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Novel 5,8-Diazabenzo[*c*]phenanthrenes: Synthesis and Mutagenicity

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Abstract

The polycyclic aromatic hydrocarbons have been recognized as carcinogens and mutagens since the early part of this century. More recently their aza and polyaza derivatives have been shown to have the same biological activity. A major source of these compounds is the combustion of fresh or metamorphosed plant materials; this contributes to the environmental burden of, and exposure to, these carcinogens. We report the synthesis and characterization of a series of novel 5,8-diazabenzo[*c*]phenanthrenes which are isosteric with the known epidermal carcinogen benzo[*c*]phenanthrene but have not yet been reported as components of soot or diesel particulate matter.

The synthesis of the compounds exploits a versatile, double Friedlander reaction between the appropriately substituted 2,2′-diaminobenzophenone and β-diketones, with yields of purified product ranging from 30–90%. The nucleophilic substitution of these diazabenzophenanthrenes with ethanolamine is also described. This strategy will enable further elaboration of these heterocyclic nuclei at a later date.

Mutagenicity testing of these agents was performed using spot tests and in Ames plate-incorporation assays using *Escherichia coli* WP2 and WP2uvrA as test organisms. The plate-incorporation assays were performed in the presence or absence of metabolic enzymes contained in the S9 liver fraction from Aroclor 1254-induced rats, to investigate whether bioactivation of the diazabenzophenanthrenes contributed to their toxicity. No differences between these two protocols were observed, with neither test showing reversion to prototrophic behaviour. Furthermore, the compounds were not toxic to the test organism.

These initial results suggest that these compounds are not mutagenic in the Ames tests employed.

It has long been recognized that the aza derivatives of carcinogenic polycyclic hydrocarbons can themselves act as carcinogens (Cook & Thomson 1945). More recently it has been shown that diaza derivatives are particularly pertinent in this context (Pai & Ranadive 1965; Buu-Hoi et al 1967, 1968). To extend our studies of such polycyclic compounds (Bloomfield et al 1986; Upton 1986, 1992; Tucker et al 1993a, b), a series of diazabenzo[*c*]-phenanthrenes has been prepared and screened, prompted by the isosteric relationship between benzo[*c*]phenanthrene **1** and its diaza analogue **2**. The carbocycle **1** has been shown to be an epidermal carcinogen (Barry et al 1935), to be present in cigarette tars (Severson et al 1977, 1978) and to occur as an airborne pollutant in industrialized countries (Lunde 1976; Lunde & Bjorseth 1977). The diaza analogue **2** has been reported to cause tumours in-vivo (Partridge & Vipond 1962) and there are indications that this polycyclic nucleus also has affinity for nucleic acids. The presence of such polycyclic derivatives in diesel emissions (Pitts et al 1978; IARC 1989) and their mutagenic nature (Barale et al 1989; Crebelli 1991) is a matter of environmental concern. We are examining diesel particulate matter for the presence of these compounds and have developed gas chromatographic (GC) and gas chromatography-mass spectrometry (GC-MS) procedures for their characterization and identification.

The reaction of diamines **3–5** with the appropriate β-diketone has given access to the series of benzophenanthrenes **6–22** which have been prepared to examine structure–activity relationships

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(Figure 1). Substituents that differ in their electronic and lipophilic properties have been selected. The electronic parameters of the selected substituents (OH, OCH_3, Cl and alkyl) vary widely when assessed using two-dimensional Craig plots (Craig 1980). Each different substituent also varies in the balance of inductive to mesomeric effects attributed to that substituent. (Theoretical log P values of selected phenanthrenes were calculated using Interactive Lab Beta 3 software produced by the Advanced Chemical Development Company. The values for the 6,7-unsubstituted compounds **2**, **11** and **16** and the acephenanthrylene **26** are $3{\cdot}18 \pm 0{\cdot}72$, $4{\cdot}68 \pm 0{\cdot}75$, $3{\cdot}68 \pm 1{\cdot}61$ and $5{\cdot}13 \pm 0{\cdot}77$, respectively.) The reaction of the 6-methyl substituent in **8** to give the styryl analogue **23** confirms the reactivity of this substituent and the preparation of the 6-hydroxyethylaminobenzophenanthrene **24** has been performed for elaboration at a later stage in subsequent synthetic work.

These polycyclic nuclei have been prepared to act as the message component of more complex structures which, with appropriate spacer fragments, have been designed to target tumour cells exploiting an address-message concept, first discussed in work describing bivalent ligands for targeting opiate receptor subtypes (Portoghese 1989; Portoghese et al 1990). We have adopted this approach successfully in work on indolocodones and indolomorphones (Maguire et al 1993) investigating the mechanism of δ-selectivity at opioid receptors.

