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N-Substituted Phenanthroimidazolamines from the Reaction of Phenanthrenequinone with Monosubstituted Guanidines

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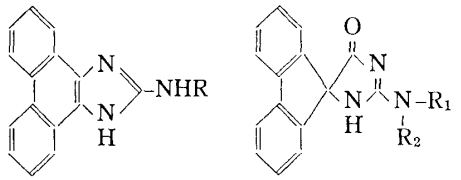
The reaction of 9,10-phenanthrenequinone (PTQ) with cyclohexylguanidine or isopropylguanidine in alkaline aqueous ethanol yielded 1*H*-phenanthro[9,10-*d*]imidazol-2-amine (PIA) as the major product and *N*-cyclohexyl-PIA or *N*-isopropyl-PIA, respectively, as a minor product. The same reaction with phenylguanidine or *tert*-butylguanidine yielded only the corresponding *N*-substituted PIAs. The yield of *N*-isopropyl-PIA was increased seven-fold when $NaBH_4$ was added to the reaction mixture of PTQ and isopropylguanidine. *N*-Substituted PIAs are fluorescent and can be separated from PIA by thin layer chromatography.

Keywords——9,10-phenanthrenequinone; 1*H*-phenanthro[9,10-*d*]imidazol-2-amine; *N*-monosubstituted guanidines; Schiff base; fluorescent products; *N*-cyclohexyl-1*H*-phenanthro[9,10-*d*]imidazol-2-amine; *N*-isopropyl-1*H*-phenanthro[9,10-*d*]imidazol-2-amine; *N*-phenyl-1*H*-phenanthro[9,10-*d*]imidazol-2-amine; *N*-*tert*-butyl-1*H*-phenanthro[9,10-*d*]-imidazol-2-amine

A fluorescent compound, 1*H*-phenanthro[9,10-*d*]imidazol-2-amine (PIA, Ia),[2] is produced when 9,10-phenanthrenequinone (PTQ) reacts with a monosubstituted guanidine such as arginine or methylguanidine in alkaline solution.[3] The fluorescence of PIA is the basis of a sensitive test for the detection of these and other guanidino compounds. The mechanism of this reaction was elucidated independently in three studies.[4] According to the proposed mechanism, condensation of the quinone with a guanidino group results in a Schiff base intermediate, which on hydrolysis yields PIA and an aldehyde; however, formation of an aldehyde is possible only when two or three hydrogens are on the α carbon with respect to the substituent group of the guanidine, as in arginine or methylguanidine. Preliminary results indicated the absence of a fluorescent product from *tert*-butylguanidine.[4c] In the present work, we show that two fluorescent products, one of which is PIA, result from compounds with one hydrogen on the α



I II

	R		R_1	R_2
Ia	H	IIa	CH_3	CH_3
Ib	$CH(CH_2)_4$	IIb	CH_3	CH_2COOH
Ic	$CH(CH_3)_2$			
Id	C_6H_5			
Ie	$C(CH_3)_3$			

Chart 1. Products in the Reactions of Guanidine and Substituted Guanidines with 9,10-Phenanthrenequinone

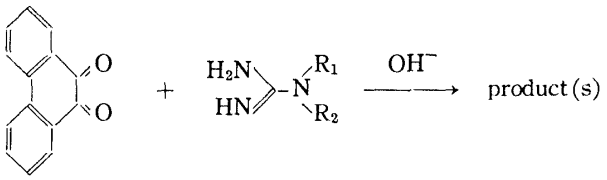
carbon and that one fluorescent product that differs from PIA results from compounds with no hydrogen on the α carbon.

