2 H), 3.31-3.80 (H4', m, 1 H), 2.04-2.38 (H2', H2'', m, 2 H), 1.16-1.60 ($(CH_2)_2CH_3$, m, 4 H), 0.78 ($(CH_2)_2CH_3$, t, 3 H).

5-n-Butyl-2'-deoxyuridine 3',5'-Cyclic Monophosphate Ammonium Salt (18). Compound 18 appeared in fractions 63-69 (yield 69%).

5-n-Pentyl-2'-deoxyuridine 3',5'-Cyclic Monophosphate Ammonium Salt (19). Compound 19 appeared in fractions 65-76 (yield 74%): ^{1}H NMR (Me_2SO-d_6) δ 7.31 (H6, s, 1 H), 6.20 (H1', dd, 1 H), 4.40-4.78 (H3', m, 1 H), 3.95-4.30 (H5', H5'', m, 2 H), 3.42-3.76 (H4', m, 1 H), 2.18-2.41 (H2', H2'', m, 2 H), 1.32 ($(CH_2)_4CH_3$, br s, 10 H), 0.87 ($(CH_2)_4CH_3$, t, 3 H).

5-n-Hexyl-2'-deoxyuridine 3',5'-Cyclic Monophosphate Ammonium Salt (20). Compound 20 appeared in fractions 68-80 (yield 45%): IR (KBr) 1235 (P=O), 1081 (POC) cm^{-1}.

5-n-Octyl-2'-deoxyuridine 3',5'-Cyclic Monophosphate Ammonium Salt (21). Compound 21 appeared in fractions 81-116 (yield 91%): ^{1}H NMR (Me_2SO-d_6/$CDCl_3$) δ 7.30 (H6, s, 1 H), 6.20 (H1', dd, 1 H), 4.38-4.75 (H3', m, 1 H), 3.92-4.35 (H5', H5'', m, 2 H), 3.45-3.90 (H4', m, 1 H), 2.10-2.40 (H2', H2'', m, 2 H), 1.25 ($(CH_2)_7CH_3$, br s, 14 H), 0.80 ($(CH_2)_7CH_3$, br s, 3 H).

Acid Hydrolysis of Compounds 15, 16, and 20. Compounds 15, 16, and 20 and for comparison cTMP, 1-(2-deoxy-α-D-ribofuranosyl)-5-isopropyluracil 3',5'-cyclic phosphate ammonium salt, and 5-isopropyl-2'-deoxyuridine 5'-phosphate diammonium salt, 9 (0.1 mM), were quickly dissolved individually in cold (0 °C) 1 M hydrochloric acid (5 mL). These solutions were then incubated at 37 °C in a thermostat. Aliquots (0.1 mL) were removed from the solutions at certain time intervals. The reactions were quenched by addition of a 1.5 M ammonium hydrogen carbonate solution (0.1 mL). The contents of these aliquots were then examined chromatographically on silica gel TLC sheets in solvent system 1. The hydrolysis products were detected by UV light at 254 nm with use of authentic samples for identification.

Biology. Antitumor assays were performed according to previously established procedures.[35] L1210/0, L1210/BdUrd, Raji/0, and Raji/TK$^-$ cell lines were characterized as described.[29] Thymidylate synthetase assays were carried out with a partially purified L1210 cell extract as indicated in ref 14.

Antiviral assays were performed as reported previously.[2b] The origin and preparation of the virus stocks have also been documented in ref 2b.

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Oxidation of 5-Hydroxytryptamine and 5,7-Dihydroxytryptamine. A New Oxidation Pathway and Formation of a Novel Neurotoxin

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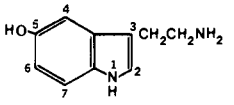
The electrochemical oxidation of 5-hydroxytryptamine (5-HT) in acidic solution proceeds through a minor route leading first to 5,7-dihydroxytryptamine (5,7-DHT) then to 4,5,7-trihydroxytryptamine and finally to 5-hydroxytryptamine-4,7-dione. The latter compound is a major electrochemical oxidation product of 5,7-DHT at pH 2 and 7 and a major autoxidation product at pH ≥6. Preliminary biological results indicate that 5-hydroxytryptamine-4,7-dione is a more potent central nervous system toxin than 5,7-DHT. These results show for the first time a chemical pathway from 5-HT to 5,7-DHT and suggest possible minor metabolic oxidative pathways for the neurotransmitter 5-HT to at least two powerful neurotoxins.

Over the past 30 years a number of reports have appeared concerned with the oxidation of the chemical neurotransmitter 5-hydroxytryptamine (5-HT) in biological systems.[1-9] However, these have been highly speculative



5-HT

reports and, in fact, neither the mechanisms nor even the products of these oxidation reactions are known. It has been suggested[1,2,4,9] that 5-HT might undergo chemical oxidation to a dihydroxytryptamine species. The possiblity of forming dihydroxy derivatives or similar species by biochemical oxidation of 5-HT is intriguing because of the known neurotoxic properties of compounds such as 5,6-dihydroxytryptamine (5,6-DHT) and 5,7-dihydroxytryptamine (5,7-DHT).[10-16] However, there is no evidence in the literature for any chemical pathway from 5-HT to such

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compounds. The discovery of an oxidative pathway from the neurotransmitter 5-HT to the neurotoxins 5,6-DHT and/or 5,7-DHT could provide a chemical basis for understanding certain neurological and psychotic disorders. It was the purpose of the work reported here to establish whether 5-HT could be oxidized to 5,6- and/or 5,7-DHT or related indolic compounds. We have employed electrochemical and other analytical techniques to study the oxidation chemistry of 5-HT. It must be emphasized that this study is focused on a very minor but potentially highly significant oxidation pathway of 5-HT. We hope to be able to provide a more detailed picture of the oxidation chemistry of 5-HT at some future time.

