Direct Acting, Highly Mutagenic, α-Hydroxy *N*-Nitrosamines from 4-Chloroindoles

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Abstract: Fava beans (Vicia fava) contain 4-chloro-6-methoxyindole, a promutagen, which on nitrosation under simulated gastric conditions forms 4-chloro-6-methoxy-2-hydroxy-N^1-nitrosoindolin-3-one oxime, a direct-acting, exceedingly potent, mutagen which may represent the putative gastric carcinogen in the high-risk area of Colombia. An analogous, equally active mutagen is produced on nitrosation of 4-chloroindole. The two mutagens appear to be the first stable α-hydroxy *N*-nitrosamines. On treatment with dilute mineral acid at slightly elevated temperatures both of these nitrosamines are irreversibly converted to the corresponding 4-formylindazoles, and it is suggested that the nitrosation of indoles to yield indazoles generally proceeds via 2-hydroxy-N^1-nitrosoindolin-3-one oximes.

Mortality from gastric cancer was found to be higher in Japan as well as in Central and Western Latin America than in Western Europe and the United States.[1] Epidemiological studies suggest that dietary factors play an important role.[2] In Columbia where fava beans (*Vicia faba*) are an important component of the diet and where nitrate intake is high,[3] the population is at especially high risk for developing gastric cancer.

We already reported on the isolation and structure determination of a promutagen from fava beans, identified as 4-chloro-6-methoxyindole, which on treatment with nitrous acid under simulated gastric conditions (pH ca. 2.5 and nitrite concentration of ca. 0.05 M) gave a highly mutagenic compound.[4] Under identical conditions 4-chloroindole was found to yield two products, one of which showed similar mutagenicity.[4] 5-Chloroindole, on the other hand, gave only nonmutagenic products.[4]

Although the nitrosation of indoles has been studied for more than a century, with most work being centered on aryl and alkylindoles, the nitrosation of halo-subsituted indoles, with the exception of one recent report,[5] remains virtually unexplored. Existing literature on the nitrosation of indoles contains many discrepancies, and it was felt that clarification should lead to a better understanding of the processes involved in the formation of the fava bean mutagen. Nitrosation of indoles can be affected by a wide variety of nitrosating agents such as nitrous acid, alkyl nitrites, nitrosyl chloride, and mixtures of nitrogen oxides. The nature of the products depends on the substitution pattern on the "pyrrole" ring, the nitrosating agent, and, often critically, the reaction conditions. Substitution prefers to occur at C_3, but if this position contains a substituent other than hydrogen, *N*-nitroso derivatives are formed. The 1-nitrosoindoles and 3-nitrosoindoles containing substituents other than hydrogen at C_1 are true nitroso derivatives (**1** and **2** respectively). However, the 3-nitroso derivatives of indole and most 2-substituted indoles with hydrogen at position 1 exist in the tautomeric oximino-3*H* form **3**.[6,7] Nitrosation of indole itself leads to complex mixtures containing among others compounds **8**, **9**, and perhaps **4**.[8-12] Reaction of 2-methylindole with nitrous acid in acetic acid was reported to give 2-methyl-3-oximino-3*H*-indole (**5**),[9,13] but other workers failed to isolate this product.[14-16] Oxime **5** can be prepared reliably by the action of isoamyl nitrite and sodium methoxide on 2-methylindole.[14] An analogous oxime **6** can be prepared from 2-aryl indoles and nitrous acid, while 3-methylindole affords the corresponding *N*-nitrosamine.[9,12,16] Under similar conditions, 2-*tert*-butylindole was reported to undergo a rather bizarre rearrangement leading to 3-pivaloylindazole (**10**).[17] This anomalous behavior has been attributed to the steric bulk of the substituent at C_2.[18] According to Hiremath et al.,[5] all 4-, 5-, and 7-substituted indoles investigated yield 3-oximino-3*H*-indoles, and it was claimed that the parent indole behaved similarly, an observation in sharp contrast to the complex mixtures obtained by many other workers using similar conditions.[8-12]

Results and Discussion

Nitrosation of 2-phenylindole with either isoamyl nitrite/sodium methoxide[14,19] or sodium nitrite/acetic acid[7,20] proceeded in accordance with the literature, giving the 3-oximino-3*H* derivative **6** as the sole isolable product. Similar results were obtained with 0.05 M aqueous sodium nitrite in water at pH 2.5.[21] The physical properties of oxime **6** agreed with those reported, and unambiguous structural assignment was made by hydrogenation to the known 3-amino-2-phenylindole.[22] In agreement with Noland et al.,[14] 2-methylindole was converted to the 3-oximino-3*H* derivative **5** when exposed to isoamyl nitrite/sodium methoxide. Although two groups[9,13] claimed that **5** could be prepared with sodium nitrite/glacial acetic acid, our findings agree with those of other investigators[14,16,23] that no pure products could be isolated when employing these conditions. Two crystalline products could now