Results

The Reaction of PTQ with Cyclohexylguanidine and Isopropylguanidine

The reaction of PTQ with cyclohexylguanidine gave two fluorescent products. The major product, which had the lower *Rf* value on thin layer chromatography (TLC) (see Table I), was identified as PIA by nuclear magnetic resonance (NMR) and mass spectral comparison with an authentic sample. The mass spectrum (MS) of the minor product showed its parent peak at *m/e* 315. The NMR spectrum of the hydrochloride of this product showed the signals of the cyclohexyl group at δ 1.33—2.10 (protons other than the proton on the carbon attached to the NH group) and δ 4.00 (the proton on the carbon attached to the NH group). Furthermore, the signals of the phenanthroimidazolamino moiety were very similar to those of PIA[4c] (see text). From these spectral data and the elemental analyses, the structure of compound Ib is *N*-cyclohexyl-PIA. In the reaction of PTQ with isopropylguanidine, PIA and a minor product

Table I. Products in the Reactions of Phenanthrenequinone with Guanidine and Substituted Guanidines



R_1	R_2	product(s)	Fluorescence (*Rf*)	Ref.
H	H	Ia	+(0.05)	3)
H	CH_2R_3[a]	Ia	+(0.05)	4)
H	CH——CH | $-(CH_2)_4-$	Ia, Ib	+(0.05), +(0.55)[b]	This work
H	$CH(CH_3)_2$	Ia, Ic	+(0.05), +(0.60)[b]	This work
H	C_6H_5	Id	+(0.49)[c]	This work
H	$C(CH_3)_3$	Ie	+(0.51)[c]	This work
CH_3	CH_3	IIa	—	5), 6b)
CH_3	CH_2COOH	IIb	—	5)

a) R_3=H, $CH_2CH_2CHNH_2COOH$, $CH_2C_6H_5$, COOH.
b) *Rf* values on thin layer chromatography with the solvent system chloroform: EtOH=23:2.
c) *Rf* values on thin layer chromatography with the solvent system cyclohexane: propanol=21:4.

Table II. Yields of Fluorescent Products in the Reactions of Phenanthrenequinone with Monosubstituted Guanidines

Guanidine	Reaction conditions	Product (yield)	
		PIA (%)	*N*-Substituted PIA (%)
Cyclohexyl	OH^-	Ia(61.0)	Ib(14.0)
Isopropyl	OH^-	Ia(68.0)	Ic(5.4)
Isopropyl	OH^-, $NaBH_4$	Ia(50.7)	Ic(36.3)
Phenyl	OH^-	—	Id(85.6)
tert-Butyl	OH^-	—	Ie(73.8)

were obtained. The mass spectrum of the minor product, obtained as crystals from isopropyl alcohol, showed its parent peak at *m/e* 275. The NMR spectrum of these crystals showed signals due to the isopropyl group attached to the NH group of phenanthroimidazolamine at δ 1.28 (two methyl groups) and δ 3.76 (NH–C<u>H</u>$(CH_3)_2$). From these spectral data, this minor product is identified as *N*-isopropyl-PIA (Ic).

The Effect of Sodium Borohydride in the Preparation of *N*-Substituted PIA

In the reaction of PTQ with *N*-substituted guanidines in alkaline aqueous ethanol solution as described above, the yield of *N*-substituted PIA was very low. Addition of sodium borohydride during the reaction of PTQ with isopropylguanidine increased the yield of *N*-isopropyl-PIA by about seven-fold (Table II).

