Nutrient Broth No. 2 (Oxoid, Wesel, FRG). The overnight cultures were centrifuged, resuspended in medium A (1.6 g/l Bacto Nutrient broth plus 5 g/l NaCl), adjusted nephelometrically to a titer of ~1.5 × 10^9 bacteria (c.f.u.)/ml and kept on ice. Within 1 h before use, the bacteria were recentrifuged and resuspended at 5-fold density in distilled water. In each experiment the presence of the R-factor pKM101 was ascertained by growing diluted cultures in ampicillin-containing and ampicillin-free, histidine-supplemented agar plates. With strains TA97, TA98, TA100 and TA104 the number of colonies was always virtually identical under the two culture conditions, whereas related plasmid-free strains (TA1538 and TA1535) grew only in the absence of ampicillin.

Mutagenicity assay. Mutagenicity in *S.typhimurium* was determined using methods similar to those described by Maron and Ames (1983). The bacterial suspension (100 μl) and the test compound [in 10 μl dimethylsulfoxide (DMSO) or 20 μl acetone:triethylamine, 1000:1, v/v] were added sequentially to a glass tube containing 500 μl of water with the indicated solutes, prewarmed at 37°C. After incubation for 20 min at 37°C, 2.0 ml of 45°C warm soft agar (0.55% agar, 0.55% NaCl, 50 μM biotin, 50 μM histidine, 50 μM tryptophan, 25 mM sodium phosphate buffer, pH 7.4) was added and the mixture was poured onto a Petri dish containing 24 ml minimal agar (1.5% agar in Vogel–Bonner E medium

