Mechanism and Products of Autoxidation of 5,7-Dihydroxytryptamine[1]

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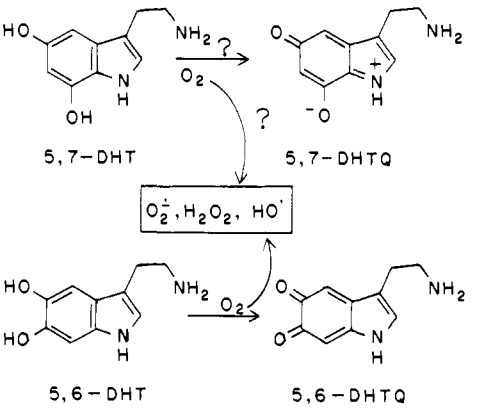
Abstract: The neurodegenerative effect of 5,7-dihydroxytryptamine (5,7-DHT) has been postulated to involve alkylation of neuronal proteins by its autoxidation product, which is believed to be the corresponding *p*-quinone imine (5,7-DHTQ). To more precisely elucidate the mechanism of autoxidation of 5,7-DHT and its molecular mechanism of action in vivo, a series of methyl-substituted 5,7-DHT's, namely, 4-Me-, 6-Me-, and 4,6-Me_2-5,7-DHT's (**21a–c**, respectively), were designed and synthesized as probes. The indole nucleus of these probes was constructed by the reductive cyclization of the corresponding 2,β-dinitrostyrene and the aminoethyl side chain was introduced via gramine methiodides. Cyclic voltammetric analysis of 5,7-DHT and **21a–c** in 0.1 M H_2SO_4 and pH 4.0 and 7.4 buffers indicated that the primary product of electrochemical oxidation from each compound is the corresponding *p*-quinone imine which is very short lived (<1 s). Like 5,7-DHT, **21a–c** exhibited phenol–keto tautomerism but **21a** and **21c** did so to a much smaller extent as determined by UV and NMR spectroscopic methods. The predominant nonphenolic tautomeric form of 5,7-DHT, out of eight possible, was shown to be **27** ($R_1 = R_2 = H$). Polarographic measurements showed that, like 5,7-DHT, **21a–c** react with O_2 in a bimolecular fashion. Consideration of all the data indicates that the initial product of autoxidation in all cases is apparently a 4-hydroperoxy derivative. Only in the case of 5,7-DHT and **21b**, the initially formed hydroperoxides break down to give colored products. These products were not stable enough to be isolated, but their UV–visible spectra correlate very well with similar known structures. The structure of this product in the case of 5,7-DHT is postulated to be the (4,7) *p*-quinone of 4,5,7-trihydroxytryptamine (4,5,7-THTQ, **35**, R = H). The results of this study further indicate that in the autoxidation of 5,7-DHT, the *p*-quinone imine (5,7-DHTQ) is almost certainly not the primary product nor is it a transient intermediate in the formation of 4,5,7-THTQ. 4,5,7-THTQ also is unstable and appears to undergo further transformations at pH 7.4.

The study of the functional organization and the role of the nervous system has been greatly aided by the discovery of chemical agents that can bring about selective degeneration of a given type of neuron.[2-7] Some of the more important examples of these chemical denervation tools include [(2,4,5-trihydroxyphenyl)-ethyl]amine (6-hydroxydopamine, 6-OHDA) for inducing degeneration in catecholamine containing neurons[4,7,8] and 5,6- and 5,7-dihydroxytryptamines (5,6-DHT and 5,7-DHT, respectively) for the denervation of 5-hydroxytryptamine (5-HT, serotonin) containing neurons.[2-7] While the selectivity of these agents is derived from their high affinity uptake by the respective neuronal membrane pumps, their ability to induce neuronal degeneration appears to be the result of an inherent chemical property, namely, autoxidizability.

In the case of 6-OHDA, an extensive body of research[4,7-10] has established that autoxidation results in the formation of electrophilic quinones and reduced-O_2 species such as H_2O_2, HO·, and O_2^{-}·. Neuronal degeneration is believed to be the result of alkylation of neuronal proteins by these quinones together with oxidation of lipids etc. by the reduced-oxygen species. Qualitatively similar mechanisms have been proposed[5,6,10-12] for both 5,6-DHT and 5,7-DHT (see Chart I) in spite of the fact that 5,7-DHT lacks a hydroquinone (as in 6-OHDA) or a catechol (as in 5,6-DHT) moiety. Indeed, there are remarkable differences in the physical and chemical properties of 5,6-DHT and 5,7-DHT. Unlike 5,6-DHT (and 5-HT), 5,7-DHT exhibits pronounced phenol–keto tautomerism[13] above pH 7. A role for this unique property of 5,7-DHT in its reaction with O_2 or in its expression of neurotoxic effects has not yet been postulated. While the autoxidation of 5,6-DHT at pH 7.4 proceeds with autocatalytic promotion[6,12] eventually producing black precipitate and 40–60 mol % yield of H_2O_2, 5,7-DHT reacts with O_2 at least 10 times faster[12] in a bimolecular fashion producing soluble products and with very little,

Chart I



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if any, production[6,10] of H_2O_2. Apparently hydroxyl radicals are also formed during the autoxidation of 5,7-DHT, as hydroxyl radical scavangers have been shown to protect peripheral sympathetic neurons against destruction by 5,7-DHT.[10] The reactivity of the respective quinone products, none of which has been characterized, but which are postulated to be 5,6-DHTQ and 5,7-DHTQ, differ significantly. By use of radioactive DHT's, it has been shown that the oxidation product(s) of 5,6-DHT undergoes extensive covalent binding with protein nucleophiles both in vitro[11b] (e.g., 17 mol of 5,6-DHT-derived product/mol of bovine serum albumin (BSA)) and in vivo[11a] while the oxidation product(s) of 5,7-DHT binds rather poorly in vitro[11b] (1 mol of 5,7-DHT-derived product/mol of BSA) as well as in vivo.[11a]

Our initial objective was to determine the relative importance of various sites on 5,7-DHTQ, postulated to be the major autoxidation product, toward alkylation by protein nucleophiles in vivo. We thought that, because of the many possible reactions which 5,7-DHTQ or any other quinoid autoxidation product of 5,7-DHT not yet postulated could undergo, direct isolation and chemical characterization of the reaction products from biological systems would be difficult and perhaps impossible. Therefore, we sought to design probes which hopefully will exhibit similar affinities for the membrane transport pump and similar redox potentials but different inherent reactivities to nucleophiles. Our previous work[9d,e,14] with 6-OHDA and 5,6-DHT suggested that these objectives might be accomplished by the incorporation of a methyl substituent at the postulated site of nucleophilic attack. Thus, the probes we required are 4-Me-5,7-DHT (**21a**), 6-Me-5,7-DHT (**21b**), and 4,6-Me_2-5,7-DHT (**21c**). Synthesis of these probes as well as the detailed comparative study of the physical chemical properties of 5,7-DHT and the probes is the subject of this paper. The results described below indicate the discovery of a unique mechanism of autoxidation of 5,7-DHT and, further, that 5,7-DHTQ is probably not formed even transiently.

Experimental Section

General Methods. Infrared spectra were obtained on a Beckman IR-33 spectrophotometer, UV–vis spectra on a Perkin-Elmer 555 spectrophotometer, and ^1H NMR spectra on a Varian T-60 or FT-80A spectrometer (with Me_4Si as internal standard). Melting points were obtained on a Thomas Hoover capillary melting point apparatus and are given uncorrected. Column chromatography was performed on silica gel 60 (70–270 mesh).

3,5-Bis(benzyloxy)-2-methylbenzaldehyde (4). To a stirred solution of anhydrous morpholine (10.5 g, 120 mmol) in 200 mL of anhydrous THF under a dry N_2 atmosphere at −78 °C (dry ice–acetone bath) was added 1.6 M *n*-BuLi in hexane (75 mL, 120 mmol). After 10 min, a solution of 3,5-bis(benzyloxy)-2-bromobenzaldehyde[15] (**2**, 39.7 g, 100 mmol) in 120 mL of anhydrous THF was added over a period of 2 min and the mixture was stirred at −78 °C for 20 min.[16] *n*-BuLi in hexane (1.6 M, 100 mL, 160 mmol) was then added over a period of 15 min, keeping the temperature at −78 °C. After stirring the mixture at −78 °C for 40 min, a solution of $(MeO)_2SO_2$ (63 g, 500 mmol) in 50 mL of anhydrous THF was added, keeping the temperature below −70 °C. After stirring at −78 °C for 2 h, the mixture was allowed to warm to 25 °C and was stirred at 25 °C for 1 h and then acidified to pH ~1 with 2 N HCl. After stirring for 15 min at 25 °C, the mixture was diluted with brine and ether, and the organic layer was collected. The aqueous layer was extracted twice with ether, and the combined organic solutions were washed, in order, with a brine–2 N HCl mixture and brine and $NaHCO_3$ solution, dried (Na_2SO_4), and then evaporated in vacuo to dryness. The syrupy residue deposited pale yellow solid on trituration with ice-cold 95% EtOH. Recrystallization of the crude solid from benzene–hexane gave 23.9 g (72%) of **4** as a colorless amorphous solid: mp 92–93 °C; NMR ($CDCl_3$) δ 2.51 (s, 3, Me), 5.07 (s, 4, OCH_2), 6.80 (d, $J_{4,6}$ = 3 Hz, 1, H-4), 7.07 (d, $J_{6,4}$ = 3 Hz, 1, H-6), 7.30–7.50 (m, 10, Ph), 10.32 (s, 1, CHO); IR (Nujol) 1665, 1600 cm^{-1}. Anal. ($C_{22}H_{20}O_3$) C, H.

3,5-Bis(benzyloxy)-4-methylbenzaldehyde (6). To a stirred suspension of $LiAlH_4$ (6.08 g, 160 mmol) in 120 mL of THF was added a solution of benzyl 3,5-bis(benzyloxy)-4-methylbenzoate[17] (**5b**, 35 g, 80 mmol) in 220 mL of THF, dropwise under an N_2 atmosphere, and the mixture was refluxed with stirring for 3 h. The mixture was cooled in an ice–water bath and 60 mL of EtOAc was added dropwise. The mixture was then diluted with ether, and 2 N HCl was added cautiously until the mixture was slightly acidic. The mixture was filtered (or decanted), the filtrate washed once with a $NaHCO_3$–NaCl solution, and the solvent removed under reduced pressure. The syrupy residue solidified upon stirring with water. The solid was collected by filtration, washed thoroughly with water, dried over P_2O_5 under vacuum, and recrystallized from benzene–hexane to give 24.3 g (91%) of 3,5-bis(benzyloxy)-4-methylbenzyl alcohol: mp 65–67 °C; NMR ($CDCl_3$) δ 2.20 (s, 3, Me), 2.38 (s, 1, OH), 4.49 (s, 2, CH_2OH), 4.97 (s, 4, OCH_2Ph), 6.50 (s, 2, H-2 and H-6), 7.16–7.42 (m, 10, Ph). Anal. ($C_{22}H_{22}O_3$) C, H.