Experimental Section

Linear sweep and cyclic voltammetry, controlled potential electrolyses, and coulometry used equipment that has been described elsewhere.[17] A pyrolytic graphite electrode (PGE, Pfizer Minerals, Pigments and Metals Division, Easton, PA) having an approximate surface area of 3 mm^2 was used for voltammetric studies. The PGE was resurfaced before running voltammograms on a sheet of 600 grit silicon carbide paper (Buehler Inc., Evanston, IL) mounted on a metallographic polishing wheel.[17] Controlled potential electrolyses were carried out with use of plates of pyrolytic graphite as the working electrode having a total surface area of about 14 cm^2. These electrodes were dipped into ca. 40 mL of buffer solution containing the indolic compound to be oxidized. 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). These latter electrodes were immersed in the same supporting electrolyte solution used in the working electrode compartment. The working, counter and reference electrode compartments were separated with a Nafion membrane (Type 117, DuPont Co., Wilmington, DE). All voltammetric measurements were made in solutions that were thoroughly deaerated with nitrogen. Controlled potential electrolyses were carried out 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 were obtained with use of a glass column 1.8m × 2mm i.d.) packed with 3% SE-30 on Chromosorb W. The carrier gas was helium at a flow rate of 30 mL min^{-1}. The GC retention times (t_R) reported were measured under the following conditions: initial temperature of 100 °C for 10 min followed by a linear temperature gradient (10 °C/min) to 280 °C. The latter temperature was then held constant for 15 min.

High-resolution fast atom bombardment (FAB) mass spectrometry (MS) was carried out on a VG Instruments Model ZAB-SE instrument. All other MS and GC–MS studies used a Hewlett-Packard Model 5985B instrument. Electron impact (EI) MS used on electron beam energy of either 12 or 70 eV. Chemical ionization (CI) MS used methane as the reactant gas at a pressure of ca. 2×10^{-4} torr in the source chamber and an electron beam energy of 150 eV.

UV–visible spectra were recorded on a Hitachi 100-80 spectrophotometer. IR spectra were recorded on a Beckman Acculab 3 spectrophotometer. ^1H NMR spectra (300 MHz) were obtained with a Varian Model 300 XL spectrometer.

Liquid chromatography used a glass column packed with Sephadex LH-20 (Pharmacia, Piscataway, NJ). The eluent used was H_2O–MeOH (9:1, v/v) adjusted to pH 2.0 with HCl. Two columns were used. A short column (60 × 2 cm) was used with an eluent flow rate of ca. 28 mL h^{-1}. A long column (100 × 2 cm) used an eluent flow rate of ca. 20 mL h^{-1}. The eluent was monitored with a Gilson Holochrome detector, which was usually set at 270 nm. Fractions of 6 mL were collected with an Isco Retriever III (Lincoln, NB) fraction collector.

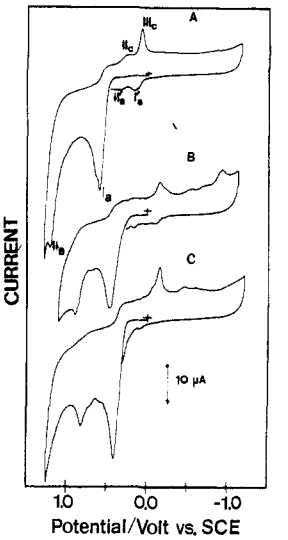

Figure 1. Cyclic voltammograms at the PGE of 1 mM solutions of (A) 5-HT, (B) 5,7-DHT, and (C) 5,6-DHT in 0.01 M HCl. Sweep rate: 200 mV s^{-1}.

5-Hydroxytryptamine hydrochloride, 5,6-dihydroxytryptamine, and 5,7-dihydroxytryptamine were obtained from Sigma. The latter two compounds were supplied as their creatinine sulfate salts. *N,O*-Bis(trimethylsilyl)acetamide (BSA) and silylation grade acetonitrile were obtained from Supelco. *N*-Methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA) and *N*-(trimethylsilyl)imidazole (TMSI) were obtained from Pierce. Deuterated dimethyl sulfoxide (Me_2SO-d_5) was obtained from Cambridge Isotope Laboratories (Woburn, MA).

All mice used were male albino animals from the ARS-HA/ICR strain and were obtained from Harlan Sprague–Dawley (Madison, WI). Each weighed ca. 40 g at the time of sacrifice. The mice were maintained on a 12-h light/12-h dark cycle, with the lights being turned on at 07:00 am, and were allowed access to food and water ad libitum. No animals were employed until at least 4 weeks after the date of arrival from the supplier. Sacrifice occurred at 10:00 to 12:00 am. Injections of the tested compounds were given intracranially[18] with all weight units referring to the free base form. The dose was contained in 5 μL of a vehicle consisting of 1 mg/mL of ascorbic acid in isotonic saline (0.9% NaCl). Compounds used in addition to 5-hydroxytryptamine-4,7-dione were obtained from readily available sources with the exception of 6-aminodopamine and α-methyl-6-aminodopamine. The latter two compounds were prepared according to Stone.[19] Determinations of endogenous neurochemicals were performed by using the liquid chromatographic method reported by Lin et al.[20] Animals were sacrificed 7 days after injection of the tested compounds.

