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Pyrroloindole Analogues of Ellipticine. The Synthesis and DNA Intercalative Properties of 5,10-Dihydro-5,10-dimethyl-2,5,10-triazadibenzo[*a*,*e*]pentalene†

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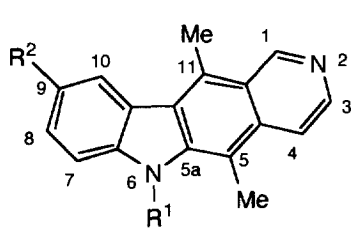
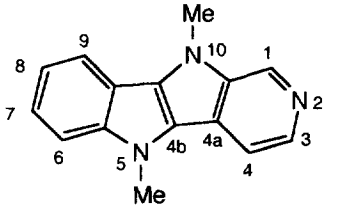
Abstract: The title compound, **2**, and its 2-methyl salt, **8**, have been synthesised from methyl 1,4-dihydro-4-methylpyrrolo[3,2-*b*]indole-2-carboxylate. The pK_a of the conjugate acid of **2** is similar to those of ellipticine and its 9-hydroxy and 9-methoxy derivatives, and intercalation studies with DNA show that **2** and **8** have association constants comparable with the anti-cancer ellipticine derivatives.

Introduction

The synthesis of the alkaloid ellipticine and its 9-oxygenated derivatives **1a** - **1c** have attracted widespread interest[1,2] as a result of their anti-tumour activities.[3] It is generally accepted that the ability of ellipticine to intercalate into DNA[4,5] accounts for its biological activity and the synthesis of many analogues of the ellipticine system have been described. In virtually all of these analogues, it is the peripheral substituents, and notably those at the 1-, 2-, 5-, 9- and 11-positions,[6] which have been modified and, although there appears to be sound evidence that substituents at these positions are significant in the overall activity of ellipticine





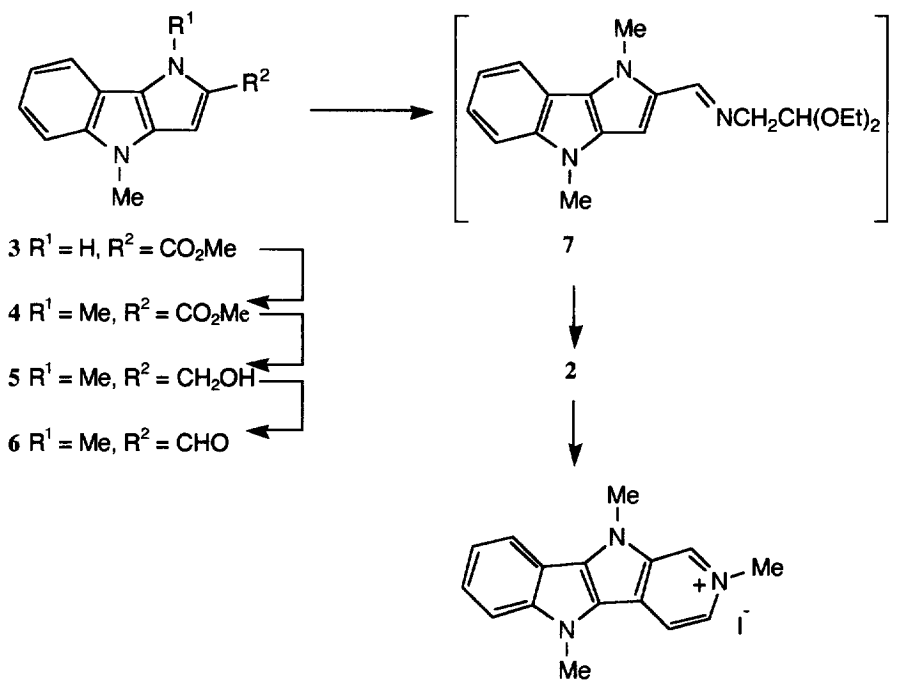
1a R^1 = H, R^2 = H; **1b** R^1 = H, R^2 = OH; **2**
1c R^1 = H, R^2 = OMe; **1d** R^1 = Me, R^2 = H;

derivatives, relatively little synthetic work has been carried out on the aza analogues,[7] and little importance has been placed on the shape of the molecule or on the basicity of the pyridine nitrogen atom.

