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Electrochemical Oxidation of 5-Hydroxytryptamine in Aqueous Solution at Physiological pH

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The electrochemical oxidation of the indolic neurotransmitter 5-hydroxytryptamine (5-HT) has been studied in aqueous solution at physiological pH using a pyrolytic graphite electrode. The initial step in the oxidation involves a $2e,1H^+$ reaction leading to a reactive carbocation intermediate (**28b**). This intermediate is attacked by 5-HT in an ion-substrate reaction to give 5,5′-dihydroxy-4,4′-bitryptamine (**1**) as the major product along with the C(4)–O–C(5′) bridged dimer **9**. Alternatively cation **28b** is attacked by water to yield, ultimately, tryptamine-4,5-dione (**5**). Many additional reactions occur between primary dimers **1** and **9** and carbocation **28b** such that a very complex mixture of ultimate products are formed. A total of 15 products have been isolated and partially or completely characterized. It is possible that the oxidation reaction pathways and product profile elucidated might have a bearing on, as yet hypothetical, aberrant oxidation reactions of 5-HT in the central nervous system which could play a role in the etiology of mental illnesses such as diseases of the Alzheimer's type.

Abnormal oxidation chemistry or biochemistry of the indolic neurotransmitter 5-hydroxytryptamine (5-HT; serotonin) has been implicated in the etiology of several psychotic diseases such as schizophrenia, major depression, and neurodegenerative Alzheimer's disease (*1*). Furthermore, it has long been known that 5-HT is oxidized in biological milieu such as human serum and ceruloplasmin (*2*) although the mechanisms and products of such oxidations remain to be elucidated. Several lines of evidence suggest that oxidation products of 5-HT have important biological activities. For example, an unidentified product of autoxidation of 5-HT is a potent inhibitor of acetylcholinesterase (*3*). Recently, it has been reported that a compound which has similar chromatographic properties to 5,6-dihydroxytryptamine (5,6-DHT) is formed endogenously in rat brain after a single dose of methylamphetamine or *p*-chloroamphetamine (*4*). Hence, it has been speculated that these amphetamine drugs alter the normal oxidative metabolism of 5-HT and that their neurodegenerative effects are mediated by the resultant formation of a dihydroxyindoleamine neurotoxin. Serotonergic abnormalities have been widely implicated in dementia of the Alzheimer's type (*1e, 5*). Analysis of cerebrospinal fluid (CSF) of Alzheimer's patients by high-performance liquid chromatography with electrochemical detection (HPLC–EC) has revealed the presence of unknown

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electroactive species which are not present in CSF of normal subjects (*1f*). At least one of these compounds has been proposed to be an endogenous but aberrant oxidative metabolite of 5-HT because it has similar chromatographic and electrochemical properties to *in vitro* electrochemically oxidized 5-HT.

The above reports indicate an important need to understand the fundamental oxidation chemistry of 5-HT. Electrochemical techniques provide a powerful approach to obtain basic insights into such oxidation chemistry. Recently the electrochemical oxidation of 5-HT in acidic aqueous (*6*) and acetonitrile (*7*) solutions has been described. In this report the electrochemically driven oxidation of 5-HT in aqueous solution at physiological pH is described. At this pH the oxidation chemistry is considerably different to that observed at low pH.

EXPERIMENTAL SECTION

N-Acetyl-5-hydroxytryptamine and *N*,*N*-dimethyl-5-hydroxytryptamine were obtained from Sigma (St. Louis, MO). Sources of other chemicals have been described elsewhere (*6*). Phosphate buffers of known ionic strength were prepared according to Christian and Purdy (*8*).

Voltammetry employed a pyrolytic graphite electrode (PGE; Pfizer Minerals, Pigments and Metals Division, Easton, PA) having an approximate surface area of 5 mm^2. The PGE was resurfaced before recording each voltammogram as described previously (*9*). Linear sweep voltammetry (LSV) and cyclic voltammetry (CV) were performed with either a conventional operational amplifier-based instrument or a BAS-100A instrument (Bioanalytical Systems, West Lafeyette, IN). All voltammograms were corrected for iR drop. Controlled potential electrolyses employed four plates of pyrolytic graphite, each having an approximate surface area of 17 cm^2, as the working electrode dipping into 40 ml of solution containing 5-HT. All potentials are referred to the saturated calomel reference electrode (SCE) at ambient temperature (22 ± 3°C).

High-performance liquid chromatography (HPLC) employed Gilson (Middleton, WI) gradient systems equipped with dual Model 302 (analytical and semipreparative columns) or Model 303 (preparative column) pumps. For analytical/semipreparative HPLC a Brownlee (Applied Biosystems, Foster City, CA) reversed-phase column (RP-18, 5 μm, 25 × 0.7 cm) was used. Mobile phase A was prepared by adding 6 ml of concentrated ammonium hydroxide to 994 ml of water; the pH was then adjusted to 4.75 with concentrated formic acid. Mobile phase B was prepared by adding 7 ml of concentrated ammonium hydroxide and 400 ml of acetonitrile to 593 ml of water; the pH was then adjusted to 4.55 with concentrated formic acid. The gradient profile was as follows: 0–4 min, linear gradient from 0 to 10% solvent B; 4–11 min, linear gradient to 13% solvent B; 11–18 min, linear gradient to 15% B; 18–19 min, linear gradient to 18% B; 19–20 min, linear gradient to 30% B; 20–32 min, linear gradient to 65% B; 32–35 min, linear gradient to 100% B; 35–38 min, 100% B. Between 0 and 11 min the flow rate was 2.5 ml min^{-1}; between 11 and 18 min the flow rate was linearly increased from 2.5 to 3.0 ml min^{-1}; between 18 and

38 min the flow rate was 3.0 ml min^{-1}. For analytical/semipreparative HPLC the maximum solution volume injected was 2.0 ml.

A reversed-phase column (J. T. Baker, Phillipsburg, NJ; C_{18}, Prep 10, 250 × 21.2 mm) was employed for preparative HPLC. The gradient profile was as follows: 0–5 min, linear gradient from 0 to 15% solvent B (flow rate 8 ml min^{-1}); 5–20 min, linear gradient to 25% B and flow rate increased to 10 ml min^{-1}; 20–30 min, linear gradient to 32% B; 30–50 min, linear gradient to 100% B; 50–60 min, 100% B. Between 20 and 60 min the flow rate was 10 ml min^{-1}. For preparative HPLC 10-ml samples were injected. For analytical/semipreparative and preparative HPLC the eluent was monitored at 254 nm with a Gilson Holochrome uv detector.

Liquid chromatography–mass spectrometry (LC–MS) was carried out with a Kratos MS 25/RFA instrument equipped with a thermospray source. The solvent was 0.1 M ammonium acetate in water, adjusted to pH 4–5 with acetic acid, at a flow rate of 0.9 ml min^{-1}. The thermospray capillary tip was maintained at 225°C and the source at 245°C. Aliquots (1–2 ml) of product solutions collected by HPLC separation and hence dissolved in the chromatographic mobile phase (pH 4.75) were injected directly into the LC–MS system using a conventional loop injector (Rheodyne Model 7125). Fast atom bombardment mass spectrometry (FAB-MS) was performed on a VG Instruments (Manchester, UK) Model ZAB-E spectrometer. ^{1}H and ^{13}C NMR spectroscopy utilized a Varian XL-300 instrument. Assignments of resonances were based on homonuclear decoupling experiments and homonuclear 2D-correlated spectroscopy (COSY) experiments. Ultraviolet–visible spectra were recorded on a Hitachi 100-80 spectrophotometer.

Product Isolation and Purification

Between 10 and 35 mg of 5-HT · HCl was dissolved in 40 ml of pH 7.2 phosphate buffer (μ = 0.2) (1.2–4 mM 5-HT) and electrolyzed at 0.270 V for about 5 h. Because of severe electrode filming electrolyses were periodically interrupted so that the electrodes could be cleaned by polishing with 600-grit silicon carbide paper. Upon termination of the electrolysis aliquots of the product solution were repetitively injected into an HPLC system and individual products were collected. Representative chromatograms are shown in Fig. 4. Compounds will be identified by the numbers assigned to peaks in this chromatogram. Several HPLC peaks are clearly overlapped and hence most products were collected and then their volume reduced by freeze-drying. The more concentrated solutions were purified by a second HPLC separation. The collected fractions were desalted by one of three methods.

In Method A 2-ml aliquots were injected onto the semipreparative HPLC column using water as the mobile phase (2.5 ml min^{-1}). Ammonium formate rapidly eluted from the column. After 6 min the mobile phase was changed to 25% MeOH in water adjusted to pH 2.8 with HCl (2.5 ml min^{-1}). This caused the organic product to elute. This process was repeated until the entire fraction was desalted. Methods B and C employed a Sephadex LH-20 column (100 × 2 cm). In Method B the mobile phase was 50% MeOH in water (25 ml h^{-1}). In Method C the mobile phase was 10% MeOH in water adjusted to pH 2.2 with HCl (25 ml h^{-1}). In both Methods

B and C the combined fractions for each component were introduced onto the column. Ammonium formate rapidly eluted followed by the organic product. Method A was used to desalt compounds **1**, **3**, **5**, **6**, and **10**; Method B for compounds **2**, **8**, **8A**, and **13**; and Method C for compounds **7**, **9**, **12**, and **14**. After being desalted the solution containing a product was freeze-dried.

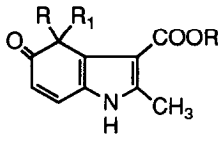
Spectral data used for structure assignments are presented below. Chemical names are provided for each compound. However, for simplicity assignments of NMR resonances refer to the numbering provided for each compound either in the text or in Schemes I–VII.

5,5'-Dihydroxy-4,4'-bitryptamine (**1**)

Compound **1** was a pale yellow solid (λ_{max} (log ε_{max}) at pH 7.2: 301 (3.91), 276 (3.90), 210 (4.47) nm). ^{1}H NMR spectra and FAB-MS for **1** and other information bearing on its structure elucidation have been reported elsewhere (*6a*).

Spiro[4H-indole-4,1'(5'H)-oxeto[3,2-e]indol]-5-one, 3,7'-Bis(2-aminoethyl)-1,5-dihydroindole (**2**)

Compound **2** was a bright yellow solid (λ_{max} at pH 7.2: 405,295 sh, 280,218 nm). FAB-MS (3-nitrobenzyl alcohol matrix) gave a pseudomolecular ion (MH^+) at *m/e* = 349.1687 ($C_{20}H_{21}N_4O_4$ calcd, *m/e* = 349.1665). ^{1}H NMR (HCONMe$_2$-d_7) δ 11.24 (s, 1H, N(1')-H), 10.69 (s, 1H, N(1)-H), 8.55 (br s, 3H, NH_3^+), 8.07 (br s, ~3H, NH_3^+), 7.38 (d, $J_{6',7'}$ = 9.7 Hz, 1H, C(7')-H), 7.16 (d, $J_{6,7}$ = 8.4 Hz, 1H, C(7)-H), 7.15 (s, 1H, C(2)-H), 6.85 (s, 1H, C(2')-H), 6.65 (d, $J_{6,7}$ = 8.4 Hz, 1H, C(6)-H), 5.67 (d, $J_{6',7'}$ = 9.7 Hz, 1H, C(6')-H), 3.52 (m, 2H, CH_2), 3.18 (m, 2H, CH_2), 2.29 (m, 2H, CH_2), 1.93 (m, 2H, CH_2). ^{1}H NMR (D_2O) δ 7.63 (d, $J_{6',7'}$ = 9.9 Hz, 1H, C(7')-H), 7.40 (d, $J_{6,7}$ = 8.7 Hz, 1H, C(7)-H), 7.33 (s, 1H, C(2')-H), 6.93 (s, 1H, C(2)-H), 6.74 (d, $J_{6,7}$ = 8.7 Hz, 1H, C(6)-H), 5.99 (d, $J_{6',7}$ = 9.9 Hz, 1H, C(6')-H), 2.47 (m, 2H, $CH_2(\alpha)$), 2.32 (m, 2H, $CH_2(\alpha')$), 2.13 (m, 2H, $CH_2(\beta)$), 1.82 (m, 1H, $CH(\beta')$), 1.67 (m, 1H, $-CH(\beta')$). The upfield shift of the C(6')-proton (d, 5.99 ppm) indicates the presence of an adjacent carbonyl group (*10*). Furthermore, the uv–visible spectrum of **2** is very similar to that of a number of 4,4'-dialkyl-5-indolones having the general structure **15** (*11*). This observation provides important support for the indolone residue in **2**. The ^{1}H NMR spectra of compounds of the type **15** are also in accord with those of **2** (*11*). CV of **2** at pH 7.2 showed an irreversible reduction peak at −0.90 V. Controlled potential electroreduction of **2** at −0.90 V yielded **1** as the sole product as expected for the electrochemical reduction of an α,β-unsaturated ketone (*12*). Compound **2** is also the major product of electrooxidation of dimer **1** at pH 7.2 (see later discussion).