Results

Electrochemical Studies. In an attempt to discover whether 5,6-DHT and/or 5,7-DHT are formed during the electrochemical oxidation of 5-HT, the cyclic voltammetric behaviors of all three compounds at the PGE were studied.

Cyclic voltammograms 5-HT, 5,6-DHT, and 5,7-DHT are most clearly defined in acidic solution. Figure 1 shows cyclic voltammograms for all three compounds in 0.01 M HCl. Voltammograms obtained in pH 2.0 phosphate

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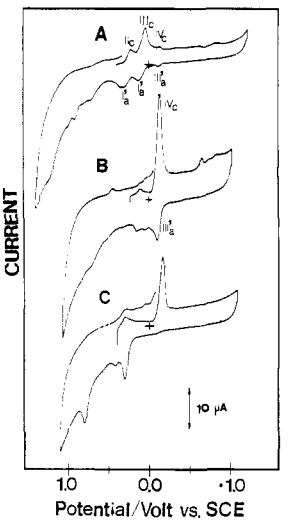

Figure 2. Cyclic voltammograms at the PGE of the product solutions formed following controlled potential electrooxidation of 1 mM (A) 5-HT, (B) 5,7-DHT, and (C) 5,6-DHT at 0.54 V in 0.01 M HCl. Sweep rate: 200 mV s^{-1}.

buffer with μ = 0.02–0.5 were virtually identical with those shown in Figure 1. 5-HT shows two voltammetric oxidation peaks (I_a and II_a, Figure 1A). On the reverse sweep, the absence of a reverse (reduction) peak coupled to peak I_a indicates that the initial product of oxidation is unstable on the time scale employed (200 mV s^{-1}) and hence is unavailable for reduction. At very fast sweep rates (>20 V s^{-1}) a very small reduction peak coupled to oxidation peak I_a can be observed at ca. pH 2. Having scanned oxidation peaks I_a and II_a of 5-HT, reduction peaks appear at more negative potentials. A small reduction peak II_c can be observed at +0.28 V, which appears to form a quasi-reversible couple with oxidation peak II_a', which is formed on the second sweep toward positive potentials. A second, larger reduction peak III_c appears at +0.10 V, which forms a quasi-reversible couple with oxidation peak I_a' observed on the second sweep toward positive potentials. Peak clipping experiments show that it is necessary to scan only oxidation peak I_a of 5-HT in order to observe the peaks II_c/II_a' couple and the peaks III_c/I_a' couple. Both 5,6-DHT (Figure 1C) and 5,7-DHT (Figure 1B) show two well-defined voltammetric oxidation peaks. On the reverse sweep there are no reduction peaks coupled to any of these oxidation peaks. After scanning the oxidation peak of 5,6-DHT, two overlapping, irreversible reduction peaks can be observed on the reverse sweep at –0.12 V (Figure 1C). However, after scanning the oxidation peaks of 5,7-DHT, subsequent sweeps show a quasi-reversible couple at –0.12 V and an irreversible reduction peak at –0.9 V (Figure 1B).

A comparison of the voltammograms for 5-HT, 5,6-DHT, and 5,7-DHT shown in Figure 1 gives no indication for formation of 5,6- or 5,7-DHT during the electrooxidation of 5-HT. However, the first oxidation peaks of 5,6-DHT and 5,7-DHT occur at potentials that are about 200 mV less positive than for peak I_a of 5-HT. Thus, if a dihydroxytryptamine species is formed as a result of the electrochemical oxidation of 5-HT, it would be immediately further oxidized. Accordingly, the intermediacy of 5,6- and/or 5,7-DHT in the electrooxidation of 5-HT can only be inferred by formation of common electrooxidation products.

Figure 2A shows a cyclic voltammogram of a product solution formed following controlled potential electrooxidation of 5-HT at 0.54 V at pH 2.0. This voltammogram shows the quasi-reversible peaks II_c/II_a' and III_c/I_a' couples. In addition, a new small reversible couple (peaks IV_c/III_a') appears at –0.12 V. The latter couple does not appear in the cyclic voltammogram of 5-HT (Figure 1A), indicating that the species responsible for reduction peak IV_c is formed in the peak I_a electrooxidation of 5-HT in very small yield. Figure 2B shows a cyclic voltammogram of the product solution formed by controlled potential electrooxidation of 5,7-DHT at 0.54 V. The peaks IV_c/III_a' couple appears but is much larger than is observed for the peak I_a oxidation product of 5-HT (Figure 2A). Cyclic voltammograms of the product solution formed after controlled potential electrooxidation of 5,6-DHT at 0.54 V at pH 2.0 show a reduction peak at potentials similar to peak IV_c but no corresponding oxidation peak III_a' (Figure 2C). These cyclic voltammograms suggest that the species responsible for reduction peak IV_c (and hence oxidation peak III_a') is formed as a result of electrochemical oxidation of 5-HT and 5,7-DHT but not from electrooxidation of 5,6-DHT.

It is obvious from the cyclic voltammograms shown in Figure 2 that several other electroactive products are formed upon electrooxidation of 5-HT, 5,7-DHT, and 5,6-DHT. Work is currently underway to identify these products.

Coulometric Oxidation of 5-HT and 5,7-DHT. 5-HT (<1 mM) was electrolyzed in 0.01 M HCl at 0.54 V. Dilute HCl was used as the supporting electrolyte solution because of its ease of removal by freeze-drying upon completion of the electrolysis. Electrolyses were terminated when cyclic voltammetry indictated that oxidation peak I_a had been eliminated. Under these experimental conditions coulometric n values ranging from 2.1 to 2.3 were measured. The electrolyzed solution had a deep red-purple color.