In this communication, we report the synthesis of the pyrroloindole analogue **2** of the ellipticine system **1d** in which the general shape of the system has been made more linear, compared with the ellipticine structure, as a result of the introduction of the pyrrole ring in place of the benzene ring.

Results and Discussion

N-Methylation of the known pyrrolo[3,2-*b*]indole system **3**[8] to give **4** was readily accomplished in high yield either by the standard procedure using sodium hydride/iodomethane or by a milder phase-transfer catalysed reaction. The ester **4** was converted into the corresponding formyl derivative **6** *via* the carbinol **5**, which was oxidised either using activated manganese dioxide or by a Swern oxidation in 72% and 19%, respectively. Reaction of **6** with aminoacetaldeyhyde diethylacetal gave the imine **7**, which was converted without isolation into the 2,5,10-triazadibenzo[*a*,*e*]pentalene **2** upon treatment with orthophosphoric acid. Quaternisation of **2** with iodomethane produced **8**.



The pKa of the conjugate acid of **2** was found to be 6.65 ± 0.1,[9] which is close to the observed values of 6.8 and 7.4 for 9-methoxyellipticine **1c**[4] and ellipticine **1a**,[10] and 6.45 and 6.1 for their respective 5-methyl derivatives.[4] Molecular modelling calculations[11] on the two systems **1a** and **2** showed an increase of 24.3° for the improper C8-C4b-N2 angle of the pyrroloindole system, compared with the corresponding improper C9-C5a-N2 angle for ellipticine, confirming the expected greater degree of linearity of the four-ring system **2**.

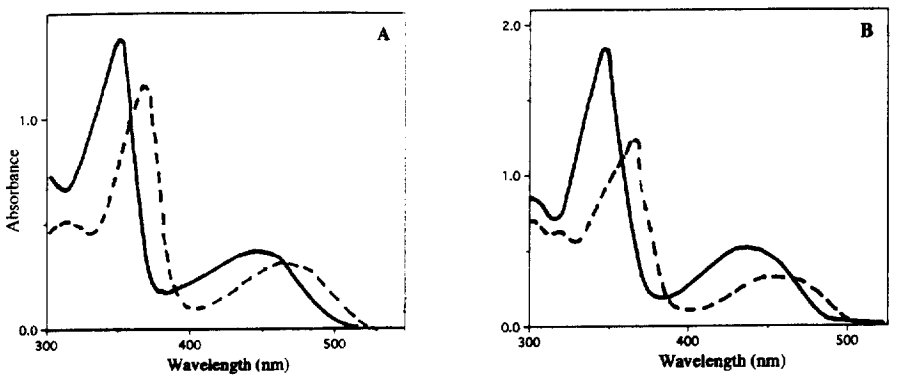


Figure 1. Electronic spectra of (A) 5,10-Dihydro-5,10-dimethyl-2,5,10-triazadibenzo[*a*,*e*]-pentalene and (B). 5,10-Dihydro-2,5,10-trimethyl-2,5,10-triazadibenzo[*a*,*e*]pentalenium iodide. Free ligand ——— Complexed with DNA – – –

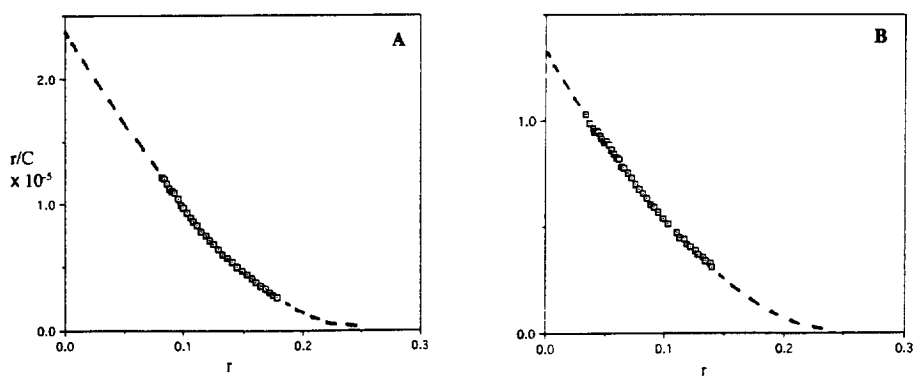


Figure 2. Scatchard plots for (A) 5,10-Dihydro-5,10-dimethyl-2,5,10-triazadibenzo[*a*,*e*]-pentalene and (B). 5,10-Dihydro-2,5,10-trimethyl-2,5,10-triazadibenzo[*a*,*e*]pentalenium iodide. [r = number of moles of bound ligand per mole of DNA. C = molar concentration of free ligand] Theoretical plot, shown as – – – , calculated from K_{ap} and n values given in Table 1.

