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PREPARATION, REACTIVITY, AND NEUROTOXICITY OF TRYPTAMINE-4,5-DIONE

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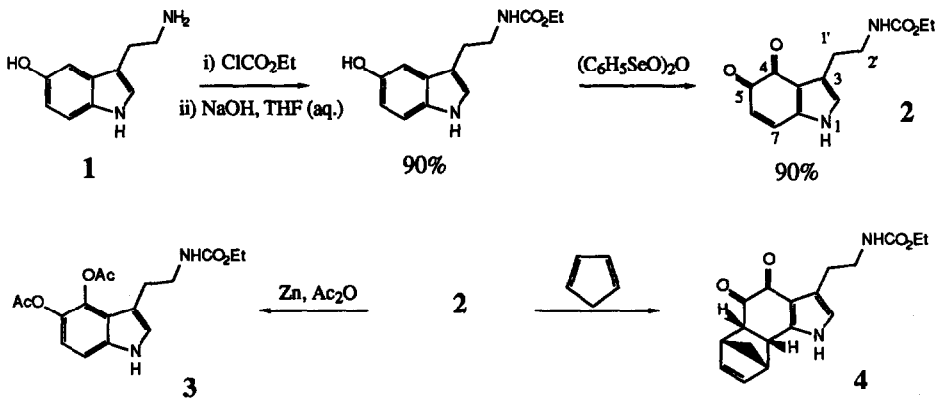
Summary: Tryptamine-4,5-dione protected as the carbamate was prepared in excellent overall yield by the oxidation of serotonin carbamate with benzeneseleninic anhydride. This *o*-quinone, which was potently neurotoxic, readily added sulfur nucleophiles under basic conditions including cysteine and glutathione in 1,4-fashion, a reactivity pattern which may reflect the mechanism of the neurotoxicity.

Abnormality in the metabolism of the neurotransmitter serotonin has been postulated to be involved in several human pyschotic diseases such as schizophrenia and major depression, as well as neurodegenerative Alzheimer's disease.[1] Several oxidized serotonin derivatives such as 5,7-dihydroxytryptamine[2] and 5,6-dihydroxytryptamine[3] are well known neurotoxins. Recently, Dryhurst and Wrona have shown that the primary electrochemical oxidative pathway of serotonin proceeds via the *p*-quinone imine to the unstable tryptamine-4,5-dione, which they more fully characterized as the quinoxaline derivative after successful trapping with *o*-phenylene diamine.[4] It has been reported that tryptamine-4,5-dione is also a potent neurotoxin,[5] but the instability of this compound casts doubt upon the exact identity of the active species in that study. More recent reports support the identity of tryptamine-4,5-dione as a neurotoxic agent produced by serotonin oxidation,[6] and suggest that tryptamine-4,5-dione may also interfere with signal transduction through specific interactions with G-proteins.[7]

We now report that tryptamine-4,5-dione can be conveniently prepared by benzeneseleninic anhydride[8] oxidation of serotonin (**1**), and that the product *o*-quinone is stable in the solid state at 0 °C (slowly decomposes in solution) as long as the terminal amino group is protected as the carbamate ester. Treatment of **1** with ethyl chloroformate in the presence of K_2CO_3 yielded the diprotected compound with reaction occurring at both the terminal amino and phenolic groups (Scheme 1). Reaction at the indole N-1 did not occur under these mild conditions. The carbonate group was selectively hydrolyzed under basic conditions, with subsequent benzeneseleninic anhydride oxidation yielding tryptamine-4,5-dione N-ethylcarbamate (**2**) in 81% overall yield.[9] This *o*-quinone was reductively acetylated to diacetate **3**, and underwent a Diels-Alder cycloaddition with cyclopentadiene, analogous to a related furano-*o*-benzoquinone,[10] to give the *endo*-adduct **4**. In preliminary studies, **2** was shown to be neurotoxic using the chicken embryo forebrain neuron bioassay,[11] with a level of activity only slightly lower than that of 5,6-dihydroxytryptamine.

