Cyclic Imides. 16. Hydroxy and Methoxy Derivatives of Aminophthalimide and Phthalhydrazide

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Treatment of *N*-alkyl derivatives of 3,6-dichlorophthalimide and 4,5-dichlorophthalimide with potassium nitrite gave 3-hydroxy-6-nitro- and 4-hydroxy-5-nitrophthalimides. The potassium salts of these phenols were alkylated by dialkyl sulfates. The products were reduced to the 3-amino-6-alkoxy- and 4-amino-5-alkoxyphthalimides, and the fluorescence emission spectra of these products were measured. Hydrazinolysis of the phthalimides in a toluene medium gave phthalhydrazides. The luminescence spectra of several aminophthalhydrazides were measured. The infrared and proton magnetic resonance spectra of these and of some nitrophthalhydrazides were measured and aspects of these spectra characteristic of phthalhydrazides were identified.

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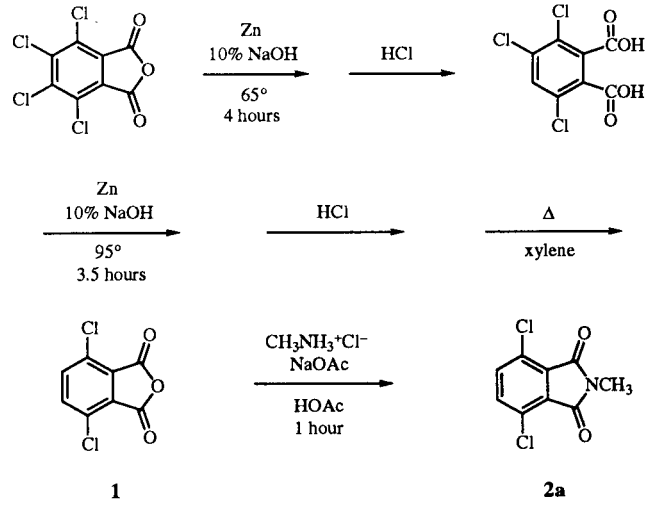
The nitrite ion in polar aprotic solvents is an ambident nucleophile which may react with an aryl halide having an electron-attracting *ortho* or *para* substituent to give either a nitro compound or a phenol [1]. The formation of the phenol apparently proceeds by initial formation of a nitrite ester, followed by an oxidation-reduction reaction in which this ester reacts with a second nitrite ion to produce nitrogen oxides and the anion of the phenol. A recent study has shown that *o*-dichlorobenzenes with an activating substituent react with sodium nitrite in dimethyl sulfoxide to give *o*-nitrophenols [2]. In the previous paper in the present series [3] it was reported that *N*-substituted 4,5-dichlorophthalimides react with potassium nitrite in *N,N*-dimethylformamide to give 4-hydroxy-5-nitrophthalimides. It has been suggested that these reactions may initiate with the replacement of the first chlorine by a nitro group, followed by a process involving neighboring group participation to form the *o*-nitrophenoxide [2].

The recent development of a synthetic pathway which affords 3,6-dichlorophthalic acid inexpensively and in good yields [4,5] has led us to a convenient synthesis of 3,6-dichlorophthalimides (Scheme 1). We can now report that an example of these compounds undergoes the nitrohydroxylation reaction as readily as the 4,5-isomers, indicating that a neighboring group interaction is not essential for the formation of the nitrophenol.

The ready availability of the hydroxynitrophthalimides from these reactions provides starting points for the synthesis of new derivatives of aminophthalimides and of aminophthalhydrazides, which are of interest because of their photochemical characteristics. The aminophthalimides are intensely fluorescent under near-ultraviolet irradiation [6], and the aminophthalhydrazides display chemiluminescence when subjected to mild oxidation in basic solution [7]. The latter property has found application in

Scheme 1

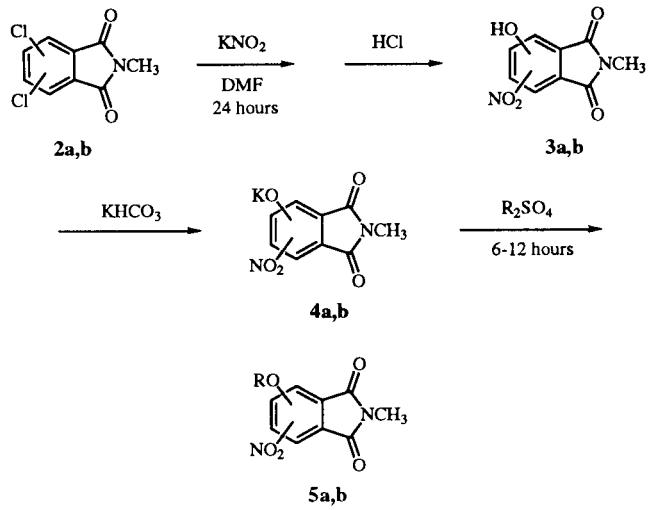


1 **2a**

clinical chemistry through the technique of luminoimmunoassay [8]. It was therefore of interest to synthesize hydroxy and alkoxy derivatives of these compounds as potential fluorescent and chemiluminescent compounds.

