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6-Hydroxycatecholine, a Choline-Mimicking Analogue of the Selective Neurotoxin, 6-Hydroxydopamine

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The synthesis and properties of 6-hydroxy-N,N-dimethylepinephrine (6-hydroxy-catecholine) are reported. This agent is intended for use as a selective presynaptic cholinotoxin and is based on previously reported neurotoxins of the same type. 6-hydroxycatecholine is a close structural analogue of the catecholaminergic neurotoxin 6-hydroxydopamine, and is expected both to be selectively reactive at cholinergic sites and to undergo less vigorous and potentially more selective inactivating reactions. It is also possible that in specific dementia-inducing pathologies, 6-hydroxycatecholine could be formed endogenously.

Quaternary ammonium alkyl-substituted catechols comprise a class of redox-reactive affinity reagents of increasing structural diversity and important selective cholinergic toxicity (1–6). Previously prepared compounds in this class are shown in Figure 1.

Agents which produce selective cholinergic neuronal inactivation are sought to trace the cholinergic contributions to neurobiologically complex pathways underlying consciousness and cognition. Such agents may be especially useful in developing animal models for the dementias as in Alzheimer's disease where selective cholinergic deficit has been established (7, 8). Here we report the synthesis, and chemical and electrochemical properties of 6-hydroxycatecholine, which is an analogue of the catecholaminergic neurotoxin, 6-hydroxydopamine (Figure 2).

Cholinomimetic catechols such as 6-hydroxycatecholine are expected to be less active and potentially more selective than hydroxylated catecholamines such as 6-hydroxydopamine. In the former, the quaternary ammonium functionality lacks the nucleophilic reactivity of the primary amine side chain in the latter. Thus, cyclization and formation of reactive indole quinones do not occur during the oxidation of catecholine or 6-hydroxycatecholine.

The reactions of 6-hydroxycatecholine with targeted neurobiological sites may be more selective than other agents in this class of redox reactive neurotoxic agents. It is proposed that 6-hydroxycatecholine may represent the most useful member of this class of agents in producing specific cholinergic neuronal inactivation.

MATERIALS AND METHODS

Materials. All chemicals were purchased from Aldrich, Pfalfz & Bauer, TCI America, and were used without purification. Pre-coated silica gel sheets (Art 5554 DC-Alufolien Kieselgel 60 F_{254}, E. Merck) were used for thin-layer chromatography. Melting points were measured on Fisher-Jones Melting Point Apparatus without correction.

Cyclic voltammetry. Measurements were conducted with a Bioanalytical Systems Inc. (BAS) 100 B electro-chemical analyzer interfaced to a Gateway 386 PC and a Houston Instruments DMP-40 digital plotter. An aqueous Ag/AgCl (3 M NaCl) electrode (BAS) and a platinum wire were used as reference and auxiliary electrodes, respectively. The working electrode was a glassy carbon disk electrode (GCE, BAS), which was polished with 0.05 μm alumina

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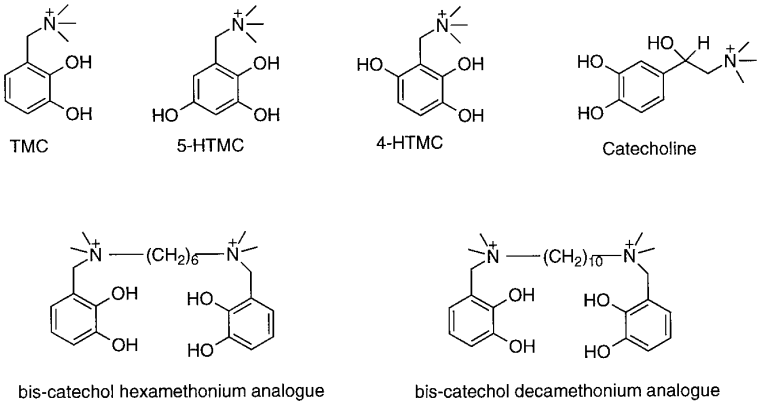

FIG. 1. Affinity-directed, redox-reactive cholinotoxic agents.

polishing powder before each measurement. All solutions were prepared from distilled and deionized water, which was purified to a resistivity of at least 18MΩ-cm by a Barnstead Organic pure water system. pH was adjusted with dilute HCl or NaOH solutions. Solutions were degassed with argon just prior to each determination. The ionic strengths were adjusted to 0.5 with solid KNO_3. pH values of the test solutions were measured to ± 0.1 after each measurement.

