°C; 8 ft × 1/4 in. 5% OV-17 packed stainless steel column with the oven temperature programmed from 50 to 250 °C at 10 deg/min. Imine **5** was isolated at a constant oven temperature of 250 °C and detector at 200 °C.

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Registry No. **1**, 1660-04-4; **2**, 87429-27-4; **3**, 110-61-2; **4**, 880-52-4; **5**, 108418-81-1; **6**, 87429-30-9; AdAd, 3732-31-8; AdCN, 23074-42-2; AA, 21245-62-5; Ad•, 2819-03-6; AdH, 281-23-2; aminoacetonitrile hydrochloride, 6011-14-9; acetonitrile, 75-05-8.

Oxidation Chemistry of 5-Hydroxytryptamine. 1. Mechanism and Products Formed at Micromolar Concentrations

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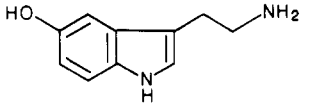
The oxidation of very low concentrations (<30 μM) of 5-hydroxytryptamine (**1**) in 0.01 M HCl has been studied by using electrochemical and other analytical techniques. The initial oxidation is a 1e,1H$^+$ reaction, giving a phenoxyl radical which exists in equilibrium with aryl, C(4)•, and N(1)• radicals. At low potentials the latter radicals can react to give dimeric products. At higher potentials, however, the primary phenoxyl radical is further oxidized (1e,1H$^+$) to a reactive quinone imine. The quinone imine is rapidly attacked by water to give 4,5-dihydroxytryptamine (**7**), which is further oxidized to tryptamine-4,5-dione (**B**). In aqueous solution at pH 2 **B** is slowly attacked by water to give 4,5,7-trihydroxytryptamine, which is further oxidized (2e,2H$^+$) to 5-hydroxytryptamine-4,7-dione (**11**). Compounds **B** and **11** then react together to give a hydroxylated tryptamine dimer. The facile oxidation of **1** to give at least two neurotoxins, **7** and **9**, might provide insight into the previously proposed anomolous oxidative metabolism of **1** as an underlying cause of various mental disorders.

5-Hydroxytryptamine (**1**) occurs naturally in the central and peripheral nervous system where it functions as a chemical neurotransmitter. Among the presumed roles of **1** in the central nervous system (CNS) are the regulation



1

of body temperature, sleep, and certain emotional states.[1] Udenfriend et al.[2] demonstrated that **1** is converted into 5-hydroxyindole-3-acetaldehyde by monoamine oxidase (MAO) and then to 5-hydroxyindole-3-acetic acid by aldehyde dehydrogenase. The major known metabolites of **1** found in urine result from the initial action of MAO.[3,4]

In the early 1950s it was suggested[5,6] that a faulty mechanism in the metabolism of **1** might be related to the onset of certain types of psychotic behavior. More recent reports suggest that depression and schizophrenia might result from such faulty metabolic pathways.[7-10] A recurring suggestion is that a defect in the metabolism of **1** leads to formation of more highly hydroxylated and therefore more reactive compounds that in some way lead to mental disorders.[2,3,11,12] Indeed, minor oxidation products of **1** in rats and rabbits have been speculated to be 4,5- or 5,6-dihydroxytryptamine.[3,12-14] Such speculations have merit in view of the subsequent discovery that dihydroxytryptamines such as 5,6-dihydroxytryptamine (5,6-DHT) and 5,7-DHT are powerful neurotoxins.[15-21] In addition, there is now considerable evidence that **1** is oxidized in biological media by routes other than the oxidative deamination pathways mentioned earlier. For example, human serum and ceruloplasmin concentrates oxidize **1** to give colored solutions although products have not been identified.[13,22-26] Hemolysates of rat erythrocytes

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oxidize 1 and other 5-hydroxyindoles to give unknown colored products.[14] Molecular oxygen and Ag^+ also oxidize 1 to similar products, one being a melanin-like pigment; another has been speculated to be a relatively long-lived but unknown dimeric intermediate.[12] Oxidations of 1 by ferricytochrome *c*,[27] alkaline permanganate,[28] autoxidation in basic solution,[29] ceruloplasmin[30] and during metabolism[31,32] appear to generate radicals. However, the reaction chemistry of these radicals and the ultimate products formed in these oxidizing systems are not known.

Thus, while the oxidative deamination pathways for metabolism of 1 are quite well understood experiments with ceruloplasmin, serum, erythrocytes, O_2, and other oxidizing enzymes[33-35] show that alternate oxidation routes are possible. However, not a single product of these oxidation reactions is known and no mechanistic pathways have been studied.

The purpose of the work described here was to employ electrochemical and other analytical approaches to study the fundamental oxidation chemistry of 1 with the expectation that the results obtained might provide a basis for better understanding the biochemical oxidations of this compound. An earlier report suggested that 1 is initially electrooxidized ($2e,2H^+$) to a very reactive quinone imine.[36] A minor reaction pathway for the latter quinone imine was proposed to be attack by water, giving 5,7-DHT which was further oxidized ($4e,4H^+$ overall) to give 5-hydroxytryptamine-4,7-dione. The latter study employed relatively high concentrations (1 mM) of 1. It has now been found that the oxidation chemistry of 1 is strongly dependent upon its initial solution concentration and pH. This report describes the electrochemical oxidation when the initial concentration of 1 is ≤ 30 μM at low pH. Subsequent reports will describe the oxidation chemistry of 1 at higher concentrations and at higher pH.

Experimental Section

5-Hydroxytryptamine hydrochloride was obtained from Sigma (St. Louis, MO). *N*-Methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA) and *N*-(trimethylsilyl)imidazole (TMSI) were obtained from Pierce (Rockford, IL). Silylation grade acetonitrile was obtained from Supelco (Houston, TX). Silylations were performed by adding 150 μL of the appropriate reagent mixture to ca. 1 mg of the compound of interest contained in a 3-mL Reacti-Vial (Pierce). The vial was then sealed and heated. Exact silylation conditions employed will be described in subsequent sections of this paper.

Sephadex LH-20 gel permeation resin was obtained from Pharmacia (Piscataway, NJ). Phosphate buffers having known ionic strengths were prepared according to Christian and Purdy.[37]

The apparatus used for electrochemical studies has been described elsewhere.[38] All voltammograms were corrected for IR drop. A pyrolytic graphite electrode (PGE, Pfizer Minerals, Pigments and Metals Division, Easton, PA) having an approximate surface area of 0.36 mm^2 was used for voltammetry and was resurfaced before recording each voltammogram.[38] Controlled potential electrolyses and coulometry employed four plates of pyrolytic graphite as the working electrode (total surface area ca. 360 cm^2) dipping into 350 mL of solution containing 1. Voltammetry and controlled potential electrolyses were carried out in conventional three-electrode cells containing a platinum counter electrode and a saturated calomel reference electrode (SCE). The latter electrodes were immersed in 0.01 M HCl. The working, counter, and reference electrodes were separated with a Nafion membrane (Type 117, DuPont Co., Wilmington, DE). All voltammetric measurements were made in solutions that had been thoroughly deaerated with nitrogen. Controlled potential electrolyses were performed on solutions that were stirred with a Teflon-coated stirring bar and with nitrogen gas bubbling vigorously through the solution. All potentials are referred to the SCE at 25 ± 3 °C.

A Hewlett-Packard Model 5880 gas chromatograph equipped with a flame ionization detector was used for gas chromatography. All GC separations used a glass column (1.8 m × 2 mm i.d.) packed with 3% SE-30 on Chromasorb W. Helium was used as the carrier gas at a flow rate of 30 mL min^{-1}. GC retention times (t_R) reported were measured under the following conditions: initial temperature 100 °C for 12 min followed by a linear temperature gradient (6°/min) to 280 °C. The latter temperature was then held constant for 60 min.