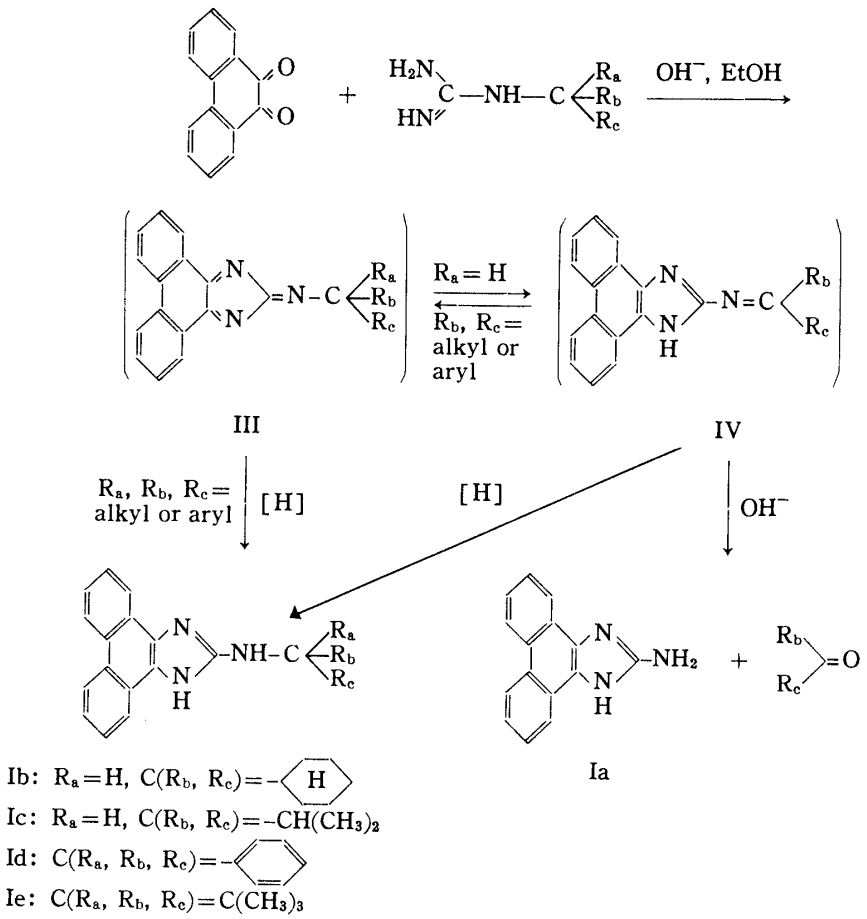
The Reaction of PTQ with Phenylguanidine and *tert*-Butylguanidine

The reaction of PTQ with phenylguanidine or *tert*-butylguanidine in alkaline solution gave only one fluorescent product. The mass spectrum of the product from the reaction of PTQ with phenylguanidine showed its parent peak at *m/e* 309. The NMR spectrum of this product shows the signals of a phenyl group in addition to those of the phenanthroimidazolamine group. The mass spectrum of the product from the reaction of PTQ with *tert*-butylguanidine showed its parent peak at *m/e* 289, and the NMR spectrum showed the signals of a *tert*-butyl group in addition to those of the phenanthroimidazolamino group. These data showed that these products were *N*-phenyl-PIA and *N*-*tert*-butyl-PIA, respectively.

Discussion

1,1-Disubstituted guanidines condense with PTQ in alkali to form spirofluoreneimidazolinones (IIa, IIb), which are stable, nonfluorescent products resulting from a benzilic acid type rearrangement.[5] When a monosubstituted guanidine reacts with PTQ, the structure of its substituent determines the nature and number of products of the reaction. If the carbon atom to which the guanidino group is bonded has two or three hydrogen atoms, the initial condensation product is a Schiff base, which hydrolyzes to PIA and an aldehyde. If this carbon atom has one hydrogen atom, PIA is the major product and an amino-substituted PIA is a minor product. The substituent on the amino group is the same as that on the guanidino group of the monosubstituted guanidine. If this carbon atom has no hydrogen, as in phenylguanidine and *tert*-butylguanidine, PIA is not produced, and the only product is the corresponding amino-substituted PIA. Formation of one or two fluorescent products and the *Rf* values of these products on thin layer chromatography can be used to detect and identify monosubstituted guanidines (Table I). The formation of an intermediate (III) in the course of this condensation reaction may be postulated. In the case of *N*-monosubstituted guanidines having at least one hydrogen on the α carbon (*e.g.* cyclohexyl- and isopropylguanidine in which R_a is H; Chart 2), tautomerism between III and IV is possible. Reduction of either III or IV gives products Ib and Ic, and hydrolysis of tautomer IV gives Ia and the corresponding ketone. On the other hand, this tautomerization is not possible with phenylguanidine or *tert*-butylguanidine derivatives that have no hydrogen on the α carbon; consequently only the reduction product Id or Ie of tautomer III is formed.