UV/Vis spectra. Spectra were recorded on a Varian DMS 200 UV-Visible Spectrophotometer at a scan rate of 200 nm/min. Aqueous stocks of 6-hydroxycatecholine (2×10^{-3} M) were prepared in HNO_3 (pH 2.0). Ceric ammonium nitrate (10 mM), ferric chloride (10 mM) were also prepared in HNO_3 (pH 2.0–6.0). Sodium dichromate stock solution was prepared in H_2SO_4 (pH 5.0). For spectral studies done at higher pH potassium phosphate buffers (pH 6–8, 0.05 M) were employed.

Chemical synthesis of 6-hydroxycatecholine. The synthesis of 6-hydroxycatecholine was under taken in two parts (Figure 3). First, in the synthesis of the key intermediate 2′,4′,5′-trimethoxyacetophenone, and second, in the conversion of the acetophenone intermediate into 6-hydroxycatecholine. Two alternative routes were developed for the synthesis of the 2′,4′,5′-trimethoxyacetophenone intermediate.

2′,4′,5′-trimethoxyacetophenone: route A. A mixture of 2,4,5-trimethoxybenzoic acid (5.0 g, 0.024 mmol), methanol (150 ml) and thionyl chloride (10 ml) was heated under reflux for 16 hrs. The solvent was evaporated *in vacuo*, and the residue was crystallized from methanol as pale yellow needles of methyl 2,4,5-trimethoxybenzoate, (4.6 gm, 86% yield), mp 90–92°C. IR(KBr) $\nu_{max.}$ 3001, 2955, 2837, 1690, 1615 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 3.88 (s, 6H), 3.91 (s, 3H), 3.95 (s, 3H), 6.54 (s, 1H), 7.42 (s, 1H); ^{13}C-NMR ($CDCl_3$): δ 51.77, 55.96, 56.32, 56.98, 97.58, 110.47, 114.28, 142.28, 142.46, 153.46, 155.58, 166.02.

The esterified product above was converted to ω-(methylsulfinyl)-2′,4′,5′-trimethoxy-acetophenone. Sodium hydride (1.54 gm, 40% mineral suspension) was placed in a three-neck round bottom flask and washed several times with light petroleum ether. The flask was fitted with a reflux condenser and two rubber septa and maintained under a positive pressure of nitrogen subsequent to removal of traces of petroleum ether *in vacuo*. Dimethyl sulfoxide (DMSO, 19.6 ml) was introduced via syringe and the mixture heated with stirring at 70–75 °C until the evolution of gas ceased (ca. 45 min). The flask was cooled in an ice bath while stirring was continued, and a solution of the ester (4.2 gm, 0.019 mol) prepared above in tetrahydrofuran (20 ml) was introduced via syringe over a period of several minutes. The ice bath was subsequently removed and stirring continued for 30 min. The reaction mixture was then poured into 3 times its volume of water, acidified with HCl to pH 3–4 and extracted with methylene chloride, which was subsequently washed several times with water and dried over anhydrous sodium sulfate. Removal of the solvent gave a white solid (4.27 gm, 85% yield), mp 141–142 °C. IR (KBr) $\nu_{max.}$: 3005, 2922, 1718, 1644, 1603, 1508 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 2.76 (s, 3H), 3.88 (s, 3H), 3.98 (s, 6H),

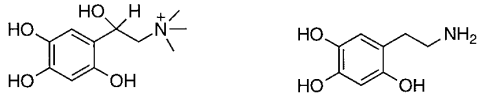

FIG. 2. Structures of 6-hydroxycatecholine and 6-hydroxydopamine.

