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Further Insights into the Oxidation Chemistry of 5-Hydroxytryptamine

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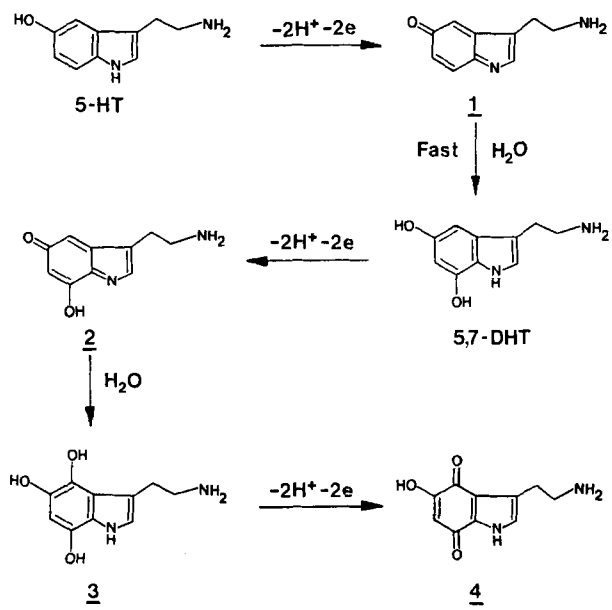
Abstract □ An important product of electrochemical oxidation of 5-hydroxytryptamine (5-HT) in acid solution is the purple compound tryptamine-4,5-dione (**6**). However, any attempt to concentrate a solution containing **6** causes it to disappear. The most important reaction of **6** is dimerization to give another purple compound 7,7′-bi-(5-hydroxytryptamine-4-one). Dione **6** can also apparently react with 2,4′-bi-5-hydroxytryptamine to give the trimer 4-[7′-(tryptamine-4,5-dione)]-2,4″-bi-5-hydroxytryptamine. Finally, **6** and other oxidation products of 5-HT react during the concentration step to yield what appears to be a trimer or perhaps a higher oligomer. This oligomer has not been identified, but it has been shown to decompose to give, in part, the neurotoxin 5-hydroxytryptamine-4,7-dione.

The neurotransmitter 5-hydroxytryptamine (5-HT) is normally metabolized in the central nervous system (CNS) first to 5-hydroxyindole-3-acetaldehyde.[1] This aldehyde is then oxidized to 5-hydroxyindole-3-acetic acid[2] or, to a much lesser extent, reduced to 5-hydroxytryptophol.[3] 5-Hydroxytryptamine can also be oxidized in vitro by a number of biological oxidants by routes other than this oxidative deamination pathways. For example, human serum, ceruloplasmin concentrates, and erythrocyte hemolysates[4–10] oxidize 5-HT to give colored, but unknown products. During metabolism, oxidations of 5-HT are claimed to generate radical intermediates, although the fate of these radicals is not known.[11,12] The latter types of oxidations are of considerable interest because it has long been speculated that a faulty or anomalous pathway in the metabolism of 5-HT might be connected to the onset of mental illness.[13,14] Some investigators have implicated such metabolic defects with schizophrenia and depression.[15–18] Furthermore, a recurring speculation is that the defective metabolic pathway for 5-HT is oxidative in nature and leads to more highly hydroxylated indoleamines which are toxic in the CNS and hence induce mental illnesses.[19–21] These speculations have some merit in view of the fact that compounds such as 5,6-dihydroxytryptamine (5,6-DHT), 5,7-DHT,[22–28] and 4,5-DHT[26,29] are powerful neurotoxins.

Recently we began to study the oxidation chemistry of 5-HT using electrochemical techniques. It was anticipated that the results of these studies would provide important insights into some of the as yet unknown biochemical oxidations of 5-HT.

Our first report[30] showed that the electrochemical oxidation of 5-HT in acid solution yields, as a minor product (≤5%), 5-hydroxytryptamine-4,7-dione (**4**). It was also found that under the same conditions **4** is a major product of the electrochemical oxidation of 5,7-DHT. Accordingly, it was concluded that a minor oxidation route for 5-HT proceeded via 5,7-DHT. The reaction pathway proposed (Scheme I) involved an initial $2e^-$, $2H^+$ oxidation of 5-HT to the quinone imine **1**, followed by a rapid attack by water to give 5,7-DHT. A series of oxidation and hydration reactions then led ultimately to **4**, as shown in Scheme I. In vivo experiments revealed that **4** is considerably more neurotoxic than 5,7-DHT.[30]



Scheme I

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In subsequent and more detailed studies,[31,32] 4 could not be detected as an oxidation product of 5-HT. These studies revealed that the electrochemical oxidation of 5-HT at potentials corresponding to its first voltammetric oxidation peak proceeds by an initial $1e^-$, $1H^+$ reaction to a radical (5, Scheme II). The subsequent reactions of 5 are highly dependent on the concentration of 5-HT oxidized and the potential used for the oxidation. At low applied potentials ($\sim E_{p/2}$) and high (millimolar) concentrations of 5-HT, radical 5 reacts to give several simple dimers. Some of these dimers are more easily oxidized than 5-HT, with the result that a very complex mixture of dimeric and oligomeric products is formed. Radical 5 can also be oxidized ($1e^-$, $1H^+$) to the quinone imine 1 which is attacked by water to give 4,5-DHT (not 5,7-DHT), which is immediately and reversibly oxidized ($2e^-$, $2H^+$) to tryptamine-4,5-dione (6), as shown in Scheme II. The latter pathway is particularly favored when low concentrations (micromolar) of 5-HT are oxidized at relatively high potentials ($\geq E_p$). The failure to detect 4 as a stable product in the latter studies was perplexing. However, one major experimental difference between the two sets of experiments was that in initial experiments where 4 was found, product solutions were always concentrated by lyophilization before the mixture was separated by column liquid chromatography. In the experiments where 4 was not found, products were separated and purified by high-performance liquid chromatography without prior concentration of the product solution. These facts suggest that formation of neurotoxin 4 occurs when the oxidation product mixture is concentrated. The concentration step also always causes the complete disappearance of 6.