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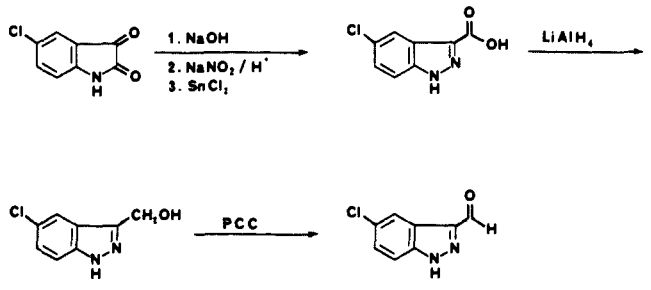
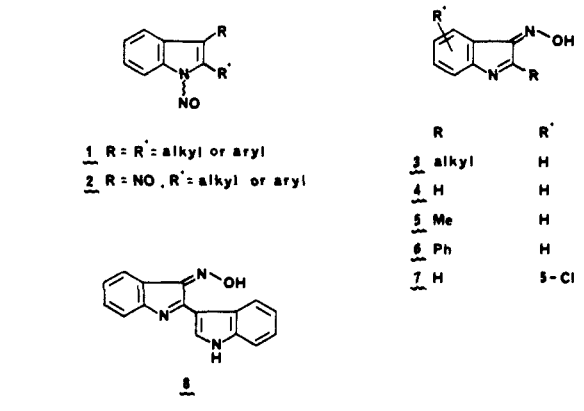
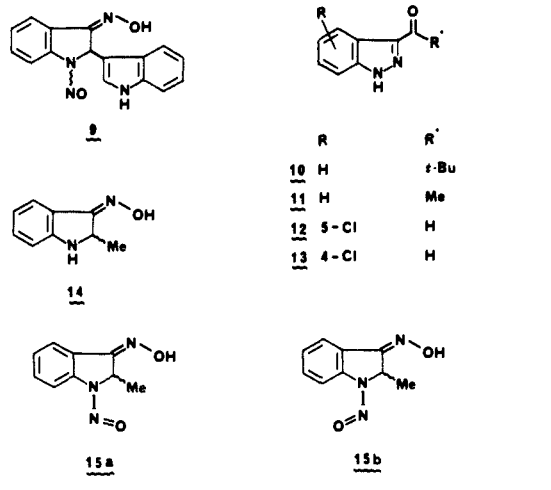
Scheme I



be isolated when nitrosations were performed under simulated gastric conditions. The minor product was identified as the oxime **5**. Reduction of **5** with sodium borohydride afforded the unstable indoline **14** whose structure was revealed by spectroscopic measurements. *Z* and *E* *N*-nitroso derivatives **15a** and **15b** were formed in a ratio of 93:7 when **14** was treated with nitrous acid. The major product turned out to be an isomer. Its NMR and mass spectra revealed the presence of an acetyl group. Infrared and particularly ultraviolet spectra were utilized for further identification and are in agreement with structure **11**.



Unlike 2-methyl and 2-phenylindole, 5-chloroindole did not react with isoamyl nitrite. Nitrosation with sodium nitrite in glacial acetic acid led to complex mixtures, and preparation of what was claimed[5] to be 5-chloro-3-oximino-3*H*-indole (**7**) with sodium nitrite in aqueous acetic acid could not be reproduced in this laboratory. Changing the nitrosating medium to an aqueous 0.05 M $NaNO_2$/pH 2.5 solution led to a crystalline product whose spectral properties agree with those anticipated for 3-formyl-5-chloroindazole (**12**). Reduction with sodium borohydride to the primary alcohol was accompanied by the proper spectral changes. An alternate synthesis of **12** (Scheme I) based on a known synthetic scheme[24] confirmed the assignment.



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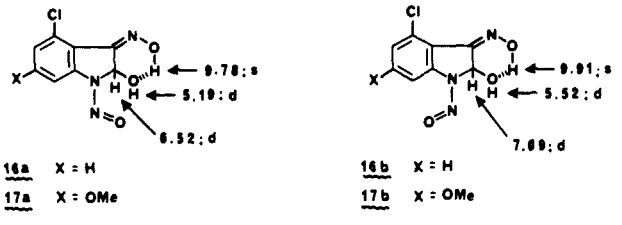
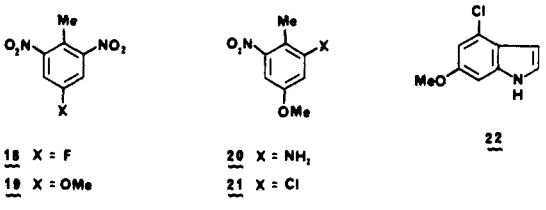
When 4-chloroindole was treated with 0.05 M aqueous sodium nitrite at pH 2.5 two crystalline products were formed. The minor, more polar (on silica), substance was identified as 3-formyl-4-chloroindazole (**13**) whose spectroscopic data and chemical behavior toward sodium borohydride paralleled those of the corresponding 5-chloroisomer (**12**). Indazole **13** was nonmutagenic when tested against *Salmonella typhimurium* Strain TM 677.[4] The less polar compound was found to have the composition $C_8H_6ClN_3O_3$. Its ultraviolet absorption spectrum with maxima at 262 and 301, and in addition a low intensity broad band at 400 nm, is incompatible with the presence of either an indole or an indazole chromophore. NMR revealed the presence of *Z* and *E* isomers of *N*-nitrosamines in a ratio of 4:1 with the C_3-proton appearing as a doublet sharpened to a singlet after D_2O exchange. Product **16** coupled readily with *N*-(1-naphthyl)ethylenediamine dihydrochloride (NEDD) at pH 8.5 to give a dye with λ_{max} 522 nm. Moreover **16** could be converted quantitatively to indazole **13** by warming it in dilute hydrochloric acid at 80 °C for 1 h. Unlike **13**, **16** turned out to be a powerful direct-acting mutagen against *Salmonella typhimurium* strain TM 677.[4] On the basis of the observed spectroscopic data and chemical reactions, structures **16a** and **16b** were assigned to the less polar product. *It represents the first stable α-hydroxy N-nitroso compound.*



Previous studies revealed their instability, and α-hydroxy dialkyl nitrosamines exhibited half-lives, under physiological conditions, of seconds to minutes.[25,26] We attribute the stability of **16** to intramolecular hydrogen bonding and to steric retardation of ring-opening to the diazohydroxide by the space-demanding C_4 substituent. Nitrosation of 4-chloro-6-methoxyindole (**22**), synthesized with the Batcho–Leimgruber[27] method, again produced a strongly mutagenic α-hydroxy N-nitrosamine which was assigned structure **17**.