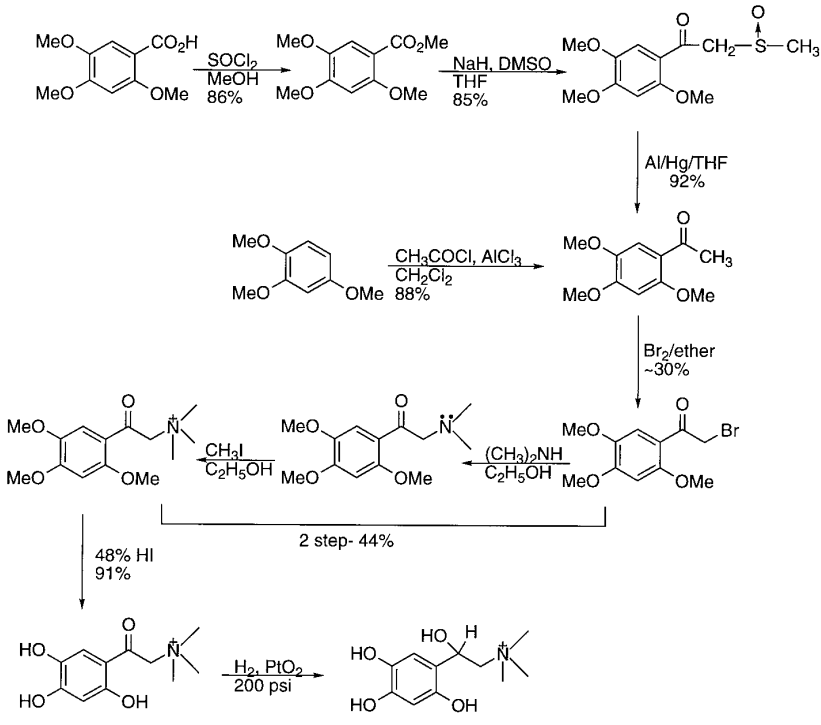

FIG. 3. Synthesis of 6-hydroxycatecholine.

4.31 (d, 1H, J = 14.9 Hz), 4.74 (d, 1H, J = 14.9 Hz), 6.51 (s, 1H), 7.41 (s, 1H); ^{13}C-NMR ($CDCl_3$): δ 39.88, 56.19, 56.24, 68.35, 95.99, 112.14, 117.16, 143.59, 155.66, 156.37, 189.84.

ω-(Methylsulfinyl)-2′,4′,5′-trimethoxyacetophenone (4.27, 0.016 mol) product from the above reaction was dissolved in 10% aqueous tetrahydrofuran (265 ml) and placed in a reaction vessel equipped with a mechanical stirrer. Aluminum amalgam was prepared by cutting aluminum foil into several large strips and immersing it all at once into a 2% solution of mercuric chloride for 15 sec, decanting and rinsing. The aluminum strips (1.56 gm, 10 gm-atoms of aluminum/mol of compound) were cut immediately directly into the reaction vessel after cooling to 0 °C and stirred for 10 min. The reaction mixture was filtered and the solids washed with tetrahydrofuran, after which ether was added. The ether phase was separated from the water, dried over anhydrous sodium sulfate and evaporated *in vacuo.* The white solid of 2,4,5-trimethoxyacetophenone was crystallized from methanol (3.04 gm, 92 % yield), mp 98–99°C. IR (KBr) $\nu_{max.}$: 2919,2837, 1647, 1606, 1515 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 2.58 (s, 3H), 3.86 (s, 3H), 3.93 (s, 3H), 6.48 (s, 1H), 7.41 (s, 1H); ^{13}C-NMR ($CDCl_3$): δ 32.02, 56.10, 56.16, 56.28, 96.51, 111.62, 119.28, 143.10, 153.95, 155.58, 197.26.

route B. Aluminum chloride (52.8 gm, 0.40 mol) was added in several portions to a solution of 1,2,4-trimethoxybenzene (60 gm, 0.36 mol) and acetyl chloride (32 gm, 0.40 mol) in 960 ml of methylene chloride at 5–8 °C. After stirring for 1 hr at 10 °C, 1.2 liters of HCl (0.5 M) was added. The organic layer was separated, washed with water, dried over anhydrous sodium sulfate and evaporated *in vacuo* to give 2,4,5-trimethoxyacetophenone, which was recrystallized from methanol (66 gm, 88% yield).