Stoichiometric condensation to a Schiff base and hydrolysis of the Schiff base to PIA and a ketone is a feasible mechanism for the reaction of PTQ with either cyclohexylguanidine or isopropylguanidine. On the other hand, formation of the *N*-cyclohexyl-PIA or *N*-isopropyl-PIA requires the incorporation of two electrons into the product. The electrons must come from the reaction medium, and ethanol is a possible source. An external reducing agent was previously suggested to account for the formation of PIA when canavanine or unsubstituted guanidine reacts with PTQ.[4c] The increase in yield of the amino-substituted product when



Ib: R_a=H, C(R_b, R_c)=-⟨H⟩
Ic: R_a=H, C(R_b, R_c)=-$CH(CH_3)_2$
Id: C(R_a, R_b, R_c)=-⟨⟩
Ie: C(R_a, R_b, R_c)=$C(CH_3)_3$

Chart 2

$NaBH_4$ was added to the reaction mixture of isopropylguanidine and PTQ is consistent with the postulated role of a reductant in this reaction. PIA is not produced when phenylguanidine or *tert*-butylguanidine reacts with PTQ, presumably because formation of a Schiff base intermediate is not possible with these compounds; these two monosubstituted guanidines yield only *N*-phenyl-PIA or *N*-*tert*-butyl-PIA. Products homologous with the rearrangement product of the condensation of 1,1-disubstituted guanidines with PTQ[5] and of both monosubstituted and disubstituted guanidines with benzil[6] are not formed from PTQ by any of the monosubstituted guanidines. All of the *N*-substituted PIAs that we prepared are fluorescent, as is unsubstituted PIA. Formation of either or both products in the reaction of a monosubstituted guanidine with PTQ depends on the nature of the substituent. These results are therefore useful for the detection and identification of substituted guanidines.

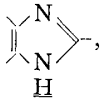
Experimental

^{1}H-Nuclear magnetic resonance spectra of samples in dimethylsulfoxide-d_6 (DMSO-d_6) solution containing internal tetramethylsilane (TMS) were recorded with a Varian EM-390 90 MHz NMR spectrometer. Mass spectra were obtained in a LKB type 9000 spectrometer by the direct inlet method at an ionizing energy of 70 eV. Fluorescence spectra were taken on an AMINCO-Bowman spectrophotofluorometer (American Instrument Co., Division of Travenol Laboratories, Inc.). Melting points were determined with Thomas Model 40 micro hot stage (Arthur H. Thomas Co., Philadelphia, PA) and are uncorrected. Precoated TLC plates (Silica Gel 60 F-254, E. Merck) were used for thin layer chromatography (TLC). SilicAR cc-7 Special (Mallinckrodt) was used for column chromatography. Elemental analyses were performed by Mr. V. Tashinian, Microlab, University of California, Berkeley. Phenylguanidine nitrate,[7] *tert*-butylguanidine nitrate,[8] cyclohexylguanidine hydrochloride,[9] and isopropylguanidine nitrate[8] were prepared according to the cited methods. Phenanthrenequinone (PTQ) was purchased from Eastman Kodak Chemicals and was

recrystallized from ethanol before use. Other compounds and solvents used in this work were of the best commercially available grades.