The purpose of the work described here was to investigate the chemistry that occurs when the oxidation products of 5-HT are concentrated, particularly the fate of the neurotoxin tryptamine-4,5-dione (6) and the origin of the neurotoxin 5-hydroxytryptamine-4,7-dione (4).

Experimental Section

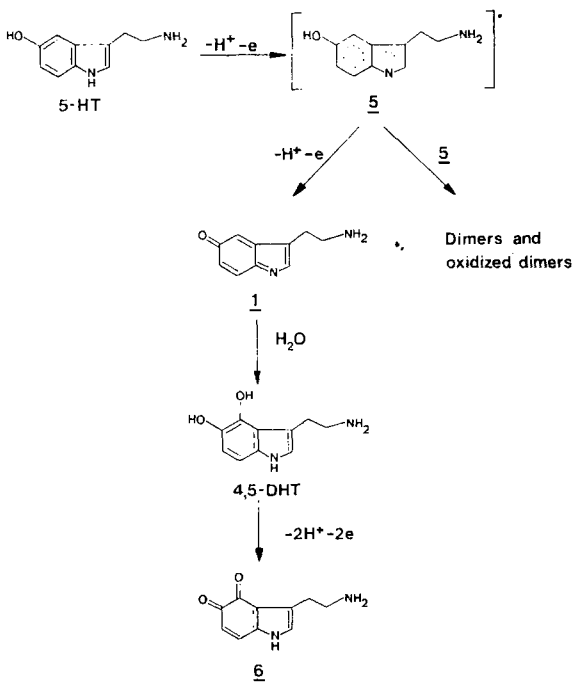
5-Hydroxytryptamine hydrochloride and *N,N*-dimethyl-5-hydroxytryptamine (monooxalate hydrate) were obtained from Sigma (St. Louis, MO). Conventional equipment was employed for electrochemical studies.[33] A pyrolytic graphite electrode (PGE, Pfizer Minerals, Pigments and Metals Division, Easton, PA) having an approximate surface area of 3.2 mm^2 was used for voltammetry. All voltammograms were corrected for IR drop. Controlled-potential electrolyses used several plates of pyrolytic graphite (total surface area ~14 cm^2) as the working electrode. Information about electrochemical cells has been presented elsewhere.[30,31] All potentials are referred to the saturated calomel electrode (SCE) at 25 ± 3 °C.

Liquid chromatography used Sephadex LH-20 (Pharmacia, Piscataway, NJ) as the stationary phase. Two columns were employed: a short column (60 × 2.0 cm) with a mobile phase flow rate of ~28 mL/h, and a long column (105 × 2.0 cm) with a mobile phase flow rate of ~20 mL/h. The mobile phase was H_2O:MeOH (9:1, v/v) adjusted to pH 2.0 with HCl. The eluant was monitored with a Gilson Holochrome detector which was usually set at 270 nm. Fractions of 6 mL were collected with an Isco model 328 (Lincoln, NB) fraction collector.

A Hewlett-Packard model 5880 gas chromatograph equipped with a flame ionization detector was used for gas chromatography. Silylating agents used were *N,O*-bis (trimethylsilyl) acetamide (BSA; Supelco), *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA), and *N*-trimethylsilylimidazole (TMSI; Pierce Chemical Company). Silylation grade acetonitrile and pyridine were obtained from Supelco. Procedures used for silylation and GC and GC-MS have been described elsewhere.[30]

Low- and high-resolution fast atom bombardment mass spectrometry (FAB-MS) was carried out on a VG Instruments model ZAB-E instrument. Liquid chromatography-mass spectrometry (LC-MS) was performed on a Kratos model MS 25/RFA instrument equipped with a thermospray source. The solvent used in LC-MS studies was 0.1 M ammonium acetate in water, and a flow rate of 0.9 mL/min was employed. Aliquots (1 or 2 mL) of products separated by liquid chromatography and dissolved in the mobile phase were injected directly into the thermospray LC-MS system using a loop injector (Rheodyne model 7125). The thermospray capillary tip was maintained at 145–155 °C and the source at 245 °C.

The UV-VIS spectra were recorded on a Hitachi 100-80 spectrophotometer. The ^1H NMR spectra (300 MHz) were obtained with a Varian model 300 XL spectrometer.