High performance liquid chromatography (HPLC) employed a Bio-Rad gradient system and a reversed phase column (Brownlee Laboratories, Santa Clara, CA, RP-18, 25 × 0.7 cm). Two mobile-phase solvents were employed. Solvent A consisted of H_2O/MeCN (100:7, v/v) containing 1.5 μM triethylamine and adjusted to pH 3.1 with formic acid. Solvent B consisted of H_2O/MeCN (100:40, v/v) containing 0.94 μM triethylamine and adjusted to pH 3.1 with formic acid. The conditions used for HPLC analysis were as follows: 0–17 min, 100% solvent A at a flow rate of 2.8 mL min^{-1}; 17–65 min, 100% solvent B at a flow rate of 2.5 mL min^{-1}.

Low and high resolution fast atom bombardment mass spectrometry (FAB–MS) was carried out on a VG Instruments ZAB-SE mass spectrometer (VG Analytical Ltd., Manchester, U.K.) or a Kratos MS-50 spectrometer at the Midwest Center for Mass Spectrometry (University of Nebraska). High resolution electron impact mass spectrometry (EI–ms) was performed on a Kratos Model MS-25/RFA instrument. Other MS and GC–MS studies were carried out on a Hewlett-Packard Model 5985B instrument. Chemical ionization (CI) GC–MS employed methane as the reactant gas (2×10^{-4} Torr) in the source chamber and an electron beam energy of 150 eV. ^1H NMR spectra (300 MHz) were obtained with a Varian Model 300 XL spectrometer. UV–vis spectra were recorded on a Hitachi Model 100-80 spectrophotometer.

Isolation and Identification of Products. In a typical electrolysis 1.5–2.0 mg of 1 dissolved in 350 mL of 0.01 M HCl was oxidized. It was impractical to separate the resulting small amounts of each product dissolved in such a large volume of solution by HPLC. Thus, product mixtures from several identical electrolyses were combined and freeze-dried and the resulting mixtures were separated by column chromatography on Sephadex LH-20 (60 × 2 cm column using a mobile phase of H_2O/MeOH (9:1, v/v) adjusted to pH 2.0 with HCl at a flow rate of 32 mL h^{-1}). The eluent was monitored with a Gilson Holochrome UV detector set at 270 nm. Column chromatography of the freeze-dried products obtained following peak I_a (0.53 V) oxidation of 1 showed that the products responsible for HPLC peaks A, E, F, and C (Figure 3) eluted at retention volumes of 267, 400, 715, and 1212 mL, respectively. The products responsible for HPLC peaks B and D were never observed in liquid chromatograms of freeze-dried peak I_a products. Additional peaks were observed in column chromatograms at retention volumes of 155, 188, 303, and 1097 mL. The compounds eluted under these peaks were

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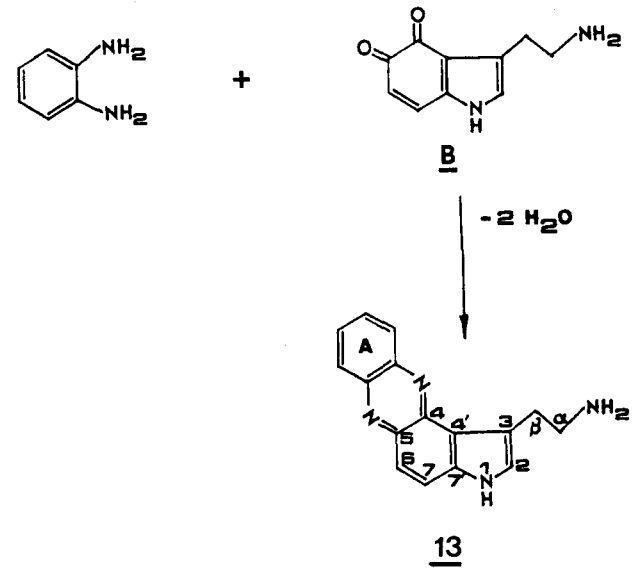
not observed by HPLC analysis of peak I_a products before freeze-drying.

Column chromatography of the freeze-dried peak II_a (0.68 V) products of oxidation of 1 gave the products corresponding to HPLC peaks E and F at retention volumes of 400 and 715 mL, respectively. The product responsible for HPLC peak B was never observed. A peak due to HPLC component C was also not observed. The additional peaks noted earlier in column chromatograms of the freeze-dried peak I_a products were also observed. However, the component responsible for the peak at a retention time of 1097 mL was significantly larger in column chromatograms of the freeze-dried peak II_a product mixtures. It was clear from the results of HPLC analysis of a fresh peak II_a product solution (which shows only HPLC peak B) taken together with column chromatography results that in the process of freeze-drying component B reacts to give secondary products. It is hoped to provide further information on these products at a later time.

4,4'-Bi-5-hydroxytryptamine (A). This product was collected from several peak I_a electrooxidations of 1 by column chromatography on Sephadex LH-20. A was purified by column chromatography on a long column of Sephadex LH-20 (100 × 2 cm) using H_2O/MeOH (9:1, v/v) adjusted to pH 2.0 with HCl as the mobile phase (18 mL h^{-1} flow rate), when it eluted at about 640 min. Between pH 2–7 A shows a characteristic UV spectrum (λ_{max} = 302, 275, 206 nm). At higher pH (10.7) this spectrum changes (λ_{max} = 350 (sh), 310, 276 nm). CVs show that A gives an oxidation peak at 0.75V in 0.01 M HCl. FAB–MS (thioglycerol matrix) gave an intense pseudomolecular ion (MH^+) at m/e 351.1826 ($C_{20}H_{23}N_4O_2$, calculated m/e 351.1821). Thus A is a dimer of 1 having a molecular formula $C_{20}H_{22}N_4O_2$. Silylation of A (MSTFA/TMSI/MeCN, 70 μL:10 μL:70 μL, 70 °C, 1 h) gave a trimethylsilyl derivative having a GC t_R = 48.2 min and a molar mass (EI- and CI–GC–MS) of 926 g. Thus A can be silylated at eight positions, indicating that the OH, N(1)-H, and $CH_2CH_2NH_2$ groups present in the two residues of 1 are intact, i.e., the two residues of 1 must be C–C linked. Before discussing the ^1H NMR spectrum of A, it is of value to summarize that of 1 (300 MHz, Me_2SO-d_6): δ 2.90 (t, 2 H, CH_2), 3.06 (t, 2 H, CH_2), 6.62 (dd, J = 9 Hz, J = 3 Hz, 1 H, C(6)-H), 6.82 (d, 1 H, J = 3 Hz, C(4)-H), 7.12 (s, 1 H, C(2)-H), 7.17 (d, 1 H, J = 9 Hz, C(7)-H), 7.81 (br s, 3 H, NH_3^+), 8.06 (br s, 1 H, OH), 10.64 (s, 1 H, N(1)-H). ^1H NMR of A (Me_2SO-d_6): δ 6.79 (d, 2 H, J = 9 Hz, C(6)-H and C(6')-H), 7.05 (d, 2 H, J = 3 Hz, C(2)-H and C(2')-H), 7.20 (d, 2 H, J = 9 Hz, C(7)-H and C(7')-H), (7.48 br s, 6 H, 2 × NH_3^+), 7.94 (br m, 2 H, 2 × OH), 10.74 (d, 2 H, J = 3 Hz, N(1)-H and N(1')-H). The region between 2.4 and 4.8 ppm was not well defined owing to the interfering signals of HOD and Me_2SO. Nevertheless, the ^1H NMR spectrum of A compared to that of 1 shows that the C(4) protons are missing. The fact that the C(6) protons of A do not give a doublet of doublets indicates that the long range spin coupling between the C(4) and C(6) protons has been lost. Thus A is 4,4'-bi-5-hydroxytryptamine (structure is shown in Figure 6).