To an efficiently stirred suspension of pyridinium chlorochromate (24.9 g, 115 mmol) in 50 mL of CH_2Cl_2 was added, at 15–20 °C, a solution of 3,5-bis(benzyloxy)-4-methylbenzyl alcohol (25 g, 75 mmol) in 120 mL of CH_2Cl_2 all at once. The mixture was then stirred at 25 °C for 2.5 h protected from moisture. After dilution with 500 mL of ether the mixture was filtered through a pad of Celite and the filtrate was passed through a column (20 × 5 cm) of silica gel in 50:50 ether–CH_2Cl_2. Further elution with the same solvent mixture and subsequent evaporation of solvent gave a pale yellow solid, which was recrystallized from benzene–hexane to give **6** as colorless plates (24 g, 96%): mp 70–71 °C; IR (Nujol) 1700, 1585 cm^{-1}; NMR ($CDCl_3$) δ 2.27 (s, 3, Me), 5.10 (s, 4, OCH_2), 7.08 (s, 2, H-2 and H-6), 7.20–7.60 (m, 10, Ph), 9.87 (s, 1, CHO). Anal. ($C_{22}H_{20}O_3$) C, H.

2,4-Dimethoxy-3-methylbenzaldehyde (7). To a stirred solution of 2,6-dimethoxytoluene (30.4 g, 200 mmol) in 300 mL of CH_2Cl_2 at 5–10 °C and protected from moisture was added 60.8 g (320 mmol) of $TiCl_4$, followed by a solution of Cl_2CHOMe (23.6 g, 205 mmol) in 30 mL of CH_2Cl_2 over a period of 20 min. The mixture was stirred at 10 °C for 1 h and then at 25 °C for 1 h. The solution was then poured into ice–water and stirred for 1 h, and the organic layer was collected and washed, in order, with 3 N HCl, brine, and $NaHCO_3$ solution. It was then dried (Na_2SO_4) and evaporated in vacuo to dryness. The residue, on recrystallization from hexanes (Skelly-B) gave **7** as long rods: mp 53–54 °C (lit.[18] mp 52–53 °C); IR (Nujol) 1692, 1600 cm^{-1}; NMR ($CDCl_3$) δ 2.17 (s, 3, Me), 3.88 (s, 3, OMe), 3.92 (s, 3, OMe), 6.73 (d, $J_{5,6}$ = 9 Hz, 1, H-5), 7.75 (d, $J_{6,5}$ = 9 Hz, 1, H-6), 10.23 (s, 1, CHO).

2,4-Dimethoxy-1-[(dimethylamino)methyl]-3-methylbenzene (8). To a stirred solution of freshly vacuum dried Me_2NH·HCl (16.62 g, 204 mmol) in 120 mL of dry MeOH, protected from moisture, was added KOH pellets (89% KOH content, 3.6 g, 57 mmol). After 10 min, **7** (29.4 g, 163 mmol) was added. After a further 20 min, a solution of $NaBH_3CN$ (3.85 g, 61 mmol) in 30 mL of MeOH was added dropwise with cooling.[19] After the addition was complete, the mixture was stirred at 25 °C for 2.5 h and then made strongly basic (pH ~12) by adding NaOH pellets with cooling. The mixture was filtered, and the filtrate was evaporated in vacuo to dryness. The oily residue was diluted with water and made acidic (pH ~1) with 6 N HCl with cooling inside a hood (**caution**: HCN is produced upon addition of HCl). The solution was washed twice with ether and the aqueous layer was collected and made strongly basic (pH ~12) by adding NaOH pellets with cooling and stirring over 30 min. The mixture was then extracted with CH_2Cl_2 (3 × 50 mL), and the combined organic layers were washed separately with H_2O and brine, dried (K_2CO_3), and evaporated in vacuo to dryness to give 30.4 g (89%) of **8** as a colorless oil: NMR ($CDCl_3$) δ 2.14 (s, 3, Me), 2.20 (s, 6, NMe_2), 3.36 (s, 2, CH_2N), 3.70 (s, 3, OMe), 3.77 (s, 3, OMe), 6.58 (d, $J_{5,6}$ = 9 Hz, 1, H-6), 7.20 (d, $J_{6,5}$ = 9 Hz, 1, H-5). Anal. ($C_{12}H_{19}NO_2$) C, H, N.

[(2,4-Dimethoxy-3-methylphenyl)methyl]trimethylammonium Iodide (9). To a stirred solution of **8** (42.6 g, 204 mmol) in 75 mL of CH_2Cl_2 was added, with cooling in an ice–water bath, a solution of MeI (37 g, 260 mmol) in 20 mL of CH_2Cl_2 over a period of 15 min. The mixture was then stirred at 25 °C for 3 h and evaporated in vacuo to dryness. To the residue was added 100 mL of acetone, and the resulting mixture was stirred briefly. Hexane (Skelly-B, 50 mL) was then added with stirring. After cooling to 5 °C the mixture was filtered and the white solid was dried under vacuum to give 68.7 g (96%) of **9**: mp 188–190 °C dec; NMR ($CDCl_3$) δ 2.16 (s, 3, Me), 3.38 (s, 9, $-N^+Me_3$), 3.79 (s, 3, OMe), 3.87 (s, 3, OMe), 4.83 (s, 2, CH_2N^+), 6.72 (d, $J_{5,6}$ = 9 Hz, 1, H-5), 7.62 (d, $J_{5,6}$ = 9 Hz, 1, H-6). Anal. ($C_{13}H_{22}INO_2$) C, H, N.

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3,5-Dimethoxy-2,4-dimethyl-1-[(dimethylamino)methyl]benzene (10). About 250 mL of anhydrous NH_3 was condensed using a dry-ice acetone bath and a dry-ice condenser in a three-neck round-bottom flask under nitrogen. $Fe(NO_3)_3 \cdot 9H_2O$ (400 mg) was added with stirring followed by Na (4.37 g, 190 mmol) in ~100-mg portions. After 30 min (or when the blue coloration disappeared) the methodide **9** (33.3 g, 95 mmol) was introduced over 5 min.[20] Additional NH_3 was condensed to maintain the original volume and then the dry-ice bath was removed. After 4 h, excess $NaNH_2$ was decomposed by carefully adding NH_4Cl (5.4 g, 100 mmol). The mixture was stirred until all NH_3 evaporated and the residue was diluted with H_2O (500 mL). The resulting mixture was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were washed separately with H_2O and brine, dried (K_2CO_3), and evaporated in vacuo to dryness to give **10** as a colorless oil (20 g, 94%). An analytical sample was obtained by chromatography on preparative thin-layer plates (silica gel, 1 mm, Analtech GF) using 9:1 Et_2O–EtOH as the developing solvent: NMR ($CDCl_3$) δ 2.14 (s, 3, Me), 2.22 (s, 6, NMe_2), 3.33 (s, 2, CH_2), 3.64 (s, 3, OMe), 3.78 (s, 3, OMe), 6.64 (s, 1, H-6). Anal. ($C_{13}H_{21}NO_2$) C, H, N.

3,5-Dimethoxy-2,4-dimethylbenzaldehyde (11). To a stirred solution of the amine **10** (18.75 g, 84 mmol) in 500 mL of reagent grade benzene was added *N*-bromosuccinimide[21] (15.3 g, 86 mmol, freshly recrystallized from water and dried over P_2O_5) in 2-g portions at 25 °C. The stoppered reaction mixture was stirred at 25 °C for 20 h, and then 300 mL of water was added. The resulting mixture was stirred vigorously under N_2 for 3 h and the organic layer was separated, washed with a brine–$NaHCO_3$ mixture, dried (Na_2SO_4), and evaporated in vacuo. The oily residue was chromatographed on a column of silica gel (60 g) using 9:1 CH_2Cl_2–hexane. Evaporation of eluents gave 9.5 g (58%) of **11** as an oil which solidified on storage at −20 °C for a few days. An analytical sample was obtained by chromatography on preparative thin-layer plates (silica gel, 9:1 CH_2Cl_2–hexane) followed by recrystallization from pentane: mp 93–94 °C; IR (Nujol) 1705, 1610 cm^{-1}; NMR ($CDCl_3$) δ 2.26 (s, 3, Me), 2.53 (s, 3, Me), 3.79 (s, 3, OMe), 3.90 (s, 3, OMe), 7.37 (s, 1, H-6), 10.08 (s, 1, CHO). Anal. ($C_{11}H_{14}O_3$) C, H.

3,5-Bis(benzyloxy)-2,4-dimethylbenzaldehyde (13). To a stirred solution of **11** (9 g, 46.4 mmol) in 180 mL of CH_2Cl_2 at −78 °C was added under an N_2 atmosphere a solution of BBr_3 (31.5 g, 140 mmol) in 20 mL of CH_2Cl_2. The mixture was stirred at −78 °C for 2 h and at 25 °C for 2 h and then poured into crushed ice containing 100 mL of concentrated HCl. After it was stirred briefly, the mixture was extracted with EtOAc (6 × 60 mL). The combined organic layers were washed, in order, with brine, $NaHCO_3$ solution, and brine and then dried (Na_2SO_4). The solution was clarified with neutral decolorizing charcoal and evaporated in vacuo to dryness. Recrystallization of the crude solid from EtOAc–hexane gave **12** as an off-white, air-sensitive solid (6.62 g, 86%): mp 177–180 °C; IR (Nujol) 3310, 1685, 1655, 1610, 1590 cm^{-1}; NMR (acetone-d_6) δ 2.18 (s, 3, Me), 2.47 (s, 3, Me), 6.20 (br s, 2, OH), 6.92 (s, 1, H-6), 10.14 (s, 1, CHO). Anal. ($C_9H_{10}O_3$) C, H. Aldehyde **12** was benzylated by heating a mixture of **12** (5.3 g, 31.9 mmol), $PhCH_2Cl$ (9 g, 72 mmol), powdered K_2CO_3 (18 g, 130 mmol), NaI (1.5 g, 10 mmol), and acetone (100 mL) under reflux in an N_2 atmosphere for 5 h. The mixture was cooled and filtered, and the filtrate was evaporated in vacuo to give a syrup which was dissolved in CH_2Cl_2. After it was washed with water and then with brine, the CH_2Cl_2 solution was dried (Na_2SO_4) and subsequently evaporated in vacuo to dryness. The syrupy residue was stirred with hexanes vigorously until solidification was complete. The crude solid was collected by filtration and chromatographed on a column of silica gel (40 g) in CH_2Cl_2. Evaporation of eluent gave 8.6 g (78%) of **13** as a fine powder. An analytical sample was prepared by recrystallization from benzene–hexane: mp 137–139.5 °C; IR (Nujol) 1675, 1595 cm^{-1}; NMR ($CDCl_3$) δ 2.28 (s, 3, Me), 2.52 (s, 3, Me), 4.78 (s, 2, OCH_2), 5.10 (s, 2, OCH_2), 7.23 (s, 1, H-6), 7.35–7.54 (m, 10, Ph), 10.27 (s, 1, CHO). Anal. ($C_{23}H_{22}O_3$) C, H.