The fact that **16** could be converted quantitatively to the indazole **13** on treatment with dilute mineral acid strongly suggests that α-hydroxy nitrosamines are intermediates in the formation of indazoles from indoles. The conversion of 4-chloro-, 5-chloro-, 4-chloro-6-methoxy- (**22**), and 2-methylindoles to indazoles on nitrosation clearly demonstrates, that bulky substituents at C_2 are not a requirement for the rearrangement to occur. The mechanism outlined in Scheme II differs from two others proposed in review articles.[18,28] The loss of nitrous acid in the transformation of the α-hydroxy nitrosamine **16** to the indazole **13** was readily demonstrated by warming **16** in the presence of diphenylamine and dilute acid. *N*-Nitrosodiphenylamine could be isolated, but whether it is formed from nitrous acid or by nitroso group transfer[29] from the C-nitroso intermediate in Scheme II remains to be clarified. Furthermore, the present mechanism requires

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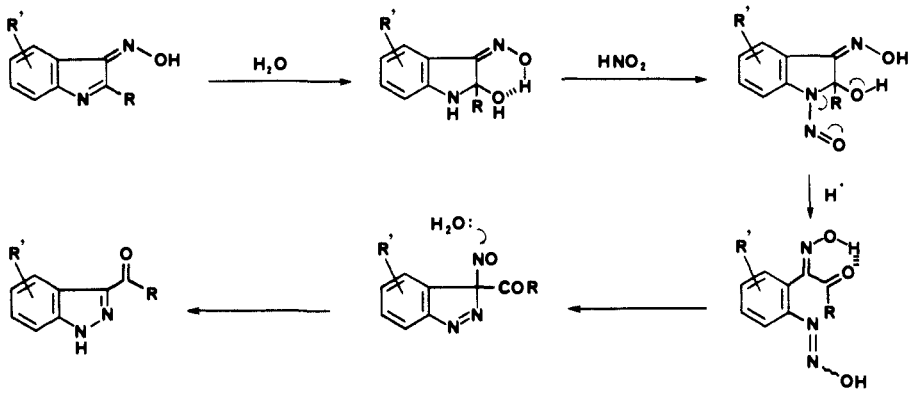
Scheme II



water which may explain why the α-hydroxy nitrosamines escaped previous investigators using glacial acetic acid as the solvent. The conversion of **16** to a diazohydroxide or the corresponding diazonium species may be relevant to the issue of how these substances interact with informational macromolecules to cause mutagenesis. Aryl diazonium ions have been proposed as the ultimate electrophilic forms of carcinogenic aryl nitrosamines.[30,31] Their generation exemplified by *N*-nitrosomethylaniline results from hydroxylation on the carbon atom adjacent to the nitroso group followed by fragmentation.[32] Data on the half-lives of aromatic diazohydroxides and/or or aryl diazonium salts[33] do not contradict the postulate that these are sufficiently long lived to cross membrane barriers to interact with cellular macromolecules. Because of this hydrolytic instability little definitive data on the carcinogenicity of aryl diazonium salts exist, but the benzenediazonium ion is an established gastric carcinogen in the mouse.[34]

Experimental Section

Melting points were determined on a Reichert hot stage microscope or in open capillary tubes on a Büchi SMP-20 melting point apparatus and are uncorrected. Ultraviolet spectra were recorded on a Perkin-Elmer Hitachi 200 and HP 8450 spectrometers. Infrared spectra were recorded on a Perkin-Elmer 283B infrared spectrophotometer and were calibrated against a polystyrene film. Nuclear magnetic resonances at 250 or 270 MHz were recorded on a Bruker WM 250 or HX270 spectrometer. Chemical shifts were reported in parts per million (δ) relative to internal standard tetramethylsilane. Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; and br, broad. Low-resolution mass spectra were determined at 70 eV on a Varian MAT-44 instrument and GC–MS were obtained with a HP-8992 system, 25 × 0.25 mm bonded OV-1 column (Alltech Associates). Flash column chromatography was performed with Woelm silica gel 0.032–0.063 mm. Analytical thin-layer chromatography (TLC) was performed on precoated 0.25-mm silica gel 60F-254 plates (EM reagent). Solvents and reagents (all are commercially available) were purified by standard procedures.[35] Petroleum ether stands for light petroleum (bp 30–60 °C) fraction.