Tryptamine-4,5-dione (B). All attempts to isolate this compound were unsuccessful. However, oxidations of very dilute solutions of 1 at peak II_a potentials give B as the sole 4e product. This is consistent with B being an oxidized dihydroxy compound, i.e., a diketone. The UV–vis spectrum of B (λ_{max} = 535, 350, 232 nm at pH 2.0, Figure 4A) and its CV (Figure 4B) are quite different to those of 5,6- and 5,7-DHT and their oxidized forms.[36] The absorption band of B at 535 nm (Figure 4A) is typical of *o*-quinones such as *o*-benzo- and *o*-naphthoquinone[21] and various indolic 4,5-quinones.[42-44] Accordingly, B was condensed with *o*-phenylenediamine, which reacts with 1,2-diketones,[45] using the following procedure: A 20 μM solution of 1 in 0.01 M HCl (350 mL) was oxidized at 0.68 V (peak II_a). Upon completion of the oxidation (~40 min), 10 mg of *o*-phenylenediamine was added

Scheme I



(0.1 mM) and the solution was stirred for about 10 s. The solution was then freeze-dried. The solid obtained was dissolved in 2 mL of water and passed through a short column (60 × 2 cm) of Sephadex LH-20 using the usual conditions. Two major fractions eluted at t_R = 320 min (unreacted *o*-phenylenediamine) and at t_R = 960 min. The latter peak was collected, freeze-dried, and purified by repeating the latter chromatographic procedure. The red-orange product showed a characteristic spectrum, λ_{max} (0.01 M HCl) = 530, 425, 275, 230 (sh), 215 nm. EI–MS (70 eV, 235 °C) gave the following major peaks, m/e (relative abundance): 262 (12, M^+), 233 (100, $M^+ - CHNH_2$), 232 (78, $M^+ - CH_2NH_2$). High resolution EI–MS on M^+ gave m/e 262.1220 ($C_{16}H_{14}N_4$, calcd m/e 262.1218). These results indicate that B is tryptamine-4,5-dione which reacts with *o*-phenylenediamine to give the quinoxaline derivative **13** (Scheme I). Structure **13** was confirmed by ^1H NMR. In Me_2SO-d_6: δ 3.39 (m, 2 H, C(α)-H_2), 3.60 (t, 2 H, C(β)-H_2), 7.48 (d, J = 2.8 Hz, 1 H, C(2)-H), 7.80 (d, J = 9 Hz, 1 H, C(6)-H or C(7)-H), 7.91 (m, 2 H, Ar protons from ring A), 8.08 (d, J = 9 Hz, 1 H, C(6)-H or C(7)-H), 8.26 (m, 4 H, NH_3^+ and 1 Ar proton from ring A), 8.43 (dd, J = 9 Hz, J = 2.6 Hz, 1 H, Ar proton on A), 12.19 (s, 1 H, N(1)-H). In Me_2SO-d_6 with D_2O added: δ 3.41 (t, 2 H, C(α)-H), 3.49 (t, 2 H, C(β)-H), 7.48 (s, 1 H, C(2)-H), 7.79 (d, J = 9 Hz, 1 H, C(6)-H or C(7)-H), 7.88 (m, 2 H, Ar protons), 8.09 (d, J = 9 Hz, 1 H, C(6)-H or C(7)-H), 8.27 (d, J = 9 Hz, 1 H, Ar), 8.42 (d, J = 9 Hz, 1 H, Ar).

4-Chloro-5-hydroxytryptamine (C). In 0.01 M HCl exhibits a typical indolic spectrum (λ_{max} = 293 (sh), 272, 215 nm). CVs of C in 0.01 M HCl (200 mV s^{-1}) showed two apparently irreversible oxidation peaks at E_p = 0.64 and 1.20 V. On the reverse sweeps a reversible couple at 0.10 V was observed. C was purified by column chromatography on Sephadex LH-20. FAB–MS (dithiothreitol/dithioerythritol matrix) gave the following results, m/e (relative abundance): 213 (MH^+, 31), 212 (11.4), 211 (MH^+, 100), 196 (23), 195 (12), 194 (83), 177 (61). Addition of NaCl to the matrix resulted in the appearance of two new pseudomolecular ions (MNa^+) at m/e 233 and 235 with the ion at m/e 235 having approximately one-third the intensity of that at m/e 233. Silylation of C (MSTFA/TMSI/MeCN, 70 μL:10 μL:70 μL, 80 °C, 45 min) followed by EI–GC–MS showed two GC peaks at t_R = 24.2 and 27.3 min having molar masses of 426 g and 498 g, respectively. These molar masses correspond to derivatives of C (MM = 210 g) containing three and four trimethylsilyl substituents, respectively. ^1H NMR spectrum of C in Me_2SO-d_6: δ 3.07 (m, 2 H, C_α-H_2), 3.17 (t, 2 H, C_β-H_2), 6.80 (d, J = 8.7 Hz, 1 H, C(6)-H), 7.14 (d, J = 8.7 Hz, 1 H, C(7)-H), 7.9 (d, J = 2.1 Hz, 1 H, C(2)-H), 7.97 (br s, 3 H, NH_3^+), 9.25 (s, 1 H, OH), 11.03 (s, 1 H, N(1)-H). In D_2O: δ 6.87 (d, J = 9 Hz, 1 H, C(6)-H), 7.21 (s, 1 H, C(2)-H), 7.25 (d, 1 H, C(7)-H); the region around 3 ppm was obscured by a large HOD peak. The FAB–MS results clearly indicate that C contains a single Cl atom while GC–MS results show that all of the silylatable sites of 1 remain intact. Comparison of the ^1H NMR spectrum of C with that of 1 reveals that in C

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the C(4) proton is missing. The signal from C(6)-H in C is only a doublet, not a doublet of doublets as in 1, indicating that long range coupling between protons at C(4) and C(6) does not occur. These data indicate that the Cl substituent in C must be located at C(4). Thus C is 4-chloro-5-hydroxytryptamine. Further support for the structure is provided from the observation that C is not formed as a result of electrochemical oxidation of 1 in phosphate buffers, i.e., in the absence of HCl.

Compound E. In 0.01 M HCl B slowly transforms into red E. The spectrum of E, λ_{max} (0.01 M HCl) = 470, 350, 285, 230 nm, exhibits features observed in the spectrum of B (λ_{max} in 0.01 M HCl 535, 350, 232) and 5-hydroxytryptamine-4,7-dione (11, Figure 7) (λ_{max} in 0.01 M HCl 458, 332, 277, 220 nm). Furthermore, when equal concentrations (20 μM) of B and 11 in 0.01 M HCl were mixed the initial spectrum of the mixture (λ_{max} 500, 341, 282, 228 nm) changed over the course of an hour and became identical with that of E. HPLC analysis of the solution at this point showed a single chromatographic peak having the same t_R as E. A CV of E showed two reversible reduction peaks at 0.10 and −0.14 V. B shows a reversible couple at 0.10 V and 11 shows a reversible couple at −0.12 V. Thus both spectra and CVs of E show features characteristic of B and 11. This suggests that E consists of residues of B and 11. Such a suggestion is supported by the FAB–MS of E (thiothreitol/thioerythritol matrix), which exhibits a pseudomolecular ion (MH^+) at m/e 395.1338 ($C_{20}H_{19}N_4O_5$, calcd m/e 395.1354). Thus E has a molar mass of 394 g and a molecular formula $C_{20}H_{18}N_4O_5$. Unfortunately, only very small amounts of E could be isolated. Furthermore, this red compound was not very stable in aqueous solution or Me_2SO and decomposed to an orange compound that has not been identified. For these reasons it has not been possible to obtain ^1H NMR spectra of E. Nevertheless, the evidence presented above indicates that E consists of residues of B and 11. At this time the exact linkage sites between the two indolic residues in E are not known.