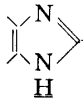
Reaction of Cyclohexylguanidine with PTQ——A solution of cyclohexylguanidine hydrochloride (400 mg) in distilled water (20 ml) and 2 N NaOH (35 ml) was added to a suspension of PTQ (470 mg) in ethanol (120 ml). The mixture was stirred for 2 h at room temperature, then poured into ice water (200 ml) and extracted twice with chloroform (200 ml and 100 ml). The combined extracts were washed thoroughly with water, dried over Na_2SO_4, filtered, and evaporated to dryness. The residue was applied to a silica gel column (5 cm i.d. × 25 cm), which was eluted with 5% acetone in chloroform followed by 10% methanol in chloroform. The 10% methanol fractions were combined and evaporated to dryness. The residue was dissolved in 50 ml of ethanol, and 1 N HCl was added until a small amount of white precipitate was seen. One drop of ethanol was then added to dissolve the precipitate, and the solution was allowed to stand at 4°C. Precipitated crystals of Ia were collected by suction filtration and dried (618.5 mg, 61%). This compound was identified as PIA from the NMR and mass spectra. A second product was obtained by elution with 5% acetone in chloroform. Evaporation of the eluting solvent gave an oil: ^{1}H NMR of the oil (DMSO-d_6) δ: 1.07—2.10 (10H, m, protons of cyclohexyl group except the proton on the carbon attached to the NH group), 3.71 (1H, m, the proton on the carbon attached to the NH group), 6.55 (1H, d, J=7.5 Hz, exchangeable with D_2O), 7.40 (2H, td, J=6.0, 1.5 Hz, H-5 and 10), 7.55 (2H, td, J=6.0, 1.0 Hz, H-6 and 9), 8.23 (2H, dd, J=6.0, 1.5 Hz, H-4 and 11), 8.70 (2H, dd, J=6.0, 1.5 Hz, H-7 and 8). This oil was dissolved in ethanol (10 ml), and 1 N HCl was added dropwise until a small amount of white precipitate was seen. Two drops of ethanol were added to dissolve the precipitate, and the solution was allowed to stand at 4°C. The precipitated crystals of *N*-cyclohexyl-1*H*-phenanthro[9,10-*d*]imidazol-2-amine (Ib) were gathered by suction filtration and dried (106.7 mg, 14%). The method described above for Ia was also used to recrystallize Ib, mp 181—183°C; fluorescence (EtOH) $\lambda_{max}^{exc.}$ 360 nm, $\lambda_{max}^{emis.}$ 395 nm (excitation at 360 nm). ^{1}H NMR of the crystals (DMSO-d_6) δ: 1.33—2.10 (10H, m, protons of cyclohexyl group except the proton on the carbon attached to the NH group), 4.00 (1H, m, the proton on the carbon attached to the NH group), 7.73 (4H, m, H-5, 6, 9 and 10), 8.43 (1H, d, J=7.5 Hz, –NH–, exchangeable with D_2O), 8.58 (2H, dd, J=7.0, 3.0 Hz, H-4 and 11), 8.90 (2H, dd, J=6.0, 3.0 Hz, H-7 and 8). MS *m/e* (relative intensity): 315 (59.3, M^+), 272 (4.0), 255 (3.3), 233 (100), 232 (22.8), 205 (22.6), 190 (8.0), 177 (4.0), 165 (4.0), 151 (4.1). *Anal.* Calcd for $C_{21}H_{21}N_3 \cdot HCl \cdot 2H_2O$: C, 65.02; H, 6.76; N, 10.83. Found: C, 65.21; H, 6.68; N, 10.99.

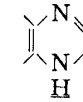
Reaction of Isopropylguanidine with PTQ——a) Isopropylguanidine nitrate (500 mg) in a solution of distilled water (10 ml) and 2 N NaOH (20 ml) was added to a suspension of PTQ (500 mg) in ethanol (120 ml). After stirring of this mixture for 2 h at room temperature, 2 N HCl (100 ml) was added. The mixture was allowed to stand overnight at 4°C, but no precipitate was obtained. The mixture was extracted twice with 300 ml of chloroform, washed with water, dried over Na_2SO_4, and filtered. The filtrate was concentrated to give an oily residue. TLC of the residue showed three spots (*Rf* 0.63, 0.60, and 0.05; solvent system $CHCl_3$: EtOH=23: 2). The residue was applied to a silica gel column. Two compounds (*Rf* 0.60 and 0.05) were separated. The product with *Rf* 0.60 contained a small amount of a contaminant with *Rf* 0.65; however, the NMR spectrum of this product showed that it was the same compound as that obtained by method b) (36 mg, 5.4%). The other compound (*Rf* 0.05) was PIA (381 mg, 68% crystals).