Nitrosation of 2-Phenylindole. (i) With Isoamyl Nitrite and Sodium Methoxide. The procedure of Spica and Angelico,[19] as modified by Noland[14] et al., was adopted. Recrystallization of the product from amyl acetate gave 104 mg (19%) of **6** as orange prisms: mp 280–281 °C dec [lit.[36] mp 276–278 °C dec, it has been reported to be yellow, mp 244 °C,[37] 250 °C,[19] 258 °C dec,[22] or orange mp 280 °C dec[7] and 282 °C[13]]; UV λ_{max} (95% EtOH) 265 nm (log ε = 4.6) 335 (3.6), 387 (3.5); IR (Nujol) 2800–2100, 1860, 1540, 1360, 1030, 760, 750, 715, and 690 cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 7.35 (t, 1 H, *J* = 6.3 Hz), 7.55 (m, 5 H), 8.14 (d, 1 H, *J* = 6.3 Hz), 8.28 (m, 2 H), and 13.86 (br, 1 H); mass spectrum, *m/e* (rel intensity) 222 (50, M$^+$), 205 (100), 103 (26), and 77 (39). (ii) **With 0.05 M Aqueous Sodium Nitrite.** A mixture of 2-phenylindole (230 mg, 1.2 mmol) and 0.05 M aqueous sodium nitrite (239 mL, 12 mmol; adjusted to pH 2.5 with dilute HCl) was stirred at room temperature for 1 h. The precipitated solid was filtered and on recrystallization from amyl acetate afforded 120 mg (45%) of **6**. (iii) **With Sodium Nitrite and Aqueous Acetic Acid.**[7] A solution of sodium nitrite (60 mg, 0.9 mmol) in water (0.2 mL) was added, with stirring, to a suspension of 2-phenylindole (352 mg, 1.8 mmol) in glacial acetic acid (3 mL) cooled at 0 °C. Yellow crystals soon started to deposit. After 20 min at room temperature, the mixture was diluted with water (10 mL). The precipitated solid was filtered and purified as above and gave 101 mg (50%) of **6**. (iv) **With Sodium Nitrite and Glacial Acetic Acid.**[20] Sodium nitrite (123 mg, 1.8 mmol) was added to a suspension of 2-phenylindole (346 mg, 1.8 mmol) in glacial acetic acid (30 mL). The mixture was stirred at room temperature for 2 h and poured into water. The precipitated solid was collected and purified as above and gave 157 mg (39%) of **6**.

Reduction of 2-Phenyl-3-oximino-3*H*-indole (6). A mixture of 2-phenyl-3-oximino-3*H*-indole (**6**) (53 mg, 0.24 mmol) and 10% palladium on activated carbon (20 mg) in ethanol (10 mL) was hydrogenated under 40 psi for 6 h and filtered through Celite. The filtrate, after being concentrated, was purified on a column of SiO_2. Fractions eluted with ethyl acetate–petroleum ether (2:3) on evaporation afforded 31.4 mg (63%) of 3-amino-2-phenylindole as glistering purple prisms: mp 173–174 °C dec [lit.[22] mp 174 °C]; UV λ_{max} (95% EtOH) 247 nm (log ε 4.3), 312 (4.1), 334 (4.1); IR (Nujol) 3200, 1600, 800, 770, 760, 740, and 700 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.50 (br, 2 H) and 7.06–7.68 (m, 10 H); mass spectrum, *m/e* (rel intensity) 208 (100, M$^+$), 180 (8), 104 (10), 103 (25), and 77 (14).

Nitrosation of 2-Methylindole. (i) With Isoamyl Nitrite and Sodium Methoxide. The procedure by Noland et al.[14] was adopted. Compound **5** was obtained (72%) as yellow prisms (ethanol–water): mp 205–206 °C dec [lit.[14] mp 198–200 °C dec; mp 198 °C dec[19]]; UV λ_{max} (95% EtOH) 240 nm (log ε 4.2), 253 (4.3), 277 (sh, 3.5), 313 (3.7), 357 (sh, 3.4); IR (Nujol) 2800–2200, 1860, 1560, 1550, 1370, 1340, 1020, 870, and 760 cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 2.51 (s, 3 H), 7.26 (m, 1 H), 7.42 (m, 2 H), 7.97 (d, 1 H, *J* = 6.3 Hz), and 13.47 (br, 1 H); mass spectrum, *m/e* (rel intensity) 160 (36, M$^+$), 143 (100), 102 (52), and 75 (28). (ii) **With 0.05 M Aqueous Sodium Nitrite.** A mixture of 2-methylindole (230 mg, 1.8 mmol) and 0.05 M aqueous sodium nitrite (351 mL, 18 mmol; adjusted to pH 2.5 with dilute HCl) was stirred at room temperature for 1 h. The precipitated solid was filtered and on recrystallization from ethanol–water afforded 32 mg (11%) of **5**, having identical physical and spectroscopic data as obtained above. The filtrate was extracted with ethyl acetate (3 × 40 mL). Evaporation of the washed and dried ($MgSO_4$) extracts afforded deep orange crystals. Flash chromatography on a column of SiO_2 with ethyl acetate–petroleum ether (1:3) afforded 84 mg (30%) of **11** as pale orange prisms: mp 180–181 °C; UV λ_{max} (95% EtOH) 236 nm (log ε 4.0), 241 (sh. 3.9), 299 (4.0) [lit.[17] UV λ_{max} (EtOH) 235 nm (3.9), 241 (3.9), 298 (4.0)]; IR (Nujol) 3200, 1650, 1210, 1160, 960, 780, and 755 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.76 (s, 3 H), 7.34 (dt, 1 H, *J* = 6.8, 1 Hz), 7.46 (dt, 1 H, *J* = 6.8, 1 Hz), 7.56 (d, 1 H, *J* = 6.8 Hz), 8.40 (dd, 1 H, *J* = 6.8, 1 Hz), and 10.55 (br, 1 H); mass spectrum, *m/e* (rel intensity) 160 (46, M$^+$), 145 (100), 117 (12), 90 (27), and 43 (47).