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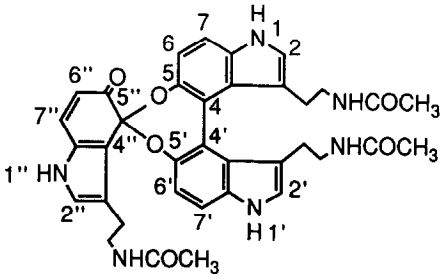
4,4' :6',4''-Ter(5-hydroxytryptamine) (**3**)

Compound **3** was a white solid (λ_{max} (log ε_{max}) at pH 7.2: 303 (4.19), 275 (4.13), 210 (3.91) nm). FAB-MS (dithioerythritol/dithiothreitol matrix) gave *m/e* = 525.2625 (MH^+, 100%; $C_{30}H_{33}N_6O_3$ calcd, *m/e* = 525.2614). Hence **3** is a simple trimer (molar mass, 524 g; $C_{30}H_{32}N_6O_3$). ^{1}H NMR ($HCONMe_2$-d_7) δ 11.06 (br s, 2H, N(1')-H and N(1'')-H), 10.93 (d, $J_{1,2}$ = 2.2 Hz, 1H, N(1)-H), 9.35 (br s, 1H, OH), 9.17 (br s, 1H, OH), 8.24 (br s, ~4H, NH_3^+ + OH), 8.07 (br s, 3H, NH_3^+), 7.91 (br s, 3H, NH_3^+), 7.39 (d, $J_{6'',7''}$ = 8.7 Hz, 1H, C(7'')-H), 7.37 (d, $J_{1',2'}$ = 2.2 Hz, C(2')-H), 7.35 (s, 1H, C(7')-H), 7.32 (d, $J_{6,7}$ = 8.7 Hz, 1H, C(7)-H), 7.29 (d, $J_{1'',2''}$ = 2.2 Hz, 1H, C(2'')-H), 7.25 (d, $J_{1,2}$ = 2.2 Hz, 1H, C(2)-H), 7.08 (d, $J_{6'',7''}$ = 8.7 Hz, 1H, C(6'')-H), 7.05 (d, $J_{6,7}$ = 8.7 Hz, 1H, C(6)-H). The resonances of **3** at ≤3.6 ppm were overlapped with large signals due to HOD and DMF. ^{1}H NMR (D_2O) δ 7.52 (d, $J_{6'',7''}$ = 8.7 Hz, 1H, C(7'')-H), 7.48 (d, $J_{6,7}$ = 8.7 Hz, 1H, C(7)-H), 7.46 (s, 1H, C(7')-H), 7.28 (s, 1H, C(2'')-H), 7.27 (s, 2H, C(2)-H and C(2')-H), 7.03 (d, $J_{6'',7''}$ = 8.7 Hz, 1H, C(6'')-H), 7.00 (d, $J_{6,7}$ = 8.7 Hz, 1H, C(6)-H), 3.20 (m, 4H, CH_2CH_2), 2.71 (m, 2H, CH_2), 2.60 (m, 2H, CH_2), 2.48 (m, 2H, CH_2), 2.39 (m, 2H, CH_2). ^{13}C NMR (D_2O) δ 150.38, 149.62, 147.95, 135.08, 134.92, 134.56, 129.55, 129.45, 129.37, 129.35, 129.22, 128.97, 128.72, 122.60, 117.05, 116.24, 115.81, 115.46, 115.22, 115.05, 114.97, 112.50, 112.44, 112.30, 43.18, 43.13, 42.66, 26.39, 26.20, 26.07. The ^{13}C NMR results indicate 6 signals in the aliphatic region (24–45 ppm) and 24 signals in the aromatic region (110–152 ppm), confirming that **3** is a trimer. The absence of ^{1}H NMR resonances for any C(4)-proton indicates that all 5-HT residues are substituted at this position. The fact that the C(7')-proton is a singlet confirms that one residue is linked at both C(4') and C(6').

Spiro[[1,3]dioxepino[5,4e :6,7-e']diindole-4,4'-[4H-indol]-5'(1'H)-one, 3',10,11-Tris(2-aminoethyl)-8,13-dihydroindole (**4**)

Compound **4** gave a bright yellow solution (λ_{max} at pH 4.8–7.0: 426, 316, 265 nm) but was unstable in both acidic and neutral solution. In acid solution **4** followed a decomposition pathway typical for acetals; in neutral solution cyclization reactions occurred (see later discussion). LC–MS on a freshly chromatographed solution of **4** gave *m/e* = 525 (MH_3^+, 0.1%), 523 (MH^+, 0.1%), 351 (**1**H^+, 4%), 193 (**5**H^+, 2%). These data suggested that **4** was a trimeric product. Compounds analogous to **4** are also obtained following controlled potential electrooxication of *N,N*-dimethyl-5-hydroxytryptamine and *N*-acetyl-5-hydroxytryptamine (NAc-5-HT). These compounds were unstable in acid solution. However, the compound analogous to **4** derived from NAc-5-HT, i.e., **16**, was stable at pH 7 and was isolated. For compound **16**, λ_{max} at pH 4.8–7.2: 427,320,266,216 nm. FAB-MS (3-nitrobenzyl alcohol matrix) gave a pseudomolecular ion (MH^+) at *m/e* = 649.2728 ($C_{36}H_{37}N_6O_6$ calcd, *m/e* = 649.2775). ^{1}H NMR (Me_2DO-d_6) δ 11.02 (d, $J_{1,2}$ = 2.1 Hz, 1H, N(1)-H), 10.89 (d, $J_{1',2'}$ = 2.1 Hz, 1H, N(1')-H), 10.79 (d, $J_{1'',2''}$ = 2.0 Hz, 1H, N(1'')-H), 7.34 (d, $J_{6'',7''}$ = 9.7 Hz, 1H, C(7'')-H), 7.30 (d, $J_{6,7}$ = 8.6 Hz, 1H, C(7)-H), 7.18 (d, $J_{6',7'}$ = 8.6 Hz, 1H, C(7')-H), 7.13 (d, $J_{1',2'}$ = 2.0 Hz, 1H, C(2')-H), 7.08 (d, $J_{1,2}$ = 2.0 Hz, 1H, C(2)-H), 6.99 (d, $J_{6,7}$ = 8.6 Hz, 1H, C(6)-H), 6.55 (s, 1H, C(2'')-H), 6.30 (d, $J_{6',7'}$ = 8.6 Hz, 1H, C(6')-H), 5.52 (d, $J_{6'',7''}$ = 9.7 Hz,

1H, C(6″)-H), 2.62 (m, 2H, CH_2), 2.19 (m, 2H, CH_2), 1.93 (m, 2H, CH_2), 1.68 (m, 2H, CH_2), 1.68 (s, 3H, CH_3), 1.59 (s, 3H, CH_3), 1.57 (s, 3H, CH_3), 1.24 (m, 2H, CH_2), 1.16 (m, 1H, CH_2), 0.98 (m, 1H, CH_2). ^{1}H NMR (D_2O) δ 7.59 (d, $J_{6',7'}$ = 8.7 Hz, 1H, C(7′)-H), 7.57 (d, $J_{6'',7''}$ = 9.7 Hz, 1H, C(7″)-H), 7.45 (d, $J_{6,7}$ = 8.7 Hz, 1H, C(7)-H), 7.35 (s, 1H, C(2″)-H), 7.27 (s, 1H, C(2′)-H), 7.15 (d, $J_{6',7'}$ = 8.7 Hz, 1H, C(6′)-H), 6.80 (s, 1H, C(2)-H), 6.71 (d, $J_{6,7}$ = 8.7 Hz, 1H, C(6)-H), 5.81 (d, $J_{6'',7''}$ = 9.7 Hz, 1H, C(6″)-H), 2.84 (m, 2H, CH_2), 2.48 (m, 4H, 2 × CH_2), 2.16 (m, 2H, CH_2), 1.84 (m, 1H), 1.80 (s, 6H, 2 × CH_3), 1.74 (s, 3H, CH_3), 1.51 (m, 1H), 1.36 (m, 1H), 1.04 (m, 1H). ^{13}C NMR (Me_2SO-d_6) δ 196.90 (C(5)=O), 177.10 (acetyl C=O), 168.75 (acetyl C=O), 165.66 (acetyl C=O), 145.68 (C-5), 142.55 (C-5′), 135.36, 135.29, 134.33, 126.29, 125.94, 125.45, 124.55, 123.89, 123.07, 121.63, 120.22, 120.07, 117.98, 116.48, 116.36, 114.08, 113.83, 110.75, 110.33, 108.46, 107.48, 38.50, 38.44, 37.11, 25.89, 24.90, 24.10, 22.48, 22.45, 22.37. The ^{13}C NMR spectrum indicates 9 resonances in the aliphatic region, 23 resonances in the region (100–150 ppm), 3 resonances assigned to acetyl carbonyl groups and 1 additional carbonyl resonance (C(5)=O) in agreement with the measured molar mass (MM = 648 g), elemental formula ($C_{36}H_{36}N_6O_6$) and proposed structure **16**.



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Tryptamine-4,5-dione (**5**)

Compound **5** has a very characteristic spectrum (λ_{max} at pH 2.0–7.2: 535, 350, 232 nm). CV shows a reversible reduction–oxidation couple at $E^{\circ\prime}$ = −0.205 V at pH 7.2. This compound is a major product of electrochemical oxidation of 5-HT at pH 2 and has been previously characterized as a quinoxaline derivative after trapping with *o*-phenylenediamine (*6d*).

Spiro[[1,3]dioxepino[5,4-e:6,7-e′]diindole-4,4′(5′H)-pyrrolo[2,3-b]indol]-5′-one, 10,11-bis(2-aminoethyl)-1′,2′,3′,8,8a′,13-hexahydroindole (**6**)

Compound **6** (λ_{max} at pH 7.2: ~400 br low, 272, 218 nm) was moderately stable in aqueous solutions at pH 2–8. However, at pH <6 it slowly decomposed to **1** and several unidentified products. Attempts to isolate **6** in a pure form were not successful apparently because of polymerization reactions. However, FAB-MS (3-nitrobenzyl alcohol matrix) of impure samples of **6** gave an intense ion at *m/e* = 523. LC–MS showed intense ions at *m/e* = 525, 351, 193, and 177. Cyclic

voltammetry at pH 7.2 showed a reduction peak at −0.11 V and, after scan reversal, a quasi-reversible oxidation peak at −0.07 V. Compound **6** is thus a very easily reduced compound and hence it may be concluded that its molar mass is 520 g (*14*) and that this compound is some form of oxidized trimer (see later discussion).