Synthesis of β-Nitrostyrenes 14a–c: General Procedure.[22] A mixture of the aldehyde **4**, **6**, or **13** (20 mmol), CH_3NO_2 (3.7 g, 60 mmol), anhydrous NH_4OAc (4.62 g, 60 mmol), and 60 mL of HoAc was refluxed for 1.5 h (or until the reaction was complete as judged by silica gel TLC in CH_2Cl_2) protected from moisture. The mixture was then cooled to 25 °C and poured into water (500 mL). The precipitated solid was collected by filtration, washed with water, and dissolved in CH_2Cl_2. The CH_2Cl_2 solution was washed with $NaHCO_3$ solution, dried (Na_2SO_4), and passed through a column of silica gel (20 g) in CH_2Cl_2. Further elution with CH_2Cl_2 and evaporation of eluent gave a yellow solid, which was recrystallized as described below: 3,5-Bis(benzyloxy)-2-methyl-β-nitrostyrene (**14a**) was recrystallized from benzene–hexane to give 6.2 g (83%): mp 124 °C; NMR ($CDCl_3$) δ 2.30 (s, 3, Me), 5.03 (s, 4, OCH_2), 6.68 (s, 2, H-4 and H-6), 7.40 (d, $J_{\alpha,\beta}$ = 14 Hz, 1, H_α), 7.39 (s, 10, Ph), 8.30 (d, $J_{\alpha,\beta}$ = 14 Hz, 1, H_β). Anal. ($C_{23}H_{21}NO_4$) C, H, N. 3,5-Bis(benzyloxy)-4-methyl-β-nitrostyrene (**14b**) was recrystallized from benzene–hexane yielding 5.6 g (75%): mp 153–155 °C; NMR ($CDCl_3$) δ 2.26 (s, 3, Me), 5.09 (s, 4, OCH_2), 6.72 (s, 2, H-2 and H-6), 7.20–7.55 (m, 11, Ph and H_α), 7.90 (d, $J_{\alpha,\beta}$ = 14 Hz, 1, H_β). Anal. ($C_{23}H_{21}NO_4$) C, H, N. 3,5-Bis(benzyloxy)-2,4-dimethyl-β-nitrostyrene (**14c**) was recrystallized from EtOH giving 6.0 g (77%): mp 148–149.5 °C; NMR ($CDCl_3$) δ 2.27 (s, 3, Me), 2.32 (s, 3, Me), 4.76 (s, 2, OCH_2), 5.08 (s, 2, OCH_2), 6.82 (s, 1, H-6), 7.21–7.49 (m, 11, Ph and H_α), 8.28 (d, $J_{\alpha,\beta}$ = 14 Hz, 1, H_β). Anal. ($C_{24}H_{23}NO_4$) C, H, N.

Synthesis of Dinitrostyrenes 15a–c. General Procedure.[23] To a stirred solution of the β-nitrostyrenes **14a–c** (10 mmol) in Ac_2O (freshly distilled from P_2O_5, 40 mL) at 60–65 °C and protected from moisture was added $Cu(NO_3)_2 \cdot 3H_2O$ (3.87 g, 16 mmol) in small portions over a period of 30 min keeping the temperature between 60 and 65 °C. The mixture was then stirred at 60–65 °C for 1 h (or until the reaction was complete as judged by TLC in 85:15 CH_2Cl_2–hexane), cooled to 25 °C, and poured into cold water (400 mL). The precipitated solid or gum was collected by filtration or decantation, washed with water, and dissolved in CH_2Cl_2. The CH_2Cl_2 solution was washed with $NaHCO_3$ solution, dried (Na_2SO_4), and then passed through a column of silica gel (20 g) in 9:1 CH_2Cl_2–hexane. Elution with the same solvent system and evaporation of solvent gave relatively pure solid in each case. The solid was further purified by recrystallization from benzene–hexane. Yields and physical properties are described below. 3,5-Bis(benzyloxy)-2-methyl-6,β-dinitrostyrene (**15a**): yield 2.56 g (61%); mp 157–159 °C; NMR ($CDCl_3$) δ 2.17 (s, 3, Me), 5.03 (s, 2, OCH_2), 5.08 (s, 2, OCH_2), 6.58 (s, 1, H-4), 7.07 (d, $J_{\alpha,\beta}$ = 14 Hz, 1, H_α), 7.20–7.41 (m, 10, Ph), 7.98 (d, $J_{\alpha,\beta}$ = 14 Hz, 1, H_β). Anal. ($C_{23}H_{20}N_2O_6$) C, H, N. 3,5-Bis(benzyloxy)-4-methyl-6,β-dinitrostyrene (**15b**): yield 2.86 g (68%); mp 163 °C; NMR ($CDCl_3$) δ 2.27 (s, 3, Me), 4.95 (s, 2, OCH_2), 5.15 (s, 2, OCH_2), 6.80 (s, 1, H-6), 7.20–7.47 (m, 11, Ph and H_α), 7.89 (d, $J_{\alpha,\beta}$ = 14 Hz, 1, H_β). Anal. ($C_{23}H_{20}N_2O_6$) C, H, N. 3,5-Bis(benzyloxy)-2,4-dimethyl-6,β-dinitrostyrene (**15c**): yield 2.1 g (48%); mp 154–155 °C; NMR ($CDCl_3$) δ 2.27 (s, 6, Me), 4.85 (s, 2, OCH_2), 4.97 (s, 2, OCH_2), 7.29–7.53 (m, 11, Ph and H_α), 8.01 (d, $J_{\alpha,\beta}$ = 14 Hz, 1, H_β). Anal. ($C_{24}H_{22}N_2O_6$) C, H, N.

Synthesis of Indoles 16a–c. General Procedure.[22] A mixture of the dinitrostyrene **15a**, **15b**, or **15c** (4 mmol), silica gel (70–270 mesh, 10 g), HOAc (24 mL), reduced Fe powder (electrolytic grade-IX, Mallinckrodt, 4 g, 71 mmol), and toluene (40 mL) was refluxed under an N_2 atmosphere for 1 h. The mixture was then cooled to 25 °C, diluted with CH_2Cl_2, and filtered. The filter cake was washed thoroughly with CH_2Cl_2 and the combined filtrates were washed, in order, with a $NaHSO_3$ solution and $NaHCO_3$ solution, dried (Na_2SO_4), and evaporated in vacuo to dryness. The light brown residue was chromatographed on a column of silica gel (15 g) using 9:1 CH_2Cl_2–hexane as eluent. Evaporation of solvent gave almost pure (air sensitive) indoles which were recrystallized from benzene–hexane. 5,7-Bis(benzyloxy)-4-methylindole (**16a**): yield 1.2 g (88%); mp 88–89 °C; NMR ($CDCl_3$) δ 2.37 (s, 3, Me), 4.90 (s, 2, OCH_2), 4.95 (s, 2, OCH_2), 6.37 (t, $J_{1,3}$ = $J_{3,2}$ = 3 Hz, 1, H-3), 6.42 (s, 1, H-6), 6.84 (t, $J_{1,2}$ = $J_{2,3}$ = 3 Hz, 1, H-2), 7.14–7.41 (m, 10, Ph), 8.02 (br s, 1, H-1). Anal. ($C_{23}H_{21}NO_2$) C, H, N. 5,7-Bis(benzyloxy)-6-methylindole (**16b**): yield 1.1 g (80%); mp 105–107 °C; NMR ($CDCl_3$) δ 2.31 (s, 3, Me), 4.89 (s, 2, OCH_2), 4.98 (s, 2, OCH_2), 6.34 (t, $J_{1,3}$ = $J_{2,3}$ = 3 Hz, 1, H-3), 6.79 (t, $J_{1,2}$ = $J_{2,3}$ = 3 Hz, 1, H-2), 6.87 (s, 1, H-4), 7.18–7.43 (m, 10, Ph), 7.85 (br s, 1, H-1). Anal. ($C_{23}H_{21}NO_2$) C, H, N. 5,7-Bis(benzyloxy)-4,6-dimethylindole (**16c**): yield 1.26 g (92%); mp 97–99 °C; NMR ($CDCl_3$) δ 2.31 (s, 3, Me), 2.44 (s, 3, Me), 4.80 (s, 2, OCH_2), 4.93 (s, 2, OCH_2), 6.48 (t, $J_{1,3}$ = $J_{2,3}$ = 3 Hz, 1, H-3), 6.91 (t, $J_{1,2}$ = $J_{2,3}$ = 3 Hz, 1, H-2), 7.25–7.58 (m, 10, Ph), 8.01 (br s, 1, H-1). Anal. ($C_{24}H_{23}NO_2$) C, H, N.

Synthesis of Indole-3-acetonitriles 19a–c. General Procedure.[24,25] Although the intermediate gramines and gramine methiodides could not be readily purified to homogeneity due, in part, to their instability, the

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crude products were sufficiently pure to give satisfactory NMR spectra. **Gramines 17a–c**: To a stirred mixture of HOAc (10 mL), EtOH (5 mL), 37% aqueous CH_2O (400 mg, 5 mmol), and 40% aqueous Me_2NH (600 mg, 5 mmol) at 0–5 °C was added a solution of the indole (**16a**, **16b** or **16c**, 2 mmol) in 5 mL of EtOH. After it was stirred at 0–5 °C for 2 h and then at 25 °C for 8 h, the mixture was diluted with 100 mL of water and made strongly basic (pH ~12) with 4 N NaOH with cooling. After stirring for 30 min, the mixture was extracted with CH_2Cl_2 (3 × 10 mL). The CH_2Cl_2 extract was washed with brine, dried (K_2CO_3), and evaporated in vacuo to dryness to give a light brown gum in each case. The NMR data of the crude products in $CDCl_3$ are described below. **Gramine 17a**: δ 2.21 (s, 6, NMe_2), 2.61 (s, 3, Me), 3.53 (s, 2, CH_2NMe_2), 4.92 (s, 2, OCH_2), 5.02 (s, 2, OCH_2), 6.48 (s, 1, H-6), 6.84 (d, $J_{1,2}$ = 2.5 Hz, 1, H-2), 7.20–7.40 (m, 10, Ph), 8.27 (br s, 1, H-1). **Gramine 17b**: δ 2.18 (s, 6, NMe_2), 2.30 (s, 3, Me), 3.52 (s, 2, CH_2NMe_2), 4.95 (s, 2, OCH_2), 5.05 (s, 2, OCH_2), 6.82 (s, 1, H-4), 6.95 (br s, 1, H-2), 7.06–7.47 (m, 10, Ph), 8.13 (br, 1, H-1). **Gramine 17c**: δ 2.23 (s, 6, NMe_2), 2.34 (s, 3, Me), 2.72 (s, 3, Me), 3.54 (s, 2, CH_2NMe_2), 4.80 (s, 2, OCH_2), 4.97 (s, 2, OCH_2), 6.85 (d, $J_{1,2}$ = 2 Hz, 1, H-2), 7.30–7.60 (m, 10, Ph), 7.82 (br s, 1, H-1). **Gramine Methiodides 18a–c**: To a stirred solution of CH_3I (10 g, 70 mmol) in 10 mL of EtOH at 0–5 °C, protected from moisture, was added a solution of the gramine **17a**, **17b**, or **17c** (1 mmol) in 10 mL of EtOAc over a period of 10 min. The mixture was refrigerated for 12 h and then evaporated at 25 °C to dryness. The residue, often a gum, was triturated with a 9:1 mixture of EtOAc–hexane and refrigerated for a few hours. The precipitated white solid was collected by filtration and dried under vacuum. In each case, NMR showed the presence of varying amounts of dimeric products, although the precise amounts could not be quantitated due to partially overlapped signals. Methiodide **18a**: mp 145 °C dec; NMR ($(CD_3)_2SO$) δ 2.43 (s, 3, Me, partly buried in the signal due to residual protons of solvent but separates on addition of D_2O), 3.02 (s, 9, NMe_3), 3.13 (s, NMe_2 of the dimer, representing approximately 20% dimer in the mixture), 4.78 (br s, 2, CH_2N^+), 5.07 (s, 2, OCH_2), 5.27 (s, 2, OCH_2), 6.84 (s, 1, H-6), 7.20–7.70 (m, 11, H-2 and Ph), 11.72 (br s, 1, H-1, exchanges with D_2O). **Methiodide 18b**: mp 168 °C dec; NMR ($(CD_3)_2SO$) δ 2.19 (s, 3, Me), 3.13 (s, 9, NMe_3), 3.05 (s, Me_2N^+ of dimer, representing presence of approximately 10% of dimer in the mixture), 4.71 (s, 2, CH_2N^+), 5.05 (s, 2, OCH_2), 5.18 (s, 2, OCH_2), 7.20–7.50 (m, 12, H-2, H-4 and Ph), 11.54 (s, 1, H-1, exchanges with D_2O). Methiodide **18c**: mp 128 °C dec; NMR ($(CD_3)_2SO$) δ 2.22 (s, 3, Me), 2.52 (s, 3, Me on C-4), 3.07 (s, Me_2N^+ of the dimer, representing approximately 20% dimer in the mixture), 3.13 (s, 9, Me_3N^+), 4.75 (br, s, 4, CH_2N^+ and OCH_2), 5.01 (s, 2, OCH_2), 7.12–7.70 (m, 11, H-2 and Ph), 11.69 (s, 1, H-1, exchanges with D_2O). **Indole-3-acetonitriles 19a–c**: To a stirred solution or suspension of the gramine methiodide **18a**, **18b**, or **18c** (1 mmol) in 5–7 mL of Me_2NCHO at 75 °C was added, immediately, a solution of KCN (260 mg, 4 mmol) in 3 mL of water. The mixture then heated with stirring at 75 °C for 1 h, cooled to 25 °C, and diluted with water (70 mL). The mixture was kept at 0 °C for 1 h and the precipitated solid (or gum) was collected by filtration (or decantation). The residue was washed with water and dissolved in CH_2Cl_2 and the CH_2Cl_2 solution was washed with brine, dried (Na_2SO_4), and evaporated in vacuo to dryness. The residue was chromatographed on a column of silica gel (20 g) using 9:1 CH_2Cl_2–hexane. Evaporation of eluent and recrystallization of the residue from benzene–cyclohexane gave a white solid in each case. **5,7-Bis(benzyloxy)-3-(cyanomethyl)-4-methylindole (19a)**: yield 76%; mp 127–128.5 °C; IR ($CHCl_3$) 2230 cm^{-1}; NMR ($CDCl_3$) δ 2.52 (s, 3, Me), 3.96 (s, 2, CH_2CN), 5.03 (s, 2, OCH_2), 5.14 (s, 2, OCH_2), 6.63 (s, 1, H-6), 7.18 (d, $J_{1,2}$ = 2.4 Hz, 1, H-2), 7.30–7.60 (m, 10, Ph), 8.37 (br s, 1, H-1). Anal. ($C_{25}H_{22}N_2O_2$) C, H, N. **5,7-Bis(benzyloxy)-3-(cyanomethyl)-6-methylindole (19b)**: yield 69%; mp 102–104 °C; IR ($CHCl_3$) 2230 cm^{-1}; NMR ($CDCl_3$) δ 2.32 (s, 3, Me), 3.71 (s, 2, CH_2CN), 4.98 (s, 2, OCH_2), 5.08 (s, 2, OCH_2), 6.80 (s, 1, H-4), 6.92 (d, $J_{1,2}$ = 2 Hz, 1, H-2), 7.23–7.48 (m, 10, Ph), 7.75 (br s, 1, H-1). Anal. ($C_{25}H_{22}N_2O_2$) C, H, N. **5,7-Bis(benzyloxy)-3-(cyanomethyl)-4,6-dimethylindole (19c)**: yield 75%; mp 110–112 °C; IR ($CHCl_3$) 2225 cm^{-1}; NMR ($CDCl_3$) δ 2.41 (s, 3, Me), 2.56 (s, 3, Me), 3.88 (s, 2, CH_2CN), 4.78 (s, 2, OCH_2), 4.97 (s, 2, OCH_2), 6.98 (d, $J_{1,2}$ = 2 Hz, 1, H-2), 7.32–7.60 (m, 10, Ph), 7.97 (br s, 1, H-1). Anal. ($C_{26}H_{24}N_2O_2$) C, H, N.