Scheme II

Results and Discussion

A representative cyclic voltammogram (CV) of 5-HT (0.5 mM) at pH 2 is presented in Figure 1. The peak I_a oxidation reaction has been discussed to a limited extent in connection with Scheme II and in detail elsewhere.[31,32] After scanning through oxidation peak I_a, two reversible couples, peaks II_c/II_a' and III_c/I_a'', are observed on the reverse sweeps (Figure 1). Peak II_c is due to the $2e^-$, $2H^+$ reduction of 5-hydroxy-3-(2-aminoethyl)-3-[4′-(tryptamine-5′-one)]-indolenine (9_{ox}) to 5-hydroxy-3-(2-aminoethyl)-3-[4′-(5′-hydroxytryptamine)]-indolenine (9_R), and peak II_a' is due to the reverse reaction. Peaks III_c/I_a' are due to the tryptamine-4,5-dione (6)/4,5-dihydroxytryptamine (4,5-DHT) couple, as shown in Figure 1. Voltammograms of 5-HT.in 0.01 M HCl are essentially identical to those shown in Figure 1 and, in fact, ultimate products of controlled-potential electrooxidations of 5-HT at peak I_a potentials are, with one exception (see later discussion), the same in phosphate buffers at pH 2 and in dilute HCl. Because inorganic phosphate complicates the separation of the complex mixture of products formed following peak I_a oxidation of 5-HT, dilute HCl at pH 2 was used as the supporting electrolyte. The latter electrolyte also has the great advantage that it is easily removed by lyophilization.

In a typical controlled-potential electrolysis experiment, 6–9 mg of 5-HT·HCl dissolved in 40 mL of 0.01M HCl (~1 mM) was oxidized. After complete oxidation of all 5-HT (as determined by CVs and HPLC[31,32] analysis) the purple solution was immediately lyophilized and the resulting dry product mixture was stored at −5 °C. The dry product mixture was subsequently dissolved in ~1.5 mL of the chromatographic mobile phase and separated on a short column of

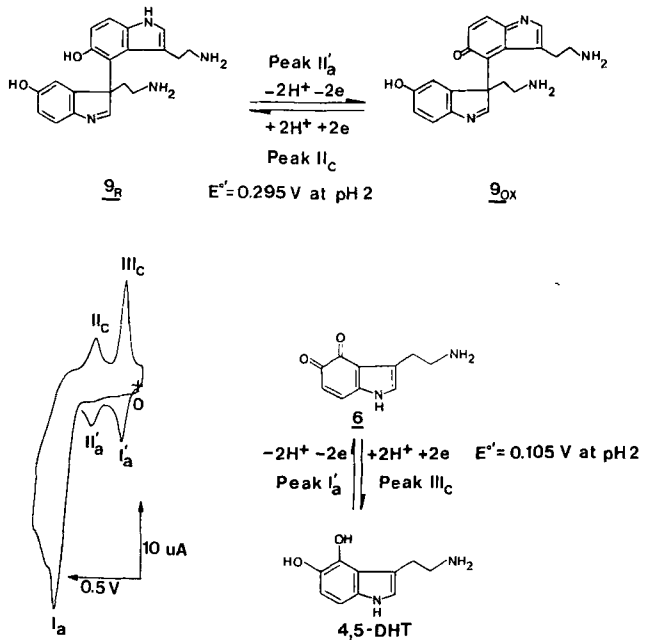


Figure 1—*Cyclic voltammogram at the PGE of 0.51 mM 5-hydroxytryptamine in pH 2.4 phosphate buffer (μ = 0.5). Sweep rate: 200 mV/s; E°' values are for the latter buffer system.*

Sephadex LH-20 (see *Experimental Section*). A typical liquid chromatogram is shown in Figure 2. Products responsible for some chromatographic peaks which were identified in previous studies[31,32] (when the product solutions were not lyophilized) are shown in this Figure and will not be discussed further. There are, clearly, four major chromatographic peaks (**A**, **B**, **I**, and **J**) and one minor peak (**C**) which correspond to products which were not observed in previous studies and therefore must be formed as a result of the concentration (i.e., lyophilization) step. 4-Chloro-5-hydroxytryptamine is responsible for chromatographic peak **K** and is the product mentioned earlier which is observed only when peak I_a electrolyses are performed in dilute HCl. With increasingly positive applied potentials, chromatographic peaks **A**, **B**, **E**, **I**, and **J** become larger. Furthermore, with increasing initial concentrations of 5-HT oxidized, chromatographic peaks **A** and **J** grow relative to other peaks.

Product Characterization and Identification—The main goal of this work was to identify the products responsible for chromatographic peaks **A**, **B**, **I**, and **J**. The relatively minor component eluted under chromatographic peak **C** was unstable and could not be isolated. Hence, this product will not be discussed further.

Compound A—After lyophilization of the solution eluted under chromatographic peak **A**, a brown solid material was obtained. The shape of the chromatographic peak suggests that more than one compound was eluted. The UV-VIS spectra of fractions corresponding to the beginning, center, and end of peak **A** all showed bands at 435 (broad and low), 300, and 275 nm in 0.01 M HCl. The only differences between these spectra were the relative heights of the two bands at 300 and 275 nm. The CVs of **A** in 0.01 M HCl showed two reversible reduction peaks at E_p = 0.30 and 0.15 V, along with an apparently irreversible oxidation peak at E_p = 0.62 V. The MS and FAB-MS (glycerol and thioglycerol matrices) analyses of **A** failed to give any useful spectra. Attempts were made to derivatize **A** using various silylating agents (BSA, MSTFA, TMSI). Gas chromatography (GC) showed several very small peaks. The GC-MS analysis indicated that these peaks corresponded to trimethylsilyl derivatives of 5-HT (molar masses of 464, 392, and 320 g) and another derivative having a molar mass of 664 g. The latter corresponds to a compound having a molar mass of 520 g substituted with two trimethylsilyl groups. The rapid elution of **A** in liquid chromatography suggests that it might be an oligomeric or