Coulometric oxidation of 5,7-DHT under identical conditions gave experimental n values ranging from 2.7 to 3.0. The electrolyzed solution had a bright orange color.

The solutions obtained after completion of such electrolyses were immediately freeze-dried. The resulting solid products were dissolved in about 2 mL of the liquid chromatographic eluent solution and injected onto a column of Sephadex LH-20 using H_2O–MeOH (9:1 v/v) adjusted to pH 2.0 with HCl as the eluent. Typical liquid chromatograms obtained with the electrooxidation products of 5-HT and 5,7-DHT are shown in parts A and B of Figure 3, respectively. Both 5-HT and 5,7-DHT clearly give a very complex mixture of products. The identity of these products will be the subject of future reports.

The electrooxidation product of 5,7-DHT eluted under liquid chromatographic (LC) peak 7 (Figure 3B) was bright orange. A cyclic voltammogram of this product at pH 2.0 (Figure 4) shows reduction peak IV_c (–0.12 V) and, on the reverse cycle, oxidation peak III_a'. Two additional oxidation peaks at 0.72 and 1.16 V also appear. The latter two peaks can be observed even if reduction peak IV_c is not first scanned. The UV–visible spectrum of the orange product at pH 2.0 shows four bands at λ_{max} 220, 277, 332, and 458 nm (Figure 5A). Reduction of this product by controlled potential electrolysis at the PGE at –0.15 V gives a colorless solution (λ_{max} 210, 250, and 287 nm, Figure 5A). This reduced form is rapidly reoxidized to the orange compound by air. In subsequent discussion the orange electrooxidation product of 5,7-DHT characterized by LC peak 7 (Figure 3B), the cyclic voltammogram shown in Figure 4 and the spectrum shown in Figure 5A, will be referred to as compound **5**.

Liquid chromatograms of the electrooxidation product mixture from 5-HT (Figure 3A) show a broad peak (LC

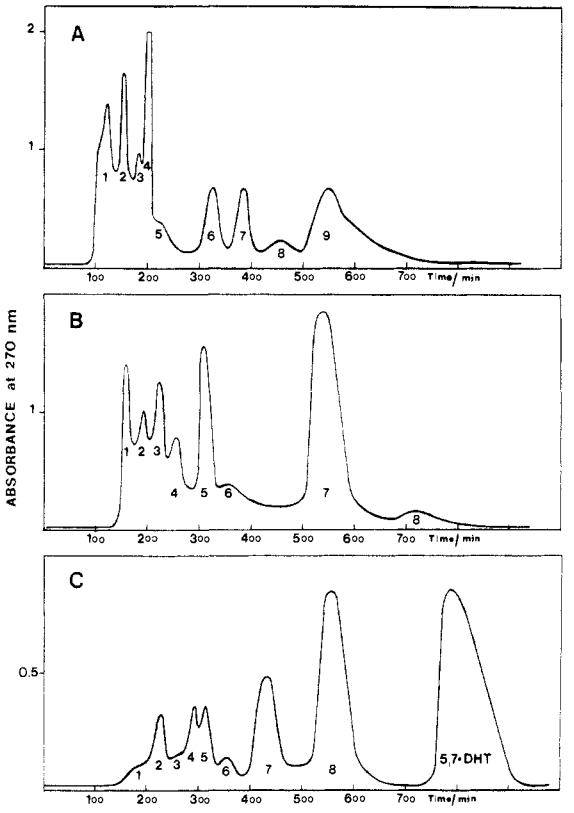

Figure 3. Liquid chromatograms of the product mixtures formed by controlled potential electrooxidation of (A) 5-HT and (B) 5,7-DHT at 0.54 V in 0.01 M HCl using a column of Sephadex LH-20 (60 × 2 cm) and an eluent of H_2O–MeOH (9:1, v/v) adjusted to pH 2.0 with HCl. Flow rate: ca. 28 mL h^{-1}. (C) Products formed by air-oxidation of 5,7-DHT in pH 7.0 phosphate buffer (μ = 0.01) using the same chromatographic conditions.

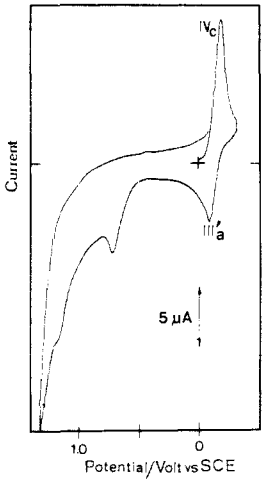

Figure 4. Cyclic voltammogram at the PGE of the orange oxidation product of 5,7-DHT dissolved in 0.01 M HCl. Sweep rate: 200 mV s^{-1}.

peak 9) at approximately the same retention time as LC peak 7 observed for the electrooxidation products of 5,7-DHT (Figure 3B). (Liquid chromatograms were obtained by using many different columns having slightly different dimensions and flow rates. Thus, some variation in retention times was always noted.) The shape of LC peak 9 in Figure 3A indicates that more than one component is eluted under this peak. The eluent and components eluted under LC peak 9 (Figure 3A) were collected, freeze-dried, redissolved in ca. 1–2 mL of the chromatographic eluent solution, and injected onto a longer column of Sephadex LH-20 (100 × 2 cm) using a slower flow rate (~20 mL h^{-1}). The resulting chromatogram showed that three components were eluted having retention times of 1000, 1430, and 1620 min. The component eluting at 1000 min was orange and exhibited exactly the same cyclic voltammogram and spectrum to that of **5** (i.e., Figures 4 and 5).