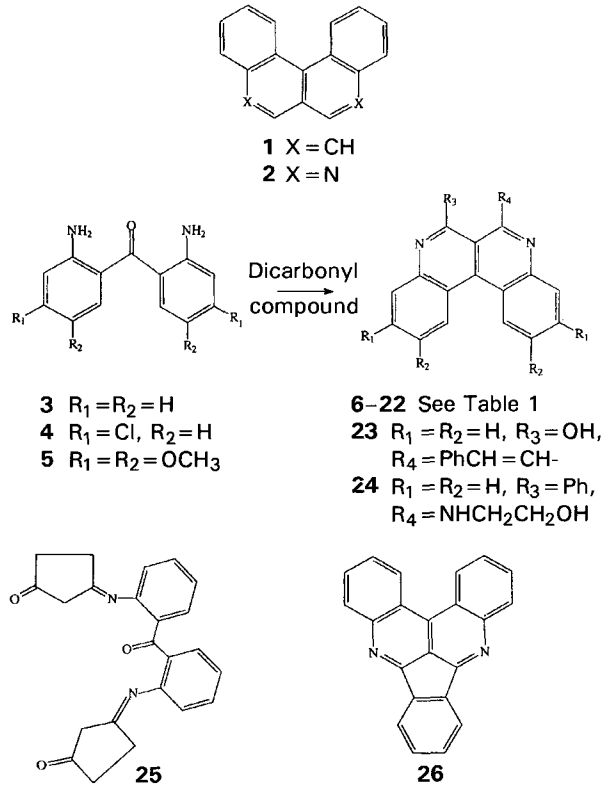

Figure 1. Synthesis of 5,8-diazabenzo[*c*]phenanthrenes.

Previous syntheses of this diazabenzophenanthrene nucleus have been reported (Kempter & Klug 1971; Walser et al 1975). These groups used oximes of 9-phenyl-1-acridones and substituted 4-(2-fluorophenyl)quinoline 3-carboxylates, respectively, but these relatively inaccessible starting materials are of limited synthetic scope and applicability and do not offer a convergent route to an extended series of these structures. In another report (Gulland & Robinson 1925) the preparation of 3,10-diamino-6,7-dimethyl-5,8-diazabenzo[*c*]-phenanthrene from the corresponding tetraamine was described. We have adopted the method reported earlier (Upton 1986) which offers access to the required range of target compounds.

Materials and Methods

Experimental

Chemistry. Melting points were determined using a Gallenkamp melting-point apparatus and are uncorrected. ^{1}H NMR spectra were recorded using a Jeol GX270MHz FT spectrometer from solutions in deuterochloroform unless otherwise stated; J values are given in Hz, signals described as br are broadened. ^{13}C NMR spectra were recorded at 67·8 MHz; signals described as t or q were tertiary or quaternary, respectively. Ultraviolet spectra were recorded using a Perkin-Elmer Lambda 3B UV/vis spectrophotometer and IR spectra with a Perkin-Elmer 782 infrared spectrophotometer. Microanalyses were performed by the University of Bath microanalysis service and mass spectrometry (electron-impact (EI) or chemical ionization (CI)) by the University of Bath Mass Spectrometry Service. Thin-layer chromatography was performed on silica gel 60 F_{254} plates from Merck; plates were visualized by illumination at 254 nm.

Hexane-2,4-dione (Adams & Hauser 1944), 2,2′-diamino-4,4′-dichlorobenzophenone (Spalding 1946) and 2,2′-diaminobenzophenone (Partridge & Vipond 1962) were prepared by published methods. During this synthesis the proton spectrum of 2,2′-dinitrobenzophenone was obtained to confirm the structure of this material; its NMR data were consistent with the assigned structure: δ 7·66 (2H ,dd, J 7·3 and 1·5, 6- and 6′-H), 7·88 (4H, m, 4-,4′-,5-,5′-H), 8·16 (2H, dd, J 8·1 and 1·6, 3- and 3′-H). Spectroscopic data for its precursor 4,4′-diamino-2,2′-dinitrodiphenylmethane dihydrogen sulphate

were δ_H (270 MHz; DMSO-d_6), 4·32 (2H, s, $ArCH_2Ar$), 6·1 (6H, br s, NH_3), 7·10 (2H, d, J 8·4, 6- and 6′-H), 7·19 (2H, dd, J 8·1 and 2·2, 5- and 5′-H), 7·59 (2H, d, J 2·2, 3- and 3′-H). Data for the title diamine were δ_H (270 MHz; $CDCl_3$), 5·4 (4H, br s, 2- and 2′-amino, disappears on deuteration), 6·63 (2H, td, J 8·0 and less than 1, 5- and 5′-H), 6·7 (2H, d, J 8·0, 3- and 3′-H), 7·24 (2H, td, J 8·1 and 1·4, 4- and 4′-H), 7·35 (2H, dd, J 8 and 1·3, 6- and 6′-H). δ_c (68 MHz; $CDCl_3$) 119·3 q, 123·2 q, 127·2, 127·7, 130·4, 130·7, all t, 135·3 q, 148·0 q, 151·3 t.