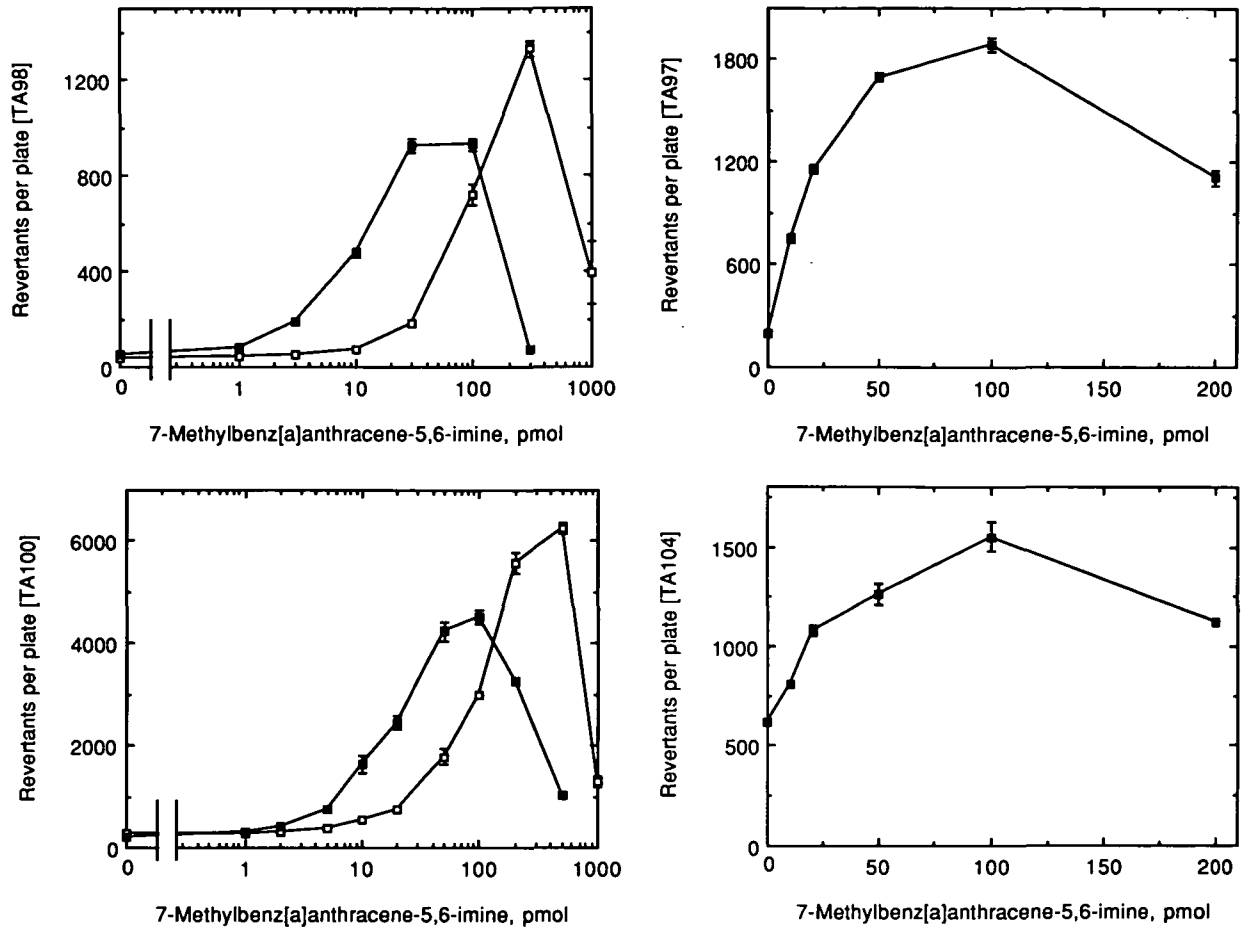


Fig. 2. Dose–response curves of the mutagenicity of 7-methylbenz[*a*]anthracene-5,6-imine in *S.typhimurium* TA98, TA100, TA97 and TA104, with exposure in water (solid symbols) or in 125 mM KCl (open symbols). Values are means and SE from two to six incubations. Where error bars are not visible, they fall within the symbol. The decreases in the numbers of colonies at high doses were accompanied by a decrease in the background lawn, indicating toxicity.

with 2% glucose). After incubation for 3 days in the dark, the colonies (*his*$^+$ revertants) were counted.

In our experience, an increase in the number of bacteria above the usual level enhances the mutagenic response with many short-lived directly-acting mutagens (e.g. Glatt *et al.*, 1991), whereas the number of mutants on control plates is insignificantly altered. However, sporadically it is increased. These increases were most often seen with strain TA104 and possibly involve higher than usual numbers of pre-existing mutants.

Specific mutagenicities, as a measure for the mutagenic activity, were calculated as described elsewhere in detail (Glatt, 1989). Briefly, mean numbers of colonies were calculated from all plates belonging to the same treatment group. Treatment groups in which these values were less than twice the value of the solvent control were excluded from further processing. In the remaining groups, the number of colonies of the solvent control plates was subtracted. The resulting value was then divided by the dose. For a perfectly linear curve, the quotients obtained with the different doses are identical and correspond to the slope of the curve. In practice this is seldom exactly true. As explained elsewhere (Glatt, 1989), we therefore use the highest quotient as a measure of the potency and term it specific mutagenicity. It reflects the number of mutations induced per dose unit at distribution of the material on an optimal number of plates.

Estimation of the half-life of test compounds under the conditions of the mutagenicity assay. The test compound, using several dose levels, was preincubated in the indicated medium for varying times, then the bacteria were added to conduct a mutagenicity experiment as described above. The amount of compound present at the end of the preexposure period was estimated from the decline in mutagenicity, as compared with incubations in which the bacteria were added immediately after the test compound. Ideally, the concentration–response curve remains unaltered in its shape, but is right-shifted, in logarithmic units, to an extent proportional to the preincubation time. The half-life ($t_{1/2}$) is then calculated from the preincubation times (t_i and t_{i+1}) and the corresponding specific mutagenicities (a_i and a_{i+1}) using the formula:

$$t_{1/2} = (t_{i+1} - t_i)\ \ln 2/\ln(a_i/a_{i+1})$$

In practice, the preincubation time was varied above a large range in the initial experiment. The results usually allowed a rough estimate of the half-life. In subsequent experiments at least three different preincubation times were used, the first one being much shorter than the estimated half-life, the second and third one being about one and two estimated half-lives, respectively.