Conversion of 2,4,5-trimethoxyacetophenone into 6-hydroxycatecholine: 2-Bromo-2′,4′,5′-trimethoxyacetophenone. 2′,4′,5′-trimethoxyacetophenone (1.0 gm, 4.76 mmol) was dissolved in anhydrous ether (25 ml) and placed in a dry three-neck flask fitted with a reflux condenser. The solution was cooled in an ice bath with stirring and bromine (250 μl) was introduced from a syringe. The ice bath was removed and the solution refluxed for 45 min. The ether was removed *in vacuo* and the solid residue recrystallized from methanol to obtain gray crystals (408 mg, 30% yield), mp 141–142 °C. IR (KBr) $\nu_{max.}$: 3025, 2954, 1658, 1607, 1578, 1522 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 3.86 (s, 3H), 3.93 (s, 3H), 3.94 (s, 3H), 4.57 (s, 2H), 6.48 (s, 1H), 7.44 (s, 1H); ^{13}C-NMR ($CDCl_3$): δ 37.92, 56.17, 56.29, 96.03, 113.04, 115.86, 143.49, 154.90, 155.49, 189.81.

α-Dimethylamino-2′,4′,5′-trimethoxyacetophenone. 2-Bromo-2′,4′,5′-trimethoxyacetophenone (1.0 gm, 3.46 mmol) was dissolved in absolute ethanol at 60 °C in a dry three- neck flask. Dimethylamine (2.2 ml, 35% solution in absolute ethanol) was introduced via syringe. The mixture was stirred at 60 °C under nitrogen for 3 hrs, then cooled to room

temperature and stirred an additional two hrs. The reaction mixture was evaporated *in vacuo* to give a solid product which was used in the subsequent reaction without purification.

α-Dimethylamino-2′,4′,5′-trimethoxyacetophenone methiodide. The product from the above reaction was redissolved in absolute ethanol and treated with excess methyl iodide (12.0 ml) and stirred for 1 hr. The mixture was allowed to stand at room temperature for 16 hrs, when the product separated as a white crystalline solid, 603 mg, 44% based on 2-bromo-2′,4′,5′-trimethoxyacetophenone), mp 188–190 °C (dec.). IR (KBr) $\nu_{max.}$: 3072, 2978, 2919, 1647, 1608, 1508 cm^{-1}; ^{1}H-NMR (D_2O): δ 3.22 (s, 9H), 3.72 (s, 3H), 3.85 (s, 3H), 4.73 (s, 2H), 6.62 (s, 1H), 7.32 (s, 1H); ^{13}C-NMR (D_2O): δ 54.99, 56.96, 57.13, 57.18, 72.42, 72.42, 97.68, 112.46, 116.29, 143.48, 156.86, 158.24, 190.57.

α-Dimethylamino-2′,4′,5′-trihydroxyacetophenone methiodide. α-Dimethyl-amino-2′,4′,5′-trimethoxyacetophenone methiodide (100 mg, 0.28 mmol) was dissolved in 2.5 ml 48% aqueous hydroiodic acid in a 10 ml round bottom flask and refluxed for 5 hrs. The reaction mixture was evaporated *in vacuo* to give a whitish-yellow solid which was washed with acetone and dried under a vacuum (81 mg, 91%) , mp 236–237 °C (dec.). IR (KBr) $\nu_{max.}$: 3433, 3106, 1645, 1521 cm^{-1}; ^{1}H-NMR (DMSO): δ 3.27 (s, 9H), 4.91 (s, 2H), 6.40 (s, 1H), 7.15 (s, 1H); ^{13}C-NMR (D_2O): δ 53.21, 69.45, 103.20, 112.10, 113.94, 139.17, 154.62, 155.24, 189.38.

