The *E* and *Z* configurations were assigned tentatively on the basis of the lower field absorption of the olefin proton in the NMR spectrum of the *Z* isomer.

7-Chloro-1,3,4,5-tetrahydro-3(*R*)-(1*H*-indol-3-ylmethyl)-5-phenyl-2*H*-1,4-benzodiazepin-2-one Hydrochloride (62). Benzodiazepine 1 (etherate, 240 mg, 0.51 mmol) was dissolved in acetic acid (10 mL) and cooled to 10 °C. To the yellow solution was added sodium cyanoborohydride (63.6 mg, 1.01 mmol) all at once. After the reaction mixture was stirred for 15 min at 10 °C, it was diluted with H_2O (10 mL), made basic with saturated Na_2CO_3 (aqueous), and extracted with EtOAc (2 × 25 mL). The combined organic extracts were washed with brine, dried over $MgSO_4$, filtered, and evaporated to dryness in vacuo. The residue was chromatographed (silica gel, 900:10:1:1 (v/v/v/v) CH_2Cl_2/MeOH/H_2O/HOAc), and the product fractions were evaporated to dryness in vacuo. The residue was dissolved in absolute ethanol, filtered, and treated with 5.4 M HCl in ethanol until the solution was acidic. The product crystallized as fine white needles, which were dried in vacuo at 82 °C: ^{1}H NMR (CD_3OD) δ 3.2 (1 H, dd, J_1 = 14 Hz, J_2 = 5 Hz, CH_{2a}), 3.63 (1 H, dd, J_1 = 14 Hz, J_2 = 9 Hz, CH_{2b}), 3.95 (1 H, dd, J_1 = 9 Hz, J_2 = 5 Hz, $CHCH_2$), 5.72 (1 H, s, CHN), 6.8–7.7 (13 H, m, aro).

Method E. 3-((2,3-Dihydro-1*H*-indol-3-yl)methyl)-5-(2-fluorophenyl)-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one (49). Compound 8 (120 mg, 0.31 mmol) was dissolved in 2 mL of trifluoroacetic acid. The resulting orange solution was treated with 0.5 mL (3.1 mmol) of triethylsilane and stirred rapidly at room temperature. After 2 h, the reaction mixture was evaporated to dryness and the residue was partitioned between water and ethyl acetate. The organic phase was washed with sodium bicarbonate solution (saturated) and brine and then dried ($MgSO_4$) and concentrated. The analytical sample was obtained via preparative thick-layer chromatography on silica gel (1:1 hexane/ethyl acetate, v/v, multiple elutions): ^{1}H NMR ($CDCl_3$) δ 2.35 (1 H, ddd, J_1 = 14 Hz, J_2 = 10 Hz, J_3 = 5 Hz), 2.98 (1 H, ddd, J_1 = 14 Hz, J_2 = 9 Hz, J_3 = 4 Hz), 3.20 (1 H, t, J = 9 Hz), 3.61 (1 H, t, J = 9 Hz), 3.70 (1 H, dd, J_1 = 9 Hz, J_2 = 5 Hz), 3.82 (1 H, br ddd, J_1 = 19 Hz, J_2 = 8 Hz, J_3 = 4 Hz), 6.66 (1 H, d, J = 9 Hz), 6.74 (1 H, t, J = 15 Hz), 7.0–7.6 (m, aro), 8.0 (1 H, s).

Method F. 3(*R*)-((1-(*N*-((1,1-Dimethylethoxy)carbonyl)-L-leucyl)-2,3-dihydro-1*H*-indol-3α-yl)methyl)-5-(2-fluorophenyl)-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one (56). Compound 49 (100 mg, 0.259 mmol), *N*-Boc-L-leucine monohydrate (64.7 mg, 0.259 mmol), 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride (EDC, 49.8 mg, 0.259 mmol), and 1-hydroxybenzotriazole hydrate (HBT, 35.0 mg, 0.259 mmol) were combined in freshly degassed dimethylformamide (DMF, 2 mL) and stirred at room temperature. The pH of the solution was adjusted to 9.0–9.5 with triethylamine (0.108 mL, 0.777 mmol), and stirring was continued for 24 h. The mixture was evaporated in vacuo, treated with 10% Na_2CO_3 (aqueous) (20 mL), and extracted with EtOAc (2 × 30 mL). The combined extracts were washed with H_2O (20 mL) and brine (20 mL), dried over $MgSO_4$, filtered, and evaporated to dryness in vacuo. The residue was chromatographed (silica gel, 30% (v/v) EtOAc in hexane) to give **56**: ^{1}H NMR ($CDCl_3$) δ 0.85 (3 H, d, J = 6 Hz), 0.90 (3 H, d, J = 6 Hz), 1.4 (9 H, s), 1.2–1.8 (3 H, m), 2.15–2.27 (br), 3.04 (br t, J_t = 12 Hz), 3.79 (dd, J_1 = 9 Hz, J_2 = 4 Hz), 3.90–3.98 (br), 4.07–4.19 (br), 4.45 (br, dt, J_1 = 9 Hz, J_2 = 4 Hz), 5.30 (d, J = 9 Hz), 7.05–7.60 (m), 8.20 (d, J = 8 Hz), 8.25 (s).