2-Methylindolin-3-one Oxime (14). To 2-methyl-3-oximino-3*H*-indole (**5**) (529 mg, 3.3 mmol) in ethanol (5 mL) was added powdered sodium borohydride (376 mg, 9.9 mmol). After 30 min, most of the solvent was removed and water (10 mL) was added. Extraction (ethyl acetate, 3 × 10 mL), drying ($MgSO_4$), and evaporation afforded an oil, which was chromatographed on a column of SiO_2. Fractions eluted with ethyl

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acetate–petroleum ether (3:7) on evaporation gave 380 mg (71%) of **14** as very pale brown prisms: mp 113–114 °C; UV λ_{max} (95% EtOH) 227 nm (log ϵ 4.3), 258 (sh, 3.9), 358 (3.7); IR (Nujol) 3350, 3300–2700, 1640, 1600, 1585, 1325, 1240, 960, 760, and 755 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.47 (d, 3 H, J = 6.5 Hz), 4.18 (b, 1 H), 4.42 (q, 1 H, J = 6.5 Hz), 6.73 (d, 1 H, J = 8.2 Hz), 6.79 (t, 1 H, J = 8.2 Hz), 7.28 (dt, 1 H, J = 8.2, 1 Hz), 8.22 (d, 1 H, J = 8.2 Hz), and 8.89 (br, 1 H); mass spectrum, m/e (rel intensity) 162 (99, M^+), 147 (100), 131 (86), 117 (41), 103 (34), 90 (10), and 77 (27).

***N*-Nitroso-2-methylindolin-3-one Oxime 15a and 15b.** A solution of 2-methylindolin-3-one oxime **14** (109 mg, 0.7 mmol) in methanol (2 mL) followed by 2 M HCl (0.4 mL, 0.8 mmol) was added to a solution of sodium nitrite (63 mg, 0.9 mmol) in water (0.4 mL) cooled at 0 °C. Yellow crystals soon started to deposit. After 1 h at 0 °C, the mixture was extracted with ethyl acetate (3 × 10 mL). Solvent removal gave a residue which was purified on a column of SiO_2. Fractions eluted with ethyl acetate–petroleum ether (3:7) on evaporation afforded 102 mg (79%) of **15a** and **15b** as yellow prisms: mp 146–147 °C dec; UV λ_{max} (95% EtOH) 219 nm (log ϵ 4.0), 260 (4.1), 302 (4.1); IR (Nujol) 3160, 1645, 1600, 1585, 1480, 1180, 1165, 1075, 975, 955, 770, and 760 cm^{-1}; ^1H NMR (Me_2SO-d_6) **15a** δ 1.44 (d, 3 H, J = 6.6 Hz), 5.18 (q, 1 H, J = 6.6 Hz), 7.43 (t, 1 H, J = 7.8 Hz), 7.64 (t, 1 H, J = 7.8 Hz), 7.97 (d, 1 H, J = 7.8 Hz), 8.35 (d, 1 H, J = 7.8 Hz), and 11.88 (s, 1 H); ^1H NMR (Me_2SO-d_6) **15b** δ 1.78 (d, 3 H, J = 6.6 Hz), 5.74 (q, 1 H, J = 6.6 Hz), 8.43 (d, 1 H, J = 7.8 Hz), 8.53 (d, 1 H, J = 7.8 Hz), and 11.99 (s, 1 H), two aromatic proton signals are hidden under those of **15a**. From the integral, **15a**:**15b** is approximately 93:7; mass spectrum, m/e (rel intensity) 191 (6, M^+), 161 (46), 144 (100), 129 (62), 117 (16), 103 (76), 102 (6), 76 (42), and 43 (35).

Nitrosation of 5-Chloroindole. The procedure for 4-chloroindole was followed. After 1 h at room temperature, the crude product was purified by a column of SiO_2. Fractions eluted with ethyl acetate–petroleum ether (1:3) recovered unreacted 5-chloroindole (35%) and 81 mg (31%) of **12** as deep yellow prisms: mp 209–210 °C dec; UV λ_{max} (95% EtOH) 213 nm (log ϵ 4.4), 239 (4.0), 246 (4.0), 287 (3.9), 305 (3.9); IR (Nujol) 3240, 1660, 1580, 1335, 1310, 1110, 1045, 930, 810, and 800 cm^{-1}; ^1H NMR (CD_3CN) δ 7.44 (dd, 1 H, J = 7.3, 1.8 Hz), 7.57 (d, 1 H, J = 7.3 Hz), 8.17 (d, 1 H, J = 1.8 Hz), 10.18 (s, 1 H), and 12.01 (br, 1 H); mass spectrum, m/e (rel intensity) 182 (33, M^+ + 2), 180 (100, M^+), 181 (26), 179 (50), 154 (11), 152 (34), 125 (22), and 90 (16).

5-Chloroindazole-3-carboxylic Acid. A literature procedure[24] was adopted. 5-chloroindazole-3-carboxylic acid was obtained (44%) as a pale yellow prisms: mp 293–294 °C; UV λ_{max} (95% EtOH) 213 nm (log ϵ 4.5), 268 (3.7), 274 (3.7), 301 (3.8), 311 (sh, 3.7); IR (Nujol) 3500–2400, 1680, 1580, 1175, 930, 805, and 780 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 7.30 (dd, 1 H, J = 8, 2 Hz), 7.60 (d, 1 H, J = 8 Hz), 7.96 (d, 1 H, J = 2 Hz), and 13.40 (br, 2 H).