1,4′-Bi-5-hydroxytryptamine (F). This is a minor peak I_a oxidation product of 1. Between pH 2 and 11 the UV spectrum of F remains virtually unchanged and shows a characteristic double band at λ_{max} = 310 and 280 nm. A CV of F shows a quasi-reversible oxidation peak at 0.3 V in 0.01 M HCl. FAB–MS of F (thioglycerol matrix) gave a pseudomolecular ion (MH^+) at m/e 351.1827 ($C_{20}H_{23}N_4O_2$, calcd m/e 351.1821). EI–MS (12 eV, 155 °C) (relative abundance): 350 (11, M^+), 333 (100, $M^+ - OH$), 316 (7, $M^+ - 2(OH)$), 303 (24, $M^+ - CH_2NH_2 - OH$), 204 (11), 147 (93), 146 (48). Derivatization of F (MSTFA/TMSI/MeCN, 70:10:70, 80 °C, 40 min) gave a single trimethylsilyl derivative having a GC t_R 50.0 min. EI- and CI–GC–MS showed the molar mass of this derivative to be 854 g. Thus F (MM = 350 g, $C_{20}H_{22}N_4O_2$) is a dimer of 1 but, since it can only be silylated at seven positions, it appears that an OH, N(1)-H, or exocyclic amino group is involved in linking together the two residues of 1. ^1H NMR of F (Me_2SO-d_6): δ 2.35, 2.69, 3.37, 3.71 (unresolved multiplets, 8 H, 2 × CH_2CH_2), 6.05 (s, 1 H, C(2)-H), 6.46 (m, 2 H, C(6)-H and C(6′)-H), 6.58 (d, J = 9 Hz, 1 H, C(7′)-H), 6.76 (d, J = 3 Hz, 1 H, C(4)-H), 7.15 (d, J = 9 Hz, 1 H, C(7)-H), 7.34 (d, J = 3 Hz, 1 H, C(2′)-H), 8.22 and 8.45 (br s, 8 H, 2 × NH_3^+, 2 × OH), 11.15 (d, J = 3 Hz, 1 H, N(1)-H). After addition of D_2O: δ 2.54, 2.72, 2.91, 3.47 (unresolved multiplets, 8 H, 2 × CH_2CH_2), 6.25 (s, 1 H, C(2)-H), 6.71 (dd, J = 9 Hz, J = 3 Hz, 1 H, C(6)-H), 6.79 (d, J = 9 Hz, 1 H, C(6′)-H), 6.85 (d, J = 9 Hz, 1 H, C(7)-H or C(7′)-H), 6.96 (d, J = 3 Hz, 1 H, C(4)-H), 7.36 (asymmetric d, J = 9 Hz, 2 H, C(7)-H or C(7′)-H and C(2)-H). A significant feature of the spectrum in Me_2SO-d_6 is the presence of only one N(1)-H, indicating that one residue of 1 is linked through the latter position. The high field shift of the C(2)-H (6.05 ppm in Me_2SO-d_6 and 6.25 ppm in D_2O) compared to C(2)-H of 1 (7.12 ppm in Me_2SO-d_6) and the loss of a signal from C(4′)-H together with the disappearance of long range coupling between C(4′)-H and C(6′)-H, which results in the C(6′)-H signal being a doublet compared to a doublet of doublets in the spectrum of 1, indicates that F must be 1,4′-bi-5-hydroxytryptamine (Figure 6).

Results and Discussion

Cyclic Voltammetry. A representative CV of 1 (20 μM) at pH 2 is shown in Figure 1A. At this very low concentration two oxidation peaks (I_a and II_a) appear on the first anodic sweep. On the reverse sweeps reduction peak III_R and oxidation peak II_a' appear as a reversible couple. At higher concentrations of 1 the CVs become more complex. This report will be concerned only with the oxidation chemistry of 1 at concentrations ≤30 μM.

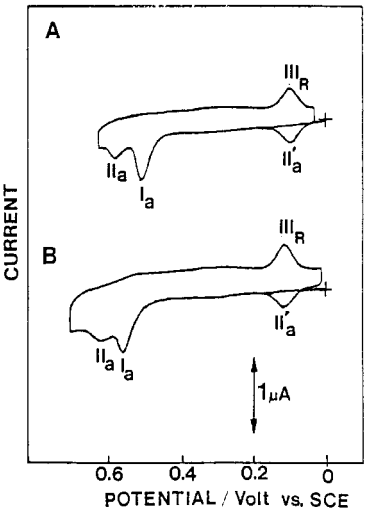

Figure 1. Cyclic voltammograms at the PGE of 20 μM 5-hydroxytryptamine in (A) pH 2.0 phosphate buffer, μ = 0.5 and (B) 0.01 M HCl. Sweep rate: 20 mV s^{-1}.

Oxidation peak I_a is pH-dependent according to eq 1 and 2. The data for these equations were obtained with 20

$$E_p = [0.615–0.047\ pH]V \text{ between pH 2.0 and 8.1} \quad (1)$$

$$E_p = [0.897–0.081\ pH]V \text{ between pH 8.1 and 10.8} \quad (2)$$

μM 1 in phosphate buffers (μ = 0.5) at a sweep rate of 20 mV s^{-1}. In some instances a small prepeak was observed at potentials more negative than peak I_a. In order to be able to isolate and identify the products of peak I_a and peak II_a oxidations of 1 extensive use was made of 0.01 M HCl as a supporting electrolyte. The following discussion, therefore, concerns primarily the electrochemical behaviors of 1 at pH 2.

CVs of 1 in phosphate buffer or in 0.01 M HCl pH 2.0 at sweep rates up to 5 V s^{-1} showed no evidence for a reduction peak coupled to oxidation peaks I_a or II_a. In phosphate buffer (μ = 0.5) the peak potential (E_p) for peak I_a shifts to more positive values with increasing sweep rate (ν). Between 5 and 500 mV s^{-1} $dE_p/d \log \nu$ = 35 mV. The peak current function for peak I_a ($i_p/C\nu^{1/2}$) increases rapidly with increasing ν (134 μA mM^{-1} L $mV^{-1/2}$ $s^{1/2}$ at 5 mV s^{-1}–468 μA mM^{-1} L $mV^{-1/2}$ $s^{1/2}$ at 500 mV s^{-1}) while the ratio $i_p/C\nu$ decreases with increasing ν (1.9 μA mM^{-1} L mV^{-1} s at 5 mV s^{-1}–0.7 μA mM^{-1} L mV^{-1} s at 500 mV s^{-1}). The ratio i_p/C also systematically decreases with increasing concentrations of 1 (63 μA mM^{-1} L at 10 μM–11 μA mM^{-1} L at 200 μM). These behaviors indicate that oxidation peak I_a is controlled to a significant extent by the adsorption of 1 at the pyrolytic graphite electrode (PGE)[39] and that under the voltammetric conditions employed it exhibits characteristics of an, overall, irreversible process.[40]