2,2′-Diamino-3,3′,4,4′-tetramethoxybenzophenone (Lawson et al 1924). The precursor for this ketone, 3,3′,4,4′-tetramethoxybenzophenone was prepared (85%) by heating 3,4-dimethoxybenzoic acid (54g, 0·296mol) and 1,2-dimethoxybenzene (41g, 0·297mol) in polyphosphoric acid (225g) for 0·5h at 80°C, pouring on to ice (3kg), neutralizing and recrystallizing from ethanol, mp 142–144°C (mp 144°C, by the methylation of 3-hydroxy-3′,4,4′-trimethoxybenzophenone (Ayres & Denney 1961)).

6-Ethyl-7-methyl-5,8-diazabenzo[c]phenanthrene **7**. A solution of 2,2′-diaminobenzophenone (2·0g, 9·4mmol) and freshly distilled hexane-2,4-dione (2·0g, 17·5mmol) in acetic acid (20cm^3) was heated at 110°C for 90min and poured into water (100cm^3). The precipitate was collected, washed (water), dried, and recrystallized from petroleum ether (bp 60–80°C) to furnish the title phenanthrene, **7**, as bright yellow needles (1·159g, 49%), mp 113–114°C (found: C, 83·76; H, 6·03; N, 10·43; $C_{19}H_{16}N_2$ requires C, 83·79; H, 5·92; N, 10·29%); λ_{max} (EtOH) nm 217, 265, 298s, 328s, 356, 374; δ_H (270 MHz; $CDCl_3$), 1·37 (3H, t, J 7·3, 7-Et), 3·27 (3H, s, 7-CH_3), 3·6 (2H, q, J 7·3, 7-Et), 7·63 (2H, t, J 8·5, 2-/11- or 3-/10-H), 7·85 (2H, t, J 8·5, 2-/11- or 3-/10-H), 8·2 (2H, d, J 8·5, 1-and 12-H), 8·8 (2H, d, J 8·5, 4- and 9-H); m/z 272 (M^+, 17%). The picrate, prepared by treating a hot ethanolic solution of the base with an equimolar weight of picric acid in ethanol, was obtained as yellow rosettes (from ethanol), mp 210–214°C. (Found: C, 59·98; H, 3·85; N, 13·94; $C_{25}H_{19}N_5O_7$ requires C, 59·87; H, 3·81; N, 13·96%). Benzophenanthrenes **6–22** were prepared in the same way or as described in the literature; analytical data for all novel compounds are listed in Table 1. The following NMR data are characteristic and representative of the remaining compounds: 6,7-dimethyl in **6**, **12**, **17** δ 3·2, s; 2,3,10,11-tetramethoxy in **16** to **22** δ 4·12, 2s; 7-ethyl in **7**, **13**, **18** δ 1·45, t, J 7·1 (CH_3), 3·47, q, J 7·1 (CH_2); 6-methyl in **8**, **14**, **19** δ 3·6, s; 7,7-dimethyl in **10**, **15**, **21** δ 1·2, s; 6H, δ 3·3, 4H, s; CH_2 at 6,8. In **9** and **20** the CH_2 at 6 and 8 resonates at δ 3·4, t, and that at 7 resonates at δ 2·3, m. Ultraviolet data are given in Table 2 and analytical data are given in Table 1.

6-(2-Hydroxyethylamino)-7-phenyl-5,8-diazabenzo[c]phenanthrene **24**. A solution of 6-chloro-7-phenyl-5,8-diazabenzo[*c*]phenanthrene (Upton 1986) (1·5g, 4·4mmol) in ethanolamine (8mL) was heated at 160°C for 0·5h and then poured into water (20mL). The residue was extracted into toluene (charcoal), and petroleum ether (bp 100–120°C) was added to the hot solution to induce precipitation. On cooling the title alcohol **24** was obtained as bright yellow needles (1·07g, 66%) mp

Table 1. Elemental analyses (% C,H,N) for novel 5,8-diazabenzo[*c*]phenanthrenes.