Results

Synthesis of alkylated benz[a]anthracene-5,6-imines

The key intermediates in the preparation of arene imines **7**, **9** and **11** were the β-azido-alcohols formed by reaction of the corresponding arene oxides **6**, **8** and **10** with sodium azide. Each of the epoxides furnished a mixture of *trans*-azido-alcohols having the azide function attached either to C5 or C6 of the 5,6-dihydrobenz[*a*]anthracene skeleton. Transformations of the various azido-alcohols to the aziridines could not be accomplished by one standard procedure. While the synthesis of 1a,11b-dihydro-11-methylbenz[3,4]anthra[1,2-*b*]azirine (**9**) was accomplished by treatment of the corresponding azido-alcohols with tri-*n*-butylphosphine (Blum *et al.*, 1978), the analogous 1a,11b-dihydro-11-ethylbenz[3,4]anthra[1,2-*b*]azirine (**11**) could be obtained only with the use of triethyl phosphite as the cyclization agent (Weitzberg *et al.*, 1980). For the preparation of 1a,11b-dihydro-5-methylbenz[3,4]anthra[1,2-*b*]azirine (**7**) neither of these methods was applicable. The reaction of the azido-alcohols **18** and **19** with triethyl phosphite gave, under various conditions, a mixture of highly stable amidophosphates **20** and **21**, which could not be converted thermally into the required aziridine. Interaction of **18** and **19** with tri-*n*-butylphosphine in