Synthesis of Dihydroxytryptamines 21a–c. General Procedure.[23-25] To a stirred suspension of $LiAlH_4$ (570 mg, 15 mmol) in 30 mL of dry Et_2O under an N_2 atmosphere was added a solution of **19a**, **19b**, or **19c** (1 mmol) in 30 mL of PhH gradually, and the mixture was then refluxed for 6.5 h. After cooling the mixture to 5–10 °C, excess $LiAlH_4$ was decomposed by carefully adding water until the inorganic materials separated as a white gel. The organic solution was collected by decantation, washed with brine, dried (K_2CO_3), and then evaporated in vacuo to dryness to give the protected tryptamines **20a**, **20b**, or **20c** in >90% yield as gums in each case. These tryptamines (pure by NMR, see below) were utilized in the next step without further purification. Tryptamine **20a**: NMR ($CDCl_3$) δ 1.72 (br s, 2, NH_2), 2.51 (s, 3, Me), 2.95 (s, 4, CH_2CH_2), 4.94 (s, 2, OCH_2), 5.05 (s, 2, OCH_2), 6.49 (s, 1, H-6), 6.86 (br s, 1, H-2), 7.22–7.50 (m, 10, Ph), 7.92 (br s, 1, H-1). **Tryptamine 20b**: NMR ($CDCl_3$) δ 1.63 (br s, 2, NH_2), 2.33 (s, 3, Me), 2.88 (t, poorly resolved, J = 4.5 Hz, 4, CH_2CH_2), 4.98 (s, 2, OCH_2), 5.07 (s, 2, OCH_2), 6.70 (d, $J_{1,2}$ = 2 Hz, 1, H-2), 6.80 (s, 1, H-4), 7.07–7.53 (m, 10, Ph), 8.05 (br s, 1, H-1). **Tryptamine 20c**: NMR ($CDCl_3$) δ 1.78 (br s, 2, NH_2), 2.34 (s, 3, Me), 2.60 (s, 3, Me), 2.97 (m, 4, CH_2CH_2), 4.78 (s, 2, OCH_2), 4.93 (s, 2, OCH_2), 6.77 (d, $J_{1,2}$ = 2.5 Hz, 1, H-2), 7.20–7.60 (m, 10, Ph), 8.17 (br s, 1, H-1). To a solution of the bis(benzyloxy)tryptamine **20a**, **20b**, or **20c** (1 mmol) in 50–75 mL of deoxygenated EtOH was added 1 M H_2SO_4 (1 mL, 1 mmol) and 200 mg of 10% Pd/C, and the mixture was shaken in a Parr shaker at 40 psi of H_2 for 6 h at ambient temperature. [All the operations described below were conducted, as far as practicable, in a positive N_2 atmosphere.] The mixture was filtered under gravity and to the filtrate was added a solution of creatinine (113 mg, 1 mmol) in 1 mL of deoxygenated water. The cloudy mixture was evaporated in vacuo at <40 °C to dryness. The residue was dissolved in deoxygenated water and filtered. To the filtrate was added deoxygenated acetone until precipitation of some solid had begun, and the mixture was stored at −20 °C overnight. White solid was obtained in each case and was collected by filtration and dried under vacuum.

5,7-Dihydroxy-4-methyltryptamine Creatinine Sulfate (4-Me-5,7-DHT, 21a): yield 89%; mp 240 °C dec; NMR (D_2O) δ 2.32 (s, 3, 4-Me), 2.91–3.07 (m, 7, CH_2CH_2, NMe), 4.01 (s, 2, CH_2 of creatinine), 6.99 (s, 1, H-2); partial NMR in $(CD_3)_2SO$ δ 6.20 (s, 1, H-6), 6.97 (s, $J_{1,2}$ = 2.5 Hz, 1, H-2). Anal. ($C_{15}H_{23}N_5O_7S{\cdot}2H_2O$) C, H, N. **5,7-Dihydroxy-6-methyltryptamine Creatinine Sulfate (6-Me-5,7-DHT, 21b)**: yield 72%; mp 235 °C dec; NMR (D_2O) δ 2.02 (2, 6-Me), 2.85–3.08 (m, 7, CH_2CH_2, NMe), 4.05 (s, 2, CH_2 of creatinine), 6.99 (s, 1, H-2); partial NMR in $(CD_3)_2SO$ δ 6.40 (s, 1, H-4), 6.98 (d, $J_{1,2}$ = 2.5 Hz, 1, H-2). Anal. ($C_{15}H_{23}N_5O_7S{\cdot}2H_2O$) C, H, N. **5,7-Dihydroxy-4,6-dimethyltryptamine Creatinine Sulfate (4,6-Me_2-5,7-DHT, 21c)**: yield 92%; mp 187 °C dec; NMR (D_2O) δ 2.02 (s, 3, 6-Me), 2.25 (s, 3, 4-Me), 2.90–3.05 (m, 7, CH_2CH_2, NMe), 4.03 (s, 2, CH_2 of creatinine), 6.96 (s, 1, H-2). Anal. ($C_{16}H_{25}N_5O_7S{\cdot}2H_2O$) C, H, N.

Cyclic Voltammetry. An electrochemical cell with a carbon paste working electrode, a standard Ag/AgCl (3N NaCl) reference electrode, and a platinum foil auxiliary electrode was used.[26] The carbon paste was prepared by mixing Ultracarbon (Ultra F purity) and hexadecane in a ratio of 2:1 by weight. Each voltammogram was generated by using a freshly prepared electrode surface. Approximate area of this surface was 1.5 mm^2. The electrolytes were 0.1 M H_2SO_4, 0.05 M acetate buffer at pH 4.0 containing 0.9% NaCl, or 0.05 M phosphate buffer at pH 7.4 containing 0.9% NaCl. These solvents were freed of dissolved O_2 by purging with N_2 for at least 1 h. The cyclic voltammograms were recorded while maintaining the test solutions quiet in an N_2 atmosphere at a scan rate of 50 mV/s by using an IBM EC 225 voltammetric analyzer and a Houston Instruments Model 2000 x–y recorder.

Polarographic Measurements of O_2 Consumption.[12,27] The rates of oxygen consumption were measured with a Clark electrode (Yellow Springs Instruments) in a closed water-jacketed cell equipped with a magnetic stirrer at 37 °C using 0.05 M phosphate buffer at pH 7.4 as the solvent. The final volume of the test solution in the cell was 1.1–1.2 mL. The oxygen consumption rates were calculated on the basis of a dissolved oxygen content, determined experimentally,[27] in the solvent at 37 °C of 188 nmol/mL.

Results

Synthesis of the Probes. Our general strategy for the synthesis of **21a–c** was to transform appropriately substituted benzaldehydes to the corresponding indoles via 2,β-dinitrostyrenes followed by introduction of the aminoethyl side chain. The manner in which the aminoethyl side chain was introduced was dictated by our need in future studies for radiolabeled probes with the radiolabel in a chemically and metabolically stable position.