[4,4'-Bi-1H-indol]-5-ol, 3,3'-Bis(2-aminoethyl)-5'-[[3-(2-aminoethyl)-5-hydroxy-1H-indol-4-yl]oxy]indole (**7**)

Compound **7** was a white solid (λ_{max} (log ε_{max}) at pH 7.2: 297 (4.08), 268 (4.08), 224 (4.69) nm). FAB-MS (3-nitrobenzyl alcohol matrix) gave a pseudomolecular ion (MH^+, 100%) at *m/e* = 525.2632 ($C_{30}H_{33}N_6O_3$ calcd, *m/e* = 525.2614). Hence **7** is a trimer (MM = 524 g, $C_{30}H_{32}N_6O_3$). ^1H NMR (Me_2DO-d_6) δ 11.02 (s, 1H, N(1)-H), 10.95 (s, 1H, N(1)-H), 10.91 (s, 1H, N(1)-H), 8.55 (br s, 1H, OH), 8.10 (br s, 1H, OH), 7.60 (br s, ~9H, 3 = NH_3^+), 7.21 (d, $J_{6,7}$ = 8.7 Hz, 1H, C(7)-H), 7.18 (s, 2H, 2 × C(2)-H), 7.11 (s, 1H, C(2)-H), 7.10 (d, $J_{6,7}$ = 8.7 Hz, 1H, C(7)-H), 7.06 (d, J = 8.7 Hz, 1H, C(7)-H), 6.89 (d, $J_{6,7}$ = 8.7 Hz, 1H, C(6)-H), 6.72 (d, $J_{6,7}$ = 8.7 Hz, 1H, C(6)-H), 6.33 (d, $J_{6,7}$ = 8.7 Hz, 1H, C(6)-H), 2.91 (m, 2H, CH_2), 2.82 (m, 2H, CH_2), 2.69 (m, 2H, CH_2), 2.33 (m, 2H, CH_2), 2.25 (m, 2H, CH_2), 2.11 (m, 2H, CH_2). Addition of a few drops of D_2O caused the resonances at 11.02, 10.95, 10.91, 8.55, 8.10, and 7.60 ppm to disappear. Thus, in trimer **7** the C(4)-protons are missing in all three 5-HT residues and only two OH residues are present in accord with the proposed structure.

[4,2'-Bi-1H-indol]-5-ol, 3,3'-Bis(2-aminoethyl)-5'-[[3-(2-aminoethyl)-5-hydroxy-1H-indol-4yl]oxy]indole (**8**)

HPLC peak **8** was collected, concentrated by freeze-drying, and desalted by Method B. Under the latter conditions two chromatographic fractions separated at retention times (t_R) of 11.5 h (**8A**) and 13.5 h (**8**). Compound **8**, a minor product, was a white solid (λ_{max} at pH 7.2: 292, 268, 219 nm). FAB-MS (3-nitrobenzyl alcohol matrix) gave *m/e* = 525.2636 (MH^+, 100%; $C_{30}H_{33}N_6O_3$ calcd, *m/e* = 525.2614). Thus **8** is a simple trimer of 5-HT (MM = 524 g, $C_{30}H_{32}N_6O_3$). ^1H NMR (Me_2DO-d_6) δ 11.00 (s, 1H, N(1')-H), 10.86 (s, 1H, N(1")-H), 10.83 (s, 1H, N(1)-H), 8.74 (br s, 1H, OH), 7.57 (br s, ca. 10H, 3 × NH_3^+, OH), 7.28 (d, $J_{6,7}$ = 8.8 Hz, 1H, C(7)-H), 7.19 (s, 1H, C(2")-H), 7.15 (s, 1H, C(2')-H), 7.06–7.02 (3 × d, J = 8.8 Hz, 3H, C(6)-H, C(7')-H, C(7")-H), 6.98 (d, $J_{4,6}$ = 2.1 Hz, 1H, C(4)-H), 6.92 (d, $J_{6",7"}$ = 8.8 Hz, 1H, C(6")-H), 6.43 (d, $J_{6',7'}$ = 8.8 Hz, 1H, C(6')-H). The region between 2.9 and 2.7 ppm consisted of several overlapping multiplets (12H) which could not be resolved. ^1H NMR (D_2O) δ 7.46 (d, $J_{6,7}$ = 8.8 Hz, 1H, C(7)-H), 7.24 (d, $J_{6",7"}$ = 8.8 Hz, 1H, C(7")-H), 7.21 (s, 1H, C(2")-H), 7.20 (d, $J_{6',7'}$ = 9.0 Hz, 1H, C(7')-H), 7.06 (s, 1H, C(2')-H), 7.03 (dd, $J_{6,7}$ = 8.7 Hz, $J_{4,6}$ = 2.4 Hz, 1H, C(6)-H), 6.91 (d, $J_{4,6}$ = 2.4 Hz, 1H, C(4)-H), 6.88 (d, $J_{6",7"}$ = 8.8 Hz, 1H, C(6")-H), 6.56 (d, $J_{6',7'}$ = 9.0 Hz, 1H, C(6')-H), 3.14 (t, 2H, CH_2), 3.07 (t, 2H, CH_2), 2.90 (t, 2H, CH_2), 2.82 (t, 2H, CH_2), 2.64 (t, 2H, CH_2), 2.53 (m, 2H, CH_2). Thus, in trimer **8** one 5-HT residue has the C(4)-, C(6)-, and C(7)-positions

unsubstituted but a C(2)-proton is missing. The two other 5-HT residues are linked via their C(4)-positions.

[4,2':4',4"-Ter-1H-indol]-5,5',5"-triol, 3,3',3"-Tris(2-aminoethyl)-6'-[[3-(2-aminoethyl)-1H-indol-4-yl]oxy]indole (**8A**)

Compound **8A** was a white solid (λ_{max} at pH 7.2: 301, 276, 221 nm). FAB-MS (3-nitrobenzyl alcohol matrix) gave *m/e* = 699.3400 ($C_{40}H_{41}N_4O_4$ calcd, *m/e* = 699.3407). ^{1}H NMR (Me_2SO-d_6) δ 11.00 (s, 1H, N(1)-H), 10.94 (s, 1H, N(1')-H), 10.91 (s, 1H, N(1")-H), 10.73 (s, 1H, N(1"')-H), 7.8 (vbr s ca. 12H, 4 = NH_3^+), 7.02 (d, $J_{6',7'}$ = 8.7 Hz, 1H, C(7')-H), 7.01 (s, 1H, C(2')-H), 6.98 (s, 1H, C(4')-H), 6.94 (dd, $J_{6',7'}$ = 8.7 Hz, $J_{4',6'}$ = 2.4 Hz, 1H, C(6')-H), 6.90 (s, 1H, C(2"')-H), 6.83 (s, 1H, C(2")-H), 6.79 (d, $J_{6",7"'}$ = 8.7 Hz, 1H, C(7"')-H), 6.70 (d, $J_{6",7"}$ = 8.7 Hz, 1H, C(7")-H), 6.62 (d, $J_{6,7}$ = 8.7 Hz, 1H, C(7)-H), 6.45 (d, $J_{6"',7"'}$ = 8.7 Hz, 1H, C(6"')-H), 6.42 (d, $J_{6",7"}$ = 8.7 Hz, 1H, C(6")-H), 6.31 (d, $J_{6,7}$ = 8.7 Hz, 1H, C(6)-H), 2.91 (m, 4H, 2 × CH_2), 2.72 (m, 4H, 2 × CH_2), 2.39 (m, 4H, 2 × CH_2), 2.04 (m, 2H, CH_2), 1.69 (m, 2H, CH_2). Addition of D_2O caused the resonances at 11.00, 10.94, 10.91, 10.73, and 7.80 ppm to disappear. The MS and NMR result clearly indicated that **8A** is a tetramer and are in accord with the structure proposed.

5-[[3-(2-Aminoethyl)-1H-indol-4-yl]oxy]-3-(2-aminoethyl)-1H-indole (**9**)

Compound **9** was a white solid (λ_{max} (log ε_{max}) at pH 7.0: 289 (3.94), 268 (4.03), 218 (4.63) nm). FAB-MS (dithioerythritol/dithiothreitol matrix) gave *m/e* = 351.1832 (100%, MH^+; $C_{20}H_{23}N_4O_2$ calcd *m/e* = 351.1821). ^{1}H NMR (Me_2SO-d_6) δ 10.87 (d, $J_{1',2'}$ = 2.1 Hz, 1H, N(1')-H), 10.84 (d, $J_{1,2}$ = 2.1 Hz, 1H, N(1)-H), 8.55 (br s, 1H, OH), 7.99 (br s, 3H, NH_3^+), 7.23 (d, $J_{6',7'}$ = 8.7 Hz, 1H, C(7')-H), 7.19 (d, $J_{1,2}$ = 2.1 Hz, 1H, C(2)-H), 7.18 (br s, 3H, NH_3^+), 7.10 (d, $J_{6,7}$ = 8.7 Hz, 1H, C(7)-H), 7.06 (d, $J_{1',2'}$ = 2.1 Hz, 1H, C(2')-H), 6.90 (d, $J_{4',6'}$ = 2.1 Hz, 1H, C(4')-H), 6.83 (d, $J_{6,7}$ = 8.7 Hz, 1H, C(6)-H), 6.68 (dd, $J_{6',7'}$ = 8.7 Hz, $J_{4',6'}$ = 2.4 Hz, 1H, C(6')-H), 2.95 (m, 4H, 2 × CH_2), 2.85 (m, 2H, CH_2), 2.78 (m, 2H, CH_2). ^{1}H NMR (D_2O) δ 7.45 (d, $J_{6,7}$ = 8.7 Hz, 1H, C(7)-H), 7.32 (d, $J_{6',7'}$ = 8.7 Hz, 1H, C(7')-H), 7.24 (s, 1H, C(2)-H), 7.17 (s, 1H, C(2')-H), 7.00 (d, $J_{6,7}$ = 8.7 Hz, 1H, C(6)-H), 6.98 (d, $J_{6',7'}$ = 8.7 Hz, 1H, C(6')-H), 6.76 (d, $J_{4',6'}$ = 2.4 Hz, 1H, C(4')-H), 3.10 (m, 4H, 2 × CH_2), 2.90 (t, 2H, CH_2), 2.75 (t, 2H, CH_2). ^{13}C NMR (D_2O) δ 155.74, 143.99, 136.45, 136.15, 135.06, 129.70, 128.70, 128.35, 123.82, 116.03, 114.07, 112.51, 111.77, 111.74, 110.57, 103.96, 43.23, 42.41, 26.56, 25.19. The ^{13}C NMR data confirm the FAB-MS result that **9** contains 20 carbon atoms (4 aliphatic and 16 aromatic). ^{1}H NMR spectra indicate the absence of a C(4)-proton in only one 5-HT residue of **9** and the absence of an OH proton in the second residue. CV of **9** at pH 7.2 (ν = 200 mV s^{-1}) showed an irreversible oxidation peak at E_p = 0.26 V. After scan reversal a reversible reduction/oxidation couple appeared at $E^{\circ\prime}$ = −0.205 V. This corresponds to the **5**/4,5-dihydroxytryptamine (**31**) couple. Controlled potential electrooxidation of **9** at 0.22 V at pH 7.2 caused the colorless solution to become pink-purple. HPLC analysis showed that **1**, 5-HT, and **5** were the major products of this electrooxidation.

Spiro[[1,3]dioxepino[5,4-e:6,7-e']diindole-4,4'(5'H)-pyrrolo[2,3-b]indol]-5-ol, 10, 11-Bis(2-aminoethyl)-1',2',3',8,8',8a',13-heptahydroindole (**10**) *and Spiro[[1,3]dioxepino[5,4-e:6,7-e']diindole-4,4'(5'H)-pyrrolo[2,3-b]indol]-5-one, 10,11-Bis(2-aminoethyl)-1',2',3',8,8a',13-hexahydroindole* (**11**)

Compound **10** (λ_{max} at pH 2.0–7.2: 293, 268, 212 nm) and compound **11** (λ_{max} at pH 7.2: ca. 400 br low, 293 sh, 268, 213 nm) comprise the components of a quasi-reversible redox system. Compound **10** exhibits an oxidation peak at 0.04 V (ν = 200 mV s^{-1}) at pH 7.2 and **11** a reduction peak at −0.05 V. Controlled potential electrooxidation of **10** at 0.30 V converted it quantitatively into **11**. Conversely, electroreduction of **11** at −0.30 V resulted in its quantitative conversion to **10**. LC–MS results were identical for solutions of **10** and **11** with major peaks at *m/e* = 525, 351, 193, and 177.