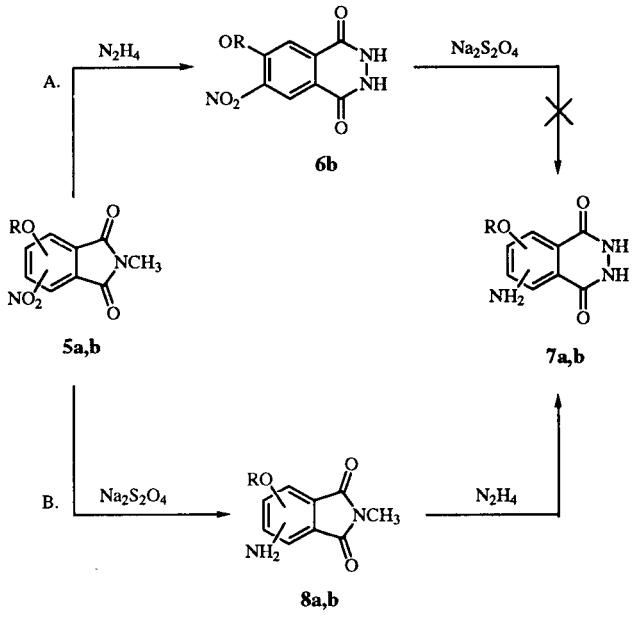
Scheme 1 shows the synthesis of 3,6-dichlorophthalic anhydride (**1**) and its conversion to 3,6-dichloro-*N*-methylphthalimide (**2a**). Scheme 2 shows the conversion of the dichloro derivatives of *N*-methylphthalimide to hydroxynitro and alkoxynitro derivatives. The reaction of **2a** with potassium nitrite in refluxing *N,N*-dimethylformamide gave 3-hydroxy-6-nitro-*N*-methylphthalimide (**3a**) in the same manner in which 4,5-dichloro-*N*-methylphthalimide (**2b**) is converted to 4-hydroxy-5-nitro-*N*-methylphthalimide (**3b**). Like **3b** [3], **3a** is a sufficiently strong acid to be soluble in aqueous sodium bicarbonate solution, and whose ultraviolet-visible absorption spectrum in ethanol solution fails to obey the Beer-Lambert law. The spectrum of **3a** in aqueous base solution

Scheme 2



a 3,6- b 4,5- **5a** R = CH_3 **5b$_1$** R = CH_3 **5b$_2$** R = C_2H_5

Scheme 3



resembles that of *p*-nitrophenol [9] under the same conditions. Both **3a** and **3b** react with aqueous potassium bicarbonate solutions to give orange precipitates of their potassium salts **4a** and **4b**.

The hydroxynitrophthalimides were resistant to alkylation. The most effective alkylation method started with their solid potassium salts which had been dried under vacuum. These were treated with dimethyl or diethyl sulfate in either anhydrous acetone or *N,N*-dimethylformamide for a brief period of refluxing, followed by a prolonged period of stirring at lower temperature. The use of acetonitrile or dimethyl sulfoxide as the solvent, or the use of prolonged reflux time, substantially decreased the yield. Procedures using methyl iodide as the methylating agent, or which used aqueous media, failed entirely.

Scheme 3 outlines two routes which were investigated for the conversion of the hydroxy- and alkoxynitrophthalimides to derivatives of luminol and isoluminol. Route A began with hydrazinolysis of the nitro derivative of the *N*-methylimide to form the nitrophthalhydrazide. This procedure gave satisfactory results for the conversion of **5b** to the corresponding 4-hydroxy- or methoxy-5-nitrophthalhydrazides **6b**. Hydrazinolysis of **5a**, on the other hand, gave the corresponding phthalic dihydrazides instead of the expected phthalhydrazides. Reduction of **6b** with sodium dithionite also gave unsatisfactory results, producing products which could not be purified to a satisfactory analysis, although they displayed the spectra anticipated for the desired products.

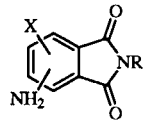
The steps of hydrazinolysis and reduction were reversed in the more successful Route B. Reduction of the nitrophthalimides **5a** and **5b** with sodium dithionite gave the corresponding aminophthalimides **8a** and **8b**. These products were deep yellow compounds with a green fluorescence. The absorption and emission spectra of these compounds are summarized in Table 1, in comparison with 3-aminophthalimide and 4-amino-*N*-methylphthalimide. Alkylation of the hydroxyl group had little effect on the absorption spectra, but produced a small redshift in the fluorescence emissions. The alkylation decreased the fluorescence intensity of the 3-amino compounds and increased it with the 4-amino compounds. The 4-amino-5-alkoxyphthalimides **8b$_1$** and **8b$_2$** showed two emission maxima instead of one.

In order to introduce the least possible amount of impurities into the product, a new hydrazinolysis method was developed for conversion of the *N*-methylphthalimides **8** to the phthalhydrazides **7**. To a dispersion of the imide in toluene was added an excess of 98% hydrazine hydrate and the mixture was refluxed with stirring 24 hours. The hydrazine hydrate provided sufficient water to catalyze the reaction. The reflux condenser was topped by a drying tube containing indicator Drierite. The deepening of the blue color of the indicator during the process was an indication of absorption of the methylamine expelled by the hydrazinolysis. Upon completion of the reaction the excess hydrazine was removed from the product by washing the precipitate with dilute hydrochloric acid.

The luminescence of basic solutions of 3-aminophthalhydrazide (luminol), 4-aminophthalhydrazide (isoluminol), 3-amino-6-methoxyphthalhydrazide (**7a$_1$**) and 4-amino-5-methoxyphthalhydrazide (**7b$_1$**) was tested using hydrogen peroxide as oxidizer and hemoglobin as catalyst [10]. The results are reported in Table 2. Although substituent groups which donate electrons by resonance have been reported [11] to enhance the luminescence of

Table 1

Absorption and Fluorescence Spectra of Aminophthalimides



Compound	Absorption [a] λ max, nm (ε max)	Fluorescence [b] λ max, nm	Approximate Intensity	
3-Aminophthalimide (3-NH_2, R = X = H)	388 (5,050), 258 (7,690), 235 (20,000), 224 (22,400)	478	1.0	[c]
8a (3-NH_2, R = CH_3, X = OH)	424 (6,580), 235 (14,600)	499	0.8	[c]
8a$_1$ (3-NH_2, R = CH_3, X = CH_3O)	424 (6,070), 229 (15,500)	504	0.4	[c]
4-Amino-*N*-methylphthalimide (4-NH_2, R = CH_3, X = H)	380 (3,870), 306 (4,140), 260 (21,600)	523	1.0	[d]
8b (4-NH_2, R = CH_3, X = OH)	395 (2,440), 315 (2,720), 266 (31,500)	517	1.8	[d]
8b$_1$ (4-NH_2, R = CH_3, X = CH_3O)	390 (2,960), 312 (2,900), 265 (32,000)	536	2.8	[d]
		443	1.2	[d]
8b$_2$ (4-NH_2, R = CH_3, X = C_2H_5O)	392 (2,860), 312 (2,930), 265 (31,800)	544	2.0	[d]
		445	1.1	[d]