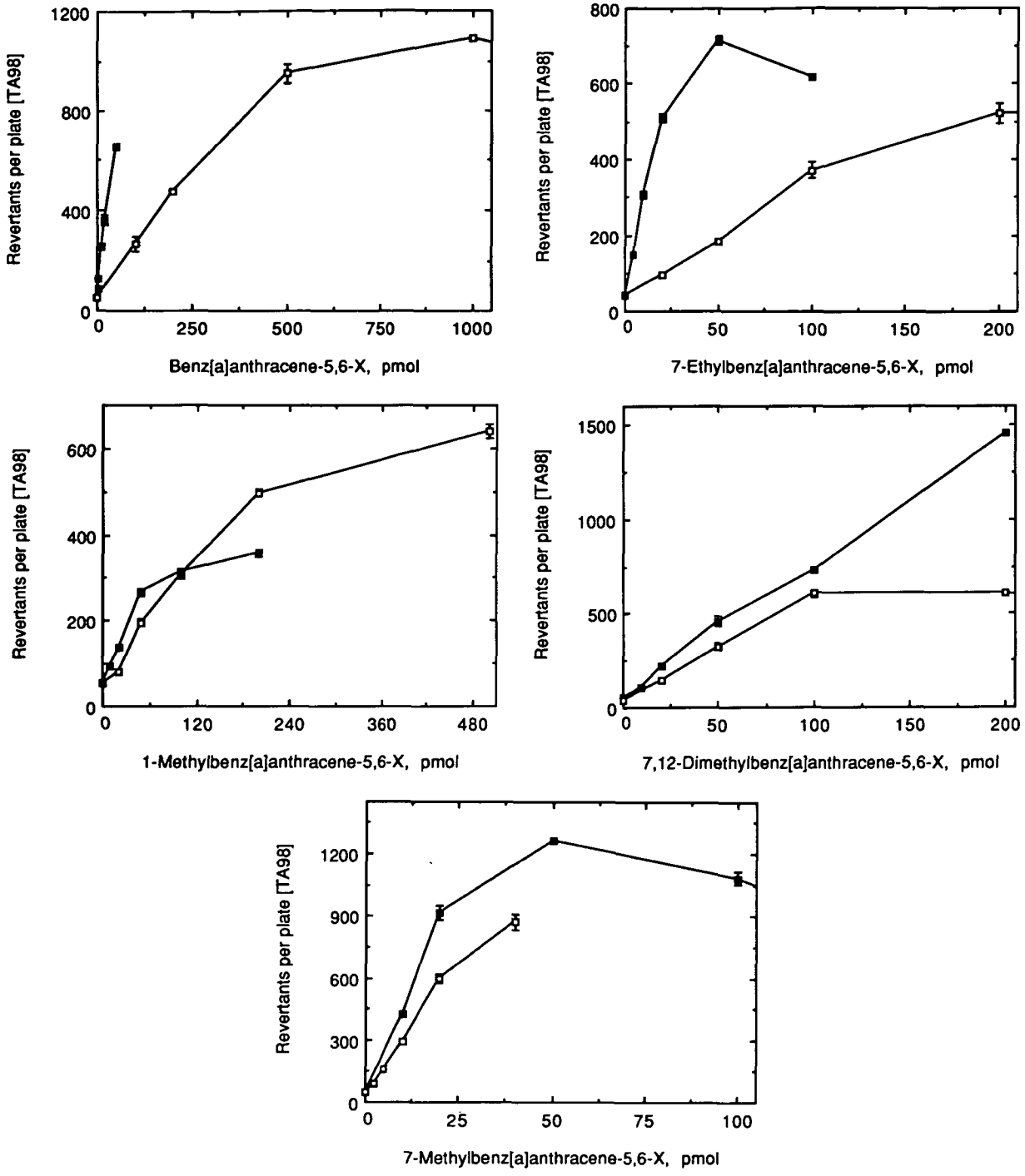


Fig. 3. Dose–response curves of the mutagenicity of the K-region oxides (X = O, open symbols) and imines (X = NH, solid symbols) of benz[*a*]anthracene, 7-ethylbenz[*a*]anthracene, 1-methylbenz[*a*]anthracene, 7,12-dimethylbenz[*a*]anthracene and 7-methylbenz[*a*]anthracene in *S.typhimurium* TA98. Exposure was in water. Values are means and SE from two to six incubations. Where error bars are not visible, they fall within the symbol.

either boiling benzene (Newman and Blum, 1964) or boiling *n*-heptane (Blum *et al.*, 1978) resulted in the formation of similar adducts. Treatment of the mixture of azido-alcohols with the reagent in *cold* ether (*cf.* Shtelzer *et al.*, 1984) afforded the two 'Staudinger adducts' **22** and **23**, of which only the former underwent thermolysis to **11** in boiling dichloromethane. Since the separation of the imine from unchanged **23** was found to be associated with heavy losses, this method proved impractical for the preparation of **7**. Transformation of the *trans*-azido-alcohols to **7** was successfully accomplished via formation of chloro-azide intermediates. Reaction of the mixture of **18** and **19** with thionyl chloride gave the chloro-azides **24** and **25** with the same stereochemistry as the starting azido-alcohols (S_Ni mechanism) together with the two dehydrohalogenated azides **28** and **29** as minor side products. It is remarkable that unlike some other cases in which polycyclic azido-alcohols were reacted with thionyl chloride (e.g. Abu-Shqara *et al.*, 1990), the formation of the aromatic azides was not accompanied by any positional rearrangement. Furthermore, the ratio between **29** and **28** was exactly the same as that between the corresponding starting azido-alcohols **18** and **19**. Lithium aluminum hydride reduction of **24** and **25** under careful exclusion of acid afforded **7** in 85% yield.

Table IV. Mutagenicity of the K-region oxides and imines of BA and alkylated congeners in four *his*⁻ strains of *S.typhimurium*[a]

Compound	Specific mutagenicity (revertants/nmol)					
	TA100, KCl	TA100, H_2O	TA98, KCl	TA98, H_2O	TA97, H_2O	TA104, H_2O
4	1900	5200	320	2200	5100	5200
	1400	8000		2600		
				1800		
5	75000	220000	5600	21000	89000	102000
				53000		
8	15000	27000	2200	17000	34000	72000
	9000	55000	4400	23000		
	11000	39000	2200	22000		
			2600	23000		
			3900	29000		
				39000		
				30000		
				39000		
9	31000	140000	6800	47000	56000	23000
	57000	280000		43000		
10	1500	4800	270	3400	3300	8600
				3300		
				5600		
11	6200	70000	1500	19000	57000	22000
				26000		
6	5200	13000	220	2900	13000	9600
				3000		
7	8000	38000	520	4300	45000	26000
				7400		
12	4400	44000	230	5600	8700	55000
13	19000	31000	2000	8300	53000	14000

[a]Exposure was in KCl (125 mM) or solute-free water. Each number represents an experiment consisting of ~25 incubations. Where several values are shown, they are from experiments carried out on separate occasions. The data for **10** are from previously published experiments (Glatt *et al.*, 1991).

Mutagenicity investigations

All 10 investigated arene oxides and imines showed strong mutagenic effects under all six experimental conditions. Detailed results have been published previously for 7-MBA-O and are presented for the corresponding arene imine in Figure 2. For the other compounds, dose–response curves are shown for experiments with strain TA98 (Figure 3). A summary of all results is given in Table IV. Some compounds were examined on several occasions under the same condition. As indicated in Table IV, the reproducibility was good.