At low concentrations of 1 (~20 μM) oxidation peak II_a can be quite clearly observed at pH 2 (Figure 1), although at higher pH values it is generally very indistinct. Thus it was not possible to accurately measure the pH dependence of E_p for peak II_a. At pH 2.0, however, peak II_a shifts to more positive potentials with increasing ν ($dE_p/d \log \nu$ = 35 mV between ν = 5 and 500 mV s^{-1}). The peak current function for peak II_a increases with increasing ν. With increasing concentrations of 1 peak II_a grows relative

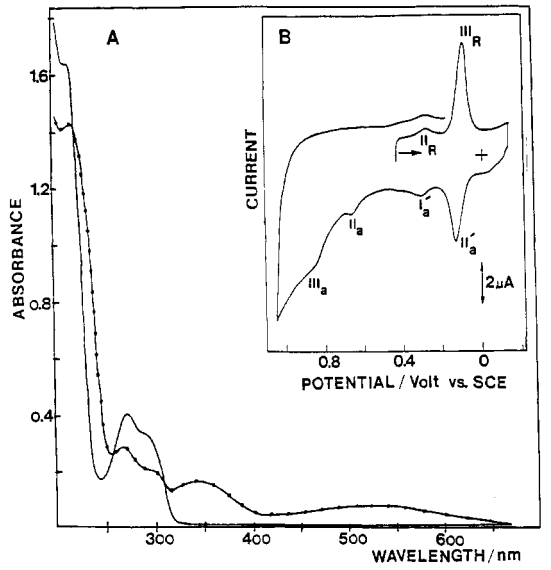

Figure 2. (A) Spectrum of 30 μM 5-hydroxytryptamine in 0.01 M HCl (—) before electrophoresis and (-·-) after electrolysis. (B) Cyclic voltammogram at the PGE after exhaustive electrochemical oxidation of 5-hydroxytryptamine; sweep rate, 200 mV s^{-1}. Controlled potential electrolyses were performed at 0.52 V (peak I_a).

to peak I_a so that at concentrations ≥1 mM peak I_a appears only as a poorly defined inflection on the rising portion of peak II_a. These data indicate that the species responsible for peak II_a is probably adsorbed at the PGE and that it may be formed as the result of a second-order chemical reaction of a primary peak I_a product.

The peaks III_R/II_a' couple, observed after scanning through oxidation peak I_a or peaks I_a and II_a (Figure 1), are highly symmetrical. Using low concentrations of **1** peaks III_R and II_a' have virtually identical E_p and I_p values. In phosphate buffers (μ = 0.5) the E_p values for peaks III_R and II_a' are pH dependent according to eq 3. This

$$E_p = [0.234–0.062\ pH]V \text{ between pH 2 and 8.3} \quad (3)$$

equation is based upon data obtained from CVs of **1** (20 μM) at a sweep rate of 20 mV s^{-1}. The highly symmetrical shapes of peaks III_R and II_a' indicate that the peaks I_a/II_a product responsible is strongly adsorbed at the PGE.[39]

The voltammetric behaviors of **1** in 0.01 M HCl are very similar to those described above in phosphate buffers except that E_p values for all peaks are shifted to slightly more positive potentials (Figure 1B).

Controlled Potential Electrolysis and Coulometry. Controlled potential electrooxidations of **1** were carried out primarily in 0.01 M HCl because of the subsequent ease of removal of this supporting electrolyte by freeze-drying. Preliminary studies revealed that the nature and yields of products were very dependent upon the initial concentration of **1** oxidized and the potential employed. In this study concentrations of **1** ≤30 μM were oxidized.

Controlled potential electrooxidation in 0.01 M HCl at 0.52 V ($\approx E_{p/2}$ for oxidation peak I_a) caused the characteristic UV band of **1** (λ_{max} 285 (sh), 272, 215 nm, Figure 2A) to slowly disappear. As the electrolysis progressed the solution first became purple and then a reddish tint developed. After complete oxidation of **1** the spectrum of the product solution was quite complex (λ_{max} 530, 350, 300, 272, 220 nm, Figure 2A). These electrolyses required approximately 10 h to remove all **1** and coulometric n values of 3 ± 0.3 (average of 10 replicate determinations) were measured. CVs recorded after such peak I_a electrolyses showed that a small peak II_a remained along with another

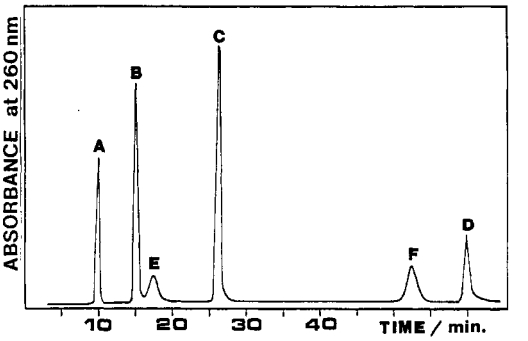

Figure 3. High performance liquid chromatogram of the product mixture formed upon electrochemical oxidation of 20 μM 5-hydroxytryptamine in 0.01 M HCl at 0.53 V ($E_{p/2}$ for peak I_a). Chromatographic conditions are given in the Experimental Section.

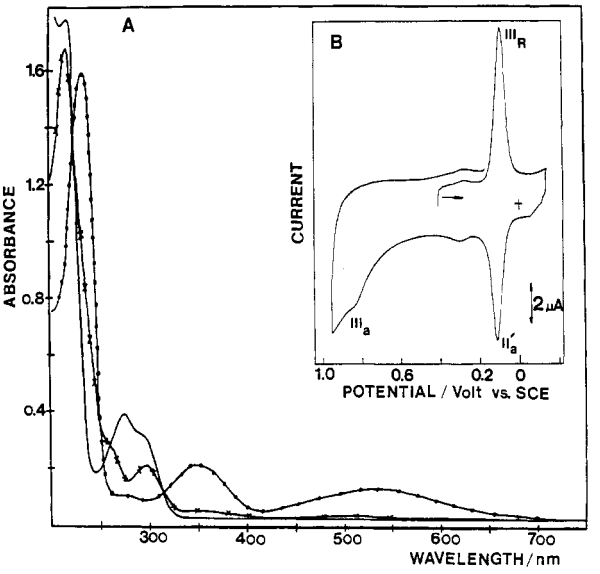

Figure 4. (A) Spectra of 26 μM 5-hydroxytryptamine in 0.01 M HCl (—), after electrooxidation at 0.68 V (-·-) and after electrochemical reduction of the oxidized product at 0.05 V (-×-). (B) Cyclic voltammogram at the PGE after electrooxidation of 26 μM 5-hydroxytryptamine at 0.68 V. Sweep rate: 200 mV s^{-1}.

small oxidation peak III_a at more positive potentials (Figure 2B). On the first cathodic sweep reversible peaks III_R/II_a' at 0.10 V appear. The reversible couple characterized by the small peaks II_R/I_a' (Figure 2B) is much larger following oxidations of higher concentrations of **1**. The species responsible for these peaks will be described elsewhere.

Figure 3 shows a high performance liquid chromatogram of the product solution formed by electrooxidation of 25 μM **1** at 0.52 V ($E_{p/2}$ for peak I_a). Three major products are formed characterized by HPLC peaks A, B, and C along with minor products responsible for peaks E, F, and D. The product responsible for HPLC peak D was unstable and will not be discussed further. When the applied potential was increased to 0.56 V (E_p for peak I_a) spectra and CVs of the resulting product solutions were similar to those shown in Figure 2A,B. However, HPLC analysis showed that peak B grew relative to all of the other peaks.