Scheme 1

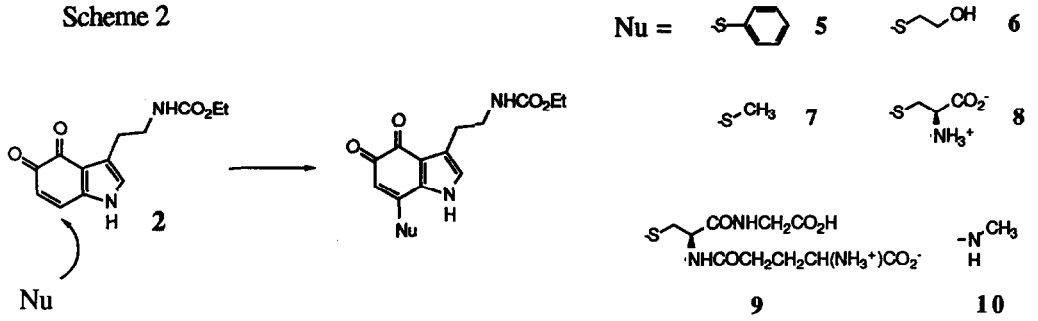


The potential interaction of **2** with possible nucleophiles located on the serotonin receptor as well as the binding site of the G-proteins was examined by the reaction of **2** with several thiols (Scheme 2). Reactions were characterized by the loss of the purple color of **2** immediately upon addition of the nucleophile. All reactions proceeded readily at room temperature and were essentially over within a few minutes under ambient conditions. The return of the purple color due to the *o*-quinone unit of the adduct signified the end of the reactions. In control experiments run under argon, the reactions of **2** with thiophenol and mercaptoethanol remained colorless until oxygen was readmitted into the reaction flask, suggesting O_2 reoxidation following the conjugate addition. The reactions also proceeded readily in the dark. The 1,4-Michael adducts were indicated by the loss of the low field H-7 resonance and retention of the H-6 resonance in the ^1H NMR spectra. Confirmation of the sulfur nucleophilic addition in **6** (rather than the oxygen) came from selective INEPT[12] experiments which revealed an enhancement of C-7 of the adduct due to three bond polarization transfer upon saturation of the resonance of the methylene protons adjacent to the sulfur.[13]

The reactions were performed by dissolving **2** (5 mg) in THF (4 mL) and adding the aqueous solution of the nucleophile. Cysteine was insoluble under these conditions, so this reaction was performed using an aqueous solution buffered to pH 9.7. In the reaction with glutathione, no reaction ensued when the aqueous solution was buffered to pH 7 or pH 8. In pH 9.7 buffer, however, the reaction with glutathione was immediate. In contrast, thiophenol reacted with **2** at every pH examined (pH 3, 7, and 9.7). When run on a larger scale (15 mg **2**) for an accurate measurement of yield, thio-phenol and mercaptoethanol gave >90% isolated yield of adducts **5** and **6**,[14] respectively.

With these results in hand, the reaction with a nitrogen nucleophile, methylamine (40% aqueous solution), was examined. This nucleophile also underwent immediate 1,4-Michael addition to form adduct **10**. In conclusion, tryptamine-4,5-dione, a reputed neurotoxin formed by the oxidation of serotonin, can be prepared in high yield by the benzeneseleninic anhydride oxidation of serotonin, and is quite

Scheme 2



stable when the terminal amino group is protected as the carbamate ester. The *o*-quinone portion of **2** functions as an active Michael acceptor toward both sulfur and nitrogen nucleophiles, reactivity which may mimic the mechanism of neurotoxicity.

Table 1. Characterization of Conjugate Adducts.