The yield of **5** from electrooxidation of 5-HT in 0.01 M HCl was <5% and from 5,7-DHT was ≥50%. The spectrum of **5** at various pH values is shown in Figure 5B. At pH 7 a solution of **5** exhibits a pale pink color (λ_{max} 232, 292, 337 (sh), and 512 nm). On the basis of the spectral shifts of **5** with pH, the pK_a of this compound is calculated to be about 4.8.

Controlled potential electrooxidations of 5-HT and 5,7-DHT in phosphate buffers (μ = 0.1–0.5) at pH 2–2.4 gave the same experimental n values reported for 0.01 M HCl. Liquid chromatograms of the resulting product mixtures were similar to those observed for the product mixtures formed in 0.01 M HCl.

Electrolysis of 5,7-DHT (<1 mM) in phosphate buffer at pH 7 at a potential corresponding to its first voltammetric oxidation peak (0.30 V) gave experimental n values ranging from 2.4 to 2.6. The resulting product solution had a bright orange-pink color. Separation of these products using the liquid chromatographic conditions described earlier shows that the major product (>50%) was **5**. However, controlled potential electrolysis of 5-HT (<1 mM) at a potential corresponding to voltammetric peak I_a (0.35V) at pH 7 proceeded extremely slowly owing to severe electrode filming by one or more oxidation products. Experimental n values between 2.6 and 3.1 were measured. Liquid chromatography of the product mixtures formed showed that at least 10 components were present. However, compound **5** was not detected.

Autoxidation of 5,7-DHT at pH 7. In phosphate buffers at pH 6 and higher ($\mu \geq 0.01$) and in unbuffered water, 5,7-DHT undergoes autoxidation and the initially colorless solution develops a bright orange-pink color within a few hours. Liquid chromatography of the freeze-dried product mixture formed in phosphate buffer pH 7 (μ = 0.01) shows that several components are present (Figure 3C). The component eluted under LC peak 8 in Figure 3C was orange (at pH 2.0) and was spectrally and cyclic voltammetrically identical with **5**. This result agrees with earlier observations[21,22] that autoxidation of 5,7-DHT gives a stable product having a more intense absorption at 300 nm than 5,7-DHT and a low, rounded absorption band at 520 nm. This product, however, was not isolated or identified.

Structure of Compound 5. Compound **5** formed by electrochemical oxidation of 5-HT and 5,7-DHT and autoxidation of 5,7-DHT was purified by repeated liquid chromatography and then studied by mass, IR, and ^1H NMR spectroscopy. FAB-MS of **5** dissolved/suspended in thioglycerol (TG) gave the following major peaks: m/e (relative abundance) 207 ($M + H^+$, 60), 315 ($M + TGH^+$, 100), 423 ($M + TG_2H^+$, 4), 529 ($M + C_9H_{23}O_6S_3^+$, 6). This information clearly indicates that the pseudo molecular ion of **5** (MH^+) has a molar mass of 207 g. High-resolution FAB-MS showed the latter ion to have an m/e value of 207.0784, which corresponds to the formula $C_{10}H_{11}N_2O_3$ (theory 207.0767). EI-MS (12 eV, 220 °C) on **5** gave the following results: m/e (relative abundance) 208 (0.5), 207 (0.6), 206 (M^+, 1.0), 192 (1.2), 191 (11.5), 190 ($M^+ - O$, 100),

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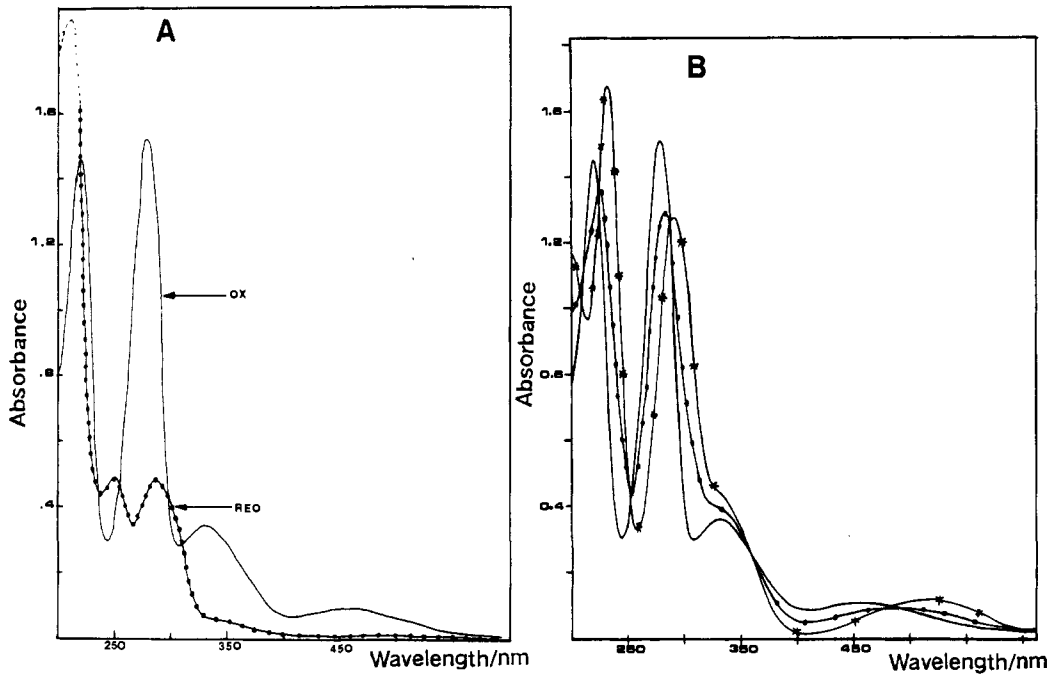
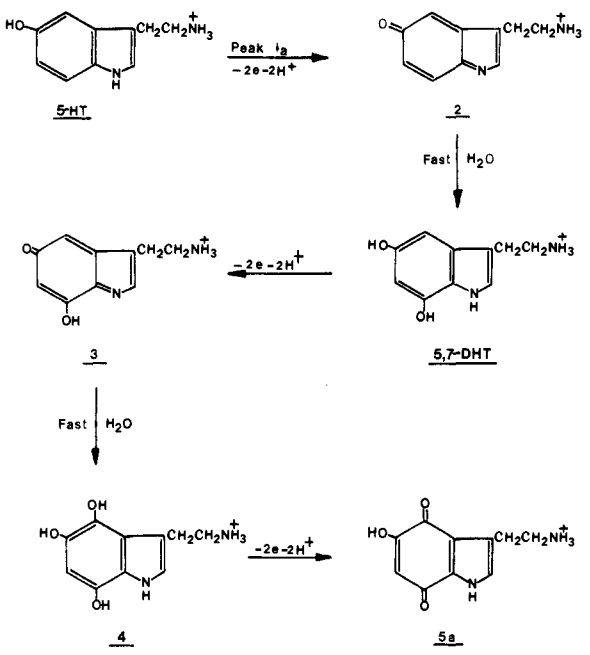