Controlled potential electrooxidations of **1** (20 μM) at 0.68 V (i.e., 50 mV positive of E_p for peak II_a) were complete in ≤1 h. During the electrolysis the UV bands of **1** disappeared and the bright purple product solution showed bands at λ_{max} 535, 350, 232 nm (Figure 4A). CVs of this product solution showed a large reversible couple at 0.10 V (peaks III_R/II_a') and a small oxidation peak III_a (Figure

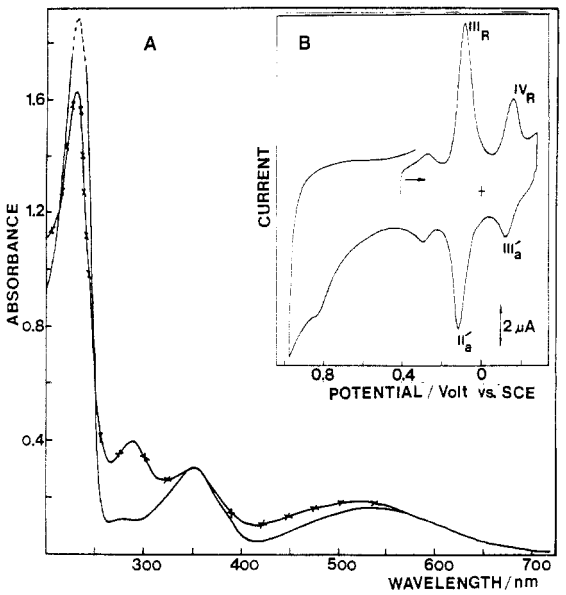

Figure 5. (A) Spectra of the purple peak II_a product of electrooxidation of 32 μM 5-hydroxytryptamine (—) in 0.01 M HCl and of the same solution 24 h later (-×-). (B) Cyclic voltammogram at the PGE of the peak II_a product after standing for 24 h at room temperature; sweep rate, 200 mV s^{-1}.

4B). Electrolyses at 0.68 V gave experimental n values of 3.9 ± 0.2 (average of 10 replicate determinations). HPLC analysis of the purple peak II_a product solution showed only chromatographic peak B. Controlled potential electrochemical reduction of the freshly formed peak II_a product solution at 0.05 V (50 mV more negative than peak III_R) caused the purple color to disappear. The spectrum of the resulting colorless solution showed UV bands at λ_{max} 293, 257, 208 nm (Figure 4A). Coulometric measurements revealed that the n value for this reduction reaction was 2.0 ± 0.3 (average of 6 replicate measurements). Oxidation of the reduced solution, either electrochemically at 0.16 V (peak II_a') or by air, resulted in the quantitative reappearance of the purple peak II_a product. These results indicate that electrooxidation of very dilute (<30 μM) solutions of 1 at peak II_a potentials is a 4e reaction to give a single purple product (λ_{max} 535, 350, 232 nm at pH 2.0) that exhibits the reversible voltammetric peaks III_R/II_a' and that the peak III_R reduction is a 2e process.

CVs and spectral studies showed that the purple compound formed upon electrooxidation of 1 at peak I_a and peak II_a potentials, responsible for HPLC peak B, is stable for only about 1 h at room temperature. After longer time periods spectral and voltammetric changes were noted. For example, after approximately 24 h the band of HPLC compound B at 232 nm increased in height, a new band appeared at 285 nm, and the absorbance between 375 and 530 nm increased (Figure 5A). These changes resulted in the bright purple peak II_a product solution assuming a red tint. Over the same time period CVs showed a decrease in the height of the peaks III_R/II_a' couple at 0.10 V and the appearance of a new quasi-reversible couple at -0.16 V (peaks IV_R/III_a', Figure 5B). HPLC analysis of a solution that exhibited the latter couple showed chromatographic peaks B and E. The former decreased in height with time and the latter increased. HPLC component E was red-brown (λ_{max} 463, 345, 290, 230 nm in 0.01 M HCl).

Reaction Scheme

The peak I_a voltammetric oxidation of 1 is a pH-dependent process that under the conditions studied appears to be, overall, irreversible. The strong adsorption of 1 at the PGE precludes measurements of voltammetric n values for the peak I_a reaction at the low concentrations of interest. CVs of 1 at concentrations <30 μM at pH 2 show that a major peak I_a product is tryptamine-4,5-dione (B). This compound is formed very rapidly and is responsible for the reversible couple at 0.10 V (peaks III_R/II_a'). Formation of B requires the transfer of 4e per molecule of 1 oxidized. Coulometric measurements, however, indicate that at peak I_a potentials approximately 3e per molecule are transferred. This observation is explained by formation of dimer A (4,4′-bi-5-hydroxytryptamine) and dimer F (1,4′-bi-5-hydroxytryptamine), which must be formed as a result of 1e oxidations, and C (4-chloro-5-hydroxytryptamine), which must be formed as a result of a 2e reaction. Controlled potential electrooxidations of 1 at peak II_a potentials results in the transfer of 4e per molecule and a single product, B, is initially formed. The two other major peak I_a products, dimer A and C, give voltammetric oxidation peaks at or close to peak II_a of 1. However, experiments have shown that A is not oxidized to B at peak II_a potentials. The fact that oxidation peak II_a grows relative to peak I_a with increasing concentrations of 1 suggests that a peak I_a product undergoes a second-order chemical reaction to yield the species responsible for peak II_a. This suggests that dimer A is a major contributor to the peak II_a process. However, controlled potential electrooxidation of 1 at peak II_a potentials does not give the peak I_a products A and F but only tryptamine-4,5-dione (B). Thus it must be concluded that at peak II_a potentials an unstable peak I_a oxidation product of 1 is further oxidized before it can chemically react to give A and F. That this is the case is supported by HPLC analyses of product mixtures formed by electrolyses of 1 at different potentials corresponding to peak I_a. Thus, at a potential corresponding to $E_{p/2}$ of peak I_a dimer A, in particular, gives a very prominent HPLC peak (Figure 3). However, upon shifting the electrolysis potential to E_p of peak I_a, the amount of A formed becomes smaller while the amount of B becomes larger.

Formation of dimers A and F strongly suggests that the initial electrooxidation product of 1 is a radical. There is literature evidence that 1 can be oxidized to a radical. For example, a slight stimulation of ceruloplasmin oxidase activity by 1 has been related to formation of a serotonin free radical.[30] It has also been argued that a phenoxyl serotonin radical is formed in the nonenzymatic mediation of 1 in the oxidation of pyridine nucleotides by cytochrome c.[27] Borg,[28] using EPR spectroscopy, obtained conclusive evidence for a serotonin radical upon permanganate oxidation in alkaline solution. The two-phase decay of the EPR signal suggested the possibility that two radical species were formed in this reaction. Autoxidation of 1 in 1 M NaOH generates a radical characterized by EPR spectroscopy as a semiquinone imine.[29] However, EPR spectra of 1 undergoing oxidation in acidic solution give no evidence for formation of a radical.[28] The products of peak I_a oxidation of 1 at pH 2, however, clearly implicate the formation of radical intermediates. It is proposed, therefore, that the primary electrooxidation of 1 is a $1e,1H^+$ reaction to give a radical. The nature of the ultimate peak I_a oxidation products indicates that at least three radical species are formed. We have concluded that the primary radical formed is the phenoxyl radical **2** (Figure 6). On the basis of the formation of dimer A, it appears that the phenoxyl radical **2** exists in equilibrium with aryl radical **3** in which the unpaired electron is located at C(4). Calculations on other phenoxyl radicals tend to support this