Adduct	(M+)	Found[a]	Calc'd	^{1}H NMR[b]
5	($C_{19}H_{18}N_2O_4S$)	370.0993	370.0987	7.68 (dd, J=7.7, 2.3 Hz, 2H), 7.62-7.61 (m, 3H), 6.93 (s, 1H), 6.20 (bs, NH), 5.15 (s, 1H), 3.99 (q, J=7.0 Hz, 2H), 3.36 (bq, J=6.8 Hz, 2H), 2.81 (t, J=6.8 Hz, 2H), 1.14 (t, J=7.0 Hz, 3H)
6	($C_{15}H_{18}N_2O_5S$)	338.0929	338.0936	6.87 (s, 1H), 6.18 (bs, NH), 5.94 (s, 1H), 3.99 (q, J=7.0 Hz, 2H), 3.90 (t, J=6.2 Hz, 2H), 3.36 (bq, J=6.3 Hz, 2H), 3.30 (t, J=6.2 Hz, 2H), 2.81 (t, J=6.3 Hz, 2H), 1.14 (t, J=7.0 Hz, 3H)
7	($C_{14}H_{16}N_2O_4S$)	308.0831	308.0831	6.86 (s, 1H), 6.21 (bs, NH), 5.82 (s, 1H), 3.98 (q, J=7.0 Hz, 2H), 3.36 (bq, J=6.5 Hz, 2H), 2.87 (t, J=6.5 Hz, 2H), 2.62 (s, 3H), 1.14 (t, J=7.0 Hz, 3H)
8	($C_{16}H_{19}N_3O_6S$)	381.1028	381.0994	6.83 (s, 1H), 5.11 (s, 1H), 3.95 (q, J=7.0 Hz, 2H), 3.28 (bq, J=6.8 Hz, 2H), 3.03 (bm, 1H), 2.91 (bm, 2H), 2.73 (t, J=6.8 Hz, 2H), 1.13 (t, J=7.0 Hz, 3H)
9	($C_{23}H_{29}N_5O_{10}S$)	567.1644	567.1635	6.78 (s, 1H), 5.15 (s, 1H), 4.71 (m, 1H), 4.00 (q, J=7.0 Hz, 2H) 3.70-3.74 (m, 3H), 3.31-3.25 (m, 3H), 2.92 (m, 1H), 2.85 (t, J=6.8 Hz, 2H), 2.38 (m, 2H), 1.90 (m, 2H), 1.17 (t, J=7.0 Hz, 3H)
10	($C_{14}H_{17}N_3O_4$)	291.1217	291.1219	7.78 (bs, NH), 7.08 (bt, J=6.7 Hz, NH), 6.90 (s, 1H), 5.07 (s, 1H), 3.95 (q, J=7.0 Hz, 2H), 3.18 (dt, J=6.7, 6.7 Hz, 2H), 2.89 (s, 3H), 2.74 (t, J=6.7 Hz, 2H), 1.13 (t, J=7.0 Hz, 3H)

a) HRMS were run using EI (70 eV), direct insertion. b) Spectra of **5**, **6**, and **7** recorded in acetone-d_6; spectra of **8** and **10** recorded in DMSO-d_6. Spectrum of **9** was recorded in D_2O/methanol-d_4. All spectra were recorded at 93.93 kG, 400 MHz for ^{1}H. Signals for N-H not observed if exchange with water is too rapid.

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9. Spectral data for **2**; IR (KBr) 3340, 3213, 1691, 1636, 1540, 1507, 1457, 1384, 1248 cm-1; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.29 (d, J=10 Hz, H-7), 7.08 (bt, J = 7.0 Hz, NH), 6.81 (s, H-2), 5.88 (d, J=10 Hz, H-6), 3.95 (q, J=7 Hz, -OCH_2-), 3.19 (bq, J=7 Hz, H-2'), 2.71 (t, J=7 Hz, H-1'), 1.13 (t, J=7 Hz, -CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 184.0 (C-5), 173.9 (C-4), 156.2 (C=O, carbamate), 136.6 (C-7a), 134.0 (C-7), 126.0 (C-3), 123.2 (C-6), 122.0 (C-2), 119.5 (C-3a), 59.5 (-OCH_2-), 38.9 (C-2', overlapped with DMSO, revealed by HETCOR), 26.2 (C-1'), 14.7 (CH_3); HRMS (EI, 70 eV) *m/z* 262.0953 (M+, calcd $C_{13}H_{14}N_2O_4$, 262.0953); UV (MeOH) λ_{max} 234 nm (ε 37 541), 353 (3 885), 542 (2 647).
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13. ^{13}C NMR data for **6** (DMSO-d_6, 100 MHz) δ 179.2 (C-5), 175.0 (C-4),156.2 (C=O, carbamate), 150.1 (C-7), 134.2 (C-7a), 125.1 (C-3), 122.3 (C-2), 118.8 (C-3a) 3a),114.2 (C-6), 59.5 (-OCH_2-), 58.5 (-CH_2OH), 38.9 (C-2', overlapped with DMSO, revealed by HETCOR), 33.5 (-SCH_2-), 26.0 (C-1'), 14.7 (CH_3).
14. ^{13}C NMR data for **5** (DMSO-d_6, 100 MHz) δ 179.4 (C-5), 174.7 (C-4),156.2 (C=O, carbamate), 151.4 (C-7), 135.7 (C-2", C-6"), 133.6 (C-7a), 131.1 (C-1"), 130.5 (C-3", C-5"), 125.7 (C-3), 125.6 (C-4"), 122.9 (C-2), 118.9 (C-3a), 114.8 (C-6), 59.5 (OCH_2), 38.9 (C-2', overlapped with DMSO, revealed by HETCOR), 26.1 (C-1'), 14.8 (CH_3).

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