5-Chloro-3-(hydroxymethyl)indazole and 5-Chloroindazole-3-carboxaldehyde (12). A mixture of 5-chloroindazole-3-carboxylic acid (396 mg, 2 mmol) and lithium aluminum hydride (380 mg, 10 mmol) in dry THF (20 mL) was refluxed for 4 h. After the mixture cooled in an ice bath, excess ethyl acetate was added dropwise to destroy the excess hydride and the mixture was poured into aqueous HCl (30 mL). Extraction (ethyl acetate), drying ($MgSO_4$), and evaporation gave 307 mg (83%) of 5-chloro-3-(hydroxymethyl)indazole as very pale brown prisms: mp 208–210 °C dec; IR (Nujol) 3200 and 805 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 4.72 (s, 2 H), 5.50 (br, 2 H), 7.25 (dd, 1 H, J = 8, 2 Hz), 7.50 (d, 1 H, J = 8 Hz), and 7.80 (d, 1 H, J = 2 Hz). Without further purification, the product from above (37 mg, 0.2 mmol) was added to a well-stirred suspension of pyridinium chlorochromate (66 mg, 3 mmol) in CH_2Cl_2 (1 mL). After 1 h at room temperature, the mixture was diluted with ether (5 mL) and filtered through Celite. The filtrate, after being evaporated, was chromatographed on a column of SiO_2. Fractions eluted with ethyl acetate–petroleum ether (1:3) on evaporation afforded 24 mg (66%) of a pale orange solid, having identical physical and spectroscopic data as **12**.

Nitrosation of 4-Chloroindole. A mixture of 4-chloroindole (201 mg, 1.3 mmol) and 0.05 M aqueous sodium nitrite (265 mL, 13 mmol, adjusted to pH 2.5 with dilute HCl) was stirred at room temperature, with protection from light, for 1 h. Extraction (ethyl acetate, 3 × 50 mL), drying ($MgSO_4$), and evaporation afforded a viscous oil which was purified on a column of SiO_2. Fractions eluted with ethyl acetate–petroleum ether (1:3) on evaporation gave 71 mg (24%) of **16a** and **16b** as deep yellow prisms: mp 128–129 °C dec; UV λ_{max} (95% EtOH) 223 nm (sh, log ϵ 4.1), 262 (4.2), 292 (3.9); IR (Nujol) 3540–3100, 1600, 1585, 1480, 1190, 1155, 1130, 940, 790, 760, and 735 cm^{-1}; ^1H NMR (CD_3CN) **16a** δ 5.19 (d, 1 H, J = 5.2 Hz, exchanged by D_2O), 6.52 (d, 1 H, J = 5.2 Hz, sharpened into a singlet on D_2O exchange), 7.40 (d, 1 H, J = 8.3 Hz), 7.51 (t, 1 H, J = 8.3 Hz), 7.87 (d, 1 H, J = 8.3 Hz), and 9.78 (1 H, s, exchanged by D_2O); ^1H NMR (CD_3CN) **16b** δ 5.52 (d, 1 H, J = 5.2 Hz, exchanged by D_2O), 7.09 (d, 1 H, J = 5.2 Hz, sharpened into a singlet on D_2O exchange), 8.32 (dd, 1 H, J = 8.3, 2.6 Hz), and 9.91 (s, 1 H), two aromatic proton signals are hidden under those of **16a**. From the integral **16a**:**16b** is approximately 4:1; mass spectrum, m/e (rel intensity) 227 (1, M^+), 197 (12), 180 (28), 163 (19), 149 (23), 137 (23), 125 (12), and 111 (13). Fractions eluted with ethyl acetate–petroleum ether (2:3) on evaporation gave 15 mg (6%) of **13** as very pale brown prisms: mp 207–208 °C dec; UV λ_{max} (95% EtOH) 242 nm (log ϵ 4.0), 248 (sh, 3.9), 296 (sh, 3.9), 301 (3.9); ^1H NMR (CD_3CN) δ 7.41 (d, 1 H, J = 7.9 Hz), 7.46 (t, 1 H, J = 7.9 Hz), 7.67 (dd, 1 H, J = 7.9 Hz), and 10.48 (s, 1 H); mass spectrum, m/e (rel intensity) 182 (32, M^+ + 2), 180 (100, M^+), 152 (24), 124 (11), and 89 (27).

Nitrosation of 4-Chloro-6-methoxyindole (22). The procedure for 4-chloroindole was followed. The product, after flash chromatography on SiO_2, was further purified by HPLC silica [Partisil PX5 5/25 (Whatman)] with ethyl acetate as the mobile phase and **17** was obtained (15%) as an orange solid after solvent removal: UV λ_{max} (MeOH) 233 nm (sh, log ϵ 3.5), 269 (3.5), 302 (3.2), 348 (3.0); IR (neat) 3400–3180, 1612, 1461, 1147, 951, and 828 cm^{-1}; ^1H NMR (CD_3CN) δ 3.66 (s, 3 H), 4.98 (d, 1 H, J = 6.9 Hz), 6.27 (d, 1 H, J = 6.9 Hz), 6.73 (d, 1 H, J = 2.2 Hz), 7.17 (d, 1 H, J = 2.2 Hz), and 9.30 (s, 1 H); mass spectrum, m/e (rel intensity) 259 (1, M^+ + 2), 257 (2, M^+), 227 (10), 210 (100), 212 (37), 193 (34), and 183 (64).