The association constants for the binding of compounds **2** and **8** to calf thymus DNA were determined spectrophotometrically by standard spectrophotomeric procedures.[12] Complexation of the ligands with the DNA results in bathochromic shifts of the long-wavelength absorption (Figure 1). Scatchard plots[13] (Figure 2) show a distinct deviation from linearity, indicating association of the ligand at more than one site on the DNA.

Analysis of the data using McGhee and von Hipple's procedures[14] provide association constant K_{ap} values (Table 1), which are comparable with those reported[4,10] for ellipticine (3.3×10^5), 9-methoxyellipticine (1.0×10^5), 9-hydroxyellipticine (2.0×10^6) and the ellipticinium cation (8.3×10^5). Similarly, the number of occluded nucleotide sites per bound molecule of **2** or **8** correspond closely to those reported for ellipticine (4.35) and the ellipticinium cation (5.26).[10]

Table 1. Association constants, K_{ap}, and number of occluded nucleotide sites per bound ligand, n, for compounds **2** and **8**.

Compound	Analytical wavelength (nm)	ε_{free}	ε_{bound}	Kap	n
2	342	16400	8600	2.35×10^5	3.75
8	346	23700	10200	1.30×10^5	3.76

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Experimental

Methyl 1,4-dihydro-1,4-dimethylpyrrolo[3,2-*b*]indole-2-carboxylate (4). *Method A*: **3**[8] (15.73 g, 69 mmol) in DMF (355 ml) was added to a stirred suspension of NaH (3.74 g, 156 mmol) in DMF (355 ml) under nitrogen at room temperature. The mixture was stirred for 60 min and iodomethane (85.24 g, 600 mmol) was then added. The mixture was allowed to stand for 12 h before water (800 ml) was cautiously added. The aqueous mixture was extracted with diethyl ether (4 x 100 ml) and the combined organic extracts were dried ($MgSO_4$) and evaporated. The residue was purified by chromatography from silica gel, using CH_2Cl_2 as the eluant, to give **4** (16.1 g, 96%) m.p. 117-120°C; δ_H ($CDCl_3$) 3.63 (s, 3 H),

3.82 (s, 3 H), 4.22 (s, 3 H), 6.70 (s, 1 H), 6.92 - 7.78 (m, 4 H); δ_C ($CDCl_3$) 30.6 (q), 34.7 (q), 51.0 (q), 96.2 (d), 109.1 (d), 114.8 (s), 117.8 (s), 118.1 (s), 123.1 (d), 124.0 (d), 127.7 (s), 133.0 (d), 143.5 (s), 162.4 (s). Anal. Calcd. for $C_{14}H_{14}N_2O_2$: C, 69.4; H, 5.8; N, 11.6. Found: 69.6; H, 5.9; N, 11.1

Method B: A heterogeneous mixture of **3** (2.28 g, 10 mmol), dimethyl sulfate (1.93 g, 15 mmol), tetra-*n*-butylammonium hydrogen sulphate (0.17 g, 0.5 mmol), aqueous NaOH (50%, 12 ml) and benzene (48 ml) was stirred at 35°C for 3 h and then allowed to stand at *ca.* 15°C for 12 h. Water (24 ml) was added and the organic phase was separated. The aqueous phase was extracted with benzene (2 x 15 ml) and the combined organic solutions were washed with water (3 x 15 ml), dried ($MgSO_4$), and evaporated. Chromatographic purification of the crude product gave **4** (2.23 g, 92%), m.p. 118 - 120°C.