(*R*,*R*)-7-Chloro-3-((2,3-dihydro-2-oxo-1*H*-indol-3-yl)methyl)-1,3-dihydro-5-phenyl-2*H*-1,4-benzodiazepin-2-one (61). The procedure of Savige and Fontana was employed.[35] Compound 1 (0.2 g, 0.5 mmol) was dissolved in Me_2SO (0.4 g, 5.1 mmol). To the stirred solution was added dropwise 12 N HCl (0.8 mL, 9.6 mmol) and glacial acetic acid (3 mL). The mixture was heated at 60 °C for 30 min and quenched in ice water (20 mL). The mixture was neutralized with saturated $NaHCO_3$ and extracted with 1-butanol (3 × 10 mL). The butanol layer was washed with water, dried over Na_2SO_4, filtered, and evaporated to dryness in vacuo. The residue was chromatographed on preparative silica gel plates eluted with 95:5 (v/v) chloroform/methanol. The title compound was isolated as a mixture of two diastereomers: ^{1}H NMR ($CDCl_3$) δ 2.41 (ddd, J_1 = 14 Hz, J_2 = 11 Hz, J_3 = 4 Hz), 2.79 (dt, J_d = 14 Hz, J_t = 8 Hz), 2.91 (dt, J_d = 15 Hz, J_t = 7 Hz), 3.13 (ddd, J_1 = 15 Hz, J_2 = 11 Hz, J_3 = 4 Hz), 3.94 (t, J = 7.5 Hz), 4.01 (dd, J_1 = 11 Hz, J_2 = 4 Hz), 4.26 (t, J = 7.5 Hz), 4.43 (dd, J_1 = 11 Hz, J_2 = 4 Hz), 6.8–7.6 (m), 7.26 (s), 7.83 (s), 8.57 (s), 8.61 (s).

Acknowledgment. We are grateful to Dr. Ralph Hirschmann and Dr. Michael Rosenblatt for their comments and support during the course of this work. We also acknowledge the contributions of Dr. Steven Pitzenberger and Joan Murphy (NMR spectra), Dr. Harri Ramjit and Brian Haggert (mass spectra), John Moreau (CHN determinations), Susan Fitzpatrick (HPLC), and Cheryl Gerson (manuscript preparation).

Supplementary Material Available: Crystallographic data including tables of the atomic positional and thermal parameters, bond distances, and bond angles for 1 (4 pages). Ordering information is given on any current masthead page.

Notes

Chemical and Enzymatic Oxidative Coupling of 5-Hydroxy-*N*,*N*-dimethyltryptamine with Amines

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As part of a program aiming to obtain a covalent labeling of serotoninergic receptors we have studied the oxidative coupling of serotonin derivatives with amino compounds. The oxidation of bufotenine (2) by MnO_2 and human ceruloplasmin followed by the Michael type addition with dansylcadaverine and dansyllysine gave a fluorescent adduct identified as fused oxazole structure 4.

Serotonin (5-hydroxytryptamine) (1) is a neurotransmitter acting in the central and peripheral nervous system. The receptors involved in the function of the serotoninergic system are still not fully known. In particular, the isolation of these sites has not yet been accomplished, since adequate methods are lacking. Many studies have

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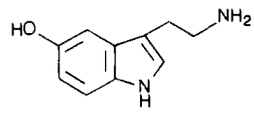