6-Hydroxycatecholine: [*α - Dimethylamino - methyl - (2′,4′,5′-trihydroxyphenyl) methiodide*]. α-Dimethylamino-2′,4′,5′-trihydroxyacetophenone methiodide (50 mg, 0.14 mmol) was dissolved in ethanol-water (3.0 ml, 4:1) in a 50 ml round bottom flask. Platinum oxide (2 mg) was added and was stirred under hydrogen in a Paar hydrogenation apparatus at a pressure of 200 lbs/in^2 for 10 hrs. The catalyst was removed by filtration. the filtrate was evaporated *in vacuo* to obtain a yellow solid (33 mg, 66% yield). ^{1}H-NMR (D_2O): δ 3.10 (s, 9H), 3.31, (dd, 1H), J_1 = 13.89, J_2 = 2.39), 3.43 (dd, 1H, J_1 = 13.89, J_2 = 9.08), 5.30 (dd, 1H, J_1 = 9.08, J_2 = 2.39), 6.34 (s, 1H), 6.75 (s, 1H); ^{13}C-NMR (D_2O): δ 55.06, 64.57, 71.15, 105.21, 115.31, 119.26, 138.29, 145.87, 147.31.

RESULTS AND DISCUSSION

6-Hydroxycatecholine has been prepared as a mixture of the R and S enantiomers by the scheme shown in Figure 3. 2′,4′,5′-Trimethoxyacetophenone is a key intermediate in the synthesis of 6-hydroxycatecholine. This compound may be prepared directly in a single step through the Friedel-Crafts acetylation of 1,3,4-trimethoxybenzene (9). Alternatively, this acetophenone intermediate may be prepared in a three step sequence in high overall yield (65–70%). 2,4,5-trimethoxybenzoic acid is first converted to the methyl ester and then to the methyl ketone following a two step alkylation with the sodium salt of dimethylsulfoxide and desulfurization with aluminum amalgam in tetrahydrofuran. Of the subsequent steps, direct bromination of the 2′,4′,5′-trimethoxyacetophenone was the most troublesome. However, the bromine was easily displaced with dimethylamine and the product easily converted to the quaternary amine with methyl iodide prior to cleavage of the trimethyl ether with hydroiodic acid and catalytic hydrogenation of the ketone.

6-Hydroxycatecholine was unstable in air and especially unstable under basic conditions in the presence of oxygen. It was converted with a variety of oxidizing agents to the hydroxy p-quinone (λ_{max} 495 nm), the stability of which was consistent with the reversibility of the oxidation reduction equilibrium studied electrochemically as reported below. Such behavior is characteristic of all of the other agents in the class of neurotoxins shown in Figure 1. However, the addition of hydroxy groups to the catechol ring both improves the ease of oxidation and shifts the λ_{max} characteristic of the visible spectrum of the quinone oxidation product to higher wavelengths.. Both the keto precursor of 6-hydroxycatecholine and 6-hydroxycatecholine itself were investigated electrochemically using cyclic voltammetry. Figure 4 shows pH-dependent anodic peak potentials (E_{pa}) determined for the two compounds. As expected, 6-hydroxycatecholine is more easily oxidized than the keto precursor. Electron withdrawing groups on the ring generally decrease ease of oxidation (10). However, the range of measured E_{pa} and their pH dependence for 6-hydroxycatecholine is quite similar to 4-HTMC and 5-HTMC in Figure 1 (5).

These electrochemical measurements also revealed differences in the relative stabilities of the two compounds in the neutral and basic pH range. For the keto precursor the character of the cyclic voltammograms could be maintained even when solutions were briefly adjusted to pH 9 and then returned to acidic pH. However, after raising pH to 10–11, the cyclic voltammogram