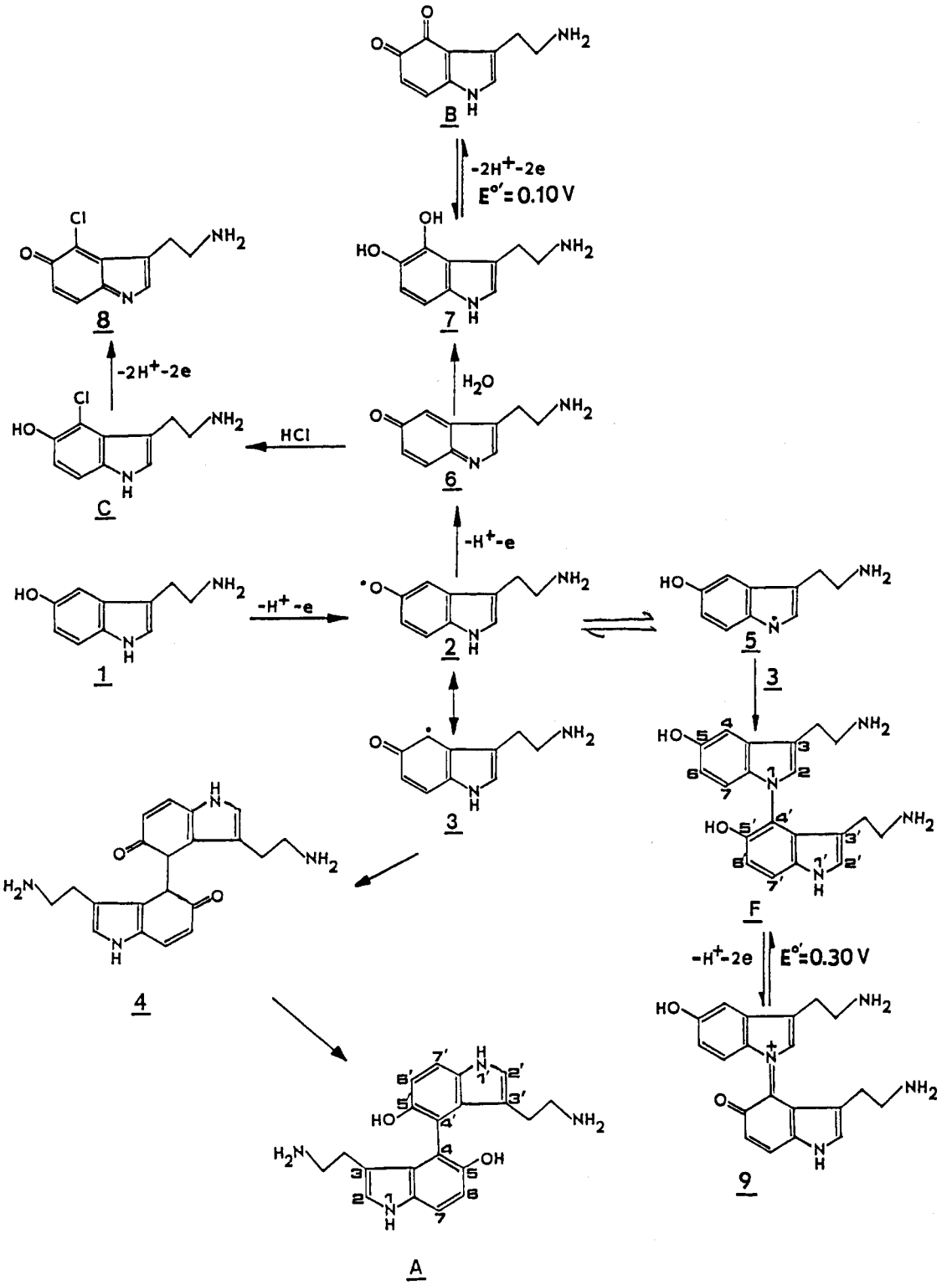

Figure 6. Proposed mechanism for the electrochemical oxidation of 5-hydroxytryptamine (1) at peak I_a potentials in 0.01 M HCl.

conclusion.[46] Coupling of two molecules of radical **3** then gives the dimer **4**, which rearranges to A (Figure 6). In order to account for dimer F phenoxyl radical **2** must also exist in equilibrium with radical **5** in which the unpaired electron is located at N(1) (Figure 6). Coupling of radicals **5** and **3** then leads to the N(1)–C(4′) dimer F.

Formation of tryptamine-4,5-dione (B) requires that the primary phenoxyl radical **2** is first oxidized (1e,1H$^+$) to quinone imine **6** (Figure 6). This step could be accomplished in at least two ways: electrochemical oxidation of radical **2** or disproportionation of **2** into quinone imine **6** and **1**.[29,47] Rapid nucleophilic attack by water on **6** then leads to 4,5-dihydroxytryptamine (**7**, Figure 6), which is immediately further oxidized (2e,2H$^+$) to tryptamine-4,5-dione (B). Nucleophilic attack of chloride on quinone imine **6** leads to C (Figure 6).

It is of interest to note that dimer F is always isolated in its reduced form yet it is reversibly oxidized at 0.30 V

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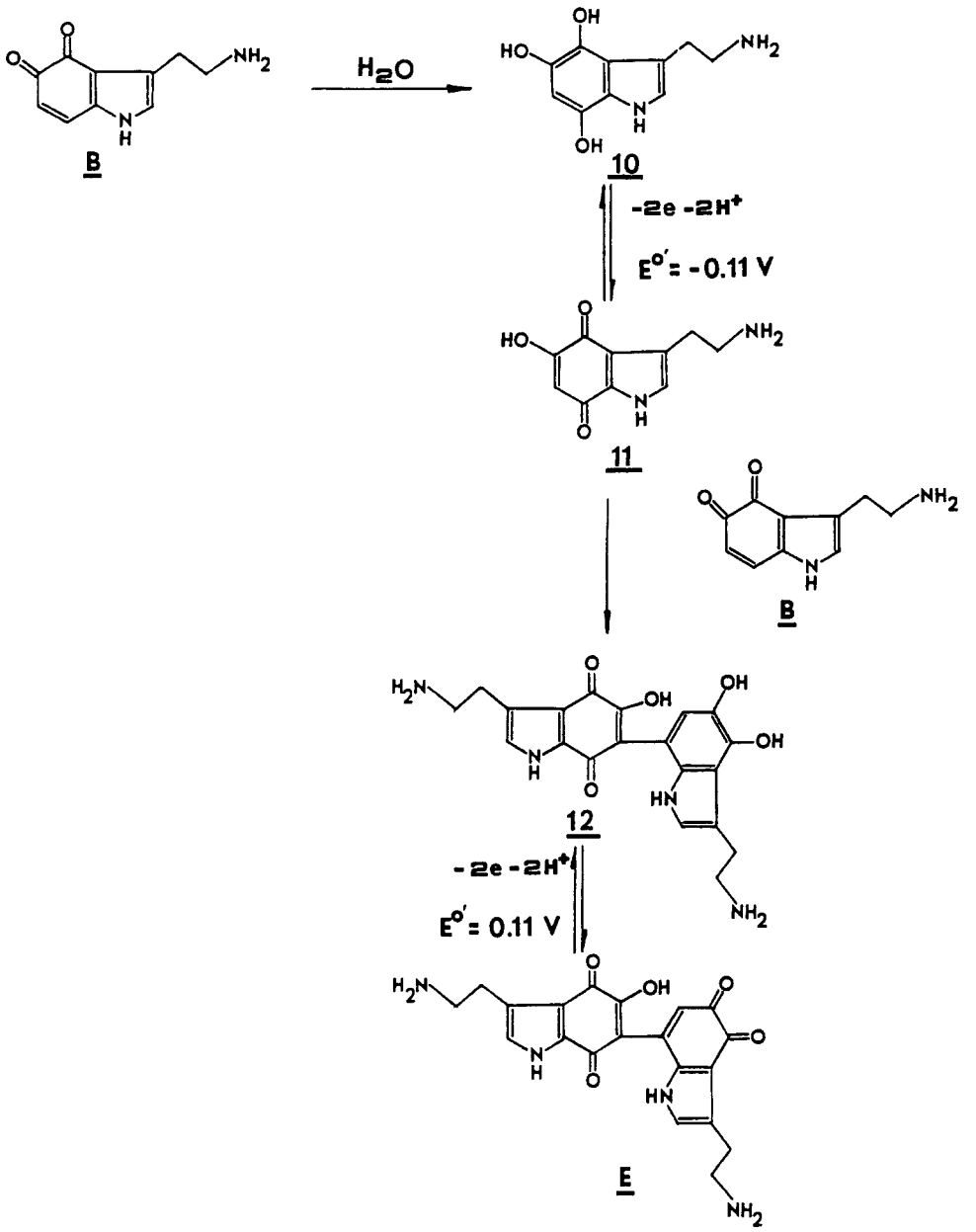