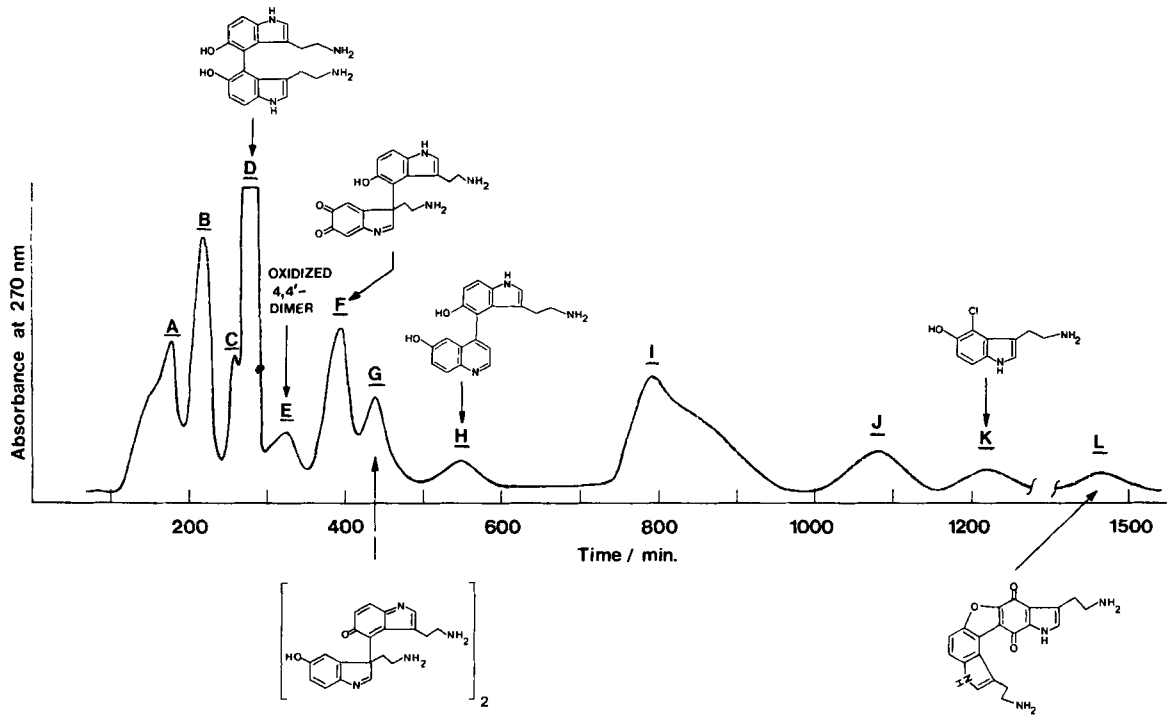


Figure 2—*Liquid chromatogram obtained for the products formed by controlled-potential electrooxidation of 0.8 mM 5-hydroxytryptamine in 0.01 M HCl at 0.530 V. The product solution was lyophilized and redissolved in ~1.5 mL of the mobile phase prior to chromatography. A column of Sephadex LH-20 (60 × 2 cm) was used with a mobile phase of H_2O:MeOH (9:1, v/v) adjusted to pH 2.0 with HCl flow rate, 28 mL/h.*

polymeric compound. The very small GC peaks observed following silylation suggest that they represent only fragments of a far larger molecule. Thus, it was concluded that A is at least a trimer and more probably a larger oligomeric compound.

4-[7'-(Tryptamine-4,5-dione)]-2,4''-bi-5-hydroxytryptamine (B)—Compound B was not chromatographically well separated from A, C, and D (Figure 2). Accordingly, the crude sample of B after lyophilization was purified by chromatography on a long column of Sephadex LH-20 (see *Experimental Section*). Using such a column, crude B gave two well-separated peaks having t_R values of 352 and 490 min. The spectrum of the component having t_R = 352 min was the same as that reported above for A. The component with t_R = 490 min was B. However, when the latter fraction was lyophilized and passed through the long chromatographic column, it again gave two peaks at t_R = 352 and 490 min. If the latter procedure was repeated several times, B was ultimately completely converted into A. Thus, B appears to be spontaneously converted into the oligomeric or polymeric compound(s) A. The electrochemical and spectral properties of B were, accordingly, recorded immediately after it emerged from the chromatographic column. Its spectrum in the chromatographic mobile phase (pH 2.0) showed bands at λ_{max} = 525(sh), 450, 335(sh), 300(sh), 275, and 219 nm (Figure 3A). The CVs showed a reversible reduction peak at E_p = 0.15 V and an irreversible oxidation peak at E_p = 0.60 V (Figure 3B). Controlled-potential reduction of B at −0.05 V caused the reddish-brown color to disappear, and the spectrum showed two close bands at λ_{max} = 308 and 275 nm (Figure 3B). Oxidation of the latter product solution at 0.20 V caused the original spectrum of B to reappear. After lyophilization of the solution containing B, all attempts to obtain useful mass spectral information were unsuccessful.