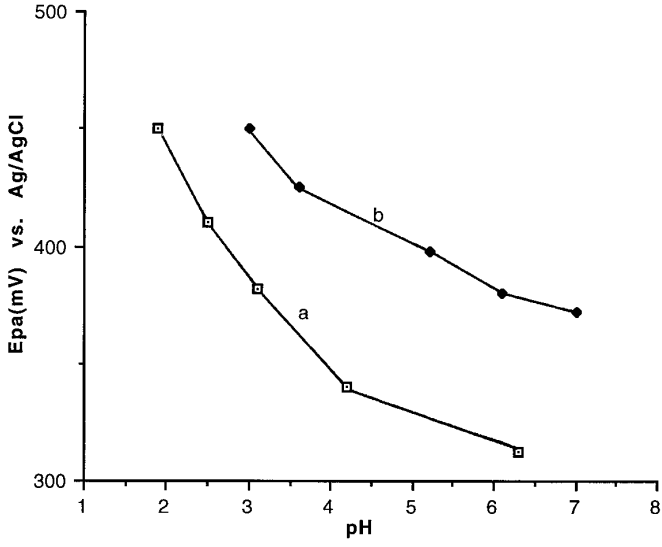

FIG. 4. pH-Dependence of the anodic peak potentials (E_{pa}) of: (a) 6-hydroxycatecholine and (b) α-dimethylamino-2′,4′,5′-trihydroxyacetophenone methiodide.

changed, indicating some oxidation and perhaps polymerization of the keto compound. For 6-hydroxycatecholine brief adjustment to pH $>$ 6 resulted in irreversible changes in the cyclic voltammogram and was accompanied by darkening of the solutions. The chemical changes occurring in these higher pH reactions have not been investigated further.

The preparation of 6-hydroxycatecholine now reported provides a more reactive analogue of catecholine, which, it is proposed, will possess a more aggressive but still selective cholinotoxic response established in earlier studies (2–4). In longer term, 6-hydroxycatecholine may provide a tool for the investigation of the effects of selective cholinergic denervation comparable in importance to what has been achieved with the use of 6-hydroxydopamine in the study of selective catecholaminergic denervation (11)

Further, it is possible that 6-hydroxycatecholine may be formed in the brains of animals and humans, whereas this is highly unlikely for 6-hydroxydopamine. The endogenous formation of 6-hydroxycatecholine remains to be investigated. Exhaustive methylation of the terminal nitrogen of neither dopamine nor epinephrine is known and under normal conditions must occur, if it occurs at all, at a vanishingly slow rate relative to both methylation and dimethylation of the terminal nitrogen in catecholamine metabolites in the brain (12–13). However, under aberrant conditions, where terminal nitrogen exhaustive methylation might occur, such as in altered substrate specificites in brain catecholamine methylases or in overproduction of methylating enzymes, it is possible that both catecholine and 6-hydroxycatecholine could be formed as metabolites in the brain. The synthesis of both catecholine and 6-hydroxycatecholine now provides authentic standards for the evaluation of the possibility of their formation and fate in the brain. While it is not likely that the endogenous formation of these neurotoxins in the brain represents a causative factor in the dementias not clearly recognized as the Alzheimer's type, a mechanism allowing formation of endogenous neurotoxins with selective cholinotoxic effects would represent an interesting mechanism of cholinergic loss in some dementias. The endogenous formation of either catecholine or 6-hydroxycatecholine could slowly and selectively accumulate at presynaptic sites through the use of the high affinity choline transporter. Should such agents slowly build up in presynaptic cholinergic sites, they would surely produce neurotoxic effects, as cholinergic neuronal sites neither possess catecholamine-O-

methyl transferase inactivating enzymes nor the H^+ and ascorbate rich storage granules which keep catecholamines reduced and effectively nontoxic at catecholaminergic synapses.

In order to pursue a more extensive study of selective neurobiological properties of 6-hydroxycatecholine we are preparing the separate R and S stereoisomers. We anticipate that neurotoxicologic properties may vary considerably as a function of stereochemistry. Further, if 6-hydroxycatecholine is formed in the brain, it is likely that the isomer will have the same relative stereochemical configuration as that of the naturally occurring epinephrine derivatives. Thus, the R and S isomers will also be useful as authentic samples to define which, if either of the 6-hydroxycatecholine stereoisomers, is formed in the brain.

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