	Compound	C Found	C Requires	H Found	H Requires	N Found	N Requires
7	$C_{19}H_{16}N_2$	83·8	83·8	6·0	5·9	10·4	10·3
11	$C_{16}H_8Cl_2N_2$	64·0	64·2	2·9	2·7	9·5	9·4
12	$C_{18}H_{12}Cl_2N_2$	66·0	66·1	3·5	3·7	8·7	8·6
13	$C_{19}H_{14}Cl_2N_2$	66·6	66·9	3·9	4·1	8·6	8·2
14	$C_{17}H_{10}Cl_2N_2O$	62·0	62·0	3·0	3·1	8·5	8·5
15	$C_{21}H_{16}Cl_2N_2$	68·7	68·9	4·1	4·4	7·6	7·7
16	$C_{20}H_{18}N_2O_4$	68·5	68·6	5·2	5·2	8·1	8·0
18	$C_{23}H_{24}N_2O_4$	70·2	70·4	6·2	6·2	7·1	7·1
20	$C_{23}H_{22}N_2O_4$	70·6	70·8	5·7	5·7	7·1	7·2
21	$C_{25}H_{26}N_2O_4$	71·7	71·8	6·3	6·3	6·4	6·7
22	$C_{22}H_{20}N_2O_4{}^1/_2H_2O$	68·1	68·6	5·6	5·5	6·9	7·3
7	Picrate, $C_{25}H_{19}N_5O_7$	60·0	59·9	3·6	3·8	13·8	14·0
14	Picrate, $C_{23}H_{13}Cl_2N_5O_8$	49·8	49·6	2·4	2·2	12·8	12·6
16	Picrate, $C_{26}H_{21}N_5O_{11}$	53·7	53·9	3·7	3·7	12·1	12·1
16	HCl: $C_{20}H_{19}ClN_2O_4$	62·0	62·2	5·0	4·9	7·4	7·2
20	Picrate, $C_{29}H_{25}N_5O_{11}$	56·5	56·2	4·1	4·0	11·0	11·3
21	Picrate, $C_{31}H_{29}N_5O_{11}$	57·8	57·5	4·6	4·5	11·0	10·8
23	$C_{24}H_{16}N_2O$	82·5	82·8	4·4	4·6	8·3	8·1
24	$C_{23}H_{20}N_2O_3$	74·2	74·2	5·5	5·4	7·5	7·5

124–126°C; δ_H (100 MHz; $CDCl_3$) 3·0–3·6 (5H, m, NCH_2CH_2OH, signal integral reduced to 4H after deuteration), 5·0 (1H, br s, NH, disappears on deuteration), 7·3–7·85 (9H, m, 7Ph, 2-,3-,10-, 1-H), 8·1-8·2 (2H, m, 1-,12-H), 8·8–8·95 (2H, m, 4-,9-H); m/z 365 (M^+,18%), 334 (37, M-CH_2OH), 320 (100, M-CH_2CH_2OH), 305 (25, M-$NHCH_2CH_2OH$); HRMS EI $C_{24}H_{19}N_3O$ found 365·1520 (M calc. 365·1528).

2,2′-Bis(3-oxocyclopentylimino)benzophenone **25**. A solution of 2,2′-diaminobenzophenone (2·8g, 13·2mmol) and cyclopentane-1,3-dione (2·0g, 20mmol) in acetic acid (20cm^3) was heated under reflux for 1h and the precipitate which formed on cooling was recrystallized from dimethylformamide to give the benzophenone **25** (2·2g, 58%), mp decomp. >·2300°C. (Found C, 74·23; H, 5·44; N, 7·50; $C_{23}H_{20}N_2O_3$ requires C, 74·19; H, 5·38; N, 7·53%; δ_H (270MHz, trifluoroacetic acid) 2·75 (8H, br s, 4-,4′-,5-,5′-CH_2), 2·97 (4H, s, 2-,2′-CH_2), 6·67 (2H, d, J 7·7, 3- and 3′-H), 7·01 (4H, m, 5-,5′-,6-,6′-H), 7·10 (2H, t, J 7·4, 4- and 4′-H); m/z (EI) 354 (100%, M-18).

*9,14-Diazabenz[*a,e*]acephenanthrylene* **26**. This phenanthrylene was prepared as described previously (Upton 1986). δ_H (270MHz; $CDCl_3$) 7·55 (2H, 6-line multiplet, 10- and 13- or 11- and 12-H), 7·71 (2H, td, J 8·1, and 1·6, 2- and 7- or 3- and 6-H), 7·82 (2H, td, J 8·0 and 1·4, 3- and 6- or 2- and 7-H), 8·17 (6-line multiplet, 11- and 12- or 10- and 13-H), 8·34 (2H, dd, J 8·1 and 1·5, 4- and 5-H), 9·0 (2H, dd, J 8·4 and 1·2, 1- and 8-H).