The reversible couple at 0.15 V observed in CVs of B and its spectral bands at 525 and 350 nm are also shown by tryptamine-4,5-dione (6) under similar solution conditions,[31] which suggested that B contains the latter residue within its structure. Accordingly, the solution containing freshly chromatographed B was reacted with *o*-phenylenediamine, which is known to condense with 1,2-diketones,[34] using a previously described procedure.[31] The resulting product (λ_{max} in 0.01 M HCl = 557, 460(sh), 300(sh), 280, 245(sh), and 218 nm) was separated and purified by chromatography on Sephadex LH-20 using the usual conditions. The FAB-MS (dithiothreitol:dithioerythritol matrix) analysis of the resulting product gave an intense pseudomolecular ion (MH^+) at *m/e* = 611.2883 ($C_{36}H_{35}N_8O_2$, calculated *m/e* = 611.3269). Thus, this product has a molar mass of 610 g and an elemental formula $C_{36}H_{34}N_8O_2$. The ^{1}H NMR (Me_2SO-d_6) of this compound is as follows: δ 11.65 (d, J = 2.4 Hz, 1H, N(1')-H), 11.42 (s, 1H, N(1)-H), 10.79 (d, J = 2.4 Hz, 1H, N(1'')-H), 8.43 (d, J = 9 Hz, 1H, protons in aromatic ring A), 8.24 (bs, 3H, N(α)-H_3^+), 8.21 (s, 1H, protons in aromatic ring A), 7.91 (m, 2H, protons in aromatic ring A), 7.70 (s, 1H, C(6')-H), 7.65 (bs, 3H, N (β)-H_3^+), 7.45 (d, J = 2.1 Hz, 1H, C(2')-H), 7.40 (s, 1H, O-H), 7.31 (d, J = 8.7 Hz, 1H, C(7)-H), 7.24 (d, J = 8.1 Hz, 1H, C(6)-H), 7.23 (d, J = 1.8 Hz, 1H, C(2'')−H), 7.11 (bs, 3H, N(γ)-H_3^+), 7.06 (s, 1H, O-H), 6.94 (d, J = 8.7 Hz, 1H, C(7'')-H), and 6.85 ppm (d, J = 8.7 Hz, 1H, C(6'')-H). The region between 2.5 and 3.8 ppm was complex and was partially overlapped with the signals from Me_2SO and HOD. In D_2O, the signals at δ = 11.65, 11.42, 10.79, 8.24, 7.65, 7.40, 7.11, and 7.06 ppm disappeared and the doublets at 7.45 and 7.23 ppm collapsed into singlets. The above mass and ^{1}H NMR spectral results are in accord with structure 8 (Scheme III). Such a structure can only be formed by reaction of *o*-phenylenediamine with a trimeric indoleamine having the structure B shown in Scheme III. The reduced form of B provides additional evidence for the assigned structure in that its UV spectrum is typical for many simple dimers and trimers of 5-HT.[31,32]

Liquid Chromatographic Peak I—The shape of chromatographic peak I indicated that more than one compound is eluted (Figure 2). After lyophilization, the compounds eluted under this peak were passed through a long column of Sephadex LH-20 (see *Experimental Section*). Three chromatographic peaks emerged from this column at t_R values of 1000, 1430, and 1630 min. The compound that eluted at t_R = 1000 min was 5-hydroxytryptamine-4,7-dione (4), while that at t_R = 1430 min was 9_R (see Figure 1). Spectral (UV-VIS, ^{1}H-NMR, and MS) and electrochemical data for the latter compounds have been presented elsewhere.[30,31] It should be noted that $9_R$was originally[31] thought to be 1,4'-bi-5-hydroxytryptamine, but subsequent studies[32] showed it to be 5-

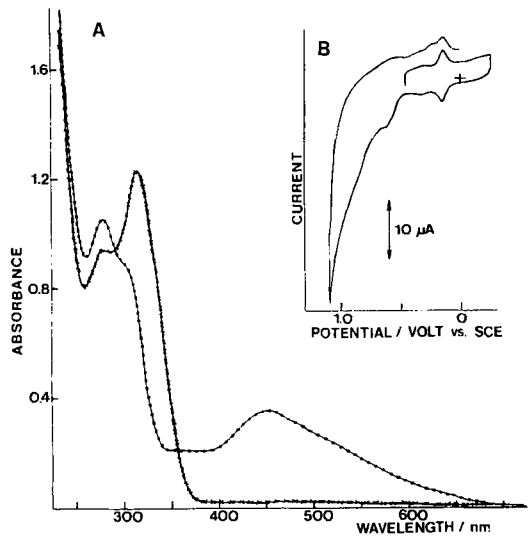
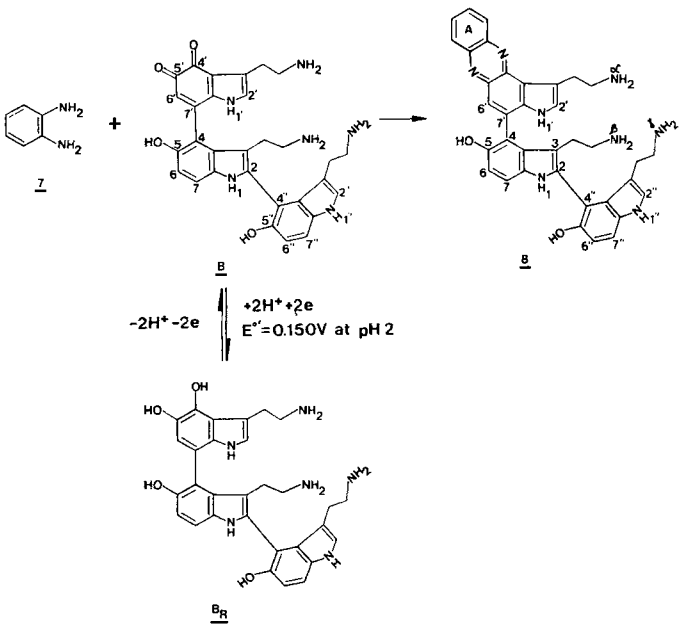


Figure 3—A: *Spectrum of* **B** *(4-[7'-[tryptamine-4,5-dione)]-2,4''-bi-5-hydroxytryptamine) in H_2O:MeOH (9:1, v/v) adjusted to pH 2.0 with HCl (····); and after electrochemical reduction at −0.05 V* (xxx). **B:** *Cyclic voltammogram at the PGE in the same solution; sweep rate, 200 mV/s.*



Scheme III

hydroxy-3-(2-aminoethyl)-3-[4'-(5'-hydroxytryptamine)]-indolenine.