b) A solution of isopropylguanidine nitrate (2.55 g) in distilled water (20 ml) and 2 N NaOH (35 ml) was added to a suspension of PTQ (2.5 g) in ethanol (200 ml). After the mixture had been stirred for 2 h at room temperature, sodium borohydride (500 mg) was added, and the solution was stirred for another hour. The reaction mixture was extracted twice with 300 ml of chloroform, and the combined extracts were washed thoroughly with H_2O, dried over Na_2SO_4, and filtered. The filtrate was concentrated to obtain an oily residue, which was applied to a silica gel column (5 cm i.d. × 25 cm; solvent system, 5% acetone in $CHCl_3$ followed by 25% acetone in $CHCl_3$). The fractions eluted with 5% acetone in $CHCl_3$ were combined and evaporated to dryness to obtain a product as an oil (1.2 g, 36.3%). This oil was dissolved in isopropyl alcohol and allowed to stand overnight at room temperature. The precipitated crystals of *N*-(isopropyl)-1*H*-phenanthro[9,10-*d*]imidazol-2-amine (Ic) were recrystallized 3 times from isopropyl alcohol, mp 129—131°C; fluorescence (EtOH $\lambda_{max}^{exc.}$ 396 nm, $\lambda_{max}^{emis.}$ 445 nm (excitation at 396 nm). MS *m/e* (relative intensity): 275 (84.2, M^+), 260 (15.7), 233 (100), 232 (52.6), 218 (11.5), 205 (39.5), 190 (12.1), 177 (8.4), 165 (8.5), 151 (8.4). NMR of the crystals (DMSO-d_6) δ: 1.05 (6H, d, J=6.0 Hz, 2×CH_3, NH–CH($C\underline{H}_3$)$_2$), 1.28 (6H, d, J=6.0 Hz, 2×CH_3, methyl groups of isopropyl alcohol), 3.76 (1H, m, NH–C$\underline{H}$(CH_3)$_2$), 4.07 (1H, m, HO–C$\underline{H}$(CH_3)$_2$), 4.28 (1H, d, J=4.5 Hz,  , exchangeable with D_2O), 6.23 (1H, d, J=9.0 Hz,  , exchangeable with D_2O), 7.43 (2H, td, J=7.5, 1.5 Hz, H-5 and 10 or H-6 and 9), 7.60 (2H, td, J=7.5, 1.5 Hz, H-6 and 9 or H-5 and 10), 8.27 (2H, br d, J=6.0 Hz, H-4 and 11, changed to dd, J=7.5, 1.5 Hz, after addition of D_2O), 8.73 (2H, dd, J=7.5, 1.5 Hz, H-7 and 8). *Anal.* Calcd for $C_{18}H_{17}N_3 \cdot HOCH(CH_3)_2$: C, 75.19; H, 7.51; N, 12.53. Found: C, 75.26; H, 7.46; N, 12.66. PIA was obtained from the fractions eluted with 25% acetone in $CHCl_3$ and crystallized as the hydrochloride salt (1.42 g, 50.7%).

Reaction of Phenylguanidine with PTQ——a) A solution of phenylguanidine nitrate (150 mg) in distilled

water (6 ml) and 2 N NaOH (12 ml) was added to a suspension of PTQ (156 mg) in ethanol (80 ml). After the mixture had been stirred for 6 h at room temperature, 6 N HCl (40 ml) was added, and the mixture was allowed to stand overnight at 4°C. The precipitate (180.2 mg, 69.5%) was obtained by suction filtration and dried in a vacuum desiccator. From the mother liquor, a second crop of crystals was obtained (31.4 mg, 12.1%) and was combined with the first. The total yield was 81.6%. The product showed one spot (*Rf* 0.49, cyclohexane: isopropanol=21: 4) on silica gel TLC.

b) A solution of phenylguanidine nitrate (150 mg) in distilled water (6 ml) and 2 N NaOH (12 ml) was added to a suspension of PTQ (156 mg) in ethanol (100 ml). The mixture was stirred for 6 h at room temperature, poured into ice water (150 ml), and then extracted with chloroform (200 ml×2). The chloroform layer was washed three times with distilled water (100 ml), dried over Na_2SO_4, filtered, and concentrated to give a syrup. The syrupy residue was dissolved in a solution of 1 N HCl (20 ml) and 99% ethanol (30 ml), and allowed to stand overnight at 4°C. The first crystals precipitated (196 mg, 75.6%) were gathered by suction filtration. The mother liquor was allowed to stand overnight at 4°C, and a second crop of crystals was obtained (20 mg, 10%). The total yield was 85.6%. This product, *N*-(phenyl)-1*H*-phenanthro[9,10-*d*]-imidazol-2-amine (Id), was recrystallized 3 times from a solution of 1 N HCl (20 ml) and ethanol (30 ml), mp 245—247°C (dec.); fluorescence (EtOH) $\lambda_{max}^{excit.}$ 360 nm, $\lambda_{max}^{emis.}$ 395 nm (excitation at 360 nm). MS *m/e* (relative intensity): 309 (100, M^+), 308 (18.0), 232 (5.6), 205 (12.8), 190 (9.4), 165 (5.3), 154.5 (15.2), 154 (8.1), 153.5 (6.5), 77 (6.6). ^1H NMR (DMSO-d_6) δ: 7.23—7.53 (6H, m, C_6H_5 and  , which disappeared after addition of D_2O), 7.71 (4H, td, J=4.5, 1.0 Hz, H-5, 6, 9, and 10), 8.60 (2H, m, H-4 and 11), 8.93 (2H, m, H-7 and 8), 10.93 (1H, s,  , exchangeable with D_2O). *Anal.* Calcd for $C_{21}H_{15}N_3 \cdot HCl \cdot 1/2H_2O$: C, 71.08; H, 4.83; N, 11.84. Found: C, 71.53; H, 4.54; N, 11.84.