Compounds **10** and **11** are minor products of electrooxidation of 5-HT. Compound **11** could not be isolated in pure form because of its apparent tendency to polymerize to a dark brown uncharacterized material. A very small quantity of **10** was isolated, however: ^{1}H NMR (D_2O) δ 7.55 (d, $J_{6'',7''}$ = 8.7 Hz, 1H, C(7'')-H), 7.36 (s, 1H, C(2')-H), 7.15 (s, 1H, C(2)-H), 7.09 (d, $J_{6',7'}$ = 8.7 Hz, 1H, C(6'')-H), 7.02 (d, $J_{6',7'}$ = 8.8 Hz, 1H, C(7')-H), 6.89 (d, $J_{6,7}$ = 8.6 Hz, 1H, C(7)-H), 6.84 (d, $J_{6',7'}$ = 8.8 Hz, 1H, C(6')-H), 6.58 (d, $J_{6,7}$ = 8.6 Hz, 1H, C(6)-H), 5.82 (s, 1H, C(2'')-H), 3.49 (m), 3.09 (m), 2.71 (m), 2.45 (m), 2.28 (m), 2.16 (m), 1.85 (m), 1.74 (m). The resonances between 3.5 and 1.7 ppm were complex and overlapped and could not be fully resolved.

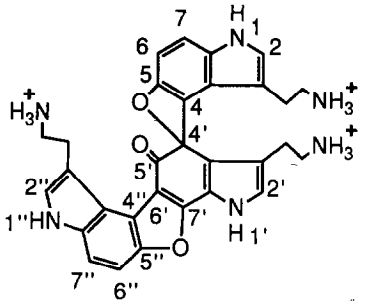
[4,2'-Bi-1H-indole]-5,5'-diol, 3,3'-Bis(2-aminoethyl)-4'-[[3-(2-aminoethyl)-1H-indol-5-yl]oxy]indole (**12**)

Compound **12** was a white solid (λ_{max} (log ε_{max}) at pH 7.2: 293 (4.17), 269 (4.21), 216 (4.69) nm). FAB-MS (3-nitrobenzyl alcohol matrix) gave *m/e* = 525.2595 (MH^+, 100%; $C_{30}H_{33}N_6O_3$ calcd, *m/e* = 525.2614). ^{1}H NMR (Me_2SO-d_6) δ 11.08 (s, 1H, N(1)-H), 10.99 (s, 1H, N(1)-H), 10.89 (s, 1H, N(1)-H), 7.23 (d, $J_{6'',7''}$ = 8.7 Hz, 1H, C(7'')-H), 7.18 (d, $J_{6',7'}$ = 8.7 Hz, 1H, C(7')-H), 7.17 (s, 1H, C(4'')-H), 7.13 (s, 1H, C(2')-H), 7.08 (s, 1H, C(2'')-H), 7.05 (d, $J_{6,7}$ = 8.7 Hz, 1H, C(7)-H), 7.02 (d, $J_{6'',7''}$ = 8.7 Hz, 1H, C(6'')-H), 6.80 (d, $J_{6',7'}$ = 8.7 Hz, 1H, C(6')-H), 6.42 (d, $J_{6,7}$ = 8.7 Hz, 1H, C(6)-H), 3.40 (m, 4H, 2 × CH_2), 2.99 (m, 4H, 2 × CH_2), 2.84 (m, 2H, CH_2), 2.68 (m, 2H, CH_2). ^{1}H NMR (D_2O) δ 7.43 (d, $J_{6'',7''}$ = 8.7 Hz, 1H, C(7'')-H), 7.23 (s, 1H, C(4'')-H), 7.20 (s, 1H, C(2')-H), 7.18 (d, $J_{6',7'}$ = 8.7 Hz, 1H, C(7')-H), 7.14 (d, $J_{6,7}$ = 8.7 Hz, 1H, C(7)-H), 7.04 (s, 1H, C(2'')-H), 7.00 (dd, $J_{6'',7''}$ = 8.7 Hz, $J_{4'',6''}$ = 2.4 Hz, 1H, C(6'')-H), 6.86 (d, $J_{6',7'}$ = 8.7 Hz, 1H, C(6')-H), 6.52 (d, $J_{6,7}$ = 8.7 Hz, 1H, C(6)-H), 3.12 (t, 2H, CH_2), 3.06 (t, 2H, CH_2), 2.88 (t, 2H, CH_2), 2.82 (t, 2H, CH_2), 2.61 (t, 2H, CH_2), 2.52 (m, 2H, CH_2). ^{13}C NMR (D_2O) δ 156.18, 147.07, 143.25, 137.05, 136.61, 136.39, 135.39, 129.68, 129.47, 128.84, 128.36, 124.44, 123.75, 116.60, 115.95, 115.63, 114.10, 113.25, 113.21, 112.14, 111.77, 110.88, 110.20, 104.34, 43.22, 42.72, 42.39, 26.56, 26.31, 25.19. The ^{13}C NMR spectrum indicates 24 aromatic carbon resonances and 6 aliphatic carbon resonances confirming that **12** is a simple trimer (MM = 524 g; $C_{30}H_{32}N_6O_3$). CV of **12** at pH 7.2 (ν = 200 mV s^{-1}) showed an oxidation peak at 0.28 V. After scan

reversal a quasi-reversible reduction/oxidation couple appeared at 0.23 V followed by a second reduction/oxidation couple at $E^{\circ\prime} = -0.19$ V. The latter occurs close to that expected for the **5/31** couple.

Spiro[furo[3,2-e:4,5-g']diindole-4(5H),1'(5'H)-oxeto[3,2-e]indol]-5-one, 3,6,7'-Tris(2-aminoethyl)-1,8-dihydroindole (**13**)

Compound **13** was a bright yellow solid (λ_{max} at pH 7.2: 385, 342 sh, 300, 275, 254 nm). In acid solution (pH ≤5) **13** was very unstable; its decomposition products remain to be identified. FAB-MS (3-nitrobenzyl alcohol matrix) gave *m/e* = 521.2324 (MH^+, 100%; $C_{30}H_{29}N_6O_3$ calcd, *m/e* = 521.2301). ^{1}H NMR ($HCONMe_2$-d_7) δ 11.48 (s, 1H, N(1)-H), 11.14 (s, 1H, N(1″)-H), 10.31 (s, 1H, N(1′)-H), 8.53 (br s, 3H, NH_3^+), 8.00 (br s, 2 × NH_3^+; DMF), 7.35 (d, $J_{1'',2''}$ = 2.0 Hz, 1H, C(2″)-H), 7.31 (d, $J_{1,2}$ = 2.0 Hz, 1H, C(2)-H), 7.23 (d, $J_{6,7}$ = 8.6 Hz, 1H, C(7)-H), 6.64 (s, 1H, C(2′)-H), 6.55 (d, $J_{6,7}$ = 8.6 Hz, 1H, C(6)-H), 3.45 (m, 2H, CH_2), 3.20 (m, 2H, CH_2). The region between 3 and 2 ppm was overlapped by peaks due to DMF. ^{1}H NMR (D_2O) δ 7.64 (d, $J_{6'',7''}$ = 9.0 Hz, 1H, C(7″)-H), 7.52 (d, $J_{6'',7''}$ = 9.0 Hz, 1H, C(6″)-H), 7.37 (s, 1H, C(2″)-H), 7.31 (d, $J_{6,7}$ = 8.7 Hz, 1H, C(7)-H), 7.11 (s, 1H, C(2)-H), 6.58 (s, 1H, C(2′)-H), 6.51 (d, $J_{6,7}$ = 8.7 Hz, 1H, C(6)-H), 3.27 (m, 2H, CH_2), 3.10 (m, 2H, CH_2), 3.03 (m, 2H, CH_2), 2.80 (m, 2H, CH_2), 2.40 (m, 2H, CH_2), 2.17 (m, 2H, CH_2). CV of **13** at pH 7.2 (ν = 200 mV s^{-1}) showed an irreversible oxidation peak at 0.31 V on the initial anodic scan. On the first cathodic scan a single, irreversible reduction peak was observed at −1.10 V. The ^{1}H NMR and MS data indicate that **13** is a trimer (MM = 520 g; $C_{30}H_{28}N_6O_3$) and that one indolic residue has no C(4)-, C(6)-, or C(7)-protons. The remaining two 5-HT residues each contain C(6)- and C(7)-protons but no C(4)-protons. The lability of **13** in acid solution and its voltammetric reduction peak are all in accord with the proposed structure. Unfortunately, only very small quantities (<1 mg) of **13** could be isolated and hence more detailed structural studies were not possible.

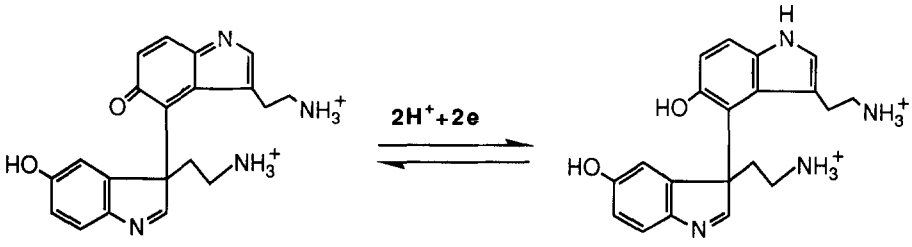


13

Spiro[3H-indole-3,9'-[9H]pyrrolo[3,2-f]quinoline]-5,6-diol, 1'-(2-Aminoethyl)-7',8-dihydroindole (**14**)

Compound **14** was a pale pink solid (λ_{max} at pH 7.2: 450, 335 sh, 293 sh, 260 nm). LC–MS showed intense ions at *m/e* = 347 and 349. CV of **14** at pH 7.3 and

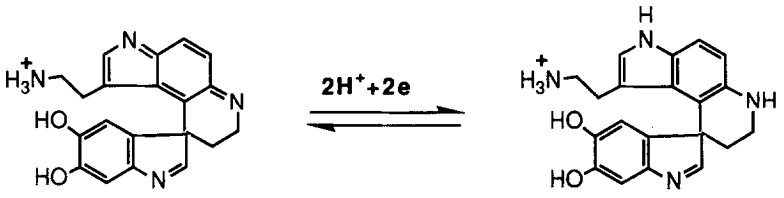
pH 4.3 showed it to be reversibly reducible with $E^{\circ\prime} = -0.022$ and 0.175 V, respectively. The ease of reduction of **14** suggests that under LC–MS conditions the ion at $m/e = 347$ is the pseudomolecular ion (MH^+) (*14*). Thus, **14** has a molar mass of 346 g. Attempts to isolate **14** were not successful because of its tendency to polymerize. Accordingly, **14**, dissolved in the HPLC mobile phase adjusted to pH 2.1 with HCl, was electrochemically reduced (−0.10 V). The reduced form of **14** was desalted using Method C. It was possible to obtain NMR spectra on this compound (**17**). However, only very small quantities (<1 mg) of **17** were obtained for such studies. ^{1}H NMR (Me_2SO-d_6) δ 11.38 (d, $J_{1',2'} = 2.1$ Hz, 1H, N(1′)-H), 11.00 (br s, 1H, N(7′)-H), 8.99 (s, 1H, OH), 8.27 (br s, 3H, NH_3^+), 7.56 (br s, 1H, OH), 7.50 (d, $J_{1',2'} = 2.1$ Hz, 1H, C(2′)-H), 7.28 (d, $J_{6',7'} = 8.7$ Hz, 1H, C(7′)-H), 6.91 (s, 1H, C(7)-H), 6.77 (d, $J_{6',7'} = 8.7$ Hz, 1H, C(6′)-H), 6.53 (s, 2H, C(2)-H + C(4)-H), 3.43 (m, 2H, CH_2), 3.25 (m, 4H, 2 × CH_2), 2.86 (m, 1H), 2.70 (m, 1H). ^{1}H NMR (D_2O) δ 7.46 (s, 1H, C(2′)-H), 7.39 (d, $J_{6',7'} = 8.7$ Hz, 1H, C(7′)-H), 6.95 (s, 1H, C(7)-H), 6.85 (d, $J_{6',7} = 8.7$ Hz, 1H, C(6′)-H), 6.72 (s, 2H, C(2)-H + C(4)-H), 3.55 (m, 2H), 3.47 (m, 3H), 3.38 (m, 1H), 2.91 (t, 2H). The MS results indicate that **14** is a dimeric species (MM = 346 g). ^{1}H NMR spectra on the reduced form of **14**, i.e., **17**, indicate that one indolic residue has lost a C(6)-proton and N(1)-proton. In addition only one NH_3^+ residue remains in the dimeric structure. One indolic residue has lost a C(4)-proton whereas in the other it remains but as a singlet (i.e., the long-range coupling with the C(6)-proton is absent). At pH 2.0 the spectrum of **14** (λ_{max}: 450, 293 sh, 260 nm) is virtually identical to that of 3-(2-aminoethyl)-3-[3′-(2-aminoethyl)-5′-carbonylindol-4′-yl]-5-hydroxyindolenine (**18**) (*6d*). Similarly, the spectrum of **17** (λ_{max} at pH 2.0: 312, 300, 283, 217 sh nm) is very similar to the reduced form of **18**, i.e., **19** (λ_{max}: 308, 281, 217 sh nm).