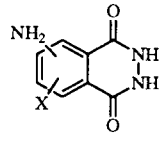
[a] Solutions in 95% ethanol. [b] 1.00 x 10^{-5} *M* in 95% ethanol. [c] Relative to 3-aminophthalimide. [d] Relative to 4-amino-*N*-methylphthalimide.

aminophthalhydrazides, this did not prove to be true in these cases of methoxy substitution. A steric effect may be invoked to explain this diminution, and also the suppression of fluorescence emission in **8a$_1$**; but such an explanation would lead to an erroneous prediction of the effect of alkoxyl groups on the fluorescence emission of the 4-aminophthalimides (Table 1).

In the course of these measurements it was found that the familiar method of lecture demonstration of luminol luminescence, using alkaline ferricyanide as the catalyst [12], actually interferes with the observation of the luminescence. The luminescence from isoluminol is not observable when this method is used because the absorption band of the ferricyanide, with its maximum at 421 nm, almost perfectly coincides with the emission band of the isoluminol, with its maximum at 419 nm. There is also significant overlap with the emission bands of luminol and of **7b$_1$**. Even with the use of a reagent which did not absorb its emitted light, the emission intensity from isoluminol and its derivative was considerably less than that from luminol, in agreement with previous observations [8a].

Table 2

Luminescence Spectra of Aminophthalhydrazides.



Compound	Emission Maximum, nm	Approximate Intensity [a]
Luminol (3-NH_2, X = H)	437	1.0
7a$_1$ (3-NH_2, X = 6-CH_3O)	445	0.8
Isoluminol (4-NH_2, X = H)	419	0.3
7b$_1$ (4-NH_2, X = 5-CH_3O	432	0.2

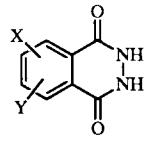
[a] Relative to luminol.

The literature contains only scattered examples of infrared spectra of phthalhydrazides, which are insufficient for drawing any general conclusions, and no examples of nuclear magnetic resonance spectra. The infrared spectra (Table 3) were found to display the carbonyl band at 1630-1665 cm^{-1}. A weaker, sharp band very close to 1600 cm^{-1} may be due to symmetric stretching of the two carbonyl groups. These two bands were merged into a single band at 1622 cm^{-1} for **7b$_1$**. Two other bands, at 1320-1395 cm^{-1} and 1450-1495 cm^{-1}, were also characteristic of the phthalhydrazides. These are probably due to mixed N-H and C-N vibrations [13] in the hydrazide group. They were submerged by the stronger nitro group bands, which occur at similar frequencies, in 4-nitrophthalhydrazide and in **6b$_1$**.

It was difficult to obtain the nmr spectra. The only solvent, other than aqueous base, in which the phthalhydrazides were found to have significant solubility, is dimethyl sulfoxide; and even in this solvent the solubilities of some of these compounds were so low that the signals were detectable only with difficulty. The results are summarized in Table 4. The broad singlet in the spectrum of phthalhydrazide at δ 11.44 is due to the hydrazide protons.

Table 3

Infrared Absorption Spectra of Phthalhydrazides [a]



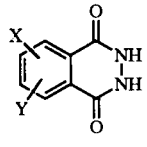
Compound	Absorption Bands, cm^{-1}
Phthalhydrazide (X = Y = H)	1663, 1603, 1495, 1331
Luminol (X = 3-NH_2, Y = H)	1655, 1603, 1495, 1323, 3428, 3345
7a$_1$ (X = 3-NH_2, Y = 6-CH_3O)	1634, 1603, 1451, 1319, 3457, 3343
4-Nitrophthalhydrazide (X = 4-NO_2, Y = H)	1655, 1599, 1350, 1553
6b (X = 4-OH, Y = 5-NO_2)	1632, 1600, 1493, 1327
6b$_1$ (X = 4-CH_3O, Y = 5-NO_2)	1653, 1619, 1362, 1535
6b$_2$ (X = 4-C_2H_5O, Y = 5-NO_2)	1655, 1616, 1362, 1537
Isoluminol (X = 4-NH_2, Y = H)	1638, 1605, 1488, 1334, 3428, 3358
7b$_1$ (X = 4-NH_2, Y = 5-CH_3O)	1622, -----, 1452, 1325, 3482, 3362

[a] Potassium bromide pellet.

For the amino compounds luminol and isoluminol, this signal is shifted upfield, to 10.46 and 11.04, respectively; and for the nitro compounds it shows a small downfield shift. In the cases of the aminomethoxyphthalhydrazides **7a$_1$** and **7b$_1$**, this signal was broadened and shifted further upfield to overlap with the signals from the aromatic protons, giving a broad bulge in the baseline, so that a specific chemical shift value could not be assigned to it.