Biology. Spot tests were performed as described by Bridges (1972). Briefly, overnight cultures of *Escherichia coli* WP2 and WP2uvrA were grown from refrigerated samples to a concentration of 1–2×10^9 bacteria cm^{-3} in standard nutrient broth, harvested and suspended in phosphate buffer. The bacterial suspension was applied to the surface of minimal agar plates, the potential mutagen applied either to the surface of the plate as a solid or to a central well as a saturated solution in aqueous dimethylformamide and after incubation for 48h at 37°C the revertant, prototrophic organisms were counted. Positive controls containing methyl methanesulphonate, a recognized mutagen for these organisms, and negative controls, which omit the test compounds, were established. Plates containing the test organism but no tryptophan demonstrated the dependence of the bacteria on this externally-supplied amino acid. All tests were performed in triplicate.

For the standard plate-incorporation assay the revised method (Maron & Ames 1983) was used. The test strain *E. coli* WP2uvrA was selected for this assay as it is the organism used by most pharmaceutical companies world-wide (Purves et al 1993). The bacteria and benzophenanthrene in soft agar were poured on to the surface of a minimal agar plate. Another plate containing an Aroclor 1254-induced liver S9 fraction was used to examine how these metabolizing enzymes affected the outcome. Positive controls, with 2-aminofluorene as diagnostic mutagen, and negative controls were again used and all tests were performed in triplicate. Incubation and revertant scores followed.

Results and Discussion

Chemistry

2,2′-Diaminobenzophenone was prepared as described previously (Partridge & Vipond 1962). In that report a discrepancy between their melting

Table 2. Absorption maxima and molar absorption coefficients for 5,8-diazabenzo[*c*]phenanthrenes*.

Compound	Absorption maxima	Molar absorption coefficients (log ε)
2	213, 268, 317 (s), 329 (s), 349, 366	4·41, 4·72, 3·96, 3·74, 3·72, 3·66
6	217, 265, 294 (s), 337 (s), 355, 372	4·51, 4·55, 4·23, 3·53, 3·48, 3·47
7	217, 265, 298 (s), 328 (s), 356, 374	4·61, 4·62, 4·28, 3·76, 3·10, 3·10
8†	241, 259, 313 (s), 366, 382	4·47, 4·57, 3·65, 3·90, 3·87
10†	217, 269, 295 (s), 329 (s), 336, 353, 371	4·14, 4·65, 4·22, 3·60, 3·60, 3·75, 3·81
11	215, 231 (s), 276, 317 (s), 352, 368	4·65, 4·12, 4·77, 4·01, 3·66, 3·68
13	220, 239, 274, 329 (s), 358, 375	4·53, 4·20, 4·51, 3·71, 3·56, 3·60
14†	227, 263, 319, 334, 354 (s), 367, 383	4·38, 4·65, 3·78, 3·77, 3·88, 4·04, 4·00
15	219, 230 (s), 277, 300 (s), 339 (s), 356, 374	4·74, 4·25, 4·82, 4·22, 3·74, 3·83, 3·93
16	226, 239 (s), 290, 321 (s), 352, 370, 389	4·62, 4·14, 4·75, 4·16, 4·74, 4·10, 4·30
17	228, 244 (s), 288, 329 (s), 355, 374, 393	4·58, 4·14, 4·64, 3·98, 3·66, 3·98, 4·12
18	228, 241 (s), 288, 356, 375, 394	4·55, 4·19, 4·57, 3·65, 3·90, 4·01
20	227, 241, 288, 355, 372, 392	4·62, 4·16, 4·73, 3·75, 4·13, 4·33
21	226, 240 (s), 288, 316 (s), 354, 372, 392	4·66, 4·17, 4·80, 4·28, 3·84, 4·20, 4·39

*Determined in 95% ethanol unless otherwise stated· †Determined in 1 : 1 ethanol–ethoxyethanol.