Figure 5. (A) (–) Spectrum of the orange oxidation product of 5,7-DHT in 0.01 M HCl. (···) Spectrum after controlled potential electrochemical reduction at –0.15 V at the PGE. (B) Spectra in phosphate buffers (μ = 0.5) at (–) pH 2.0, (·–·) pH 4.4 and (*–*) pH 6.

Scheme I

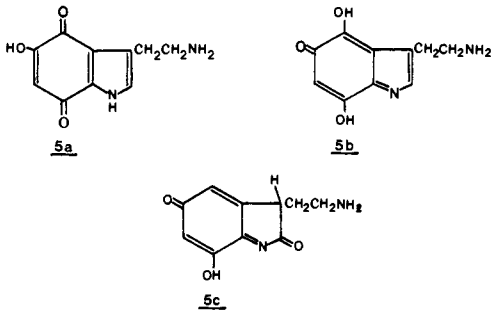


189 (19.5), 188 ($M^+ - H_2O$, 23.2), 187 (4.4), 186 (21.7), 178 (1.5), 177 ($M^+ - CH_2NH$, 12.2), 175 (3.9), 174 ($M - O_2$, 14.9), 163 (2.0), 162 ($M^+ - CH_2CH_2NH_2$, 13.3) 161 (8.7), 160 (6.7).

Compound **5** (ca. 2 mg) was silylated with either BSA (80 μL) in acetonitrile (80 μL) at 80 °C for 60 min or with a mixture of MSTFA/TMSI (100:20, v/v, 120 μL) in acetonitrile (100 μL) at 75 °C for 50 min in sealed vials. GC on the resulting solution showed five peaks with t_R values of 27.6, 28.1, 29.0, 30.2, and 31.0 min. EI- and CI-MS on the components eluted under these GC peaks showed those at t_R = 27.6 and 30.2 min to be due to a compound of molecular weight 206 derivatized with 3- and 4-trimethylsilyl groups, respectively. The GC peaks at t_R = 28.1, 29.0, and 31.0 min were due to a compound of molecular weight 208 derivatized at the 4-, 5-, and 6-positions, respectively. These results suggest that in acetonitrile under the silylation conditions employed both the oxidized (M_r 206) and reduced (M_r 208) forms of **5** exist in equilibrium and can be derivatized.

The IR spectrum of **5** (KBr pellet) showed the following bands (cm^{-1}): 2860–3160 (broad and strong, O–H and N–H stretch), 1640 (s, C=O), 1610 (s, sh), 1480 (m), 1370 (s), 1350 (m), 1300 (w), 1275 (w), 1235 (w), 1195 (s), 1120 (w), 1040 (w), 965 (m), 910 (w), 840 (w), 810 (m), 765 (m), and 710 (m).

The mass and IR spectra indicated that **5** has a molecular weight of 206 and contains one or more carbonyl groups. The EI-mass spectrum indicates that the $CH_2C-H_2NH_2$ side chain remains intact (i.e., the large peak at m/e 162 indicates loss of the $CH_2CH_2NH_2$ fragment). High-resolution MS shows that the formula of **5** is $C_{10}-H_{10}N_2O_3$. There are three quinoidal structures, **5a–c**, that could be derived from both 5-HT and 5,7-DHT and that correspond to this formula. Compounds **5a–c** have not



been reported in the literature. However, at about pH 2 compound **5** shows bands in the UV–visible region at λ_{max} 458, 332, 277, and 220 nm. 2-Oxyindole structures, similar to **5c**, are generally characterized in acidic and neutral solution by an intense band at 248–252 nm with a pronounced shoulder at ca. 285 nm.[23] The spectrum of **5** is quite similar to that of 1,4-benzoquinone (λ_{max} 240, 285,

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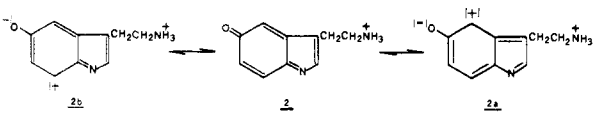
434 nm) but very different from that of 1,2-benzoquinone (λ_{max} 240, 375, 568 nm).[24] In addition, 2-hydroxy-1,4-naphthoquinone, which has considerable structural similarity to **5a**, is orange (λ_{max} 459 nm).[24] These data tend to provide support for structure **5a**.