Table 4

Proton Magnetic Resonance Spectra of Phthalhydrazides [a]



Compound	δ, ppm
Phthalhydrazide (X = Y = H)	7.77-8.03 (multiplet), 8.03-8.19 (multiplet), 11.44 (s)
Luminol (X = 3-NH_2, Y = H)	6.89-7.04 (2 triplets), 7.30 (s), 7.40-7.57 (triplet), 10.46 (s)
7a$_1$ (X-3-NH_2, Y = 6-CH_3O)	3.81 (s), 6.87-6.97 (d), ~ 7.0 (broad singlet), 7.28-7.38 (d)
4-Nitrophthalhydrazide	8.18-8.32 (d), 8.57-8.67 (d), 8.71 (s), 10.8 (s)
6b (X = 4-OH, Y = 5-NO_2)	7.60 (s, 1H), 8.44 (s, 1H), 11.50 (s, 2H)
6b$_1$ (X = 4-CH_3O, Y = 5-NO_2)	4.10 (s, 3H), 7.72 (s, 1H), 8.48 (s, 1H), 11.71 (s, 2H)
6b$_2$ (X = 4-C_2H_5O, Y = 5-NO_2)	1.37 (s, 3H), 4.36 (q, 2H), 7.70 (s, 1H), 8.45 (s, 1H), 11.68 (s, 2H)
Isoluminol (X = 4-NH_2, Y = H)	6.11 (s), 6.96-7.07 (d), 7.07 (s), 7.72-7.80 (d), 11.04 (s)
7b$_1$ (X = 4-NH_2, Y = 5-CH_3O)	3.83 (s, 3H), 5.72 (s, 2H), 7.18 (s, 1H), 7.33 (s, 1H)

[a] Solutions in dimethyl sulfoxide-d_6, tetramethylsilane reference.

Nitration of 4-hydroxy-5-nitro-*N*-methylphthalimide (**5b**) with concentrated nitric and sulfuric acids gave 4-hydroxy-3,5-dinitro-*N*-methylphthalimide, which was reduced by sodium dithionite to 3,5-diamino-4-hydroxy-*N*-methylphthalimide. This product was not fluorescent. In the solid state it displayed an unusual dull-green color, perhaps the result of a charge-transfer effect, which disappeared when the compound went into solution. 4-Methoxy-5-nitro-*N*-methylphthalimide (**5b$_1$**) did not undergo nitration under the conditions used to nitrate **5b**. Compound **5b** was readily acetylated. The product, 4-acetoxy-5-nitro-*N*-methylphthalimide, underwent hydrolysis of the ester group during the process of reduction of the nitro group.

EXPERIMENTAL

General.

Melting points were measured using an "Electrothermal" apparatus and are not corrected. Elemental microanalyses were done by Atlantic Microlab, Inc. Ultraviolet and visible absorption spectra were measured with a Cary 15 spectrophotometer, nmr spectra with a Varian EM-390 spectrometer, and ir spectra with a Midac FT-IR spectrophotometer.

The reference sample of 3,6-dichlorophthalic anhydride was purchased from Fluka Chemical Co. The reference sample of 3-aminophthalimide was purchased from Eastman Kodak. Reference samples of phthalhydrazide, 4-nitrophthalhydrazide, 3-aminophthalhydrazide, 4-aminophthalhydrazide hydrate, and 4-amino-*N*-methylphthalimide were purchased from Aldrich Chemical Co. 3,4,6-Trichlorophthalic acid was synthesized by the procedure of O'Reilly and co-workers [4]. 4-Hydroxy-5-nitro-*N*-methylphthalimide (**3b**) was synthesized by the method of Caswell and co-workers [3].

3,6-Dichlorophthalic Anhydride (**1**).

A modification of the procedure of Fertel and co-workers [5] for the synthesis of 3,6-dichlorophthalic acid was used. 3,4,6-Trichlorophthalic acid (20 g, 74.2 mmoles) was dissolved in 10% sodium hydroxide solution (200 ml) and the solution was heated to 98° in a 600-ml beaker equipped with a mechanical stirrer. Zinc dust (28 g, 428 mmoles) was added and the mixture was maintained at 95-98° for 3.5 hours with stirring and occasional addition of hot water to maintain constant volume. After cooling to room temperature, the mixture was filtered. The precipitate was washed on the filter with water (3 x 50 ml) and discarded. To the vigorously stirred combined filtrate was gradually added concentrated hydrochloric acid (50 ml). The resulting solution was extracted with ethyl acetate (5 x 100 ml). The extracts were dried over magnesium sulfate and evaporated to dryness in a rotary evaporator. To the residue was added xylene (150 ml) and the mixture was distilled until 85 ml of distillate was collected. The hot concentrate was filtered by gravity, and the white precipitate, which was inorganic material, was dis-

carded. The filtrate was chilled to give 9.88 g (61%) of dense, glittering crystals, mp 188-191°; no depression of the mp of authentic 3,6-dichlorophthalic anhydride; uv (cyclohexane): λ max 221 nm (ε 33,000), 231 nm (ε 26,400), 318 nm (ε 3,220), 330 nm (ε 3,650); ir (potassium bromide): 1773, 1221, 1860, 907, 3096 cm^{-1}; ^1H nmr (dimethyl sulfoxide-d_6): δ 8.05 (s). The spectra were identical with those of authentic 3,6-dichlorophthalic anhydride.

3,6-Dichloro-*N*-methylphthalimide (**2a**).

The following were added to a 100-ml roundbottom flask with a stirring bar in this order: 3,6-dichlorophthalic anhydride (8.66 g, 39.9 mmoles), anhydrous sodium acetate (4.12 g, 50.2 mmoles), methylamine hydrochloride (3.38 g, 52.4 mmoles) and glacial acetic acid (35 ml). The mixture was refluxed with stirring for one hour, cooled to room temperature and slurried with water (200 ml). The mixture was filtered and the white crystals were washed on the filter with water (8 x 25 ml), dried in air and recrystallized from 95% ethanol, yield, 8.88 g (97%), mp 165-166°; uv (95% ethanol): λ max 225 nm (ε 53,000), 320 nm (ε 2,670); ir (potassium bromide): 1719, 1775, 1433, 1013, 3090, 2924 cm^{-1}; ^1H nmr (dimethyl sulfoxide-d_6): δ 3.00 (s, 3H), 7.92 (s, 2H).