point for 2,2′-dinitrobenzophenone and that reported by Rose (1932) was noted. Repeating their work we have shown that the structure proposed by Partridge & Vipond is supported by its proton spectrum, as are all the intermediates in this sequence. A double Friedlander condensation of equimolar amounts of diamine and β-diketone in acetic acid furnished the required phenanthrenes **6**–**22** (Table 3). Some compounds prepared for the biological part of the programme have been reported previously but without supporting NMR data; these are included here and representative spectra are discussed. The ultraviolet spectra of fourteen of these diazabenzophenanthrenes were determined and are presented in Table 2. As a group, these 5,8-diazabenzophenanthrenes show the characteristic spectral bands expected of angular polycyclic compounds (Corbett et al 1962). It has been shown (Badger & Walker 1956) that the UV spectra of many aza and polyazapolycyclic compounds closely resemble their carbocyclic analogues, the main changes being a shift in the absorption to longer wavelength, a loss of fine structure and an increase in intensity at the longer wavelengths. When compared with the published spectrum of benzo[*c*]phenanthrene **1** (Clar & Stewart 1952) an increase in intensity was observed at longer wavelengths but in the unsubstituted 5,8-diazabenzophenanthrene **2** and its dichloro analogue **11** slight hypsochromic shifts are noted (λ 213 and 215 nm, respectively, compared with 218 nm in the carbocycle). Corbett et al (1961) also noted slight hypsochromic shifts in some polycyclic cinnolines. In the tetramethoxy analogue **16** a general and expected bathochromic shift in the spectrum is seen, along with marked hyperchromic effects at longer wavelengths.

The reactivity of the 7-methyl substituent in phenanthrene **8** was confirmed by its condensation with benzaldehyde to give 6-hydroxy-7-styryl-5,8-diazabenzo[*c*]phenanthrene **23**. The strongly deshielded alkenic protons resonated at δ 8·91 and 8·89, J 17·2 Hz, confirming the ene geometry as E. 2,2′-Diaminobenzophenone **3** reacted smoothly with cyclohexane-1,3-dione and dimedone as did diamines **4** and **5**. However, unlike **5** it failed to condense with cyclopentane-1,3-dione to give the corresponding acephenanthrylene. The reaction gave a single product (thin-layer chromatography, TLC) with an elemental analysis for $C_{23}H_{20}N_2O_3$, indicating that two moles of dione had condensed with one of diamine; the electron-impact (EI) mass spectrum confirmed this (m/z 372 (M^+, 8%), 354, (100%, M - H_2O)) and the IR spectrum indicated two strong carbonyl groups (1655 and 1725 cm^{-1}). A structure **25** is proposed and is comparable with the result reported by Parfitt (1966). This failure to cyclize under a variety of conditions also contrasts with the successful condensation of this amine with indane-1,3-dione (described below).

We were unable to convert the 6-hydroxyphenanthrene **8** directly to the 6-amino compound for subsequent coupling reactions using phenylphosphorodiamidate, as described by Rosowsky & Papathanasopoulos (1972). The authors suggested direct replacement of oxygen by nitrogen of the diamidate. However, other reports of success with this reagent with cytosines and

Table 3. Synthesis of 5,8-diazabenzo[*c*]phenanthrenes.

Diamine	Dicarbonyl compound	Product	R_1	R_2	R_3	R_4	Reaction time (min)	Yield (%)	Mp (°C)	Recryst. solvent*	Elemental anal. or lit. ref.
3	1,3-Malonaldehyde[b]	(**2**)	H	H	H	H	60	64	170–172	p	Partridge & Vipond (1962)
	Pentane-2,4-dione	(**6**)	H	H	Me	Me	60	57	162–163	a	Partridge & Vipond (1962)
	Hexane-2,4-dione	(**7**)	H	H	Me	Et	90	49	113–114	p	CHN[c]
	Ethyl acetoacetate	(**8**)	H	H	Me	OH	60	79	333–336 (d)	d	Parfitt (1966)
	Cyclohexane-1,3-dione	(**9**)	H	H	1,3-Propano		30	69	179–181	e	Parfitt (1966)
	Dimedone	(**10**)	H	H	2,2-Dimethyl-1,3-propano		90	72	170–172	a	Partridge & Vipond (1962)
4	1,3-Malonaldehyde[b]	(**11**)	Cl	H	H	H	60	51	278–281	e	CHN[d]
	Pentane-2,4-dione	(**12**)	Cl	H	CH_3	CH_3	60	46	207–208	a	CHN
	Hexane-2,4-dione	(**13**)	Cl	H	CH_3	Et	30	48	117–119	b	CHN
	Ethyl acetoacetate	(**14**)	Cl	H	CH_3	OH	60	45	318–320	e	CHN[e]
	Dimedone	(**15**)	Cl	H	2,2-Dimethyl-1,3-propano		90	48	216–218	e	CHN
5	1,3-Malonaldehyde[b]	(**16**)	OCH_3	OCH_3	H	H	60	63	270–274	t	CHN[f]
	Pentane-2,4-dione	(**17**)	OCH_3	OCH_3	CH_3	CH_3	60	88	228–229	e	Ayres & Denney (1961)
	Hexane-2,4-dione	(**18**)	OCH_3	OCH_3	CH_3	Et	30	51	185–187	e	CHN
	Ethyl acetoacetate	(**19**)	OCH_3	OCH_3	CH_3	OH	60	90	320–326 (d)	d	Ayres & Denney (1961)
	Cyclohexane-1,3-dione	(**20**)	OCH_3	OCH_3	1,3-Propano		30	36	244–245	e	CHN[g]
	Dimedone	(**21**)	OCH_3	OCH_3	2,2-Dimethyl-1,3-propano		80	60	246–249	a	CHN[h]
	Cyclopentane-1,3-dione	(**22**)	OCH_3	OCH_3	1,2-Ethano		240	36	284–290 (d)	e	CHN