18 **19**

CV of **18** at pH 7.3 and pH 4.3 shows reversible reduction/oxidation couples at $E^{\circ\prime} = -0.035$ and 0.163 V, respectively. Compound **14**, however, is quite stable in solution between pH 2 and pH 7.2, whereas **18** slowly decomposes to a mixture of products at low pH (*6d*), a process which becomes very fast at neutral pH. The major product of decomposition of **18** is 4-[4′-(6′-hydroxyquinolyl)]-5-hydroxytryptamine (*6d*), formed by a rather complex reaction pathway which involves opening of the N(1) = C(2) double bond and subsequent cyclization of the aminoethyl side chain. Compound **14** does not decompose in acid or neutral solution to an analogous quinoline–indole dimer and, upon the basis of ^{1}H NMR data, this is probably because it lacks the necessary aminoethyl

side chain. These observations are in accord with the properties expected for the structure proposed for **14**.



14 **17**

Reduction of **14** would be expected to yield **17**. It must be stressed, however, that while the spectral and chemical properties of **14** and **17** are in agreement with the structures proposed, these structures can only be regarded as tentative at this time.

RESULTS

Linear Sweep and Cyclic Voltammetry

At a slow sweep rate (ν = 5 mV s^{-1}) and low concentration (<50 μM) linear sweep voltammetry of 5-HT at pH 7.2 shows oxidation peak I_a followed at more positive potential by peak III_a (Fig. 1A). At higher 5-HT concentrations peak II_a appears and grows relative to peak I_a (Figs. 1B and 1C). At 5-HT concentrations <50 μM peak I_a can be observed without interference from peak II_a at values of ν as high as 100 V s^{-1}. However, at higher 5-HT concentrations peak II_a appears and merges with peak I_a at much smaller values of ν. For example, using 1.0 mM 5-HT peak I_a can be observed as a separate peak at $\nu < 50$ mV s^{-1}. Thus, there is only a very limited range of experimental conditions (i.e., C and ν) where peak I_a can be observed. Under such conditions the experimental peak I_a current function ($i_p/AC\nu^{1/2}$) (*17*) increased with $\nu^{1/2}$ at all 5-HT concentrations studied (35 μM to 6 mM) and the i_p/C ratio decreased, indicating adsorption of 5-HT at the PGE (*18*). Cyclic voltammograms of a very dilute solution of 5-HT (35 μM) are shown in Fig. 2. Under such conditions interferences due to peak II_a are minimal. At relatively slow sweep rates (ν = 200 mV s^{-1}; Fig. 2A) after scanning through oxidation peak I_a a well-defined reduction/oxidation couple (peaks V_c/I_a') appears after scan reversal. With an increasing sweep rate, however, reduction peak I_c coupled to peak I_a appears and grows. Correspondingly the peaks V_c/I_a' couple decreases and disappears (Figs. 2B and 2C). These results indicate that the species responsible for peak I_c is the precursor of that responsible for the peaks V_c/I_a' couple. Strong adsorption of 5-HT precluded more detailed analysis of these cyclic voltammograms. A cyclic voltammogram of a solution containing much higher concentration of 5-HT is shown in Fig. 3. Under these conditions peaks I_a and II_a are merged. After scan reversal many reduction peaks appear, several of which

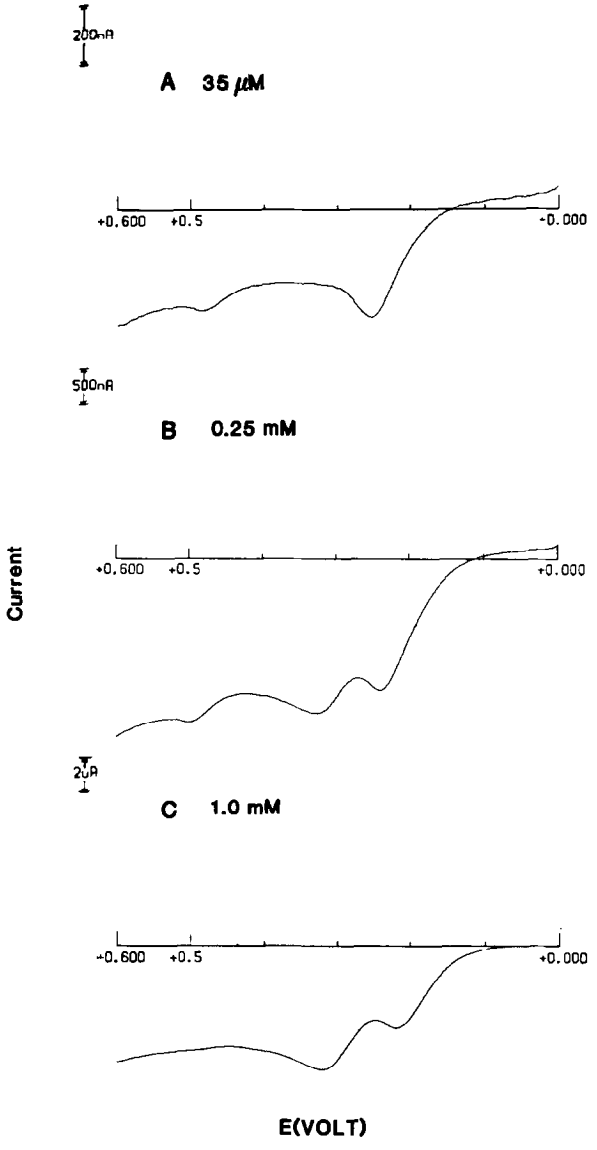


FIG. 1. Linear sweep voltammograms at the PGE of (A) 35 μM, (b) 0.25 mM, and (C) 1.0 mM 5-hydroxytryptamine in pH 7.2 phosphate buffer (μ = 0.5). Sweep rate: 5 mV s^{-1}.

form reversible couples with oxidation peaks observed on the second anodic sweep.

Controlled Potential Electrooxidation of 5-HT

Electrooxidation at peak I_a potentials causes the characteristic uv bands of 5-HT (λ_{max} at pH 7.2: 290 sh, 273, 214 nm) to decrease and new bands grow in at 370 and 244 nm. The solution becomes increasingly yellow as the oxidation

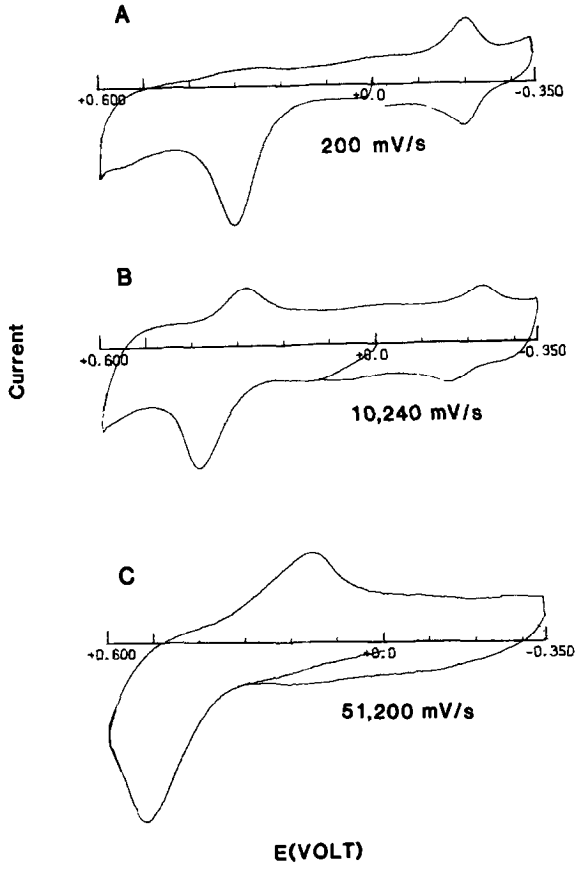


FIG. 2. Cyclic voltammograms at the PGE of 35 μM 5-hydroxytryptamine in pH 7.2 phosphate buffer (μ = 0.5) at sweep rates of (A) 200, (B) 10,240, and (C) 51,200 mV s^{-1}. All voltammograms were initiated at 0.00 V with the initial sweep toward positive potentials.

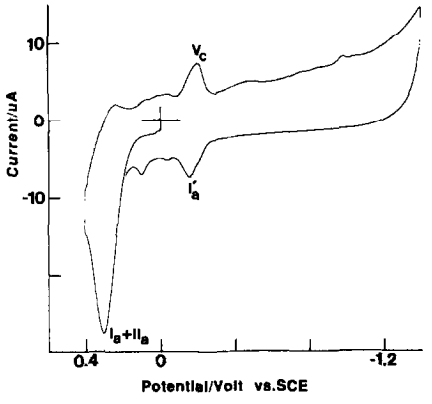


FIG. 3. Cyclic voltammogram at the PGE of 0.25 mM 5-hydroxytryptamine in pH 7.2 phosphate buffer (μ = 0.5). Sweep rate: 200 mV s^{-1}.

TABLE 1

Coulometric n Values for Electrooxidation of 5-Hydroxytryptamine in pH 7.2 Phosphate Buffer (μ = 0.5)

Concentration[a] of 5-HT (mM)	Applied potential (V)	n-Value[b]
0.04	0.22	1.12
0.10	0.22	1.08
0.40	0.22	1.06
1.00	0.22	1.18
0.04	0.26	1.19
0.60	0.26	1.14
1.00	0.26	1.16

[a] 5-HT was only partially oxidized (5–45 min). The unreacted 5-HT was determined by HPLC analysis.

[b] Average of at least duplicate measurements. All results were ±0.1.

progresses. At all potentials corresponding to peak I_a electrolyses were slow because of severe electrode filming by oxidation products. Accordingly, it was necessary to periodically interrupt electrolyses in order to clean the working electrode. Electrolyses of ≥ 1 mM 5-HT solutions resulted in formation of some black, presumably polymeric material, which was insoluble in water or any common organic solvent. Following exhaustive electrooxidation of 1 mM 5-HT at 0.210 V (E_p for peak I_a at ν = 5 mV s^{-1}) LSV revealed that peak I_a had disappeared whereas peak II_a remained. Coulometric n values were measured by partially electrolyzing solutions of 5-HT for periods ranging from 5 to 45 min. Unreacted 5-HT was assayed by HPLC analysis. Experimental results (Table 1) revealed that under these conditions 1.1–1.2 electrons per molecule of 5-HT oxidized are transferred. Precise n values could not be measured when exhaustive electrolyses were carried out because of the necessity for periodic electrode resurfacing. However, it was apparent that relatively long exhaustive electrolyses (~24 h) resulted in an appreciable increase in the experimental n value.