Synthesis of 4-Chloro-6-methoxyindole (22). 4-Fluoro-2,6-dinitrotoluene (18). Fuming nitric acid (55 mL, 1.3 mol) was added dropwise to a solution of 4-fluoro-2-nitrotoluene (100 g, 0.65 mol) in fuming sulfuric acid (137 mL) with external cooling (temperature <60 °C). After warming at ca. 80 °C for 3 h, another equivalent of fuming nitric acid (25 mL) and fuming sulfuric acid (75 mL) was added, followed by stirring at ca. 90 °C for another 3 h. On cooling, the mixture was poured into ice whereby the product separated out as a yellow semisolid. Extraction (CH_2Cl_2), drying ($MgSO_4$), and evaporation gave a yellow oil. Crystallization from hexane afforded 45 g (45%) of **18**: mp 44–47 °C; UV λ_{max} (MeOH) 232 nm (log ϵ 4.2), 299 (3.6); ^1H NMR (CD_3CN) δ 2.47 (s, 3 H), 7.96 (d, 2 H, J = 8.3 Hz); mass spectrum, m/e (rel intensity) 200 (18, M^+), 183 (100), 137 (33), and 107 (60).

2,6-Dinitro-4-methoxytoluene (19). 4-Fluoro-2,6-dinitrotoluene (**18**) (30 g, 0.15 mol) was added dropwise to a solution of sodium methoxide (0.2 mol; freshly prepared from 4.5 g of sodium and 450 mL of absolute methanol) at room temperature. After 2 h of reflux, the solution was concentrated to a small volume and filtered. Recrystallization from ethanol gave 18 g (57%) of **19**: mp 105–106 °C; UV λ_{max} (MeOH) 248 nm (log ϵ 4.0), 329 (3.6); ^1H NMR (CD_2Cl_2) δ 2.45 (s, 3 H), 3.90 (s, 3 H), and 7.53 (s, 2 H); mass spectrum, m/e (rel intensity) 212 (32, M^+), 195 (100), 165 (15), and 120 (30).

2-Amino-4-methoxy-6-nitrotoluene (20). Sodium bicarbonate (11.1 g, 0.13 mol) was added to a solution of sodium sulfide nonahydrate (31.3 g, 0.13 mol) in water (60 mL) at room temperature. After solution occurred, methanol (78.2 mL) was added, followed by standing at room temperature for $^1/_2$ h, and the solution was filtered. The filtrate, which was sodium hydrosulfide in methanol, was refluxed with a solution of 2,6-dinitro-4-methoxytoluene (**19**) (16.3 g, 77 mmol) in the same solvent (130 mL) for 1 h. After solvent removal, the residue was boiled with HCl (0.1 M) and filtered. On neutralization (pH 7), 5 g (35%) of pure **20** was obtained: mp 87–88 °C; UV λ_{max} (MeOH) 233 nm (log ϵ 3.9), 284 (3.0), 345 (2.9); ^1H NMR ($CDCl_3$) δ 2.18 (s, 3 H), 3.78 (s, 3 H), 3.93 (br, 2 H), 6.42 (d, 1 H, J = 3.3 Hz), and 6.73 (d, 1 H, J = 3.3 Hz); mass spectrum, m/e (rel intensity) 182 (32, M^+), 165 (18), 137 (33), 136 (37), 135 (28), 121 (39), and 120 (55).

2-Chloro-4-methoxy-6-nitrotoluene (21). A solution of 2-amino-4-methoxy-6-nitrotoluene (**20**) (12.5 g, 69 mmol) in water (10 mL) and 12 M HCl (25.4 mL) was diazotized, using a solution of sodium nitrite (4.74 g, 69 mmol) in water (13.8 mL), in the usual manner at 0–5 °C, followed by pouring the mixture into a solution of copper(I) chloride (6.8 g, 69 mmol) in 12 M HCl (23.8 mL) and water (8.5 mL) at the same temperature. After gradual warming to room temperature (10 min) and then over a steam bath, the mixture was left in the refrigerator overnight. The solid thus separated out was filtered and recrystallization from 40% aqueous ethanol, affording 8 g (58%) of **21**: mp 74–75 °C; UV λ_{max} (MeOH) 242 nm (log ϵ 3.8), 269 (3.6), 324 (3.5); ^1H NMR (CD_2Cl_2) δ 2.43 (s, 3 H), 3.80 (s, 3 H), 7.22 (d, 1 H, J = 2.1 Hz), and 7.26 (d, 1 H, J = 2.1 Hz); mass spectrum, m/e (rel intensity) 203 (15, M^+ + 2), 201 (48, M^+), 186 (22), 184 (71), 156 (100), 154 (24), and 140 (12).

4-Chloro-6-methoxyindole (22). The procedure developed by Batcho and Leimgruber[27] was modified as noted. A mixture of 2-chloro-4-methoxy-6-nitrotoluene (**21**) (1.2 g, 6 mmol), *N*,*N*-dimethylformamide dimethyl acetal (1.2 mL, 9 mmol; freshly distilled over sodium), pyrrolidine (0.73 mL, 9 mmol), and DMF (3 mL) was refluxed for 3 h. The residue, after solvent removal, was resuspended in benzene (5 mL) and hydrogenated, in the presence of 5% palladium on charcoal (ca. 50 mg),

under 4 atm of hydrogen overnight. After filtering through Celite, the filtrate was washed (2 M HCl, 2 × 50 mL), dried ($MgSO_4$), and evaporated down to give **22** as a yellow oil, which was further purified by a SiO_2 column with $CH_2Cl_2/CHCl_3$ (1:1).