With strains TA98 and TA100, KCl solution (125 mM, final concentration) was used as an exposure medium. Under these conditions the arene imines were always more active than the corresponding arene oxides. Replacement of the KCl solution by water resulted in increases in the mutagenic activities. With all compounds, the increase was larger with strain TA98 (4.2- to 24-fold) than with strain TA100 (1.6- to 11-fold) (Table V). The weakest influence of the exposure medium was observed with DMBA-I. Thus, with exposure in water, the activity of this compound in strain TA100 was lower than that of the corresponding arene oxide, in sharp contrast to the activities observed in the presence of KCl.

Mutagenicity was also studied with strains TA97 and TA104, but only with exposure in water. Both strains were highly responsive towards all test compounds. In general, the arene imines were more active than the corresponding arene oxides. The differences were largest in the BA-O/BA-I pair, as was the case in the other strains, TA98 and TA100. In strain TA104, however, 7-MBA-I and DMBA-I were less active than their arene oxide analogs.

The stability of the test compounds in the exposure medium was studied by adding the test compound to the medium at varying times before the bacteria. Dilute sodium phosphate buffer (10 mM, pH 7.4) was added to the media, in order to avoid potential influences of accidental variation of the pH. Detailed results were previously published for 7-MBA-O (Glatt *et al.*, 1991) and are presented in Figure 4 for the corresponding aziridine. The half-lives of the biological activities of all compounds are listed in Table VI. Extrapolation to zero-time preincubation gave figures for the specific activities of the arene imines (in dilute buffer), which were similar to those found in the standard assay (with exposure in solute-free water). For the arene oxides, however, the extrapolated figures were lower. For example, in three independent experiments with BA-O figures

Table V. Factors by which the specific mutagenicity were increased when the test compounds were exposed in H_2O instead of KCl solution (125 mM): comparison of *S.typhimurium* strains TA98 and TA100[a]

Compound	Factor	
	TA98	TA100
4	6.9	4.0
5	6.6	2.9
8	9.1	3.5
9	6.6	4.8
10	15.2	3.2
11	15.0	11.3
6	13.4	2.5
7	11.2	4.8
12	24.3	10.0
13	4.2	1.6

[a]Calculated from the means of Table IV.

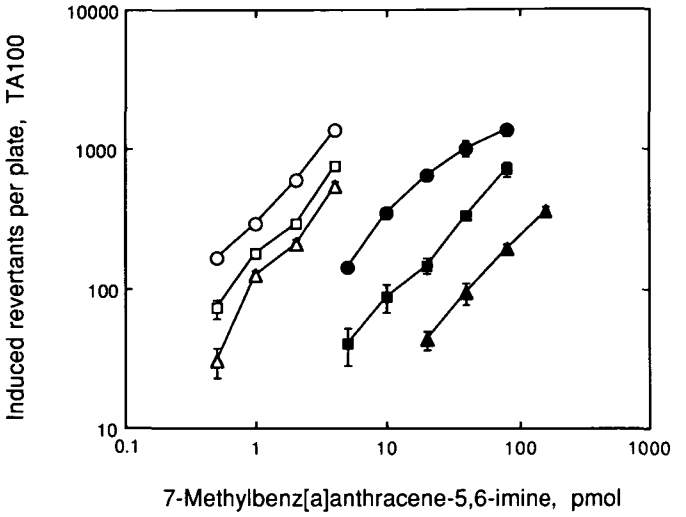


Fig. 4. Stability of 7-methylbenz[*a*]anthracene-5,6-imine in 10 mM sodium phosphate buffer (pH 7.4) without further solutes (open symbols) or containing 150 mM KCl (solid symbols), as determined from the decline in mutagenicity after preincubation of the epoxide in the exposure medium before addition of the bacteria. The bacteria were added to the incubation mixture 1 min (circles), 8 min (triangles) or 15 min (squares) after the bacteria. Values are means and SE from three incubations, minus the value for the solvent controls (137 ± 10 colonies in the absence of KCl, 112 ± 5 colonies in its presence). Where error bars are not visible, they fall within the symbol.