Anal. Calcd. for $C_9H_5Cl_2NO_2$: C, 46.99; H, 2.19; N, 6.09; Cl, 30.82. Found: C, 46.96; H, 2.18; N, 6.11; Cl, 30.75.

3-Hydroxy-6-nitro-*N*-methylphthalimide (**3a**).

3,6-Dichloro-*N*-methylphthalimide (2.16 g, 9.40 mmoles), potassium nitrite (3.20 g, 37.6 mmoles) and anhydrous *N,N*-dimethylformamide (40 ml) were added to a 100-ml roundbottom flask fitted with magnetic stirrer, reflux condenser and drying tube. The mixture was refluxed with stirring for 24 hours *in a hood* (nitrogen dioxide is evolved during the reaction), cooled to room temperature, and added gradually with vigorous stirring to 0.2 *M* hydrochloric acid (200 ml). The mixture was filtered and the filtrate was diluted with 100 ml of water and chilled to recover additional product; total yield, 1.71 g (96%), mp 203.5-204.5°; visible spectrum (0.10 *M* sodium hydroxide): λ max 394 nm (ε 16,800); ir (potassium bromide): 3202, 3086, 2963, 1775, 1707, 1535, 1348 cm^{-1}; ^1H nmr (dimethyl sulfoxide-d_6): δ 3.00 (s, 3H), 7.32 (d, 1H), 8.09 (d, 1H). The sample for microanalysis was prepared by dissolving in dilute sodium bicarbonate solution, filtering, precipitating with dilute hydrochloric acid and drying. This procedure did not alter the melting point.

Anal. Calcd. for $C_9H_6N_2O_5$: C, 48.66; H, 2.72; N, 12.61. Found: C, 48.64; H, 2.70; N, 12.63.

Potassium Salts of Hydroxynitro-*N*-methylphthalimides (**4**).

To a stirred solution of potassium bicarbonate (2.0 g, 19.9 mmoles) in water (45 ml) was added 3-hydroxy-6-nitro-*N*-methylphthalimide or 4-hydroxy-5-nitro-*N*-methylphthalimide (2.0 g, 9.0 mmoles). When the foaming subsided, the mixture was filtered and the precipitate was washed on the filter with ice-water (5 ml), ethanol (10 ml), and absolute ether (10 ml). The resulting orange powder was stored in a vacuum desiccator; yields ranged 2.2-2.3 g (94-98%), mp >350°. No further purification was needed for use in the alkylation reactions.

3-Methoxy-6-nitro-*N*-methylphthalimide (**5a**, R = CH_3).

Compound **4a** (1.0 g, 3.8 mmoles), dimethyl sulfate (1.1 ml, 11.6 mmoles) and *N,N*-dimethylformamide (7 ml) were placed in a 25-ml roundbottom flask equipped with magnetic stirrer, reflux condenser and drying tube. The mixture was heated at reflux for 30 minutes, then the temperature was gradually decreased to room temperature during 2 hours, followed by chilling in an ice bath. The chilled mixture was poured slowly with stirring into cold 7% sodium bicarbonate solution (50 ml) and filtered. The precipitate was washed on the filter with cold sodium bicarbonate solution (15 ml), followed by 0.02 *M* hydrochloric acid, yield 0.59 g (66%), mp 162-163°; uv (95% ethanol): λ max 234 nm (ε 19,500), 385 nm (ε 5,500); ir (potassium bromide): 1722, 1294, 1537, 1437, 1362, 1493, 1605, 1777 cm^{-1}; ^1H nmr (dimethyl sulfoxide-d_6): δ 2.99 (s, 3H), 4.06 (s, 3H), 7.63 (d, 1H), 8.28 (d, 1H).

Anal. Calcd. for $C_{10}H_8N_2O_5$: C, 50.86; H, 3.41; N, 11.86. Found: C, 50.81; H, 3.45; N, 11.71.

3-Ethoxy-6-nitro-*N*-methylphthalimide (**5a**, R = C_2H_5).

Compound **4a** (2.0 g, 7.7 mmoles), diethyl sulfate (2.5 ml, 20.0 mmoles) and *N,N*-dimethylformamide (10 ml) were placed in a 25-ml roundbottom flask equipped with magnetic stirrer, reflux condenser and drying tube. The mixture was refluxed 2.5 hours, then cooled to room temperature, chilled in an ice bath, and poured slowly into ice-cold 7% sodium bicarbonate solution (75 ml). The mixture was filtered, and the precipitate was washed on the filter with cold 7% sodium bicarbonate solution (75 ml), followed by cold 0.02 *M* hydrochloric acid (25 ml), yield, 1.28 g (66%), mp 176-177°; uv (95% ethanol): λ max 235 nm (ε 21,500), 285 nm (ε 6,170), 336 nm (ε 3,960); ir (potassium bromide): 1715, 1352, 1294, 1535, 1439, 1599, 1267, 1383, 1013, 1069, 1773 cm^{-1}; ^1H nmr (dimethyl sulfoxide-d_6): δ 1.39 (t, 3H), 2.98 (s, 3H), 4.35 (q, 2H), 7.62 (d, 1H), 8.26 (d, 1H).

Anal. Calcd. for $C_{11}H_{10}N_2O_5$: C, 52.80; H, 4.03; N, 11.20. Found: C, 52.68; H, 4.04; N, 11.07.

4-Methoxy-5-nitro-*N*-methylphthalimide (**5b$_1$**).