An alternate reductive cyclization step was to add a 20% titanium(III) chloride HCl solution and twice that volume of ammonium acetate (4 M) to the crude nitrostyrene in a minimum volume of acetone. After 10 min at room temperature, extraction (ether) and purification as above gave **22** as a yellow oil: UV λ_{max} (MeOH) 220 nm (log ϵ 4.3), 270 (3.5), 295 (3.5); IR (KBr) 3410–3430, 1624, 1574, 1507, 1149, 908, 836, 759, and 716 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 3.75 (s, 3 H), 6.30 (t, 1 H, $J_{H_2H_3}$ = 2.6 Hz), 6.74 (d, 1 H, J = 1.9 Hz), 6.89 (d, 1 H, J = 1.9 Hz), and 11.2 (br, 1 H). Yields varied between 40% and 60%.

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Photochemical Reaction of 1,4-Naphthalenedicarbonitrile with Alkylbenzenes and Bibenzyls

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Abstract: The photochemical reaction of 1,4-naphthalenedicarbonitrile with some alkylbenzenes and bibenzyls has been examined. A unitary mechanistic picture is formulated on the basis of product study, deuteration experiments, and fluorescence and reaction quantum yield measurements. Proton transfer within the singlet radical ion pair followed by in-cage cycloaddition of the two radicals yields stereoselectively 5,11-methanodibenzo[*a*,*e*]cyclooctene derivatives (**8**). Reaction of benzyl radicals (formed by protolysis or, for radical cations having no benzylic proton, by C–C bond cleavage) with unprotonated $NDN^{\bullet-}$ leads, again stereoselectively, to 2-benzyl-1,2-dihydronaphthalenes (**9**). Escape of the donor radical cation and following C–H or C–C bond cleavage leads to a different product; thus, benzyl radicals are trapped by NDN to yield substitution products (**11**) or recombine. Benzyl cations are trapped by nucleophiles.

The synthetic usefulness of photochemical electron transfer is demonstrated by the number and variety of the applications reported in the last decade.[1] As it has been recognized by Arnold in his pioneering studies,[2] this process requires that electron transfer (e.g., eq 1 for the case of a singlet excited acceptor, A, and a benzene derivative, PhX, as the donor) be exothermic.[3]

$$A^{1*} + PhX \rightarrow A^{\bullet-} + PhX^{\bullet+} \quad (1)$$

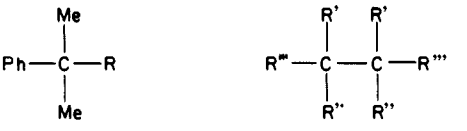
Whether this condition is met is easily ascertained on the basis of the redox potentials of the two compounds and of the excitation energy of A (Weller equation[4]). Of course, the efficiency of the ensuing chemical reaction is not as easily predicted, the only possible generalization being that back electron transfer with or without energy dissipation (eq 2) is the fate of the pair of radical

$$A^{\bullet-} + PhX^{\bullet+} \rightarrow A^* + PhX \quad (2)$$

$$A^{\bullet-} + PhX^{\bullet+} \rightarrow A + PhX$$

ions of opposite sign, unless some fast process, e.g., a molecular vibration,[5] starts a concurrent pathway, leading to chemical transformation. This explains why reactions involving radical ions of aromatic olefins (X = >C=C<) form by far the larger class of reactions within electron-transfer photochemistry of benzene derivatives (compare ref 6), since in this case deformation of the carbon–carbon bond opens exactly such a pathway.

Chart I. Organic Substrates under Study



1. R = H
1'. R = D
2. R = Me
3. R = $CHMe_2$
4. R' = H, R'' = Ph, R''' = H
5. R' = Me, R'' = Ph, R''' = Me
6. R' = H, R'' = H, R''' = C_6H_4-*p*-OMe
7. R' = Ph, R'' = Ph, R''' = H

Conceivably, another fast process initiating a chemical reaction is proton transfer, e.g., from the radical cation of an alkylbenzene. Indeed, thermochemical calculations show that the toluene radical cation has a pK_a value of ca. −10,[7] and we reported a photochemical reaction between 1,4-naphthalenedicarbonitrile (NDN) and toluene and other methylbenzenes.[8] This intriguing reaction (the main product is a bicyclo[3.3.1]nonane derivative) involves electron transfer followed by base-mediated proton transfer within the geminate radical ion pairs and stereoselective addition between naphthyl and benzyl radicals before they diffuse apart.[9]

A partial mechanistic analogy to the above reaction can be found in the NDN-sensitized photooxygenation of bibenzyl derivatives, recently reported by Griffin and Das,[10,11] in that benzyl

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(2) Arnold, D. R.; Maroulis, A. J. *J. Am. Chem. Soc.* **1976**, *98*, 5931.

(3) However, in certain cases an *endothermic* electron transfer is involved in an efficient photoreaction. See, e.g.: Schaap, A. P.; Lopez, L.; Gagnon, S. D. *J. Am. Chem. Soc.* **1983**, *105*, 663. See also ref 6.

(4) (a) Rehm, D.; Weller, A. *Isr. J. Chem.* **1970**, *8*, 259. (b) Weller, A. *Nobel Symp.* **1967**, *5*, 413. (c) Rehm, D.; Weller, A. *Ber. Bunsenges. Phys. Chem.* **1969**, *73*, 834.

(5) Other mechanisms increasing the efficiency of an overall process initiated by photochemical electron transfer, such as secondary electron transfer or chain mechanisms, have been discussed in detail by Farid; see ref 6.

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(8) Albini, A.; Fasani, E.; Oberti, R. *Tetrahedron* **1982**, *38*, 1027.

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