The compound(s) responsible for the chromatographic peak at t_R = 1630 min will be referred to as **I-3**. This compound was not stable in the chromatographic mobile phase and slowly decomposed over the course of many hours, as shown by changes in its UV-VIS spectrum. After lyophilizing, however, a brown solid was obtained, Liquid chromatography of this solid showed a peak due to 5-hydroxytryptamine-4,7-dione (**4**; t_R = 1000 min), several small peaks at short retention times, and a smaller peak due to **I-3**. If the latter peak was again lyophilized, the same effect was noted. Thus, **I-3** is the unstable compound which decomposes, partially, into neurotoxin **4**. We were unable to identify any of the several other minor decomposition products of **I-3**. A freshly chromatographed solution of **I-3** dissolved in the chromatographic mobile phase showed a very characteristic UV-VIS spectrum with λ_{max} = 450, 304, 275, and 220(sh) nm (Figure 4A). The CVs of **I-3** in the same solution showed a reversible reduction peak at 0.01 V and an apparently irreversible oxidation peak at 0.78 V (Figure 4B). Comparison of the spectral and CV properties of **I-3** with all of the other identified products of oxidation of 5-HT[31,32] suggest that it must be at least a dimeric compound containing additional oxidized hydroxyl groups. It was not possible to obtain meaningful ^{1}H NMR spectra of **I-3** owing to its decomposition to numerous products during the necessary prior lyophilization step. Furthermore, after silylating **I-3** (MSTFA, TMSI), GC showed only small peaks and GC-MS indicated that these corresponded to species having molar masses of 366, 364, 192, and 190 g. The FAB-MS (dithiothreitol:dithioerythritol: nitrobenzyl alcohol:HCl matrix) analyses gave a relatively intense ion at *m/e* = 551, which indicates that **I-3** must be at least a trimer of 5-HT containing two extra oxygen atoms.

7,7'-Bi-(5-hydroxytryptamine-4-one) (J)—In acid solution, **J** exhibited a bright purple color (λ_{max} in 0.01 M HCl = 558, 360, 295, and 217 nm; Figure 5A). At pH > 6.5, the solution of **J** became blue (λ_{max} at pH 6.5 = 565, 310(sh), and 225 nm). The CVs of **J** in 0.01 M HCl (Figure 5B) show two reversible couples at 0.27 and 0.025 V and an apparently irreversible oxidation peak at 1.14 V. Controlled-potential electrochemical reduction of **J** in 0.01 M HCl at −0.05 V caused the solution to become colorless (λ_{max} = 318, 260(sh), and 210 nm; Figure 5A). The colorless product was rapidly air-oxidized or electrochemically oxidized (0.05–0.10 V) back to

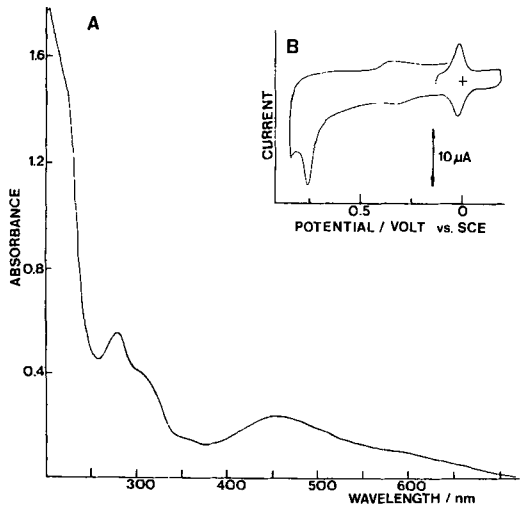


Figure 4—A: *Spectrum of* **I-3** *in H_2O:MeOH (9:1, v/v) adjusted to pH 2.0 with HCl.* **B:** *Cyclic voltammogram at the PGE of* **I-3** *in the latter solvent; sweep rate, 200 mV/s.*

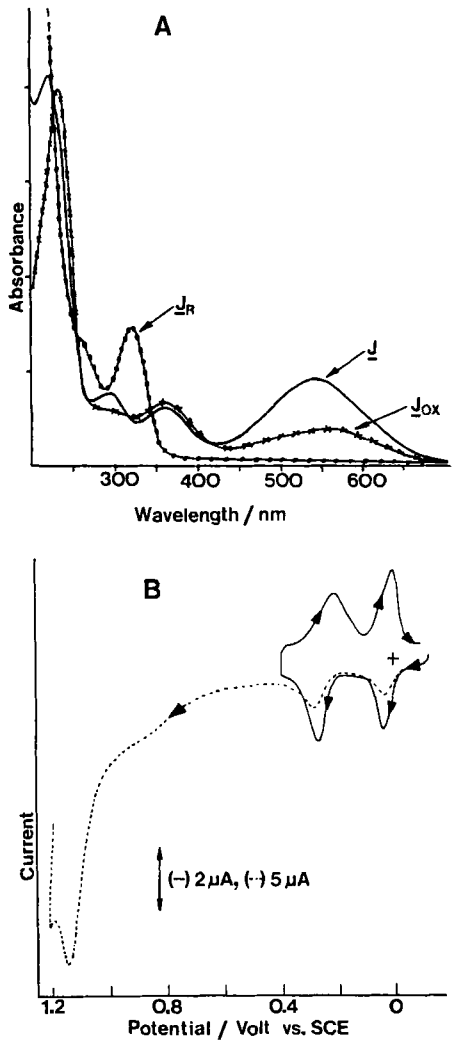
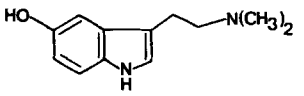