*a = Acetone; b = 1-butanol; d = dimethylformamide; e = ethanol; p = light petroleum (bp 100–120°C); t = toluene. [b]As 1,1,3,3-tetramethoxypropane, [c]Mp picrate 210–215°C (decomp. CHN), [d]high resolution EIMS $C_{16}H_8Cl_2N_2$ found 298·0060 (M calc 298·0064), [e]mp picrate 345–350°C (decomp.), [f]mp picrate 290–295°C (CHN), HCl 266–269°C (CHN), [g]mp picrate 243–246°C (CHN), [h]mp picrate 247–249°C (decomp. CHN).

Table 4. Results of reversion assay with 5,8-diazabenzo[*c*]-phenanthrenes (**2** and **6–23**).

Compound	Concn* (% w/v)	Average number of revertants (colonies/plate †)	
		WP2	WP2uvrA
Methyl methane-sulphonate	1·0 μL	104	118
Controls	0·5 mL	7	9
2	$4{\cdot}9 \times 10^{-2}$	1	2
6	$2{\cdot}4 \times 10^{-2}$	3	6
7	$4{\cdot}9 \times 10^{-2}$	6	11
8	$3{\cdot}4 \times 10^{-2}$	1	3
9	$2{\cdot}8 \times 10^{-2}$	5	6
10	$5{\cdot}1 \times 10^{-2}$	8	10
11	$0{\cdot}9 \times 10^{-2}$	2	4
12	$1{\cdot}3 \times 10^{-2}$	2	6
13	$1{\cdot}8 \times 10^{-2}$	3	5
14	$1{\cdot}4 \times 10^{-2}$	2	3
15	$1{\cdot}4 \times 10^{-2}$	3	4
16	$3{\cdot}8 \times 10^{-2}$	1	2
17	$2{\cdot}2 \times 10^{-2}$	2	4
18	$4{\cdot}4 \times 10^{-2}$	5	8
19	$2{\cdot}5 \times 10^{-2}$	3	4
20	$1{\cdot}3 \times 10^{-2}$	3	5
21	$5{\cdot}0 \times 10^{-2}$	7	10
22	$0{\cdot}8 \times 10^{-2}$	1	2
23	$4{\cdot}0 \times 10^{-3}$	2	5

*As a saturated solution in 28% (v/v) aqueous dimethylformamide. †Mean of three experiments.

quinazolines (Kroon & van der Plas 1974) supported the contention that the introduced amino group is derived, in part at least, from one of the two endocyclic nitrogens and so indirect methods of replacement of oxygen by nitrogen have been developed on the basis of previously prepared compounds. We have shown (Upton 1986) that the halogen in 6-chloro-7-phenyl-5,8-diazabenzo[*c*]-phenanthrene can undergo nucleophilic displacement by aromatic amines and have extended this reactivity to include aliphatic amines by reaction with 2-aminoethanol. These derivatives represent the starting point for later elaboration into bidentate ligands for DNA, similar to those described by Kang & Rokita (1996).