Reaction of *tert*-Butylguanidine Nitrate with PTQ——A solution of *tert*-butylguanidine nitrate (200 mg) in distilled water (10 ml) and 2 N NaOH (20 ml) was added to a suspension of PTQ (250 mg) in ethanol (200 ml). After the mixture had been stirred for 3 h at room temperature, distilled water (200 ml) was added, and this solution was extracted 3 times with 150 ml of chloroform. The combined extracts were washed thoroughly with water, dried over Na_2SO_4, filtered and concentrated to give a syrupy residue that showed one main fluorescent spot on silica gel TLC (cyclohexane: isopropanol=21: 4). The residue was applied to a silica gel column (2 cm i.d.×20 cm), which was eluted first with chloroform and then with 5% acetone in chloroform. The 5% acetone effluent was concentrated to obtain the fluorescent product; NMR of $C_{19}H_{19}N_3$ (DMSO-d_6) δ: 1.47 (9H, s, $-C(CH_3)_3$), 3.13 (1H, s,  , exchangeable with D_2O), 5.73 (1H, s,  , exchangeable with D_2O), 7.40 (2H, td, J=7.5, 1.0 Hz, H-5 and 10), 7.57 (2H, td, J=7.5, 1.0 Hz, H-6 and 9), 8.12 (2H, br d, J=7.5 Hz, H-4 and 11), 8.70 (2H, dd, J=7.5, 1.0 Hz, H-7 and 8). This product from the chromatographic effluent was then dissolved in a solution of 1 N HCl and ethanol (2: 3, 70 ml) and allowed to stand overnight at 4°C. A first crop of fine crystals was obtained (189.3 mg, 52.7%). From the mother liquor, a second crop of crystals was obtained (75.5 mg, 21.2%). The total yield was 73.8%. The product, *N*-(*tert*-butyl)-1*H*-phenanthro[9,10-*d*]imidazol-2-amine (Ia) was recrystallized 3 times from 1 N HCl–ethanol (2: 3). mp 283—284°C (dec.); fluorescence (EtOH) $\lambda_{max}^{excit.}$ 360 nm, $\lambda_{max}^{emis.}$ 392 nm (excitation a 360 nm). MS *m/e* (relative intensity): 289 (30.5, M^+), 233 (100), 232 (17.1), 205 (17.7), 190 (4.3). *Anal.* Calcd for $C_{19}H_{19}N_3 \cdot HCl$: C, 70.04; H, 6.10; N, 12.90, Found: C, 69.81; H, 5.88; N, 12.90.

Yields of products obtained in these reactions are summarized in Table II. Fluorescence of PIA was observed under an ultraviolet lamp with peak transmission at 366 nm.[3] *N*-substituted derivatives of PIA also fluoresce at this wavelength and are separable from PIA by thin layer chromatography. Thus, of the compounds we have examined, only 1,1-disubstituted guanidines do not yield fluorescent products with PTQ. These findings are shown in Table II.

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References and Notes

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2) Chemical names currently preferred in Chemical Abstracts are used for this compound and its *N*-substituted derivatives in this paper.
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