Efficient synthesis of the aldehydes, none of which has been described in the literature, is presented in Schemes I and II. For the synthesis of aldehyde **4**, 3,5-dihydroxybenzoic acid (**1**) was first converted to the bromobenzaldehyde **2** in four steps (Scheme I) as described earlier.[15] The aldehyde function of **2** was protected in situ to give amino alkoxide **3** which upon Br–Li exchange

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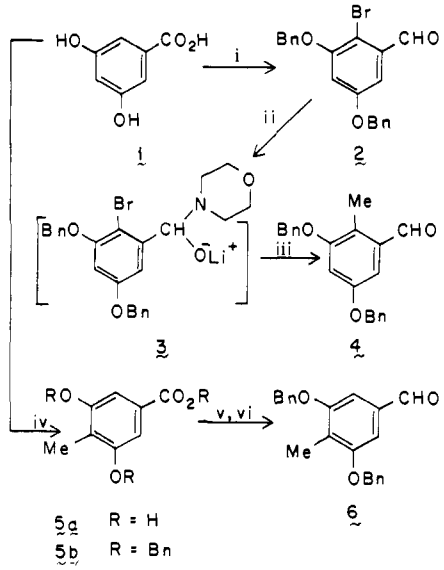
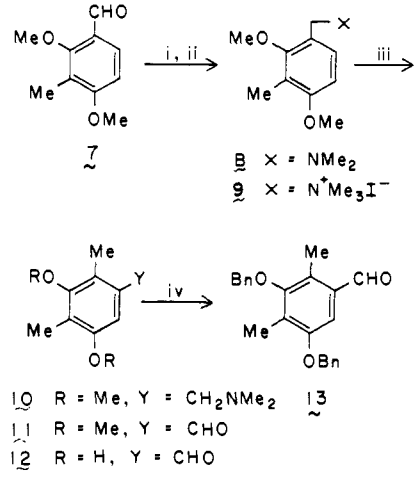
Scheme I[a]



[a] Reagents: (i) ref 15; (ii) lithium morpholide, THF, −78 °C; (iii) BuLi, −78 °C then $(MeO)_2SO_2$ then H_3O^+; (iv) ref 17; (v) $LiAlH_4$, THF; (vi) pyridinium chlorochromate, CH_2Cl_2.

Scheme II[a]

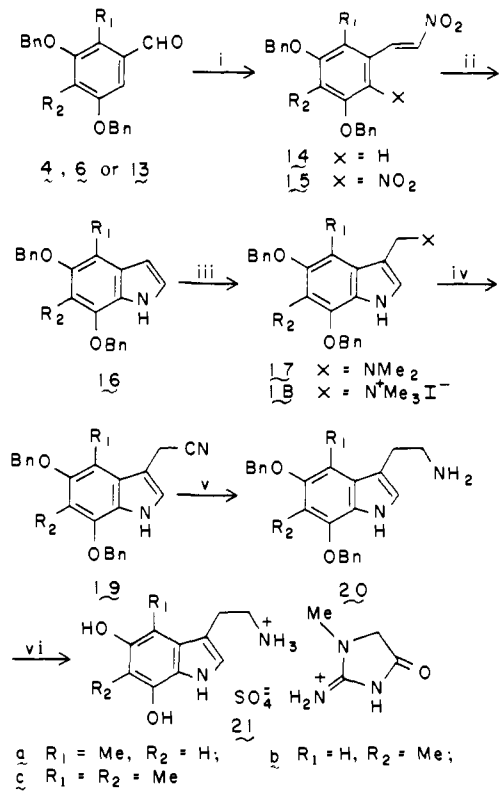


[a] Reagents: (i) 7 → 8, $Me_2NH{\cdot}HCl$, KOH, MeOH, $NaBH_3CN$; (ii) 8 → 9, MeI, CH_2Cl_2; (iii) 9 → 10, $NaNH_2$, liquid NH_3; 10 → 11, NBS, PhH; 11 → 12, BBr_3, CH_2Cl_2; (iv) 12 → 13, $PhCH_2Cl$, K_2CO_3, NaI, acetone.

followed by treatment with $(MeO)_2SO_2$ gave, after acidic work-up,[15,16] aldehyde **4**. TLC suggested formation of only one compound, and its structure as **4** was confirmed from NMR spectral data which displayed characteristic meta coupling with $J_{4,6} = 3$ Hz. For the synthesis of aldehyde **6** (Scheme I), we had to devise a selective method for the conversion of **1** to 4-methyl-3,5-dihydroxybenzoic acid[17] (**5a**) which on benzylation gave benzoate **5b**. Ester **5b** was then converted to the aldehyde **6** by a standard reduction–oxidation sequence. For the synthesis of the dimethylbenzaldehyde (**13**, Scheme II), we started with aldehyde **7** after encountering difficulty with a number of seemingly more simple pathways. Reductive amination[19] of **7** followed by quaternization of the resulting amine gave methiodide **9**. Sommelet–Hauser rearrangement[20] of **9** proceeded essentially quantitatively to give amine **10**. Oxidation of amine **10** to aldehyde **11** was accomplished using NBS.[21] Interchange of the protecting groups of **11** then afforded aldehyde **13**.

The sequence of reactions that was used for the conversion of aldehyde **4**, **6**, and **13** to the tryptamines **21a**, **21b**, and **21c**, respectively, is shown in Scheme III. Nitromethylenation of the

Scheme III[a]



[a] Reagents: (i) 4, 6, or 13 → 14, CH_3NO_2, NH_4OAc, HOAc; 14 → 15, $Cu(NO_3)_2{\cdot}3H_2O$, Ac_2O; (ii) 15 → 16, Fe, HOAc, silica gel, $PhCH_3$; (iii) 16 → 17, CH_2O, Me_2NH, EtOH, HOAc; 17 → 18, MeI, EtOH, EtOAc; (iv) 18 → 19, KCN, DMF, H_2O; (v) $LiAlH_4$, Et_2O, PhH; (vi) H_2SO_4, Pd/C, H_2, EtOH then creatinine.

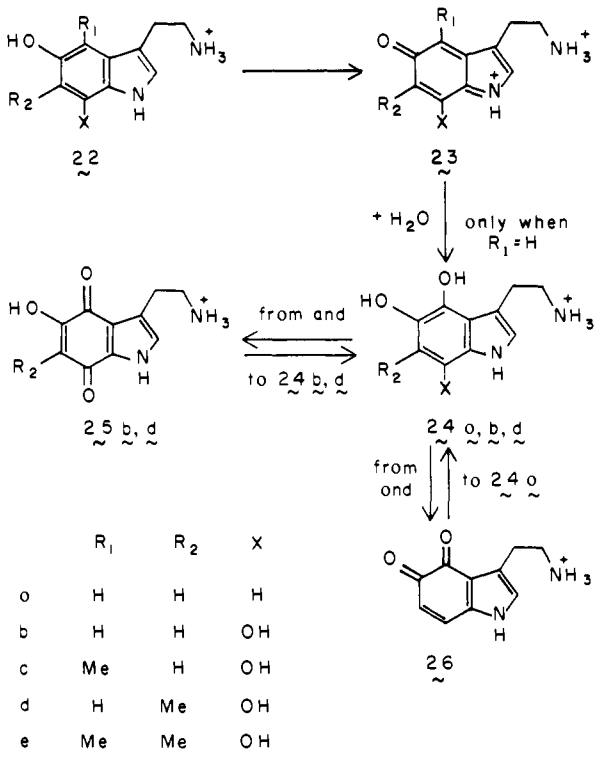
aldehydes followed by ring nitration gave the dinitrostyrenes **15**. Attempted reductive cyclization of **15** to indoles using Fe/HOAc gave disappointingly poor yields. This led us to modify the method.[22] Using the modified procedure, which involved use of Fe/HOAc in toluene in the presence of silica gel, the indoles **16a**, **16b**, and **16c** were produced in 88%, 80%, and 92% yields, respectively. The indoles were then converted to indole-3-acetonitriles **19** via reaction of methiodides **18** with KCN. Reduction[24] of nitriles **19** to the tryptamines **20** could be effected satisfactorily only in very dilute solutions of 1:1 Et_2O–PhH by using 15-fold excess of $LiAlH_4$. Catalytic debenzylation of the hydrogen sulfate salts of **20**, followed by treatment with creatinine furnished the target DHT's **21a–c**. The synthetic intermediates as well as the targets **21a–c** were characterized by NMR spectroscopy and elemental analysis.

Cyclic Voltammetric Studies. Previous cyclic voltammetric studies with 5-HT have indicated that it undergoes electrochemical oxidation to produce the corresponding *p*-quinone imine (5-HTQ, **23a**, Chart II).[28] The initial oxidation of 5-HT to 5-HTQ appears to be a 2e process.[29] Although supporting evidence was provided[28] for the subsequent chemical and electrochemical steps, outlined in Chart II for 5-HT, direct proof is lacking for any of these steps. These observations on 5-HT led us to expect that it might be possible to generate 5,7-DHTQ, which has been postulated to be the primary autoxidation product of 5,7-DHT, electrochemically and compare its chemical and electrochemical properties with the corresponding *p*-quinoneimines generated from the methyl-substituted 5.7-DHT's.

The voltammograms in the present study were generated by using the standard three-electrode configuration with a carbon paste working electrode at pH 7.4 and 4.0 and in 0.1 M H_2SO_4.

(28) Feinberg, B. A. Ph.D. Dissertation, The University of Kansas, Lawrence, KS, 1971.
(29) Blank, L. E., personal communication.

Chart II



	R_1	R_2	X
o	H	H	H
b	H	H	OH
c	Me	H	OH
d	H	Me	OH
e	Me	Me	OH

These are shown in Figure 1. On the first anodic scan a peak is observed in each case. The values of the peak potentials for 5-HT, 5,7-DHT, 4-Me-5,7-DHT, 6-Me-5,7-DHT, and 4,6-Me_2-5,7-DHT were (in mV vs. Ag/AgCl) at pH 7.4, 430, 230, 110, 190, and uncertain, respectively, at pH 4.0, 570, 460, 330, 370, and 300, respectively, and in 0.1 M H_2SO_4, 650, 560, 420, 510, and 390, respectively. The value for 4,6-Me_2-5,7-DHT at pH 7.4 is uncertain as it exhibited essentially a vanishingly small peak at 240 mV but a major peak at 620 mV. The reason for this phenomenon remained a puzzle until it was found that the pH 4.0 test solution, from which voltammogram 2 of Figure 1e was generated, produced voltammogram 2a after ~5-s exposure to air. Qualitative similarity of (1) and (2a) in Figure 1e suggests that traces of residual O_2 present in the "deoxygenated" test solution was enough to oxidize the 40 μM solution of this DHT essentially completely due to its extremely rapid rates of reaction with O_2 (vide infra). However, the species responsible for the peak at 620 mV still remains a mystery. In the case of 5,7-DHT and 4- and 6-Me-5,7-DHT, exposure to air (up to 5 min) resulted in complete disappearance of the peaks obtained at pH 4.0 and 7.4 but did not result in the appearance of any new peaks while voltammograms for 5-HT remained essentially unchanged even after exposure to air for 30 min. In analogy to 5-HT, the first anodic peak for 5,7-DHT and its methylated derivatives corresponds to the transformation **22b–e** → **23b–e** (Chart II). It should be emphasized that at the present time we do not have any direct evidence that this peak is solely due to the formation of the DHTQ's **23b–e**. The number of electrons involved in each of these oxidations also remains to be determined.