Figure 7. Proposed reaction scheme for the formation of dimer E from tryptamine-4,5-dione.

in 0.01 M HCl. Thus, during oxidations of 1 at peak I_a potentials (≥0.52 V) F should be oxidized as soon as it is formed. By analogy with the oxidation of 1, it is likely that the oxidized form of F is the *o*-quinone imine species **9** (Figure 6). The oxidized form of F shows UV bands at λ_{max} 307, 280 nm in 0.01 M HCl and these bands are clearly present in the spectrum of the peak I_a oxidation product of 1 (Figure 2A). Thus, during the isolation procedures employed, the oxidized form **9** must be chemically reduced to F. The actual reductant in this process is not known at this time.

Dimer E consists of residues of B and 5-hydroxytryptamine-4,7-dione (**11**, Figure 7). This minor peak I_a product must therefore be formed by a reaction pathway similar to that shown in Figure 7. Thus, slow reaction of water with B gives 4,5,7-trihydroxytryptamine (**10**, Figure 7), which is immediately oxidized ($2e,2H^+$) electrochemically or by air to **11**. Compounds **11** and B then react to give dimer **12**. This is then immediately oxidized to E. For the reasons outlined earlier, the exact linkage sites between the two indolic residues in E are not known.

At peak II_a potentials only B is formed upon oxidation of 1, which slowly reacts to give E. This implies that at such potentials the primary phenoxyl radical **2** is electrochemically oxidized to quinone imine **6** at a rate that is much faster either than its rearrangements to radicals **3** and **5** and/or than the further reactions of the latter radicals.

Voltammograms of 4-chloro-5-hydroxytryptamine (C) show that its E_p (0.64 V in 0.01 M HCl occurs at only slightly more positive potentials than peak I_a of 1 (E_p = 0.60 V). Furthermore, CVs of C show that after scanning through its oxidation peak the characteristic reversible couple of B at 0.10 V appears. This behavior provides an explanation of the decreasing yield of C when 1 is oxidized at increasingly positive potentials corresponding to peak I_a and the absence of C as a peak II_a oxidation product. Thus, with increasing positive potentials C must be oxidized to quinone imine **8** (Figure 6), which is attacked by water with loss of HCl to give **7**, which is immediately oxidized to B. At peak II_a potentials the latter reaction sequence results in the quantitative conversion of C to B.

Conclusions

The initial electrochemical oxidation of 1 appears to be a $1e,1H^+$ reaction, which results in formation of at least

three free radical species. The primary phenoxyl radical can be further oxidized (1e,1H$^+$) to a very reactive quinone imine, which is attacked by water to give 4,5-dihydroxytryptamine. This is then further oxidized (2e,2H$^+$) to tryptamine-4,5-dione. Since a peak corresponding to the reduction of radicals **2**, **3**, or **5** or to reduction of quinone imine **6** (Figure 6) cannot be observed in CVs of **1** at sweep rates as high as 5 V s^{-1}, the lifetimes of these intermediates must be significantly less than 10 ms. In a recent report[36] it was concluded that at low pH a minor route for the electrochemical oxidation of **1** proceeds via 5,7-DHT to **10** (Figure 7) and finally to **11**. We arrived at this conclusion because **11** was isolated as a minor product of oxidation of **1** and major product of oxidation of 5,7-DHT. However, this conclusion is clearly incorrect and the first dihydroxytryptamine to appear in the oxidation of **1** is 4,5-dihydroxytryptamine. The earlier study employed much higher concentrations of **1** than in this report and the appearance of **11** as a minor product is now thought to be due to chemical decomposition of one or possibly more oligomeric products.[48]

This study provides the first unequivocal evidence that 4,5-dihydroxytryptamine (**7**) and its oxidized form B are major oxidation products of **1**. In addition, evidence is presented for the formation of trihydroxytryptamines (**10** and **11**, Figure 7) along with several hydroxylated dimers (A, F/**9**, Figure 6, and E, Figure 7). While this study has been restricted to the oxidation of very low concentrations of **1** at low pH the information obtained might be of relevance to the role of this chemistry in mental illnesses. This is so because it has been known for some time that 5,6- and 5,7-DHT[15–21] and 4,5-DHT (**7**)[49,50] are powerful neurotoxins. An even more powerful neurotoxin is 5-hydroxytryptamine-4,7-dione (**11**).[36] Thus oxidation of **1** in very dilute solution at low potentials leads to formation of at least two neurotoxins, **7** and **11**. It seems very possible that other products identified in this study might also have neurotoxic properties. Thus, the suggestion that a faulty mechanism in the oxidative metabolism of **1** might lead to diseases such as schizophrenia and depression[7–10] seems to have significant justification based upon the oxidation chemistry of this neurotransmitter.

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Dediazoniation of Arenediazonium Ions. 24. Dual and Triple Substituent Parameter Evaluation of Competitive Heterolytic and Homolytic Dediazoniations of Diazonium Ions Complexed with 18-Crown-6 Ether[1]

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The dediazoniation of eight para-substituted benzenediazonium ions in 1,2-dichloroethane is a heterolytic reaction. The corresponding 18-crown-6 ether complexes of these diazonium ions, however, react also homolytically. The rate constants for heterolytic dediazoniation of the free diazonium ions (k_1), the rate constants for competitive heterolytic and homolytic dediazoniation of the crown ether complexed diazonium ions (k_3 and k_4, respectively), and the equilibrium constants for formation of the diazonium ion–crown ether complexes (K) were correlated with dual substituent parameter (DSP) treatments. A comparison has been made with the following substituent constants: Taft's original DSP substituent constants, Taft's triple substituent parameter constants (TSP) based on gas-phase reactions (1987), Charton's modified DSP constants, and the original (1968) and revised (1983) constants proposed by Swain. The reaction constants with the best (smallest) standard deviations and confidence limits and the highest regression coefficients are obtained with the substituent constants of Taft (both the original and the new gas-phase constants) and those of Charton. The heterolytic dediazoniation is characterized by clearly opposing signs of the field and resonance reaction constants (ρ_F and ρ_R), in contrast to the homolytic dediazoniations. The DSP treatments currently available do not provide meaningful results with homolytic dediazoniation.

The dediazoniation of meta- and para-substituted benzenediazonium ions is probably the best known example of the failure of the classical Hammett equation, i.e., of a single-parameter approach. Dickson and Eaborn[2] first pointed out that the rates of these dediazoniations could be described by a dual substituent parameter equation (DSP). This suggestion was taken up in 1973 by Taft and co-workers[3] and 2 years later by Swain et al.[4] Their treatment of experimental results demonstrates that dediazoniation is characterized by inverse signs of the resulting two reaction parameters for inductive (field) and

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