1,4-Dihydro-2-hydroxymethyl-1,4-dimethylpyrrolo[3,2-*b*]indole (5). Lithium aluminium hydride (1.71 g, 30.8 mmol) was added portionwise with vigorous stirring over 10 min to **4** (6.0 g, 24.8 mmol) in dry THF (350 ml) under nitrogen at 0°C. The mixture was stirred for a further 65 min at 0°C and the reaction monitored by TLC over a period of *ca.* 2h until all of the ester had been reduced. The reaction was then cautiously quenched by the addition of aqueous NH_4Cl (10%, 300 ml) and mixture was extracted with AcOEt (4 x 50 ml). Evaporation of the dried organic extracts gave **5** (4.61 g, 88%), m.p. 230 - 233°C; δ_H (DMSO-d_6) δ 3.70 (s, 3 H), 3.95 (s, 3 H), 4.64 (d, J = 7.0 Hz, 2 H), 5.50 (t, J = 7 Hz, 1 H), 6.06 (s, 1 H), 6.94 - 7.82 (m, 4 H). Anal. Calcd for $C_{13}N_{14}N_2O$: C, 72.9; H, 6.6; N, 13.1. Found: C, 72.5; H, 6.7; N, 13.5.

2-Formyl-1,4-dihydro-1,4-dimethylpyrrolo[3,2-*b*]indole (6). *Method A*: Activated manganese dioxide (82.0 g) was added under nitrogen to a stirred solution of **5** (16.65 g, 77.5 mmol) in CH_2Cl_2 (500 ml) under nitrogen. The stirred mixture was monitored by TLC over a period of *ca.* 4 h, until all of the hydroxymethyl compound had been consumed, and the mixture was then filtered through Kieselguhr. The filter pad was washed with hot CH_2Cl_2 (50 ml), followed by hot acetone (3 x 30 ml). The combined organic solutions were evaporated and the residue was purified by chromatography from silica gel using CH_2Cl_2 as the eluant to give **6** (11.77 g, 72%), as pale yellow needles, m.p. 103.3 - 104.4°C; *m/z* 212 (M^+, 100); δ_H ($CDCl_3$) δ 3.69 (s, 3 H), 4.27 (s, 3 H), 6.61 (s, 1 H), 7.10 - 7.35 (m, 3 H), 7.78 (d, J = 7.0 Hz, 1 H), 9.55 (s, 1 H); δ_C ($CDCl_3$) δ 30.8 (q), 34.6 (q), 101.4 (d), 109.3 (d), 114.1 (s), 118.5 (d), 118.9 (d), 124.6 (d), 130.6 (s), 133.9 (s), 134.1 (s), 144.6 (s), 179.6 (d). Anal. Calcd for $C_{13}H_{12}N_2O$: C, 73.6; H, 5.7; N, 13.2. Found: C, 73.3; H, 5.7; N, 13.1.

Method B: DMSO (2.45 g, 31 mmol) in CH_2Cl_2 (4.7 ml) was added dropwise to oxalyl chloride (2.03 g, 16 mmol) in CH_2Cl_2 (74.2 ml) at -78°C. The temperature of mixture was

kept below -70°C, while being stirred for 15 min, and **5** (2.14 g, 10 mmol) in CH_2Cl_2 (37 ml) was then added and the mixture was stirred for a further 30 min at -60 to -70°C. Triethylamine (10.13 g, 100 mmol) was added and the temperature of the mixture was allowed to rise to -10°C. Aqueous $NaHCO_3$ (5%, 250 ml) was added and the aqueous mixture was extracted with diethyl ether (4 x 50 ml). The combined extracts were dried ($MgSO_4$) and evaporated. The crude product was purified by chromatography from silica, using CH_2Cl_2 as the eluant, to give **6** (0.41 g, 19%).

5,10-Dihydro-5,10-dimethyl-2,5,10-triazadibenzo[*a*,*e*]pentalene (2). Amino-acetaldehyde diethyl acetal (11.8 g, 88.5 mmol) was added to **6** (11.7 g, 56.0 mmol) in dry toluene (1000 ml). Initial azeotropic distillation from A4 molecular sieve in a Dean-Stark assembly over 5 h was followed by the addition of a further volume of dry toluene (100 ml) and continued distillation for 17h. Subsequent evaporation of the solvent under reduced pressure gave the crude imine **7** (18.0 g, 100%).