On the first cathodic scan, at scan rates of up to 400 mV/s, none of the test compounds displayed peaks corresponding to the reduction of **23b–e** to **22b–e**, respectively, at any pH tested (Chart II, Figure 1). The only cathodic peaks observed were those for 5,7-DHT (peak Ic) and 6-Me-5,7-DHT (peak Ic) in 0.1 M H_2SO_4 at −20 and −90 mV, respectively. In analogy to 5-HT, which displays a similar cathodic peak (Figure 1a, curve 3) thought to be due to the transformation **26** → **24a** at 200 mV, these peaks would be expected to correspond to the reduction of **25b** and **25d**, respectively (Chart II). Each of these peaks, thus, amounts to an ECE process (**22b,d** → **23b,d** → **24b,d** → **25b,d** → **24b,d**) with the chemical step being Michael addition of H_2O to **23b** and **23d**,

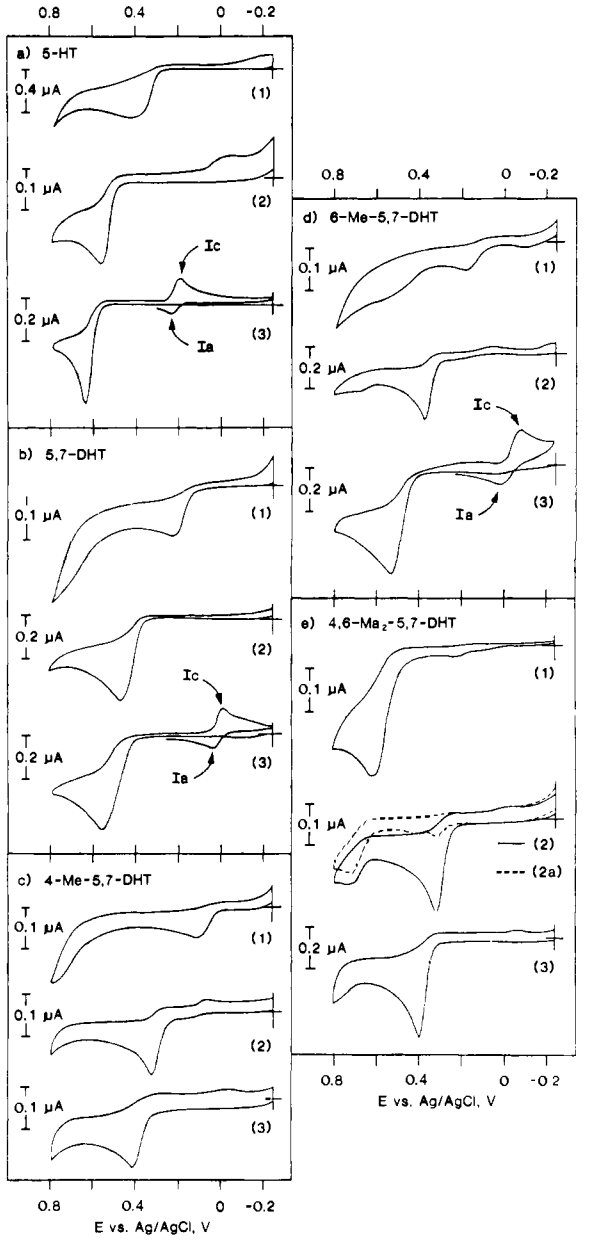


Figure 1. Cyclic voltammograms of (a) 5-HT, (b) 40 μM 5,7-DHT, (c) 25 μM 4-Me-5,7-DHT, (d) 30 μM 6-Me-5,7-DHT, and (e) 40 μM 4,6-Me_2-5,7-DHT in (1) phosphate buffer at pH 7.4, (2) acetate buffer at pH 4.0, and (3) 0.1 M H_2SO_4 at a scan rate of 50 mV/s at ~25 °C. The concentrations of 5-HT were (1) 50, (2) 30, and (3) 40 μM. The scan in each case was initiated at −0.25 V. For 4,6-Me_2-5,7-DHT (e) voltammogram 2a was obtained after bubbling air for ~5 s into the solution from which voltammogram 2 was generated.

respectively. Absence of similar cathodic peaks for 4-Me- and 4,6-Me_2-5,7-DHT supports the involvement of the 4-position in the chemical follow-up step for 5,7-DHT and 6-Me-5,7-DHT. The fact that both 4-Me- and 4,6-Me_2-5,7-DHT failed to display peaks corresponding to the process **23** → **22**, suggests that the 4-position of **23** is not the only site where nucleophilic attack by H_2O or the amino group of the aminoethyl side chain can take place. These other sites might be position 2 and the ring junctions (positions 3a and 7a) of **23**.

On the first anodic follow-up scan in 0.1 M H_2SO_4 a peak is observed only with 5-HT (peak Ia), 5,7-DHT (peak Ia), and 6-Me-5,7-DHT (peak Ia) at 240, 30, and 20 mV, respectively. Each of these peaks correspond to a simple oxidation process with the oxidation step being **24a** → **26** and **24b,d** → **25b,d**, respectively.

NMR Spectroscopic Studies. Schlossberger[13] first showed that the H-4 and H-6 of 5,7-DHT undergo rapid exchange with deuterium in D_2O; however, relative rates of exchange and the

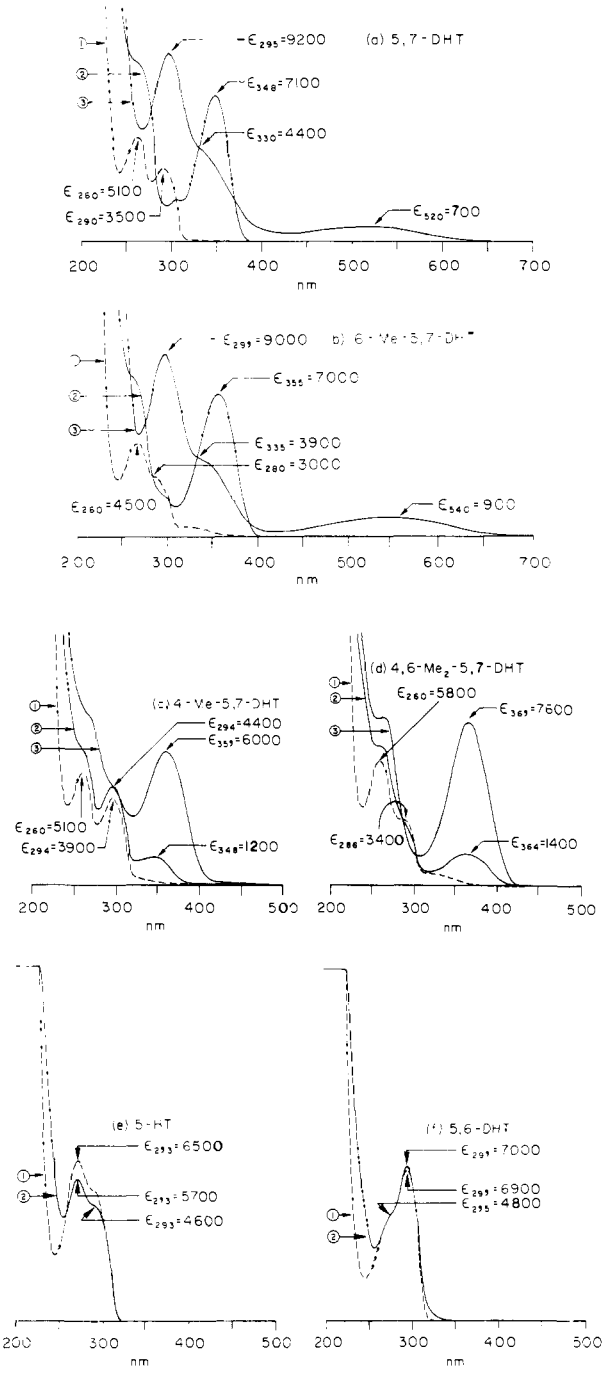


Figure 2. UV–visible absorption curves of 5,7-DHT and its methylated derivatives 5-HT and 5,6-DHT at 25 °C in (1) 0.05 M O_2-free HCl–KCl buffer at pH 2.0, (2) 0.05 M O_2-free phosphate buffer at pH 7.4, and (3) 0.05 M phosphate buffer saturated with air at pH 7.4. Curves 1 and 2 were recorded immediately after sample preparation (~2 min), while curve 3 was recorded after (a) 12 h, (b) 1 h, (c) 2 min, and (d) 2 min.

chemical shift values were not reported. We have found that in a 25 mM solution of 5,7-DHT in D_2O at 25 °C, H-6 exchanges approximately twice as fast as H-4. Complete exchange of H-6 takes about 5 min. There is also a substantial difference in the chemical shift values for the two protons; while H-6 occurs at 6.13 ppm, H-4 appears at 6.30 ppm downfield from Me_4Si. We ascribe this lower value for H-6 to the shielding effect of the two electronegative oxygen atoms on C_5 and C_7. The H-6 (δ 6.20) and H-4 (δ 6.45) signals of 4-Me-5,7-DHT and 6-Me-5,7-DHT, respectively, also underwent exchange although at slightly reduced rates (7 and 13 min for complete exchange, respectively). The shielding effect extends to the methyl substituents as well: thus, 4-Me of **21a** occurs at δ 2.32, 6-Me of **21b** at δ 2.02, and 4- and 6-Me of **21c** at δ 2.25 and 2.02, respectively.

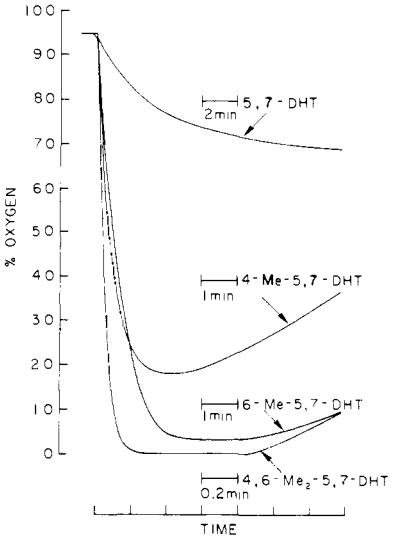


Figure 3. Oxygen electrode tracings of reaction of O_2 using a 0.5 mM test solution in phosphate buffer at pH 7.4 and 37 °C.

UV–Visible Spectroscopic Studies. The UV–visible spectra were recorded in the presence or absence of O_2 at pH 2.0, 4.0, 6.0, 7.0, 7.4, and 8.0. At every pH value the spectra were recorded at regular intervals over a period of time. Selected examples of absorption curves for each compound are shown in Figure 2. At pH 2.0 in the absence of O_2 the absorption maxima for 5,7-DHT and **21a–c** were found to be qualitatively similar to each other (Figure 2a–d, Curve 1) and also similar to those for 5-HT and 5,6-DHT (Figure 2e,f). With increasing pH in the absence of O_2, the absorption at >340 nm for each compound increased in intensity. 5-HT and 5,6-DHT show essentially no pH dependence between pH 2.0 and 8.0 (Figure 2e,f). Schlossberger[13] first found that at pH 2.0–8.0, 5,7-DHT displays several isosbestic points. Among the probes only 6-Me-5,7-DHT displayed the presence of such isosbestic points, although there were deviations for pH 4.0 and 6.0 curves at one point (data not shown). At pH 7.4 in the absence of O_2, 5,7-DHT and 6-Me-5,7-DHT displayed similar absorption curves (ϵ_{348} 7100 and ϵ_{355} 7000, respectively, Figure 2a,b, curve 2), while the corresponding peaks for 4-Me-5,7-DHT and 4,6-Me_2-5,7-DHT were much smaller in intensity (ϵ_{348} 1200 and ϵ_{364} 1400, respectively, Figure 2c,d, curve 2).[30] At pH 7.4 in the presence of O_2 at 25 °C after 2 min (sample preparation time), 5,7-DHT and all its methylated derivatives displayed qualitatively similar absorption curves. In the case of 5,7-DHT and 6-Me-5,7-DHT at this short time the curves (not shown) were essentially those obtained at pH 7.4 in the absence of O_2. But, in the case of 4-Me-5,7-DHT and 4,6-Me_2-5,7-DHT these curves (curve 3 in Figure 2c,d) were both qualitatively and quantitatively different from those obtained in the absence of O_2. The striking observation was that, while 4-Me-5,7-DHT and 4,6-Me_2-5,7-DHT did not display any further time-dependent change when monitored for up to 24 h, both 5,7-DHT and 6-Me-5,7-DHT showed the time-dependent appearance of low flat peaks at 520 and 540 nm, respectively. These peaks reached maximum intensity after 12 and 1 h, respectively.