A mixture of **4b** (1.0 g, 3.8 mmoles), dimethyl sulfate (1.0 ml, 10.5 mmoles), and 12 ml of anhydrous acetone was refluxed with stirring 2 hours in a 25-ml roundbottom flask fitted with a reflux condenser and drying tube, followed by 12 hours at 35-40°. The mixture was chilled, poured with stirring into 25 ml of cold 7% sodium bicarbonate solution, and filtered. The precipitate was washed on the filter with cold 0.02 *M* hydrochloric acid (15 ml) and dried, yield, 0.71 g (78%), mp 227-228°; uv (95% ethanol): λ max 232 nm (ε 29,600), 333 nm (ε 1,700); ir (potassium bromide): 1713, 1539, 1011, 1288, 1624, 1383, 3082, 1775 cm^{-1}; ^1H nmr (dimethyl sulfoxide-d_6): δ 3.05 (s, 3H), 4.10 (s, 3H), 7.83 (s, 1H), 8.35 (s, 1H).

Anal. Calcd. for $C_{10}H_8N_2O_5$: C, 50.85; H, 3.41; N, 11.86. Found: C, 50.90; H, 3.42; N, 11.82.

4-Ethoxy-5-nitro-*N*-methylphthalimide (**5b$_2$**).

A mixture of **4b** (2.0 g, 7.7 mmoles), diethyl sulfate (2.75 ml, 21 mmoles), and *N,N*-dimethylformamide (10 ml) was stirred at 91-95° for 30 minutes, followed by reduction of the temperature to ambient during a period of one hour. The mixture was then chilled, poured slowly into ice cold 6% sodium bicarbonate solution (75 ml), and filtered. The precipitate was rinsed on the filter with cold 6% sodium bicarbonate solution (25 ml) and dried, yield, 1.48 g (77%), mp 197-198°; uv (95% ethanol): λ max 233 nm (ε 29,800), 334 nm (ε 2,100); ir (potassium bromide): 1715, 1535, 1443, 1379, 1275, 1630, 1778 cm^{-1}; ^1H nmr (dimethyl sulfoxide-d_6): δ 1.35 (t, 3H), 3.05 (s, 3H), 4.42 (q,

2H), 7.81 (s, 1H), 8.34 (s, 1H).

Anal. Calcd. for $C_{11}H_{10}N_2O_5$: C, 52.80; H, 4.03; N, 11.20. Found: C, 52.81; H, 4.07; N, 11.17.

3,5-Dinitro-4-hydroxy-*N*-methylphthalimide.

A mixture of **3b** (1.11 g, 5.0 mmoles), concentrated nitric acid (35 ml) and concentrated sulfuric acid (1.0 ml) in a 100-ml roundbottom flask was warmed gradually under reflux with stirring until the mixture was deep red and bubbling, about 1.5 hours. After cooling, the mixture was poured onto 200 ml of crushed ice and the resulting mixture was diluted with 200 ml of cold water and filtered. The precipitate was recrystallized from methanol to give fine yellow needles, yield, 1.18 g (88%), mp 202-203°; vis (0.1 *N* sodium hydroxide): λ max 413 nm (ε 6,230); ir (potassium bromide): 1559, 1732, 1389, 1431, 1329, 1645, 1269, 1782 cm^{-1}; ^1H nmr (dimethyl sulfoxide-d_6): δ 2.93 (s, 3H), 7.54 (s, 1H), 8.10 (s, 1H).

Anal. Calcd. for $C_9H_5N_3O_7$: C, 40.46; H, 1.89; N, 15.74. Found: C, 40.74; H, 1.94; N, 15.57.

4-Acetoxy-5-nitro-*N*-methylphthalimide.

A mixture of **3b** (2.2 g, 10 mmoles), acetic anhydride (10 ml) and concentrated sulfuric acid (0.4 ml) in a 125-ml flask was heated in a boiling water bath for 15 minutes. Ice water (60 ml) was added in 10-ml portions, and the mixture was chilled and filtered. The product was recrystallized from methanol, yield, 2.4 g (92%), mp 152.5-153.5°; uv (95% ethanol): λ max 218 nm (ε 26,700); ir (potassium bromide): 1717, 1788, 1553, 1180, 1373, 1437, 1008, 978 cm^{-1}; ^1H nmr (dimethyl sulfoxide-d_6): δ 2.37 (s, 3H), 3.08 (s, 3H), 8.11 (s, 1H), 8.52 (s, 1H).

Anal. Calcd. for $C_{11}H_8N_2O_6$: C, 50.01; H, 3.05; N, 10.60. Found: C, 50.17; H, 3.08; N, 10.62.

General Procedure for the Reduction of Nitrophthalimides.

Sulfur dioxide is evolved during this reaction. The procedure should be carried out in a hood by a person who is not susceptible to asthma!

To a solution of sodium bicarbonate (2.52 g, 30.0 mmoles) in distilled water (25 ml) in a 200-ml Berzelius beaker was added 5.0 mmoles of hydroxynitrophthalimide **3a** or **3b**, or alkoxynitrophthalimide **5a** or **5b**. When the **3a** or **3b** had dissolved, or the **5a** or **5b** was thoroughly slurried, sodium dithionite (4.19 g, 24 mmoles) was added, and the mixture was vigorously stirred with a stirring rod to stir down the thick foam which was produced. When the evolution of gas had subsided, the mixture was heated to boiling and boiled 5 minutes to complete the reaction. Glacial acetic acid (5 ml) was then added and the mixture was cooled to room temperature and filtered. The bright-yellow powders were washed with ice cold water (5-10 ml), air-dried and recrystallized from methanol. The ultraviolet-visible absorption and fluorescence emission spectra of the products are summarized in Table 1.

3-Amino-6-hydroxy-*N*-methylphthalimide (**8a**, R = H).

The yield was 0.80 g (83%), mp 249-250°; ir (potassium bromide): 1670, 3354, 1489, 1736, 1458, 1277, 1003, 1383, 3470 cm^{-1}; ^1H nmr (dimethyl sulfoxide-d_6): δ 2.92 (s, 3H), 5.94 (s, 2H), 6.95 (d, 2H), 9.89 (s, 1H).

Anal. Calcd. for $C_9H_8N_2O_3$: C, 56.25; H, 4.20; N, 14.59. Found: C, 55.97; H, 4.23; N, 14.42.