Biology

There has been no systematic study of the mutagenic and genotoxic properties of this series of diazabenzophenanthrenes. Partridge & Vipond (1962) reported that **2** and **26** cause tumours in-vivo. In a recent report by Mersch-Sundermann et al (1992) results of a theoretical study of 37 aromatic polycycles, designed to identify molecular fragments related to mutagenic potential, demonstrated that compound **1** was the second most potent in its ability to elicit activity in the *E. coli* PQ37 SOS test. This compound contained six of the nine molecular fragments associated with this activity and was only marginally less active than dibenzo[*a,l*]pyrene, the most active in the series. The isosteric relationship between **1** and **2** provides the rationale for this work. The Ames test (Ames et al 1975) and its modifications (Maron & Ames 1983) have become well established as reliable indicators of mutagenesis; Bridges (1981) has demonstrated that *E. coli* WP2uvrA is comparable with the Ames *Salmonella* strains for detecting mutagens and carcinogens. It is for this reason that we have chosen *Escherichia coli* for this work. Others have recently used this strain or its equivalent in this context (Barrueco et al 1991; Procinska et al 1991; Niphadkar et al 1996). Ames et al (1975) recommended the spot test as an initial and rapid means of screening larger numbers of compounds. This test also gives a preliminary indication of the toxicity of the compounds under test; it is manifested as a zone of inhibition of growth of the background bacterial lawn around the point of application of the putative mutagen. The tester strains used in this part of the work were *E. coli* WP2 (excision repair proficient) and its

Table 5. Mutagenicity results using benzophenanthrenes **2**, **11**, **16** and **26** against *E. coli* WP2uvrA in plate-incorporation assay.

Sample		In the presence of S9			In the absence of S9		
2	Dose (μg/plate)*	565	56	0·56	565	56	0·56
	Revertant organisms†	7·3±2·5	5·3±3·1	7·0±2·0	1·3±1·2	3·7±1·5	6·3±0·6
11	Dose	35·7	5·1	0·51	35·7	5·1	0·51
	Revertants	1·7±0·6	3·7±2·5	4·0±1·0	2·3±·6	2·7±1·5	3·3±0·6
16	Dose	144	24·0	0·48	144	24·0	0·48
	Revertants	2·7±0·6	5·3±4·6	4·7±1·5	2·7±1·5	2·7±0·6	1·0
26	Dose	33·5	6·7	0·67	33·5	6·7	0·67
	Revertants	3·7±2·8	7·0±1·0	5·67±1·2	3·3±1·5	4·0±1·7	7·0±1·0
Methyl methanesulphonate‡	1·0 μL	47·3±11·7	–	–	50·0±6·2	–	–
Dimethylsulphoxide alone§	10 μL	7·7±1·5	–	–	3·3±0·6	–	–

*The first dose for each compound represents the precipitating dose (as described by Gatehouse et al (1994)). †Results of triplicate determinations at each dose used; a uniform background bacterial lawn was observed in all cases. ‡ Used as a positive, diagnostic mutagen. §Negative control plate to determine spontaneous reversion rates.

derivative *E. coli* WP2uvrA (excision repair deficient), first described by Witkin (1956). A reversion assay for tryptophan dependence is exploited (Bridges 1972) in which the mutagen is applied to a minimal agar plate containing the tester strain. The benzophenanthrenes were applied either as solids to the surface of the agar immediately after pouring or as a saturated solution in 28% (v/v) aqueous dimethylformamide (this solvent was identified by Maron et al (1981) as acceptable in such tests). The results for both series of tests were very similar (those for the reversion assay test are shown in Table 4) and suggest that these diazabenzophenanthrenes are non-mutagenic in these tests. No significant increases in the numbers of revertants were seen in any of the test plates when compared with the negative controls for spontaneous revertants. No evidence of toxicity of these benzophenanthrenes appeared, as the background lawn was regular and consistent in each case and no zones of inhibition in the lawn appeared. The positive controls showed a large number of prototrophic organisms, confirming their susceptibility to the diagnostic mutagen and an absence of any growth on the plates that were tryptophan-deficient demonstrated that the organisms were tryptophan-dependent. However, as Ames et al (1975) notes, negative results in spot tests alone are not a sufficient indicator of non-mutagenicity and increased sensitivity can be achieved in a plate-incorporation assay. Selected benzophenanthrenes (**2**, **11**, **16** and **26**) were screened using this method both in the presence and absence of Aroclor-induced mammalian S9 liver fraction. This activating enzyme was included because of the report (Partridge & Vipond 1962) of the in-vivo carcinogenicity of **2** and **26** which suggests that metabolic activation might play a role in this activity. The results are shown in Table 5 and again suggest that these tetra- and hexacyclic benzophenanthrenes are not mutagenic in this test system, either in the presence or absence of the metabolizing enzymes contained in S9. Evidence of their non-toxicity is demonstrated by the presence of a background lawn seen in all plates, testing them up to the dose at which visible signs of precipitation occurs as recommended by Gatehouse et al (1994). The sensitivity of the organism is confirmed by the inclusion of positive control plates where high numbers of revertants are seen when exposed to methyl methanesulphonate. However, the report of in-vivo tumorogenicity suggests that further work might reveal mutagenic properties in this series.

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