Before discussing the ^{1}H NMR spectrum of **5**, it is of value to summarize that of 5-HT (300 MHz in Me_2SO-d_6). The spectrum of 5-HT shows triplets at δ 2.92 and 3.06 (CH_2CH_2), doublets at δ 6.62 and 6.64 ((C(6)-H), a doublet at δ 6.82 (C(4)-H), singlet at δ 7.13 (C(2)-H), doublet at δ 7.19 (C(7)-H), and broad singlets at δ 7.28 (NH_3), 8.66 (OH), and 10.66 (N(1)-H).[25-27] The ^{1}H NMR spectrum of **5** (300 MHz, in Me_2SO-d_6) shows triplets at δ 2.95 and 3.05 (CH_2CH_2), a singlet at δ 5.86 (C(6)-H), a doublet at δ 7.05 (C(2)-H), and broad singlets at δ 7.83 (NH_3), 11.15 (OH), and 12.51 (N(1)-H). The signal from the proton located at the indolic nitrogen (N(1)-H) along with the absence of signals from protons of C(4) and C(7) indicates that **5** has structure **5a**. The broad singlet at δ 7.83 shows that **5a** is isolated as its hydrochloride salt. Elemental analysis gave the following results: C, 46.34; H, 4.69; N, 10.37; Cl, 13.31. This indicates that the compound is isolated as its monohydrate monohydrochloride (theory, C, 46.07, H, 4.99; N, 10.75; Cl, 13.63.

Discussion

The results presented above show that the electrochemical oxidation of 5-HT at potentials corresponding to its first voltammetric oxidation peak in acidic solutions leads to the formation of a small but significant amount of **5a**. Under the same conditions, 5,7-DHT yields **5a** as a major electrooxidation product. However, electrooxidation of 5,6-DHT does not, in fact cannot, lead to **5a**. These results suggest, therefore, that a minor electrooxidation route for 5-HT proceeds through the intermediary formation of 5,7-DHT. A rational explanation for such a reaction is shown in Scheme I. Thus, it appears that an initial $2e - 2H^+$ electrooxidation of 5-HT leads to the quinone imine **2**. Since a reverse reduction peak coupled to oxidation peak I_a can only be observed at very fast sweep rates (>20 V s^{-1}) at pH 2,[28] **2** must be extremely reactive and disappears in a chemical follow-up reaction. Formation of quinone imine intermediates similar to **2** upon oxidation of hydroxyindole derivatives has been suggested by other investigators.[29-31] Compound **2** would also be expected to be reactive since it can form resonance structures carrying positive charge in the indole ring,[32] e.g., 2a and 2b. Both **2a** and **2b** should be very susceptible



to attack by available nucleophiles including H_2O, 5-HT, and **5a**. Thus it would be expected that a considerable number of products would be formed, which is in fact observed.[28] Nucleophilic attack by water on **2b** would lead to formation of 5,7-DHT. In principle, attack of water on **2** could also lead to 5,6-DHT or 4,5-DHT. However, since **5a** is not a product of electrooxidation of 5,6-DHT, the latter compound cannot be an intermediate in the oxidation of 5-HT. There is no way, at this time, to decide whether **2** is attacked by water to give 4,5-DHT although the intermediacy of the latter compound in the oxidation of 5-HT has been postulated.[33,34] However, the fact that **5a** is formed as a major electrooxidation product of 5,7-DHT lends strong support to the intermediacy of the latter compound in the oxidation of 5-HT. Since 5,7-DHT is more easily electrooxidized than 5-HT, it is immediately further oxidized. It seems reasonable to conclude that 5,7-DHT is oxidized in a $2e - 2H^+$ reaction to the quinone imine **3**. The absence of a reverse reduction peak coupled to the first oxidation peak of 5,7-DHT(Figure 1B) indicates that **3** is very unstable. Nucleophilic attack by water must then give 4,5,7-trihydroxytryptamine 4. Compound 4 is undoubtedly more easily oxidized than 5-HT and hence undergoes an immediate $2e - 2H^+$ reaction to **5a**.

It must be emphasized that Scheme I represents only a minor route for the electrochemical oxidation of 5-HT which accounts for no more than 5% of the 5-HT oxidized. The reaction sequence from 5-HT to **5a** shown in Scheme I requires the transfer of 6e per molecule of 5-HT oxidized. Experimental n values averaged about 2.2. This indicates that other electrooxidation routes of 5-HT must involve, overall, transfer of less than 2e per molecule. In fact, a number of oligomeric oxidation products are formed that support this conclusion.[28] In the case of 5,7-DHT, the pathway to **5a** shown in Scheme I accounts for approximately 50% of the 5,7-DHT that is electrooxidized or autoxidized.

Preliminary Biological Results. Since 5-HT is a naturally occurring chemical neurotransmitter and 5,7-DHT is a powerful and somewhat selective neurotoxin,[16] the neurotoxic properties of their common oxidation product **5a** has been briefly investigated.