3-Amino-6-methoxy-*N*-methylphthalimide (**8a**, R = CH_3).

The yield was 0.35 g (34%), mp 219-222°; ir (potassium bromide): 1686, 3343, 1470, 1271, 1740, 1644, 3474, 1445, 1373 cm^{-1}; ^1H nmr (dimethyl sulfoxide-d_6): δ 2.89 (s, 3H), 3.80 (s, 3H), 5.98 (s, 2H), 6.96 (d, 1H), 7.21 (d, 1H).

Anal. Calcd. for $C_{10}H_{10}N_2O_3$: C, 58.25; H, 4.89; N, 13.59. Found: C, 58.12; H, 4.90; N, 13.60.

4-Amino-5-hydroxy-*N*-methylphthalimide (**8b**, R = H).

The yield was 0.59 g (61%), mp 313-315°; ir (potassium bromide): 1686, 3362, 1445, 1620, 1578 cm^{-1}; ^1H nmr (dimethyl sulfoxide-d_6): δ 2.90 (s, 3H), 5.70 (s, 2H), 6.95 (s, 1H), 6.98 (s, 1H).

Anal. Calcd. for $C_9H_8N_2O_3$: C, 56.25; H, 4.20; N, 14.58. Found: C, 56.16; H, 4.23; N, 14.55.

4-Amino-5-methoxy-*N*-methylphthalimide (**8b**, R = CH_3).

The yield was 0.50 g (49%), mp 223-224°; ir (potassium bromide): 1713, 1429, 1630, 1753, 1333, 3381, 1022, 743, 1379, 3480 cm^{-1}; ^1H nmr (dimethyl sulfoxide-d_6): δ 2.92 (s, 3H), 3.88 (s, 3H), 5.91 (s, 2H), 6.97 (s, 1H), 7.13 (s, 1H).

Anal. Calcd. for $C_{10}H_{10}N_2O_3$: C, 58.25; H, 4.89; N, 13.59. Found: C, 57.99; H, 4.90; N, 13.52.

4-Amino-5-ethoxy-*N*-methylphthalimide (**8b**, R = C_2H_5).

The yield was 0.58 g (53%), mp 164-165°; ir (potassium bromide): 1717, 1437, 1383, 1223, 743, 1620, 1323, 1044, 1591, 1767, 3329, 1505, 3418 cm^{-1}; ^1H nmr (dimethyl sulfoxide-d_6): δ 1.37 (t, 3H), 3.94 (s, 3H), 4.15 (q, 2H), 5.89 (s, 2H), 6.99 (s, 1H), 7.17 (s, 1H).

Anal. Calcd. for $C_{11}H_{12}N_2O_3$: C, 59.99; H, 5.49; N, 12.72. Found: C, 60.09; H, 5.47; N, 12.76.

3,5-Diamino-4-hydroxy-*N*-methylphthalimide.

The reduction of 3,5-dinitro-4-hydroxy-*N*-methylphthalimide (1.36 g, 5.0 mmoles) was carried out in the same manner as the above syntheses, except that a double quantity of sodium dithionite (8.38 g, 48 mmoles) was used, yield, 0.66 g (64%), mp 262-264°. The sample for analysis was recrystallized from methanol, giving a gray-green powder, mp 265-266°; uv (95% ethanol): λ max 372 nm (ε 5,550), 346 nm (ε 26,400); ir (potassium bromide): 1699, 3389, 1451, 3349, 1649, 1256, 1746, 3472, 1379, 1503 cm^{-1}. This compound was not adequately soluble in dimethyl sulfoxide to provide a satisfactory nmr spectrum.

Anal. Calcd. for $C_9H_9N_3O_3$: C, 52.17; H, 4.38; N, 20.28. Found: C, 51.96; H, 4.42; N, 20.11.

4-Hydroxy-5-nitrophthalhydrazide (**6b**, R = H) [14].

4-Hydroxy-5-nitro-*N*-methylphthalimide (**3b**) [3] (6.66 g, 30 mmoles) was added to a solution of 90% hydrazine hydrate (6.0 g) in 95% ethanol (300 ml) in a 500-ml roundbottom flask and the mixture was refluxed 2 hours. The mixture was cooled to room temperature and evaporated to dryness in a rotary evaporator. The residue was dispersed in 0.3 *M* hydrochloric acid (25 ml) and heated to 50-55° with stirring for 15 minutes. The mixture was cooled to room temperature and filtered. The pale-yellow product was recrystallized from acetone to give 5.25 g (78%), mp >350°; uv (0.10 *M* sodium hydroxide): λ max 262 nm (ε 21,300). The ir and nmr spectra are reported in Tables 3 and 4, respectively.

Anal. Calcd. for $C_8H_5N_3O_5$: C, 43.06; H, 2.26; N, 18.83. Found: C, 42.95; H, 2.28; N, 18.78.

General Procedure for the Hydrazinolysis of 4-Alkoxy-5-nitro-

N-methylphthalimides.

A mixture of the alkoxynitro-*N*-methylphthalimide (**5**) (5.9 mmoles), 98% hydrazine hydrate (1.0 ml, 20.6 mmoles), and 95% ethanol (83 ml) was refluxed with stirring 2.5 hours, then allowed to stand with stirring at room temperature 24-48 hours. The mixture was filtered and the precipitate was dried. The crude product was pulverized, mixed with 2 *M* hydrochloric acid, heated at 55-60° for 10-15 minutes, cooled and filtered. The ir and nmr spectra of the products are reported in Tables 3 and 4, respectively.

4-Methoxy-5-nitrophthalhydrazide (**6b$_1$**, R = CH_3).

The yield was 0.92 g (66%), mp 301-302°. The sample for analysis was recrystallized from acetone, mp 304-305°; uv (0.10 *M* sodium hydroxide): λ max 236 nm (ε 22,700).