HPLC Analysis

HPLC analysis of a partially electrooxidized solution of 5-HT at peak I_a shows a complex mixture of products (Fig. 4). Analysis of product mixtures formed at potentials ranging from 0.20 to 0.30 V revealed that with increasingly positive potentials the yields of **2–7**, **11**, and **13** increased. Electrolysis of 5-HT at concentrations >1 mM resulted in a significant increase in the yields of **3**, **4**, **9**, **12**, and **13**.

Product Identifications and Reaction Pathways

The major product of electrooxidation of 5-HT at peak I_a potentials is 5,5′-dihydroxy-4,4′-bitryptamine (**1**). Approximate yields of **1** range from ≥70% (electrolysis of 15 μM to 0.2 mM 5-HT) to ≥25% (electrolysis of ≥2 mM 5-HT). Dimer

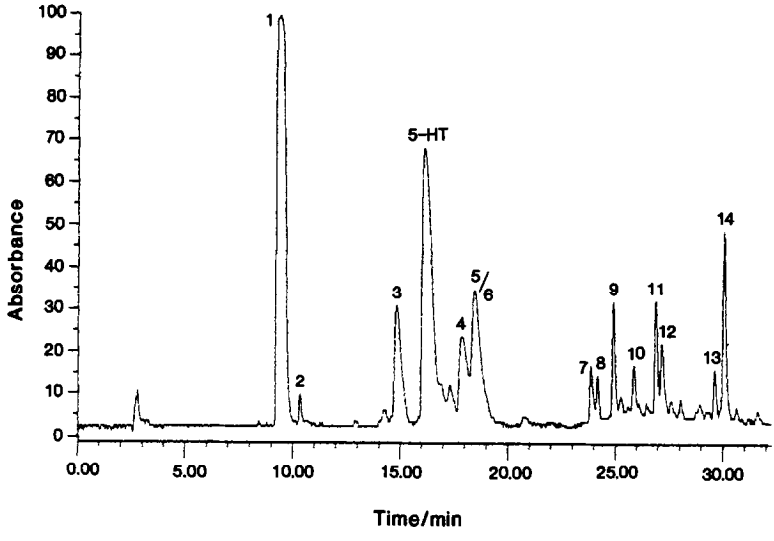
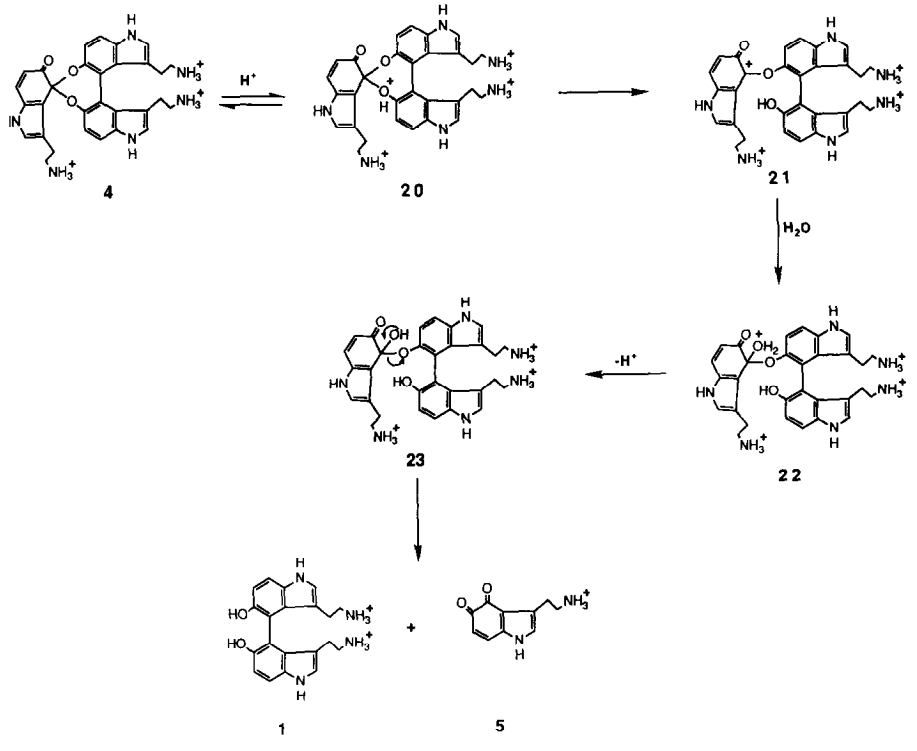


FIG. 4. High-performance liquid chromatogram of the product mixture formed as a result of controlled potential electrooxidation of 1.1 mM 5-hydroxytryptamine in pH 7.2 phosphate buffer (μ = 0.2) at 0.280 V. Chromatographic conditions are given under Experimental Section.

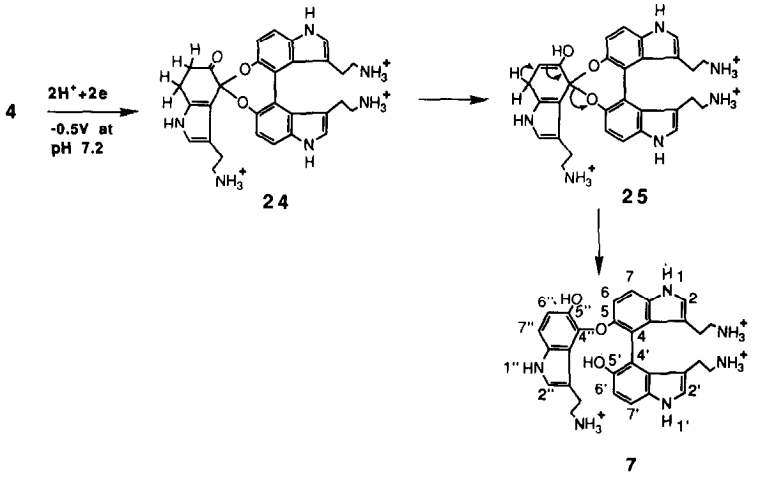
1 shows a voltammetric oxidation peak at E_p = 0.38 V (pH 7.1; ν = 5 mV s^{-1}). This is a broad peak and hence at all potentials employed to electrooxidize 5-HT some oxidation of **1** was inevitable. Controlled potential electrooxidation of **1** (0.35 V, pH 7.2) caused the colorless solution to become bright yellow. The major product of this oxidation was **2** (30–40% yield) along with **5** and several other minor products which have yet to be identified.

Compound **4** is not stable in acidic aqueous solution and attempts to isolate this product were unsuccessful. However, a compound having similar properties to **4** is formed upon electrooxidation of NAc-5-HT and has structure **16**. Confirmation that **4** has such a dioxepin structure is provided by the products formed as a result of its acid hydrolysis and electrochemical reduction. At pH ≥4 the yellow solution of **4** develops a pink color (≤30 min). HPLC analysis reveals that **1** and **5** are the major products formed. Acid hydrolysis of **4** hence follows a route typical of structurally related acetals (*13*). Protonation of **4** results in cleavage of one spiro ether linkage yielding cation **21** (Scheme I). Nucleophilic attack by water yields **23** which decomposes to the observed mixture of **1** and **5**. Controlled potential electroreduction of **4** (−0.50 V; pH 7.2) caused the yellow solution to become colorless. The product of this reaction (≥80% yield) was trimer **7** formed by a route expected for reduction of such an α,β-unsaturated ketone (*12*) and outlined in Scheme II.

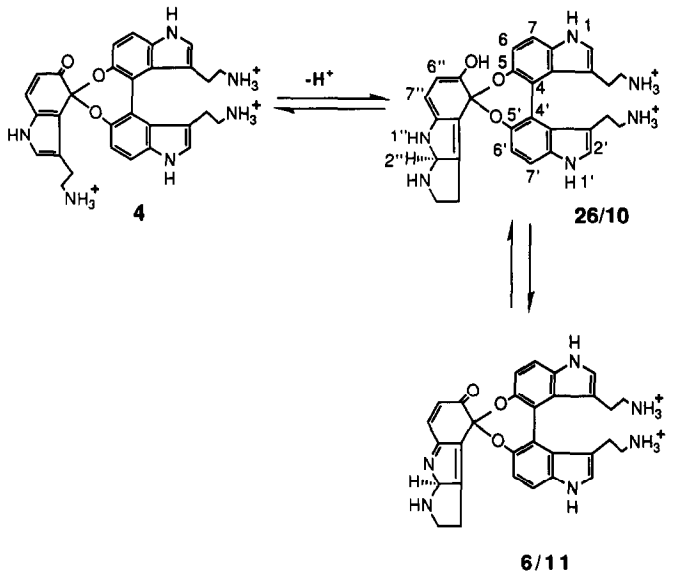
Compounds **6** and **11** could not be isolated but are both formed by spontaneous transformation of **4** in neutral solution. Compound **6** (λ_{max} at pH 7.2: ~400, 272, 218 nm) and **11** (λ_{max} at pH 7.2: ~400, 268, 213 nm) have very similar spectra. Compound **6** exhibits a quasi-reversible reduction peak ($E^{\circ\prime}$ = −0.09 V at pH



SCHEME I



SCHEME II



SCHEME III

7.2); compound **10** exhibits a quasi-reversible reduction peak ($E^{\circ\prime}$ = −0.005 V at pH 7.2). Controlled potential electroreduction of **6** (−0.25 V; pH 7.2) gives a product with λ_{max} = 293, 275 nm. Controlled potential electroreduction of **11** (−0.30 V; pH 7.2) gives **10** (λ_{max}: 293, 268 nm). The spectra of the reduced forms of **6** and **11** are typical of many simple dimers and trimers of 5-HT. LC–MS of both **6** and **11** were identical. The uv–visible spectra of **6** and **11** exhibit very weak bands at ca. 400 nm and very intense bands at about 270 nm at pH 7.2, characteristic of compounds containing a quinone imine residue (*19*). At pH ≤4 compound **6** slowly decomposes to give dimer **1** as the major identified product. In addition LC–MS of both **6** and **11** using an acidic mobile phase show intense peaks characteristic of **1** (*m/e* = 351). Taken together these results indicate that **6** and **11** are isomers (MM = 520 g) which differ from **4** (MM = 522 g), from which they are formed, by two mass units. Thus, at pH 7.2 there must be sufficient unprotonated side chain amino group in the indolone residue of **4** such that cyclization occurs to give diastereomers **10** and **26** which are autoxidized to diastereomers **6** and **11**, respectively (Scheme III). Molecular models of the latter four structures reveal that the two indolic residues are not equivalent. As a result of severe steric crowding these two residues are twisted about the C(4)–C(4′) bond. Thus, in trimers **26/10** and **6/11** there are two asymmetric carbon centers at C(4″) and C(2″) and hence diastereomeric pairs are expected. Compound **16** at pH ≥7.5 showed no tendency to cyclize to structures analogous to **6** and **11**. While only a limited amount of ^{1}H NMR data is available for **10** it is fully compatible with the structure proposed. Acid hydrolysis of **6** and **10** to give **1** would be expected to follow a route similar to that shown in Scheme I.