Preliminary studies show that **5a**, given intracranially in mice, exhibits two very interesting results. First, the general toxicity of **5a** is somewhat greater than that of previously studied neurotoxins that are believed to involve oxidation/reduction mechanisms in their mode of action. For example **5a** at a dosage of 20 μg produced death in 19 of 40 animals, indicating an LD_{50} very near 20 μg. Comparable LD_{50} values for similar compounds are 6-hydroxydopamine, 80 μg,[35] 6-aminodopamine, 50 μg,[35] α-methyl-6-aminodopamine, 45 μg,[36] and, most importantly, 5,7-DHT, 55 μg.[37] The second interesting result concerns the depletion of endogenous transmitters by **5a**. Seven days after an intracranial injection of 20 μg of 5a, whole mouse brain norepinephrine levels were lowered to 87 ± 1% of control values (mean ± SEM, n = 27). This

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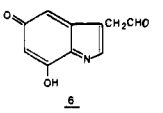
result was significant ($p < 0.001$) and, further, completely reminiscent of the norepinephrine depletion of ~80% of controls 10 days after injection reported by Massotti et al.[37] using a 21-μg dose of 5,7-DHT. However, the 20-μg dose of **5a** did not produce any substantial decline of 5-HT levels (mean ± SEM = 96 ± 1% of controls) in contrast to those observed with the parent 5,7-DHT.[37] This latter result is presumed to be the direct result of the lack of selective uptake of **5a** by serotonergic neutrons. Indeed, destruction of neurons by these neurotoxins has uniformly been shown to involve fairly selective uptake of the toxin by the targetted neurons. And, seemingly minor changes in the structure of a compound are well known to severely alter its uptake. Thus, we assume the addition of the 4-oxo group of **5a** compared to 5,7-DHT has effected such a change in its uptake properties with respect to 5-HT neurons. Nonetheless, **5a** certainly exhibits a number of biological properties that support its role as an active intermediate in the observed neurotoxicity of 5,7-DHT. First, it displays a general toxicity, leading to death of the intracranially injected animal, that is more potent than 5,7-DHT. Secondly, its long-term depletion of norepinephrine is completely comparable to that produced by a similar dose of 5,7-DHT.

Conclusions

The suggestion has been made that under certain circumstances a defect in the metabolism of 5-HT might lead to the formation of more reactive, more highly hydroxylated but unspecified derivatives[1,2,4,9] that in some fashion leads to mental disorders. Minor oxidation products of 5-HT in rats and rabbits have been speculated to be 4,5- or 5,6-DHT.[3,4,6,9] However, formation of di- or trihydroxy derivatives of tryptamine from 5-HT has never previously been demonstrated either in vivo or in vitro. The work reported here provides strong evidence that 5-HT is electrochemically oxidized to 5,7-DHT, which in turn is immediately further oxidized to **5a**. These reactions have been shown to occur in acidic solution. It has not been possible to detect **5a** as an electrooxidation product of 5-HT at physiological pH. However, the liquid chromatographic techniques employed in this investigation probably would not permit the detection of very small amounts of **5a** that might be formed in such pH regions particularly if it was coeluted with one of the many other colored oxidation products of 5-HT. Formation of **5a** as an oxidation product of 5-HT in vivo even in trace amounts might result in serious neurological consequences because of its powerful neurotoxic properties.

It has also been shown that the neurotoxin **5a** is formed by electrochemical oxidation of 5,7-DHT at pH 2 and 7 and by autoxidation of 5,7-DHT at pH 7. It is well known that 5,7-DHT has a profound lesioning effect on 5-HT containing neurons and, to a lesser extent, that it can also damage noradrenergic neurons.[16,23,28,29] However, the mechanism of neurotoxic action of 5,7-DHT is not well understood. Since the neurotoxicity of 5,7-DHT is prevented by inactivation of monoamine oxidase, it has been suggested[40] that in vivo in the presence of the latter enzyme autoxidation occurs, giving the quinone imine aldehyde **6**.



6

The aldehyde residue in **6** and, probably, the C(4) position provide two electrophilic sites that might be attacked by nucleophiles such as thiol residues on nerve ending proteins, leading to irreversible cross-linking of the proteins.[40]

The results reported here show that **5a**, formed by oxidation of 5-HT and 5,7-DHT, is a very powerful neurotoxin. This raises the possibility that at least part of the neurotoxicity of 5,7-DHT might be due to the in vivo formation of **5a**. In additoin, our results indicate for the first time that an oxidative metabolic route for 5-HT proceeding through 5,7-DHT to **5a** is *chemically* feasible. This, in turn, suggests that certain neurological disorders might be related to the 5-HT reaction pathway shown in Scheme I, which proceeds via the neurotoxin 5,7-DHT to the neurotoxin **5c**.

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On the Significance of Clusters in the Graphical Display of Structure-Activity Data

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A method is presented to evaluate the statistical significance of an apparently clustered group in the graphical display of structure-activity data. Two variations are described; each is implemented by means of a computer program. The first is applicable in situations with relatively small sets of compounds where a complete enumeration of all possible clusters can be accomplished reasonably on a high-speed electronic computer. The second is applicable in cases where such a calculation would be too time consuming. This latter variation uses random sampling of the set of all possible clusters. An application for each variation is given: for the smaller case a reevaluation of a study on aminotetralin and aminoindan monoamine oxidase inhibitors; for the larger case the discovery of some physical parameters that influence mutagenicity among some aminoacridine derivatives. It is proposed that this new technique be called cluster significance analysis (CSA).

Graphics are used in analyzing structure-activity data because the visual display of the information often affords insights that are not obvious otherwise. Notable examples in recent years include the work of Cramer and co-workers in their study of the antiallergic pyranenamines,[1] the study of antimicrobial activity in tuberlin analogues by Harrison et al.,[2] and the work of Morgan et al. on the carcinogenicity

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