Anal. Calcd. for $C_9H_7N_3O_5$: C, 45.58; H, 2.98; N, 17.72. Found: C, 45.62; H, 3.11; N, 17.48.

4-Ethoxy-5-nitrophthalhydrazide (**6b$_2$**, R = C_2H_5).

The yield was 0.92 g (62%), mp 308-309°. The sample for analysis was recrystallized from acetone, mp 319-320°; uv (0.10 *M* sodium hydroxide): λ max 236 nm (ε 24,200).

Anal. Calcd. for $C_{10}H_9N_3O_5$: C, 47.81; H, 3.61; N, 16.73. Found: C, 47.99; H, 3.71; N, 16.75.

Hydrazinolysis of 3-Alkoxy-6-nitro-*N*-methylphthalimides.

The same hydrazinolysis procedure was used, but with smaller quantities: the 3-alkoxy-6-nitro-*N*-methylphthalimide (4.0 mmoles), 98% hydrazine hydrate (0.5 ml, 10.3 mmoles) and 55 ml of 95% ethanol.

3-Methoxy-6-nitrophthalic Dihydrazide.

The product was purified by extraction of impurities with boiling methanol, in which it was insoluble, yield, 0.90 g (83%), mp 200-201°; uv (saturated solution in acetonitrile): λ max 302 nm; ir (potassium bromide): 1657, 3302, 1524, 1335, 1277, 3220, 1574, 3198, 1466, 1063, 1429, 1132, 3030 cm^{-1}; ^1H nmr (dimethyl sulfoxide-d_6): δ 3.91 (s, 3H), 4.40 (s, 4H), 7.32, (d, 1H), 8.24 (d, 1H), 9.20 (s, 2H).

Anal. Calcd. for $C_9H_{11}N_5O_5$: C, 40.15; H, 4.12; N, 26.01. Found: C, 40.32; H, 4.14; N, 25.90.

3-Ethoxy-6-nitrophthalic Dihydrazide.

The product was recrystallized from methanol, yield, 0.90 g (80%), mp 197-198°; uv (95% ethanol): λ max 303 nm (ε 8,500); ir (potassium bromide): 1653, 3275, 1524, 1628, 1335, 1277, 3179, 1260, 1059, 1460, 3028 cm^{-1}; ^1H nmr (dimethyl sulfoxide-d_6): δ 1.30 (t, 3H), 4.07-4.34 (overlapping quartet and broad singlet, 6H), 7.28 (d, 1H), 8.18 (d, 1H), 9.11 (broad singlet, 2H).

Anal. Calcd for $C_{10}H_{13}N_5O_5$: C, 42.41; H, 4.63; N, 24.73. Found: C, 42.32; H, 4.58; N, 24.77.

General Procedure for Hydrazinolysis of Aminophthalimides.

To a 50-ml roundbottom flask were added in the following order: the aminophthalimide (1.00 g), toluene (10 ml), 98% hydrazine hydrate (1.00 ml, 21 mmoles) and toluene (10 ml). To the reflux condenser was attached a drying tube containing indicator "Drierite", and the mixture was refluxed with stirring 24 hours during which time the intense yellow color of the aminophthalimide faded nearly to white. The cooled mixture was filtered and the precipitate was washed on the filter with methanol (5 ml). The dried product was pulverized and mixed with water (10 ml); 0.1 *M* hydrochloric acid (10 ml) and water (10 ml) were added, and the mixture was allowed to stand 30 minutes with occasional swirling. The mixture was filtered and air-dried. The luminescence, ir and nmr spectra are reported in Tables 2, 3 and 4, respectively.

3-Aminophthalhydrazide (Luminol).

The yield was 0.81 g (74%) from 3-aminophthalimide, mp 335-336°; reported [15] mp 329-332°; uv (0.10 *M* sodium hydroxide): λ max 301 nm (ε 6,610), 348 nm (ε 7,600). All spectra were superimposable on the spectra of the authentic material.

3-Amino-6-methoxyphthalhydrazide (6-Methoxyluminol) (**7a$_1$**).

The yield was 0.60 g (68%) from **8a**, mp 243-244°; reported [10] mp 242°; uv (0.10 *M* sodium hydroxide): λ max 309 nm (ε 7,010), 370 nm (ε 7,500).

4-Aminophthalhydrazide (Isoluminol).

The yield was 0.83 g (86%) from 4-amino-*N*-methylphthalimide; mp >350°; uv (0.10 *M* sodium hydroxide): λ max 223 nm (ε 24,000), 271 nm (ε 20,300). All spectra were superimposable on the spectra of the authentic material.

4-Amino-5-methoxyphthalhydrazide Monohydrate.

The yield was 0.72 g (79%) from **8b**, mp 321-322°; uv (0.10 *M* sodium hydroxide): λ max 239 nm (ε 32,300), 275 nm (ε 21,900).

Anal. Calcd. for $C_9H_9N_3O_3 \cdot H_2O$: C, 48.00; H, 4.92; N, 18.66. Found: C, 47.92; H, 4.90; N, 18.62.

Measurement of Fluorescence and Luminescence Spectra.

Fluorescence and luminescence spectra were obtained with a Shimadzu RF5000U spectrofluorophotometer. The fluorescence spectra of the aminophthalimides were measured with 1 x 10^{-5} *M* solutions in 95% ethanol. The exciting light in each case was at the wavelength of the lowest-energy absorption band of the compound being measured.

The luminescence spectra of the aminophthalhydrazides were measured using a modification of the procedure of Gundermann and Drawert [10]. To 2 ml of a 8 x 10^{-3} *M* solution of the aminophthalhydrazide in 0.03 *M* sodium hydroxide in the spectrofluorophotometer cell was added one drop of a solution of 0.13 g of hemoglobin in 50 ml of 0.01 *M* sodium hydroxide, followed by one drop of 3% hydrogen peroxide. The emission spectrum was measured immediately.

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