Polarographic Measurement of O_2 Consumption. These measurements were made using a Clark O_2 electrode at 37 °C in 0.05 M phosphate buffer at pH 7.4. Representative O_2 electrode tracings for the oxidation of 0.5 mM 5,7-DHT and its methylated derivatives are shown in Figure 3. Surprisingly, in the case of the methylated derivatives at concentrations including and below 0.5 mM, within a short time after the initial O_2 consumption had ceased, levels of O_2 in solution started to increase. The rates at

(30) Because of essentially instantaneous reaction of 4-Me-5,7-DHT and 4,6-Me_2-5,7-DHT with O_2 (vide infra) at pH 7.4 and because of the technical difficulty of maintaining a 100% O_2-free atmosphere during the entire process of recording an absorption curve, it is not certain that the absorptions at 348 and 364 nm are solely due to unoxidized 4-Me-5,7-DHT and 4,6-Me_2-5,7-DHT, respectively.

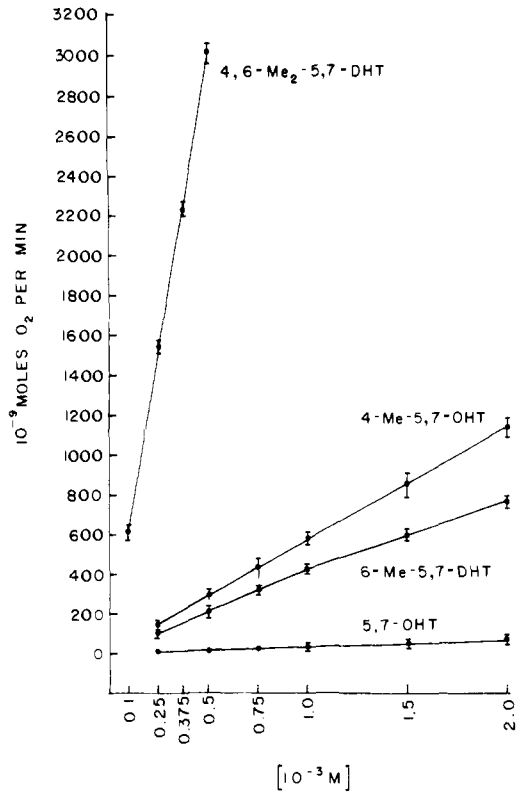


Figure 4. Plots of O_2 consumption rates in 0.05 M phosphate buffer at pH 7.4 and 37 °C, measured during the initial linear phase of the reaction as a function of concentration of the test compound. Bars denote mean ± SD for 3–5 independent determinations.

which O_2 was liberated after initial consumption varied from run to run but were 4-Me- ≃ 4,6-Me_2- > 6-Me-5,7-DHT. The amount of O_2 returned to solution also varied but was generally <30% of the amount originally consumed for 6-Me-5,7-DHT and >50% for 4-Me- and 4,6-Me_2-5,7-DHT after 30 min. A 0.5 mM solution of 5,7-DHT was found to return O_2 very slowly after 45 min, about the same time at which initial consumption had stopped. At higher concentrations of the methylated DHT's, return of O_2 started at 15–25 min after initial O_2 consumption had ceased.

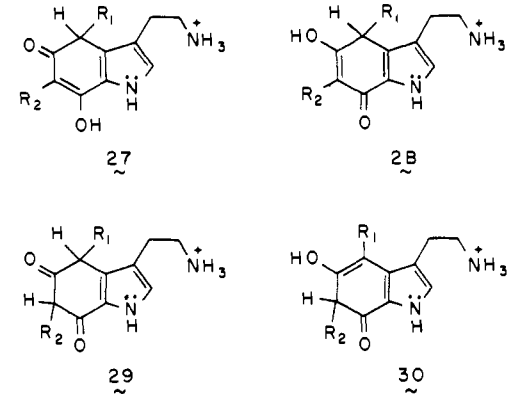
The rate of O_2 consumption for each compound at each concentration was measured during the initial linear phase of the reaction. The plots of these rates vs. concentration are shown in Figure 4. Because of technical difficulty associated with reliably measuring rates >3500 nmol/min in the present setup, rates of O_2 consumption by 4,6-Me_2-5,7-DHT at concentrations greater than 0.5 mM could not be measured. The relative initial rates of O_2 consumption by a 0.5 mM solution of the test compound (in nmol/min) were 4,6-Me_2- (3008) > 4-Me- (299) > 6-Me-5,7-DHT (214) > 5,7-DHT (16.9).[31] Thus, the 4-Me substituent causes a rate increase by 17.7-fold, 6-Me by 12.7-fold, and 4-Me and 6-Me jointly by 178-fold. The 178-fold increase in the rates for 4,6-Me_2-5,7-DHT over 5,7-DHT is 79% of the strictly additive effect (225-fold) of the two methyl substituents. It should be noted that, except for 6-Me-5,7-DHT, all the test compounds displayed a linear dependence of rate on concentration. The deviation with 6-Me-5,7-DHT, however, was less than 6% at 1.5 mM and less than 10% at 2 mM concentration.

Discussion

Nature of 5,7-DHTQ. The fact that none of the test compounds in cyclic voltammetric studies displayed the reverse of the reaction **22b–e** → **23b–e** at any pH studied and at scan rates of up to 400 mV/s suggests that 5,7-DHTQ and its methylated derivatives (**23b–e**) are highly unstable and undergo complete chemical transformation in less than 1 s.[32] These chemical transformations could conceivably involve nucleophilic attack of DHTQ's by H_2O and/or by the amino group of the aminoethyl side chain. Only in the case of 5,7-DHT and 6-Me-5,7-DHT, one of these chemical transformations could be identified, that being Michael addition of H_2O at the 4-position of **23b** and **23d**, respectively. The products of these Michael additions, 4,5,7-trihydroxytryptamine (4,5,7-THT, **24b**) and 6-methyl-4,5,7-trihydroxytryptamine (6-Me-4,5,7-THT, **24d**), respectively, apparently underwent immediate electrochemical oxidation to form the corresponding quinones **25b** (4,5,7-THTQ) and **25d** (6-Me-4,5,7-THTQ), respectively. This is a classical case of an ECE mechanism with a rapid, intervening chemical reaction.[33] Subsequent detection of the reversible couple 4,5,7-THT ⇌ 4,5,7-THTQ and 6-Me-4,5,7-THT ⇌ 6-Me-4,5,7-THTQ may be taken as evidence that these THTQ's are more stable than the corresponding DHTQ's (**23b** and **23d**, respectively) at least in 0.1 M H_2SO_4. It is not clear, however, why the THT-THTQ couple could not be detected at pH 4.0 and 7.4. These observations very strongly suggest that 5,7-DHTQ exists only transiently when generated in aqueous solution at pH 7.4.

Nature of Phenol–Keto Tautomers. Existence of phenol–keto tautomerism in 5,7-DHT is characterized by the appearance of a pH-dependent absorption maximum at 348 nm, while free reversibility among the different tautomeric forms is indicated by the presence of several isosbestic points. Among the methylated DHT's, only 6-Me-5,7-DHT behaved similar to 5,7-DHT. However, substitution of the 4-position of 5,7-DHT, as in 4-Me- and 4,6-Me_2-5,7-DHT, sharply reduced the extent of phenol–keto tautomerism. This is evidenced by the absence of isosbestic points and by the sharply reduced intensity of the absorption maxima at 348 nm for 4-Me-5,7-DHT and at 364 nm for 4,6-Me_2-5,7-DHT (Figure 2c,d, curve 2), as compared to 5,7-DHT and 6-Me-5,7-DHT at pH 7.4. These results suggest that the predominant keto (tautomeric) forms of 5,7-DHT, responsible for the absorption at 348 nm, are those that involve proton transfer to the 4-position. NMR data on the rates of exchange of H-4 and H-6 of 5,7-DHT, on the other hand, indicate that proton transfer to position 6 is twice as fast as it is to position 4. Thus, proton transfer to position 4, although less favorable kinetically, leads to intermediates that are thermodynamically more stable.

In theory, 5,7-DHT can exist in eight different keto (tautomeric) forms, two of which are diketo and six are monoketo. In each set of three monoketo forms, arising from the ketonization of one hydroxyl group, there is one form with a maximum of 10e conjugation and two forms with (8 + 2)e cross conjugation. Only three of these eight forms involve proton transfer to position 4. These are **27–29**. Among these three forms, all of which maintain



(31) The values obtained in the present study for 5,7-DHT agree very closely with those described in ref 12 and in: Creveling, C. R.; Lundstrom, J.; McNeal, E. T.; Tice, L.; Daly, J. W. *Mol. Pharmacol.* **1975**, *11*, 211–222.

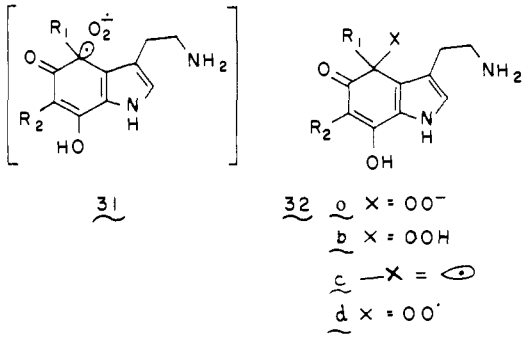
(32) This number was derived as follows: with the assumption that the cathodic peak in 0.1 M H_2SO_4 representing **23b–e** + 2e → **22b–e** could occur anywhere in the first two-thirds of the reverse sweep: range of reverse sweep of the triangular potential (1025 mV ÷ 2) × 2/3 ÷ maximum sweep rate (400 mV/s) = 0.85 s.

(33) Bard, A. J.; Faulkner, L. R. "Electrochemical Methods: Fundamentals and Applications"; Wiley: New York, 1980, p 461.

aromaticity in the pyrrole ring,[34] only **27** still maintains the maximum 10e conjugation[35] and hence is expected to be the most stable keto tautomer of 5,7-DHT. It may be argued that although **28** and **29** are less stable than **27**, they might contain sufficient delocalization energy to provide significant absorption at a wavelength as high as 348 nm. If this was the case, then tautomer **30** (R_1 = Me, R_2 = H), which also contains the same 8e conjugated chromophore as **28** and **29** should have given rise to a prominent absorption band at 348 nm for 4-Me-5,7-DHT.[36] Compared to 5,7-DHT (ϵ_{348} 7100), 4-Me-5,7-DHT exhibits only a weak absorption at 348 nm (ϵ 1200). This suggests that tautomeric forms **28** and **29** for 5,7-DHT (R_1 = R_2 = H) either do not absorb at 348 nm or have very low extinction coefficients at this wavelength. Thus, it appears very likely that the predominant keto (tautomeric) form of 5,7-DHT at pH 7.4 is **27** (R_1 = R_2 = H). Similarly, the predominant keto form of 6-Me-5,7-DHT is also **27** (R_1 = H, R_2 = Me). Results of the present study, however, do not allow determination of the relative proportion of phenol vs. keto forms present in solution.

Autoxidation: Products and Mechanism. The reaction of each of the DHT's with O_2 is first order in O_2, first order in the DHT, and, thus, overall second order, at least at the initial stages. These results are consistent with a mechanism of autoxidation involving incorporation[37] of O_2 into the DHT nucleus and are not consistent with the transformation of the DHT's to the corresponding DHTQ's (cf. **22b–e** → **23b–e**). The initial products of autoxidation at pH 7.4 from each of the DHT's displayed an UV–visible absorption curve that is strikingly similar to the absorption curves of 5,7-DHT and 6-Me-5,7-DHT in the absence of O_2 at pH 7.4 (Figure 2). Since the latter absorption curves, which are similar to each other, are due mostly to tautomer **27** (R_1 = R_2 = H for 5,7-DHT and R_1 = H, R_2 = Me for 6-Me-5,7-DHT, respectively) we conclude that for each of the DHT's, the initial incorporation of O_2 takes place primarily in the 4-position.