The crude imine (7.20 g, 21.9 mmol) in 88% orthophosphoric acid (24.0 g) was heated at 170°C for 3 h. The mixture was cooled to room temperature and diluted with water (300 ml) The aqueous mixture was filtered and the solid residue was washed with aqueous phosophoric acid (5%, 100 ml).The filtrate and the acidic washings were made alkaline (pH 12 - 13) with aqueous ammonia (10%) and extracted with AcOEt (5 x 30 ml) until the extracts were no longer fluorescent. The combined extracts were dried ($MgSO_4$) and evaporated to give a crude product which was recrystallised from ethanol to give **2** (3.75 g, 75%) m.p. 208 - 210°C; *m/z* 235 (M^+), 205, 158 (100); δ_H (DMSO-d_6) 4.09 (s, 3 H, 10-Me), 4.21 (s, 3 H, 5-Me), 7.09 (d, J = 1.3 Hz, 1 H, 7-H), 7.16 (d, J = 1.3 Hz, 1 H, 6-H), 7.27 (dd, J = 2.0, 1.5 Hz, 1 H, 8-H), 7.42 (dd, J = 2.0, 1.3 Hz, 1 H, 4-H), 7.63 (s, 1 H,9-H), 8.03 (d, J = 1.5 Hz, 1 H, 3-H), 8.12 (s, 1 H,1-H); δ_C (DMSO-d_6) δ 31.3 (q), 31.5 (q), 110.3 (s), 113.5 (d), 117..2 (s), 118.3 (s), 118.6 (s), 123.4 (d), 124.3 (s), 128.4 (d), 132.9 (s), 137 (d), 142.1 (s), 142.9 (d), 178.5 (d). Anal. Calcd for $C_{15}H_{13}N_3$: C, 76.6; H, 5.6; N, 17.9. Found: C, 76.3; H, 5.5; N, 17.8.

5,10-Dihydro-2,5,10-trimethyl-2,5,10-triazadibenzo[*a*,*e*]pentalenium iodide (8). The free base **2** (0.03 g, 0.1 mmol) and iodomethane (0.04 g, 0.3 mmol) in ethanol (10 ml) were heated under reflux for 2 h. The solution was cooled and concentrated and the precipitated product was collected and recrystallised from methanol-CH_2Cl_2 to yield **8** (0.03 g, 63%) as yellow crystals, m.p. > 330°C; *m/z* 250 (M^+), 142 (100); δ_H (DMSO-d_6) δ 4.12 (s, 3 H, 2-Me), 4.33 (s, 3 H, 5-Me), 4.39 (s, 3 H,10-Me), 7.22 - 7.77 (m, 4 H, 4-,6-, 7- and 8-H), 8.17 - 8.44 (m, 2 H, 3- and 9-H), 9.52 (s, 1 H, 1-H); δ_C (DMSO-d_6) δ 29.9 (q), 34.1 (q), 51.8 (q), 109.1 (s), 110.2 (d), 110.7 (s), 117.3 (s), 119.6 (d), 119.9 (d), 121.1 (s), 121.4 (d), 123.3 (s), 142.3 (s), 142.4 (d), 160.5 (d), 161.5 (d). Anal. Calcd for $C_{16}H_{16}N_3I$: C, 50.9; H, 4.3; N, 11.1. Found: C, 51.0; H, 4.4; N, 10.8.

DNA Association Measurements. Calf thymus DNA (Sigma) was dissolved in tri-HCl buffer (50 mmol) containing aqueous sodium chloride (15 mmol) and the pH adjusted to 7.5. DNA concentrations were determined spectrophotometrically based on the nucleotide phosphate at 260 nm using a molar extinction coefficient of 6600 cm^{-1} M^{-1}.

The association constants for compounds **2** and **8** were determined spectrophotometrically in 10 cm cells using a Hewlett Packard 8452A Diode Array spectrophotometer following the procedure described by Wilson and Lopp.[12] Titrations, performed either by addition of DNA stock to the dilute solution of **2** or **8**, or the reverse procedure, gave identical results. All measurements were performed at the most intense long-wavelength absorbance maxima of the non-bonded ligand (Table 1) and the experimental data were analysed by the "SOLO statistical system" programme,[15] using the McGhee and von Hipple equation.[14] The apparent association constant, K_{ap}, and the number of occluded nucleotides per bonded ligand, n, are those obtained with the lowest standard deviation using a single set of points that fall within the 20 - 80% bound ligand.

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† 5,10-Dimethyl-5*H*,10*H*-pyrido[4',3':4,5]pyrrolo[3,2-*b*]indole may be used as an acceptable alternative nomenclature for the title compound.

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