DISCUSSION

Peak I_a corresponds primarily to the oxidation of 5-HT in an electrodimerization reaction because dimer **1** is the major electrooxidation product. The value of $E_p - E_{p/2}$ for peak I_a under conditions where it is readily distinguished from peak II_a is 42 ± 3 mV (35 μM to 6 mM 5-HT; $\nu \leq 20$ mV s^{-1}). This value is only slightly larger than that expected for an electrodimerization mechanism (*20*). In addition, with increasing 5-HT concentration (35 μM to 6 mM; $\nu = 5$ mV s^{-1}) E_p shifts to more negative potentials by an average of 22 mV/log C. This shift is consistent with an electrodimerization reaction (*21*). There are three principal coupling pathways for electrodimerization reactions. Two involve initial one-electron abstractions followed either by coupling of the resultant radicals (radical–radical coupling) or attack by the radical on the parent compound (radical–substrate coupling). The third possibility involves a two-electron abstraction to give a divalent ion which reacts with the parent compound (ion–substrate coupling). Diagnostic criteria have been developed to distinguish between these mechanisms and nuances resulting from protonation and disproportionation reactions, based upon the influence of C, pH, and ν on E_p in linear sweep voltammetry (*21*). Such criteria assume that all electron-transfer reactions are nernstian, the homogeneity of the chemical reaction, that all chemical reaction steps are very rapid, and the absence of adsorption. At physiological pH 5-HT is strongly adsorbed at the PGE; electrochemical and coupled chemical reactions lead to products other than simple dimers. Furthermore, at relatively modest sweep rates quasi-reversible reduction peak I_c coupled to peak I_a appears (Figs. 2B and 2C). Such a peak should not be observed if the assumptions employed by Savéant and co-workers (*21*) to elucidate criteria to diagnose electrodimerization reactions are valid. Accordingly, mechanistic conclusions must be based upon a more qualitative interpretation of the voltammetric behaviors of 5-HT, the identity of the ultimate electrooxidation products, and coulometric measurements.

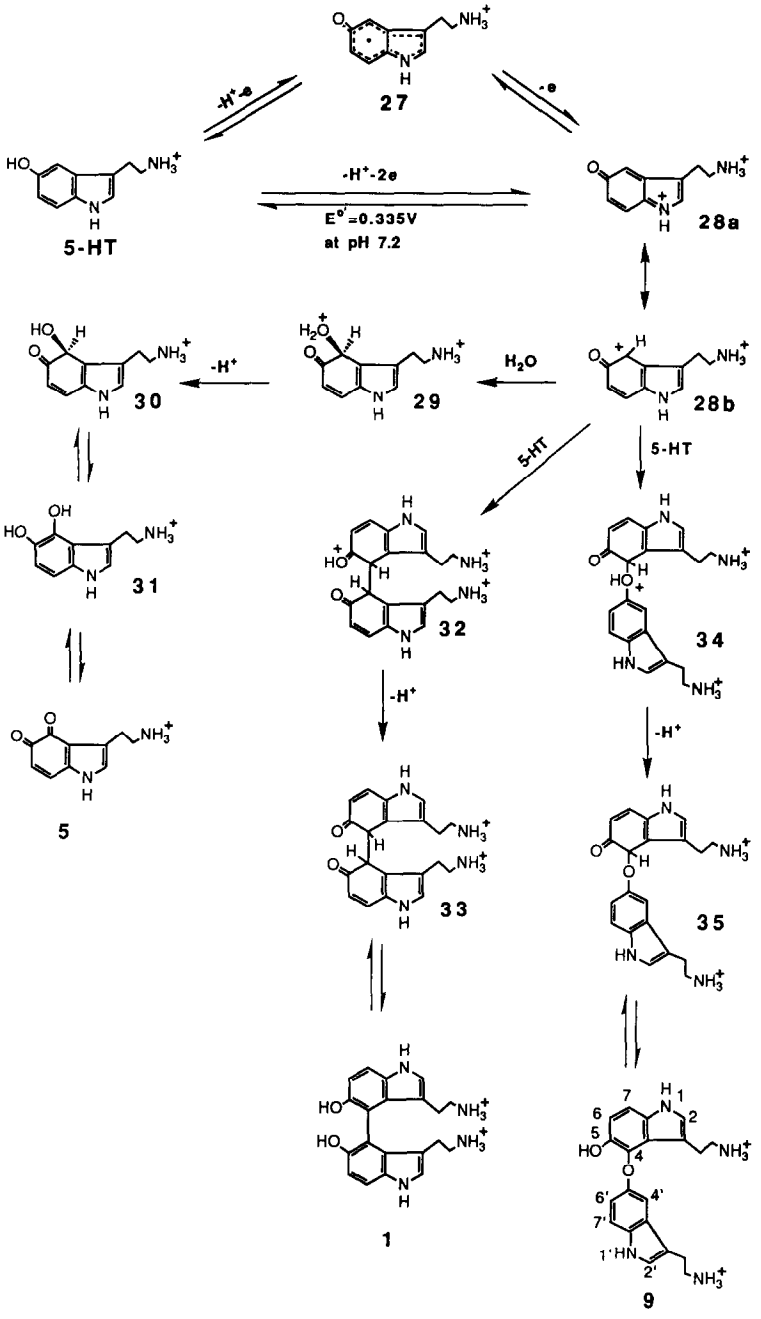
In acidic aqueous (*6d*) and acetonitrile (*7*) solutions 5-HT is oxidized via a radical–substrate mechanism to yield a variety of carbon–carbon linked dimers as the major products. Calculations have revealed that the primary radical intermediate generated in acetonitrile reacts with 5-HT at a rate close to the diffusion limit (*7*). At physiological pH in aqueous solution dimer **9**, which contains the C(4)–O–C(5′) linkage, appears as a major product along with C(4)–C(4′)-linked dimer **1**. The state of ionization of 5-HT ($pK_{a_1} = 10.0$ and $pK_{a_2} = 11.1$, where pK_{a_1} refers to dissociation of the exocyclic NH_3^+ group and pK_{a_2} to dissociation of the OH) (*22*) is virtually the same as pH 2 and pH 7.2. This suggests that it is rather unlikely that at pH 7.2 5-HT is electrooxidized to a significant extent to a phenoxy radical which ultimately yields oxygen-bridged dimeric and trimeric products since such products are not formed at lower pH. A feature common to all of the identified dimeric and trimeric electrooxidation products is the presence of at least one 5-HT residue linked at its C(4)-position. Furthermore, the only monomeric product, dione **5**, has been substituted at C(4). Thus, peak I_a electrooxidation of 5-HT at pH 7.2 clearly results in activation of the C(4)-position. An important clue to the identity of the key intermediate in the oxidation reaction is provided by cyclic

voltammetry experiments (Fig. 2). These indicate that the unstable intermediate responsible for reduction peak I_c is the precursor of **5** (the species responsible for the peaks V_c/I_a' couple). This same intermediate must also serve as the precursor to dimers **1** and **9**. Thus, the intermediate giving peak I_c must be an electrophilic species capable of nucleophilic attack by water, to ultimately yield dione **5**, or by 5-HT to yield dimers **1** and **9**. Previous results (*7*) suggest that 5-HT radical species are too reactive to be detected by cyclic voltammetry ($\nu \leq 100\ V\ s^{-1}$). Accordingly, we propose that at physiological pH 5-HT is initially electrooxidized in a 2e reaction to quinone imine **28a** (Scheme IV). This reaction no doubt proceeds via transient radical intermediates such as **27**. And, it must be noted that it is not possible to discount the possibility that some products might in part be formed by reactions of such intermediary radical species. Nevertheless, activation of the C(4)-position toward nucleophilic attack results from carbocation intermediate **28b**. Nucleophilic attack by water on **28b** leads to 4,5-dihydroxytryptamine (**31**) which at peak I_a potentials is immediately electrooxidized to **5**. At fast-sweep rates in cyclic voltammetry peak I_a corresponds to a 2e electrooxidation of 5-HT to **28a/28b** and peak I_c to the reverse reaction. At slow sweep rates, however, approximately one-sixth of **28a/28b** is attacked by water to yield **31** and then dione **5** such that on the reverse sweep the peaks V_c/I_a' couple appears (the area under peak V_c corresponds to about one-sixth that under peak I_a). Nucleophilic attack by 5-HT on **28b** yields dimers **1** and **9** as conceptualized in Scheme IV.

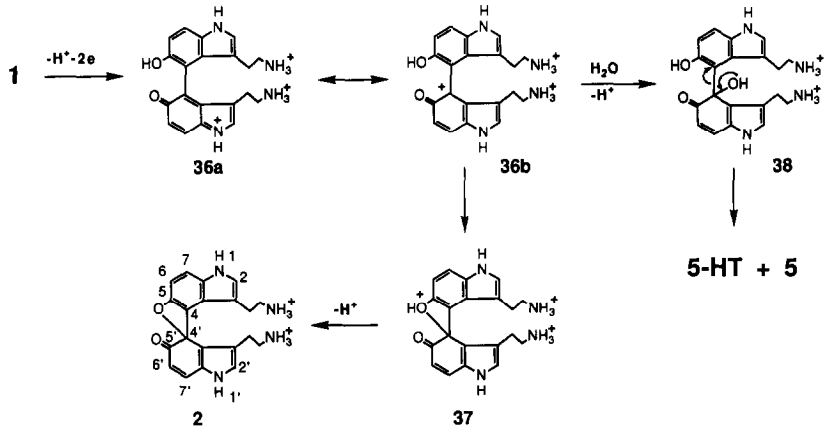
Dimer **1** exhibits an oxidation peak at 0.38 V (1.3 mM **1**; pH 7.2; $\nu = 5\ mV\ s^{-1}$). In fact, peak II_a observed in CV and LSV of 5-HT is predominantly due to oxidation of **1** formed as the major product of the peak I_a reaction. The voltammetric peak of **1**, however, is very broad such that at all peak I_a potentials employed to oxidize 5-HT **1** is also partially electrooxidized. The major electrooxidation product of **1** is **2**. By analogy with the mechanism proposed for electrooxidation of 5-HT we propose that **1** is oxidized ($1e,2H^+$) to cationic intermediate **36a/36b**. Intramolecular cyclization and deprotonation leads to **2** as outlined in Scheme V. While details of the oxidation chemistry of **1** remain to be elucidated another product of electrooxidiation is dione **5** which is expected as a result of nucleophilic attack by water on putative intermediate **36b** (Scheme V).

There are several plausible routes which could lead to trimer **3**. In view of the fact that 5-HT is more easily oxidized than dimer **1** it is likely that carbocation **28b** attacks **1** to form **3** by the route shown in Scheme VI. Trimer **3** exhibits an oxidation peak at $E_p = 0.29\ V$ ($\nu = 5\ mV\ s^{-1}$; pH 7.2) indicating that it must contribute to peak II_a observed in voltammograms of 5-HT.

Trimers **7**, **8**, and **12** all consist of a residue of dimer **9** coupled at different sites to a residue of 5-HT. In principal all three trimers could result from attack of carbocation **28b** on dimer **9**. Indeed, electrolyses of a mixture of **9** and 5-HT results in an increase in the yields particularly of trimers **8** and **12**. Thus, it is proposed that **28b** attacks **9** to yield **8** and **12** by the route shown in Scheme VII. In view of the fact that **7** contains a residue of **1** and because the latter dimer is such a major product it is probable that **7** is largely formed by attack of **28b** on **1** (Scheme VII). Indeed, electrolysis of a mixture of **1** and 5-HT results in an enhanced yield of **7** as predicted from the proposed reaction pathway.