Table VI. Half-lives of the K-region oxides and imines of BA and alkylated congeners in aqueous media[a]

Compound	$t_{1/2}$ (min)	
	H_2O[b]	150 mM KCl[b]
4	110	100
5	32–40[c]	—[d]
8	11–14	9–13
9	7–9	4–4
10	30[c]	—[d]
11	12–15	7
6	30–32[c]	—[d]
7	7–8	4
12	4–5	3
13	0.5	0.6

[a]Determined from the decrease in mutagenicity after preincubating the test compound in the indicated medium for varying periods before the addition of the bacteria. In an initial experiment suitable preincubation times were sought, followed by one or several experiments in which this information was used. When several experiments gave conclusive results, the ranges of the results are shown. Detailed data for **9** are presented in Figure 4. The data for **10** are from Glatt *et al.* (1991).

[b]The exposure medium additionally contained 10 mM sodium phosphate buffer (pH 7.4), except for **10** which was investigated in the absence of any buffer.

[c]The decrease in mutagenicity did not follow first-order kinetics, the initial decrease being faster than the later decrease. The presented values refer to preincubation periods ranging from 1 min to 1 or 2 $t_{1/2}$. These compounds were not further investigated using a KCl-containing exposure medium.

[d]Not determined.

of 650, 760 and 750 revertants/nmol were extrapolated, whereas activities of 1800–2600 revertants/nmol were observed. When the bacteria were added to dilute buffer before BA-O, 820 to 1400 revertants/nmol were found. Thus, a part of the difference can be attributed to an influence of the buffer. The other part (reflecting the influence of whether the bacteria or the test compound is first added to the medium) is not elucidated (and may be related to potentially different modes of precipitation and adsorption to bacteria/glass tube of the hydrophobic chemicals). Further complications result from the observation that the shift of the dose–response curves was not always proportional to the difference in preincubation time, indicating deviations from first-order kinetics. For these reasons, the figures shown in Table VI should be used with some caution. Nevertheless, the data may allow the following conclusions: (i) the arene imines were shorter-lived than the corresponding arene oxides, the difference being the largest in the DMBA-O/DMBA-I pair; (ii) all alkyl substitutions reduced the stabilities; (iii) methylation at position 7 exerted stronger influence than methylation at position 1; (iv) 7-methyl substitution exerted stronger influence than 1-ethyl substitution; (v) the DMBA derivatives were the most reactive compounds; and (vi) addition of KCl (150 mM) appeared to reduce the half-lives of several compounds by up to a factor of 2. The influence was most clearly demonstrated with 7-MBA-I, which showed very consistent half-lives in four independent experiments with KCl-free medium (7–9 min) and two independent experiments with KCl-containing medium (4 min in both experiments). In contrast, addition of KCl showed little, if any, influence on the half-lives of the longest-lived compound (BA-O) and the shortest-lived compound (DMBA-I).

Discussion

The specific mutagenicities observed in the present study are among the highest ones reported for any compounds and any modifications of the Ames assay. In part, the high values can be explained by the fact that optimized assay conditions were used. For BA-O, BA-I, 7-MBA-O and 7-MBA-I, data from standard plate-incorporation assays with strains TA98 and TA100 are available (Glatt *et al.*, 1985, 1991). Specific mutagenicities under these conditions only amounted 0.3–5% of the corresponding values of the present study (using water as the exposure medium). Even if this circumstance is taken into account, the compounds have to be classified as potent mutagens in *Salmonella*. Moreover, they appear to induce a broad spectrum of mutations, as they were highly active in substitution-mutated strains (TA100 and TA104) and frameshift-mutated strains (TA97 and TA98), as well as in strains with hot spots in GC-rich sequences (TA97, TA98 and TA100) and AT-rich sequences (TA104).

The composition of the exposure medium strongly affected the mutagenic activities. We have already reported this observation for 7-MBA-O (Glatt *et al.*, 1991). Using various solutes and permutating anions and cations, it was found that monovalent cations were critical and that K^+ was the strongest antimutagen. Since KCl had the stronger antimutagenic effect with strain TA98 than with strain TA100, and since it had little influence on the stability of 7-MBA-O, we concluded that the medium exerted its influence on the bacteria rather than on the test compound. This notion is supported by results of the present study. With all investigated compounds, the influence of the medium was again stronger in strain TA98 than in strain TA100. Moreover, some of the test compounds were relatively stable in both media used. For example, the half-life of BA-O exceeded the usual exposure time of the assay 5-fold.