Figure 5—A: *Spectra of* **J** *in 0.01 M HCl (——); after oxidation of* **J** *at 0.30 V (xxx); and after reduction of* **J** *at −0.05 V (···).* **B:** *Cyclic voltammogram at the PGE of* **J** *in 0.01 M HCl; sweep rate, 200 mV/s.*

purple **J**. Controlled-potential electrooxidation of **J** in 0.01 M HCl at 0.30 V caused the solution to become somewhat less intensely purple in color (λ_{max} = 552, 361, 289, and 234 nm; Figure 5A). Thus, the main effect of oxidizing **J** was to cause the original visible band of **J** (λ_{max} = 538 nm) to decrease in height by ~60% and to shift to a slightly longer wavelength. This new purple compound could be easily transformed back into **J** by reduction at 0.20 V (i.e., at a potential between the two reversible reduction peaks observed in CVs of **J**; Figure 5B). It was also found that **J** is formed when solutions of tryptamine-4,5-dione (**6**) are concentrated, for example, by lyophilization [a virtually pure solution of **6** can be obtained by controlled-potential electrooxidation of 5-HT (≤30 μM) at 0.68 V in 0.01 M HCl[31]]. This observation explains why the yield of **J** from electrooxidation of 5-HT increases with increasingly positive applied potentials, since at more positive potentials, more **6** is formed.[31] The FAB-MS (dithiothreitol:dithioerythritol matrix) analysis of **J** gave a low intensity pseudomolecular ion (MH^+) at *m/e* = 383.1702 ($C_{20}H_{23}N_4O_4$, calculated *m/e* = 383.1719). In some parallel experiments it has been found that electrooxidation of *N,N*-dimethyl-5-hydroxytryptamine (**10**) under exactly the same conditions used for 5-HT, followed by lyophilization and chromatography, gives a purple product which has virtually the same spectral and electrochemical properties as **J**. The LC-MS



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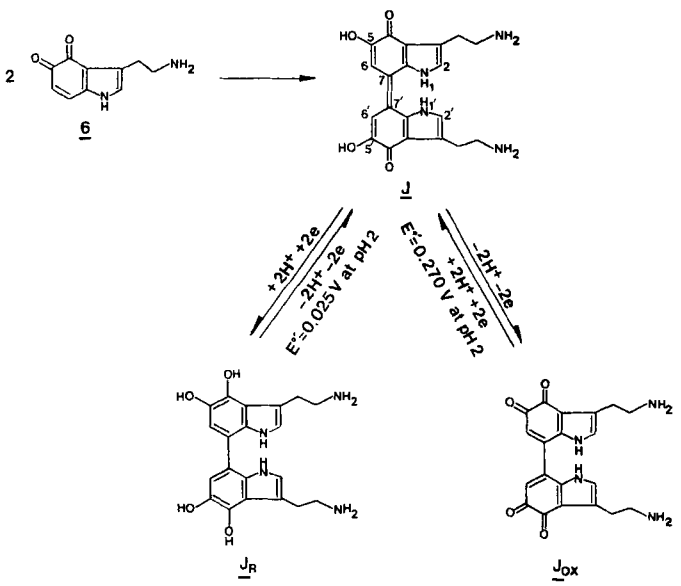
analysis of this purple compound gave an intense pseudomolecular ion (MH^+) at m/e = 439. These results indicate that **J** is a simple dimer of 4,5-DHT (molar mass 382 g). However, such a simple dimer could not have the spectral and electrochemical properties reported for **J**. It has recently been noted[35,36] that FAB-MS analysis of easily reducible compounds in matrices such as dithiothreitol:dithioerythritol causes reduction to occur. Similarly, LC-MS analysis of, for example, tryptamine-4,5-dione (**6**); molar mass 190 g) shows a peak (MH^+) at m/e = 193, indicating that this compound is reduced to 4,5-DHT under the conditions used.[31] Thus, it may be concluded on the basis of MS and UV-VIS spectra and electrochemical behaviors that **J** has a molar mass of 380 g.

Compound **J** reacted very rapidly with Me_2SO-d_6 to give a black solution and so it was not possible to obtain an NMR spectrum in this solvent. The ^1H NMR spectrum in D_2O is as follows: δ 6.73 (s, 2H, C(2)-H and C(2′)-H), 6.22 (s, 2H, C(6)-H and C(6′)-H), 3.24 (m, 4H, 2 × CH_2), and 2.87 ppm (m, 4H, 2 × CH_2). These results support the conclusion that **J** is a highly symmetrical dimer, 7,7-bi-(5-hydroxytryptamine-4-one). The structure and numbering of this compound are shown in Scheme IV.