27
-H+-e
-e
5-HT
-H+-2e
E°'=0.335V
at pH 7.2
28a
28b
-H+
H2O
30
29
31
5
5-HT
32
34
-H+
33
35
1
9

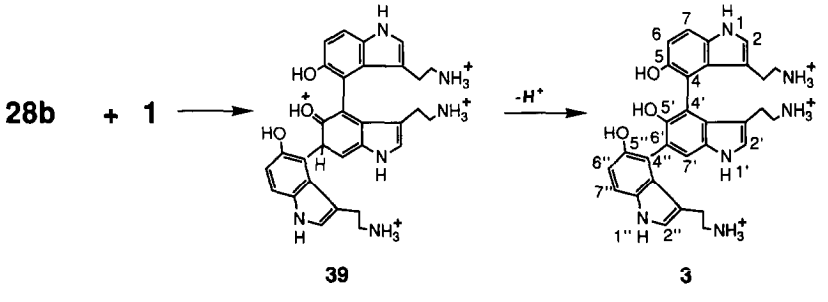
SCHEME IV



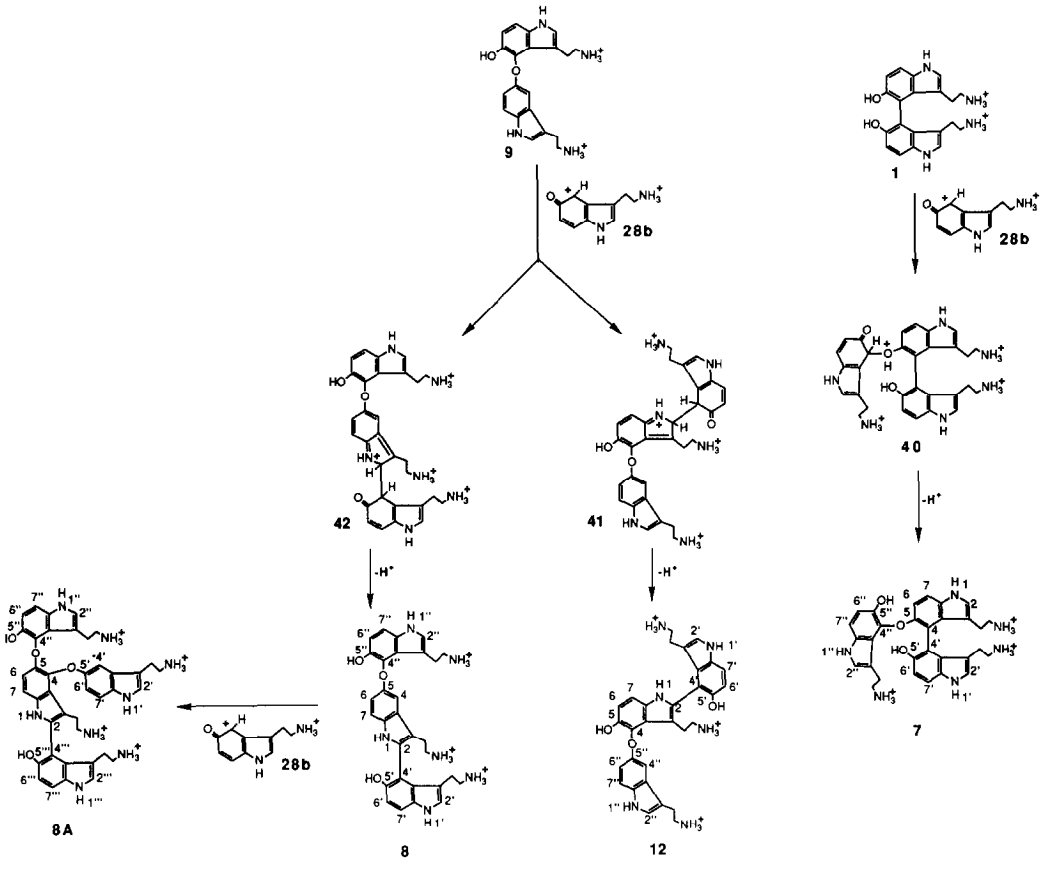
SCHEME V

Cyclic voltammetry of **7** at pH 7.2 (ν = 200 mV s^{-1}) shows an oxidation peak at 0.12 V. After scan reversal a quasi-reversible reduction peak appears at 0.07 V followed by a second reduction peak at −0.45 V. Thus **7** ($E^{\circ\prime}$ = 0.095 V at pH 7.2) is more easily oxidized than 5-HT ($E^{\circ\prime}$ = 0.335 V at pH 7.2). Controlled potential electrooxidation of **7** at 0.26 V at pH 7.2 gives **4** as the major product. Dioxepin **4** is responsible for the reduction peak observed at −0.45 V yielding **7** as the product (see Scheme II). Electrooxidation of **7** (2e,1H^+) yields cation **43**. Intramolecular cyclization of carbocation **43b** results in formation of dioxepin **4** as outlined in Scheme VIII.

Potential-step cyclic voltammetry of 5-HT reveals that the yield of dione **5**, characterized by the peaks V_c/I_a' couple, increases as the initial potential is made more positive. There are several contributions to this observation. Compounds **1**, **8**, **9**, and **12** exhibit voltammetric peaks at 0.38, 0.38, 0.24, and 0.28 V, respectively (ν = 200 mV s^{-1}; pH 7.2). Furthermore, CV studies and controlled potential electrolyses followed by HPLC analyses reveal that these compounds are oxidized partially to dione **5** or a compound having cyclic voltammetric behaviors similar



SCHEME VI

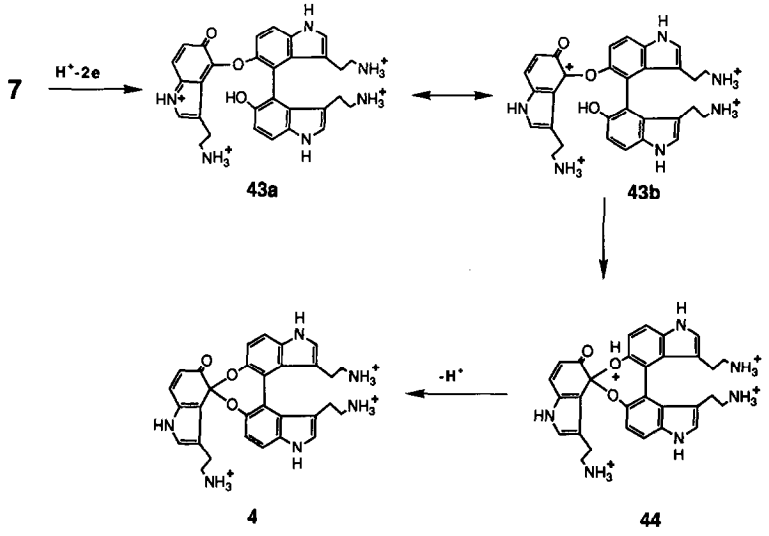


SCHEME VII

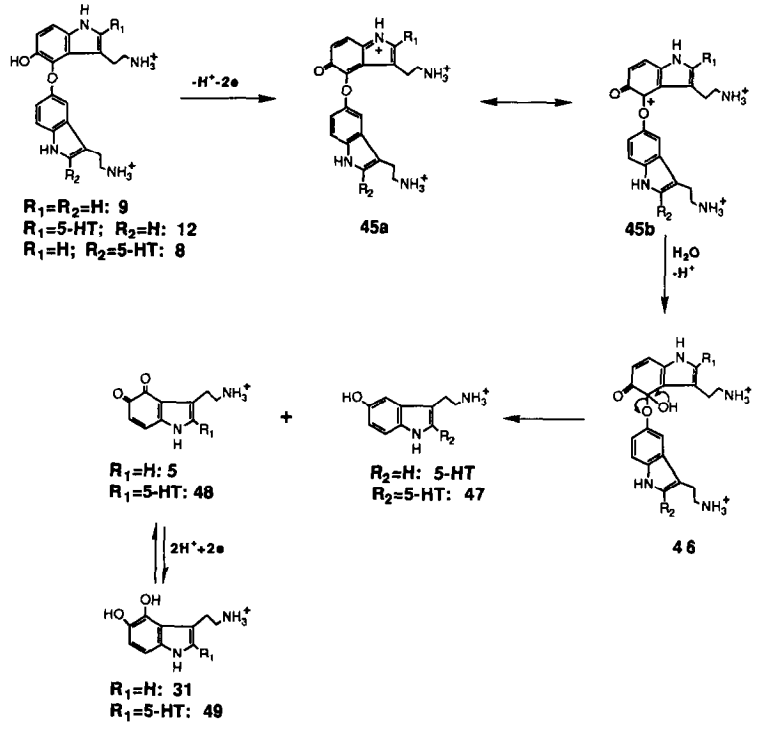
to **5**. In the case of compounds **8**, **9**, or **12** the reason for these observations are evident from Scheme IX. Thus, electrooxidation of **8**, **9**, or **12** yields the corresponding quinone imine (**45a**)/carbocation (**45b**) intermediate. Nucleophilic attack by water on **45b** results in formation of either **5** or a dimer containing a 4,5-dione residue (**48**) and 5-HT or dimer **47**. Previous studies (*15*) have shown that indolic dimers containing a 4,5-dione residue exhibit cyclic voltammetric behaviors similar to **5**.

CONCLUSIONS

The results presented above indicate that at peak I_a potentials 5-HT is electrooxidized at physiological pH to a reactive carbocation intermediate (**29b**). The primary reaction of this intermediate is with 5-HT to yield 5,5′-dihydroxy-4,4′-bitryptamine (**1**) as the major product. A second oxygen-bridged dimer **9** is also formed by a



SCHEME VIII



SCHEME IX

similar reaction pathway. Nucleophilic attack by water on the primary carbocation intermediate leads to 4,5-dihydroxytryptamine (**31**) and then tryptamine-4,5-dione (**5**). The chemistry at the electrode surface becomes extremely complex as a result of secondary reactions between carbocation **28b** and dimer **1** to yield trimers **3** and **7** and between **28b** and dimer **9** to yield trimers **8** and **12**. One tetrameric product **8A** is formed as a result of further reaction between **28b** and trimer **8**. It is likely that many other similar oligomerization reactions lead to products responsible for the very minor peaks noted in chromatograms of the crude electrolysis products of 5-HT. The black, insoluble polymeric material formed as a result of electrooxidation of relatively high concentrations of 5-HT no doubt represents the ultimate product of these types of reactions. Trimer **7** is the key precursor to dioxepin **4** which spontaneously transforms into diastereomers **26** and **10** and then via an electrochemically mediated or autoxidation reaction yield the oxidized diastereomers **6** and **11** (Scheme III). Additional, somewhat structurally more complex products such as **13** and **14** are also formed. The reaction pathways leading to these products remain to be elucidated.

These studies reveal, therefore, that the indolic neurotransmitter is a relatively easily oxidized compound. Upon the basis of these electrochemical investigations it appears that it is not inconceivable that 5-HT might under certain pathological conditions be oxidized in the central nervous system. If this in fact does occur it seems probable that reactive radical (*6*) or quinone imine/carbocation intermediates might be formed. While the reactions of such intermediates with endogenous components have not yet been investigated it is very probable that they could inflict serious damage to the central nervous system. This study reveals however, that one fate of putative carbocation **28b** is reaction with water to yield 4,5-dihydroxytryptamine (**31**), a known neurotoxin (*23*). The latter compound undergoes very facile electrochemical or autoxidation to tryptamine-4,5-dione (**5**). The latter compound or dimers containing this residue can also be generated by the facile oxidation of dimer **9** or trimers **1**, **8**, and **12** (Schemes V and IX) or by hydrolysis of dioxepin trimer **4** (Scheme I). Recently, Chen *et al.* (*24*) used *in vitro* superfusion experiments to demonstrate that **5** (prepared by an electrochemical method (*6a*)) is probably a neurotoxin in view of the fact that it evoked 5-HT efflux from rat brain fragments in a similar manner to the known serotonergic neurotoxin 5,6-dihydroxytryptamine. Analysis of the cerebrospinal fluid (CSF) of individuals suffering from diseases of the Alzheimer's type using HPLC with an electrochemical detector have failed to detect **5** although very many easily electrooxidized but unknown compounds were detected (*25*). However, if in fact dione **5** is a toxin of significance in the etiology of Alzheimer's disease or related dementias it is very unlikely that it would exist in the free state in CSF owing to its avid reactions with nucleophiles such as thiol groups and because of its relatively short life time ($\leq$1 h) at physiological pH (*26*). Electroactive compounds detected by HPLC–EC analysis of CSF of Alzheimer's patients exhibit similar chromatographic and electrochemical behaviors to partially electrooxidized solutions of 5-HT (*1f*). These results appear to imply that abnormal oxidation chemistry or biochemistry of 5-HT does indeed occur in the CNS of Alzheimer's patients. This suggests that the mechanistic conclusions and product profiles reported here for the electrochemi-

cally driven oxidation of 5-HT might provide valuable guidance concerning *in vitro* enzyme-mediated and *in vivo* oxidative transformations of the neurotransmitter which could have a bearing on an understanding of the fundamental reactions leading to diseases of the Alzheimer's type. We hope to report shortly on the enzyme-mediated oxidation chemistry of 5-HT and on the neurodegenerative properties of its many novel oxidation products.

ACKNOWLEDGMENTS

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