It is conspicuous that alkyl substitutions of BA-O and BA-I strongly influenced mutagenic activity and stability. For example, 7-MBA-O was 5–14 times more active under all conditions than either BA-O or 7-EBA-O. Also the bay region *anti*-diol-epoxide

of 7-MBA was ~10 times as mutagenic as its 7-EBA analog using various genetic end points in bacterial and mammalian cells (Glatt *et al.*, 1989).

The observation that methyl substitution of BA-O may enhance the rate of solvolysis was recently also reported by Nashed *et al.* (1991). They determined the second-order rate constants for the acid-catalyzed reaction and the first-order constant for spontaneous hydrolysis, as well as the structures of the reaction products. Moreover, using theoretical calculations on the stability of the carbocations, they showed that steric factors are important in determining the relative reactivity and the types of products formed from substituted BA-O. Steric factors were especially strong with substitutions in the bay region (positions 1 and 12), leading to pronounced distortion of the planarity of the aromatic systems, as can also be deduced from the NMR spectra. The half-lives determined in the present study for the arene oxides are consistent with the rate constants of Nashed *et al.* (1991). The half-lives of the arene imines were shorter than those of the corresponding arene oxides, but followed the same order with regard to substituent effects.

Interestingly, Nashed *et al.* (1991) found that the rates of hydrolysis of 7-MBA-O and DMBA-O were somewhat enhanced in the presence of a high concentration of KCl (0.5 M) than in 0.1 M $NaClO_4$. They propose that at high Cl^- concentrations a chlorohydrin intermediate may be formed which then may decompose to the *cis*-dihydrodiol. However, it is unlikely that this mechanism was a major factor determining the mutagenic response in the present study, since (i) $CaCl_2$ did not affect the mutagenicity of 7-MBA-O (Glatt *et al.*, 1991), (ii) KCl had little, if any, effect on the half-lives of several investigated compounds under our experimental conditions, (iii) KCl diminished the activity of rather stable compounds too and (iv) the extents of the influence of KCl varied with the bacterial target strain used.

In this study, we have demonstrated that an unobtrusive medium component can markedly influence mutagenic activities in *S.typhimurium*. The presence of KCl diminished the mutagenic activity of many other lipophilic mutagens too (H.R.Glatt, unpublished results), but strongly potentiated that of some ionized mutagens, in particular of sulfate esters (Glatt *et al.*, 1990; Enders *et al.*, 1993). In these cases, a chemical mechanism appears to be involved, i.e. the substitution of chloride for sulphate.

It was also demonstrated that a minor structural alteration, i.e. the substitution of a methyl or ethyl group on the aromatic system, may strongly influence the chemical and biological activities of arene oxides and imines. It is known that BA, its 12 possible monomethyl derivatives, 7-EBA and DMBA differ in their carcinogenic activity from apparently inactive to very potent, and also differ in their mutagenic activity (Stevenson *et al.*, 1965; Huggins *et al.*, 1967; Coombs *et al.*, 1976; Glatt *et al.*, 1981; Wislocki *et al.*, 1982; Utesch *et al.*, 1987). A number of mechanisms may be involved in these differences: (i) methylarenes may be activated to benzylic sulfate esters (Watabe *et al.*, 1982; Surh *et al.*, 1990, 1991), (ii) alkyl substituents may shift the region of metabolic attack (Thakker *et al.*, 1979; Yang *et al.*, 1981), (iii) alkyl substituents may affect the reversible binding of the active metabolites to the DNA, e.g. for steric reasons (Urano *et al.*, 1988), and (iv) alkyl substituents may alter the chemical reactivity of homologous metabolites (Melikian *et al.*, 1988; Nashed *et al.*, 1991; this study).

On the one hand, such complex influences by unobtrusive experimental and structural factors may impede the prediction of carcinogenic and mutagenic activities. On the other hand, they may potentially be exploited, e.g. in the development of drugs and in treatments to minimize carcinogenic and mutagenic activities.

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