While meta disposition of the hydroxyl groups is essential for the existence of phenol–keto tautomerism and for the type of reactivity toward O_2 under discussion,[38,39] it is not clear whether it is the 5-phenoxy form of 5,7-DHT and its methylated derivatives or the carbanion of the keto tautomer **27** that is the primary electron donor to O_2. The difference is subtle but the outcome is the same, namely, formation of free radical superoxide complex **31**. Recombination[38,40] of superoxide with the incipient carbon



free radical would produce hydroperoxide anion **32a**, which upon proton abstraction would produce hydroperoxide **32b**. Direct formation of **32a** by a concerted interaction between the ground state (singlet) 5-phenoxy form of 5,7-DHT and its methylated derivatives and ground state (triplet) O_2 is unlikely[40] on the basis of the usual spin conservation rules. For the same reason, formation of **32a** by the direct reaction between O_2 and the carbanion generated from **27** is also unlikely.[40] Indeed, essentially additive effects of methyl substitution on the rates of O_2 consumption strongly suggest a mechanism involving free radical–superoxide complex **31**. While the stability of these hydroperoxides is not known, these are secondary and tertiary hydroperoxides and, as such, are expected to be moderately stable.[41,42] However, oxygen electrode tracings also show relatively rapid return of O_2 to the solution soon after initial consumption had ceased for all the methylated DHT's. The precise reason for the return of O_2 to the solution is not known at the present time, but the following possibilities are suggested. The carbon free radical in **31** could dissociate some of the time from the complex (to form **32c**) and undergo reaction with O_2 to give the peroxide radical **32d**. This could react with the parent phenoxide of the DHT to produce **32c**, setting up a chain reaction.[40] Disproportionation[41,42] of the peroxide radical, possibly at the later stages of the reaction when sufficient concentration has built up, will produce O_2, as would dismutation[43] of $O_2^{-}\cdot$ and reaction[43] of $O_2^{-}\cdot$ with hydroperoxides. These types of reactions are probably not important at the initial stages, from which rates were calculated, but would be expected to become significant as the reaction proceeds to completion. Clearly, the key step in these processes is the formation of free radical **32c**. The stability of **32c** and the feasibility of its detection (by, e.g., ESR spectroscopy) remain to be determined.

From the foregoing discussion, it may appear that in the case of 5,7-DHT and its methylated derivatives (in contrast to 6-OHDA,[44] for example), it does make a difference whether, during oxidation, it is the electrode or O_2 that acts as an electron acceptor. In fact there is probably no fundamental difference in the two modes of oxidation. Thus, in both modes of oxidation, initial electron transfer step may produce the same intermediate, namely, a 5-phenoxy radical. During electrochemicl oxidation, which is carried out in the absence of O_2, the phenoxy radical is simply converted to the *p*-quinone imines **23b–e** by disproportionation of the corresponding semiquinone radicals, whereas, during autoxidation, the phenoxy radical, on its way to becoming a semiquinone radical, is trapped as **31** and/or **32d**. The fact that 5,7-DHT reacts with O_2 in a bimolecular fashion clearly suggests that very little, if any, of the carbon free radical in **31** (R_1 = R_2 = H) or **32c** escapes to become 5,7-DHTQ.

While the initial autoxidation products from 4-Me- and 4,6-Me_2-5,7-DHT did not display any noticeable changes in UV–vis absorption spectra nor any change in color, even after 24 h, both 5,7-DHT and 6-Me-5,7-DHT showed time-dependent appearance of pink color and absorption in the visible region at 520 and 540 nm, respectively. As the hydroperoxides **33** (R = H or Me, Chart III) from the latter two compounds are secondary, they would be expected[40,41] to break down to generate a 4-keto function as in **34** (R = H or Me, respectively). Tautomerization of *o*-quinones **34** (R = H or Me) would readily produce the corresponding *p*-quinones **35** (R = H or Me), which, fortuitously, are identical with the postulated secondary products of electrochemical oxidation (Chart II) of 5,7-DHT (**25b**) and of 6-Me-5,7-DHT (**25d**), respectively. Lack of formation of colored compounds from 4-

(34) The only other keto tautomer that maintains aromaticity in the pyrrole ring is **30**.

(35) The only other keto tautomer that maintains 10e conjugation arises from the ketonization of 7-OH and proton transfer to position 7a. This form does not maintain aromaticity in the pyrrole ring and hence is expected to be less stable than **27**. In addition, if this form made a significant contribution, then tautomerism of 5,7-DHT would not have been significantly affected by the substitution of methyl groups on position 4 and/or 6.

(36) Facile exchange of H-6 of 4-Me-5,7-DHT is best explained by invoking the intermediacy of tautomer **30** (R_1 = Me, R_2 = H). This exchange of H-6 is also possible through the ketonization of 5-OH. However, the resulting ketone, an (8 + 2)e cross-conjugated system, is expected to be less stable than **30** as it does not maintain aromaticity in the pyrrole ring.

(37) Unpublished observations of H. G. Schlossberger have been cited in ref 6 and 12 to suggest that oxygen may be incorporated into the indole ring.

(38) (a) Haynes, R. K.; Musso, H. *Chem. Ber.* **1974**, *107*, 3723–3732. (b) Haynes, R. K.; Hess, H.; Musso, H. *Ibid.* **1974**, *107*, 3733–3748.

(39) Sosnovsky, G.; Zaret, E. H. In "Organic Peroxides"; Swern, D., Ed.; Wiley: New York, 1969; Vol. 1, Chapter VII.

(40) Guthrie, R. D. In "Comprehensive Carbanion Chemistry—Part A, Structure and Reactivity"; Buncel, E., Durst, T., Eds.; Elsevier: Amsterdam, 1980; Chapter 5.

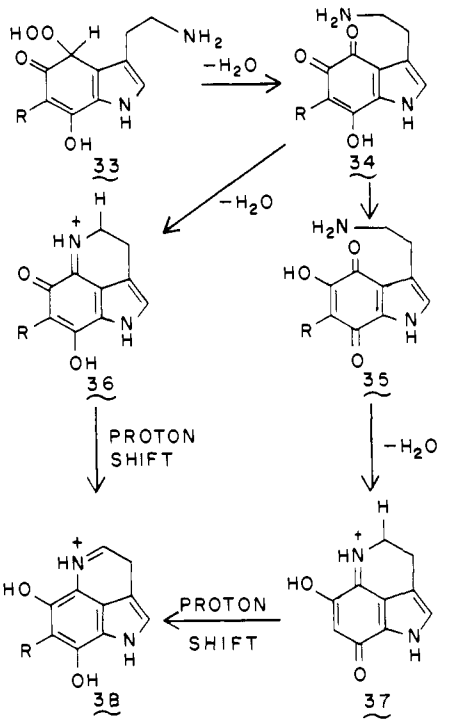
(41) Hiatt, R. In "Organic Peroxides"; Swern, D., Ed.; Wiley: New York, 1971; Vol. 2, Chapter I; Vol. 3, Chapter I.

(42) "The Chemistry of Peroxides"; Patai, S., Ed.; Wiley: New York, 1983.

(43) (a) Frimer, A. A. In ref 42, Chapter 14. (b) Sawyer, D. T.; Valentine, J. S. *Acc. Chem. Res.* **1981**, *14*, 393–400. (c) Nanni, E. J., Jr.; Stallings, M. D.; Sawyer, D. T. *J. Am. Chem. Soc.* **1980**, *102*, 4481–4485.

(44) Dryhurst, G.; Kardish, K. M.; Scheller, F., Renneberg, R. "Biological Electrochemistry"; Academic Press: New York, 1982; Vol. 1, Chapter 2.

Chart III

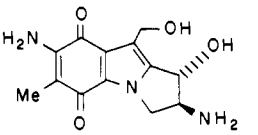


Me-5,7-DHT supports the conclusion that the primary site of O_2 incorporation in all the DHT's is the 4-position. Thus, if O_2 incorporation did also take place in position 6, then, in the case of 4-Me-5,7-DHT, 6-hydroperoxy-4-Me-5,7-DHT would have been a side product which could have undergone decomposition to an *o*-quinone of 4-Me-5,6,7-trihydroxytryptamine with the concomitant appearance of color in solution. The observed shielding of C-6 substituents (H or Me) in NMR spectra suggests that the lack of O_2 incorporation on C-6 is almost certainly due to the electronegative environment created by the oxygen atoms in C-5 and C-7.

Unfortunately, attempts to isolate either of the quinones **34** or **35** (R = H or Me) have not yet been successful, suggesting that these initially produced quinones are reactive intermediates in the formation of related structures. In analogy to the intramolecular cyclization reaction of 6-hydroxydopaminequinone,[44] the quinones **34** and **35** may condense with the respective 4-keto function resulting in the formation of **36** and **37**, respectively. Proton shift from both **36** and **37** would produce **38**. Hydrolysis of the iminium moiety of **38** followed by autoxidation of the resulting aminophenol should lead to another series of compounds containing the UV-active chromophores similar to that present in **35**. Additional reactions of **34** and **35** may involve attack at position 2 by the amino group of the aminoethyl side chain.

The view that the intramolecular reaction involving the amino group of the aminoethyl side chain is the major reason for the instability of **35** is supported by the demonstrated stability of **39**, a cleavage product of mitomycins, which contains a very similar quinone structure but lacks the aminoethyl side chain.[45] The absorption maxima displayed[45] by this compound (ϵ_{530} 700, ϵ_{350} 3200, ϵ_{309} 10 000) are strikingly similar to those observed with **35** and their degradation products. These data lend further support to our postulation that the primary quinone products from the autoxidation of 5,7-DHT are **34** and **35** (R = H).

To minimize the above-mentioned intramolecular reactions involving the amino group, we now plan to utilize a suitable amino-acyl derivative of 5,7-DHT in which nitrogen has been rendered essentially nonnucleophilic. Autoxidation as well as electrochemical oxidation of such an amino-acyl derivative of 5,7-DHT might lead to isolable products. In addition, unambiguous generation of **35** (R = H) and its amino-acyl derivative and the study of their solution properties are planned. Results of these studies will be reported in due course.



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Conclusions

The results of this investigation lead to the following conclusions: (1) Autoxidation of 5,7-DHT does not produce the postulated *p*-quinone imine, 5,7-DHTQ. This *p*-quinone imine, however, is the initial product of electrochemical oxidation of 5,7-DHT and is highly unstable and exists less than 1 s in aqueous solutions ranging in pH from 0.1 M H_2SO_4 to pH 7.4; (2) at pH 7.4 the predominant keto tautomeric form of 5,7-DHT is **27** ($R_1 = R_2 = H$). (3) 5,7-DHT reacts with O_2 in a bimolecular fashion to initially produce the 4-hydroperoxy intermediate **32b** ($R_1 = R_2 = H$) essentially exclusively. Relative contributions of mechanisms involving recombination of superoxide with the incipient free-radical **31** ($R_1 = R_2 = H$) vs. chain reaction with O_2 involving **32c** ($R_1 = R_2 = H$) toward the formation of the 4-hydroperoxide could not be determined; (4) the eventual colored products formed from the autoxidation of 5,7-DHT appear to be the ortho and *p*-quinones of 4,5,7-trihydroxytryptamine (**34** and **35**, respectively, R = H) and their degradation products (Chart III).

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