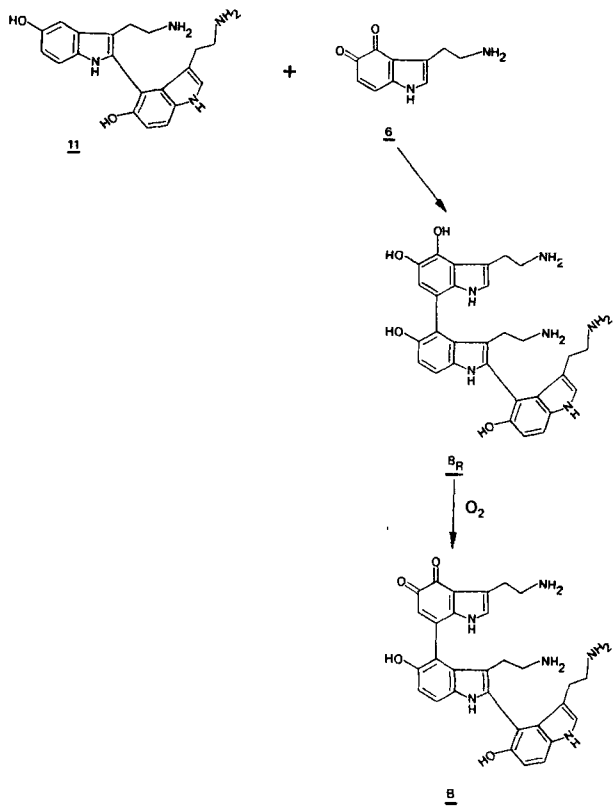
Reaction Schemes—The results described above indicate that concentration of the product mixture formed upon electrochemical oxidation of 5-HT at pH 2 leads to the disappearance of tryptamine-4,5-dione (**6**) and the appearance of three new major products.

It is only possible to speculate about the reactions which lead to trimer **B** based on the identity of compounds known to exist in the product solution prior to its concentration. Dione **6** is certainly a very reactive species. The chromatogram shown in Figure 2 does not show the presence of 2,4′-bi-5-hydroxytryptamine (**11**), although this compound is present before the product mixture is concentrated.[32] Thus, it appears that dione **6** reacts with dimer **11** to yield trimer $\mathbf{B_R}$, as shown in Scheme V.

The latter trimer is easily air-oxidized to **B** ($E^{\circ\prime}$ = 0.15 V at pH 2), as shown in Scheme V.



Scheme IV



Scheme V

5-Hydroxytryptamine-4,7-dione (**4**) is quite clearly formed as the result of the decomposition of the oligomeric compound **I-3** and is not present as an oxidation product of 5-HT before the product solution is lyophilized. Unfortunately, the precursor of **4**, formed as a result of concentration of the product solution, is not sufficiently stable to permit its identification. However, spectral and electrochemical information indicate that it is either a trimeric or perhaps a larger oligomeric compound.

A major reaction of tryptamine-4,5-dione (**6**) when it is concentrated is to form the purple dimer **J**. This compound is reversibly oxidized at 0.27V and reversibly reduced at 0.025 V. The UV-VIS absorption spectrum of **J**, particularly the intense band at 538 nm, indicates that this compound has a highly conjugated structure. In order to account for these observations, it is proposed that upon concentration tryptamine-4,5-dione (**6**) dimerizes to give the *para*-quinonoid structure **J** (Scheme IV). This highly symmetrical and conjugated structure explains the intense visible absorption band at 538 nm and the simple four-line ^1H NMR spectrum in D_2O. Many compounds having similar extended *p*-quinonoid structures to **J** are purple colored and exhibit similar UV-VIS spectra.[37,38] As expected, by analogy with, for example 4,[30] the *p*-quinonoid **J** is reversibly reduced to 7,7′-bi-(4,5-dihydroxytryptamine), $\mathbf{J_R}$ (Scheme IV). The UV spectrum of $\mathbf{J_R}$ is, as would be expected, quite similar to that of 4,5-DHT (λ_{max} in 0.01 M HCl = 293, 257(sh), and 208 nm[31]). Oxidation of **J** yields 7,7′-bi-(tryptamine-4,5-dione; $\mathbf{J_{ox}}$), which is spectrally very similar to tryptamine-4,5-dione (λ_{max} in 0.1 M HCl = 535, 350, and 232 nm[30]).

Conclusions

The electrochemical oxidation of 5-HT at pH 2 in aqueous solution always leads to the formation of tryptamine-4,5-dione (**6**). The yield of **6** depends on the initial concentration of 5-HT oxidized and the applied potential.[31,32] After concen-

trating solutions containing **6** by, for example, lyophilization, the latter compound completely disappears. The most important reaction of purple **6** is a dimerization process giving purple **J**. However, alternative reactions can occur. For example, it appears that **6** can attack 2,4′-bi-5-hydroxytryptamine (**11**) to give, ultimately, trimer **B** (Scheme V), a quite stable compound. In addition **6** and/or other oxidation products react during the concentration process to give an oligomeric compound that is responsible in part for liquid chromatographic peak **I**. This compound is unstable and hence we have been unable to ascertain its chemical structure. Nevertheless, it has been unequivocably established that this oligomer decomposes, in part, to the neurotoxin 5-hydroxytryptamine-4,7-dione (**4**). Thus, our initial report[30] that the electrochemical oxidation of 5-HT proceeds through a minor route leading first to 5,7-DHT and then to **4**, as shown in Scheme I, is incorrect. *p*-Quinone **4** can only be observed as an oxidation product of 5-HT after the initially formed product solutions are concentrated. In the concentration step, an unstable oligomer is formed which subsequently decomposes to give, in part, **4**.

References and Notes

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