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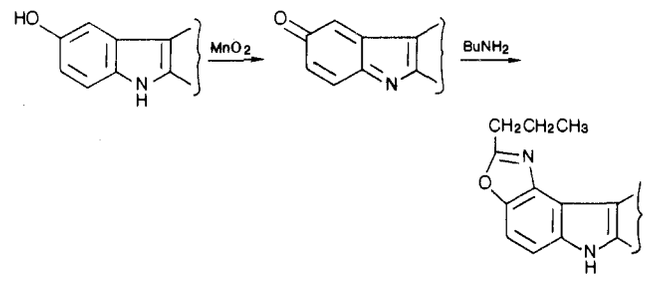
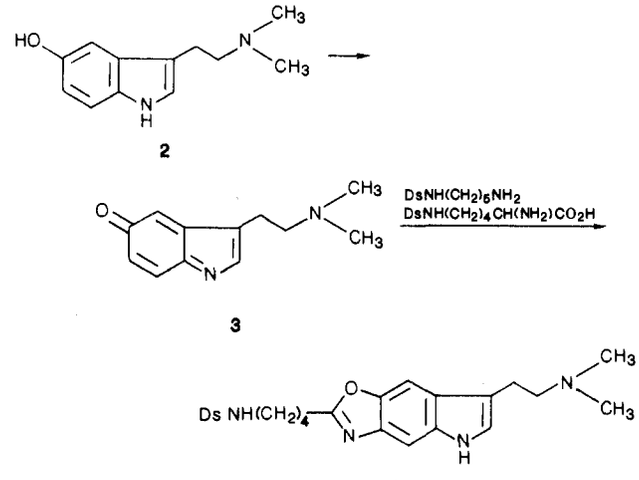
been made to synthesize agonists or antagonists of 5-hydroxytryptamine[1-3] and numerous examples in the literature have illustrated that irreversible ligands are necessary for the isolation of this receptor.[4-6] As part of a program of affinity covalent binding of the serotoninergic receptor, we have previously described a photoactivatable compound,[7] 3-(azidoethyl)5-hydroxyindole, which gave a specific covalent binding with a serotonin receptor. However, confronted with the difficulty of preparing its tritiated derivative, we decided to investigate the covalent binding of serotonin itself with its receptor. This approach was based on previous publications in the ellipticine series.[8-11] The fused bicyclic indole antitumor drug 9-hydroxyellipticine gave, upon oxidation, a quinone imine that reacted by a Michael type addition with a variety of nucleophiles including amino acids and nucleosides to give a series of annulated oxazoles. These reactions were also demonstrated in the case of 1,4-dimethyl-6-hydroxycarbazole,[12] a 5-hydroxyindole-containing molecular framework (Scheme I).

Moreover it has been suggested that ceruloplasmin, a copper-containing protein, with oxidase activity, may play a role in the regulation of serotonin and biogenic amines levels within the brain via a quinone imine.[13,14] The biochemical oxidation of the aromatic hydroxyl group of biogenic amines is less studied[15] as compared to the well-known oxidation of the amino side chain with monoamine oxidases. In a preliminary investigation, we decided to start with bufotenine, 5-hydroxy-*N*,*N*-dimethyltryptamine (**2**) (K_D = 0.2 μM for serotonin receptors[7]), instead of serotonin (**1**) because it was expected that its dimethylamino group would not react with the 5-quinone intermediate, avoiding the formation of self-condensation polymers. In order to test the Michael addition of an amine, we used *N*-dansylcadaverine, a fluorescent compound that facilitated the isolation of the final adduct. The oxidation was conducted by two means: chemical oxidation with MnO_2 and enzymatic oxidation with ceruloplasmin.

Scheme I



Chemical Oxidation with MnO_2. In a one-pot synthesis of **4**, bufotenine (**2**) was oxidized by MnO_2, in the conditions described[12] as giving the best yield of adducts in the fused polycyclic indole series. The formation of the postulated quinone imine **3** was assumed by the kinetic appearance of a visible absorption at 410 nm accompanied by the reduction of the bufotenine absorption at 275 and 295 nm. The Michael addition of *N*-dansylcadaverine occurred at position 6 and not at carbon 4 as for the fused oxazole previously isolated,[9,12] leading to the adduct **4**.



2

3

4

Ds = dansyl

The structure of oxazole **4** was unambiguously assigned on the basis of its mass spectrum (FAB$^+$, M^+ = 534.5) and ^{1}H NMR spectrum. The site of addition was determined by high-field NMR (500 MHz) ^{1}H–^{1}H 2D COSY (pulse sequence 45°): H_2 and H_7 indole protons were identified at 7.30 ppm. The spectral integration agreed with two protons and ^{1}H–^{1}H 2D COSY did not show observable coupling of these protons. The remaining H_4 indole proton appeared at 7.20 ppm. This was confirmed by the absence of a large coupling constant of 8–9 Hz observed in the oxazolo[4,5-*e*]indole identified in previous works.[9]

The oxidative coupling of bufotenine with N^ϵ-dansyl-L-lysine gave the same compound **4** as identified by TLC and HPLC. This could be easily explained by the oxidative decarboxylation mechanism proposed by Potier et al.[9]

Enzymatic Oxidation with Ceruloplasmin. Bufotenine (**2**), dansylcadaverine, and human ceruloplasmin were stirred overnight at room temperature, and the blue color of the copper-containing protein disappeared. A great amount (≈80%) of bufotenine was recovered, but we isolated material that was identical with compound **4** by TLC, HPLC, UV, and mass spectra (FAB$^+$).

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As a consequence of this work, it may be possible to isolate the serotoninergic receptor by the covalent binding of a labeled serotonin under oxidative conditions (e.g., H_2O_2/horseradish peroxidase). The results reported here with ceruloplasmin offer new perspectives on the possible role of this oxidizing protein in neurophysiological disorders.

Experimental Section

Proton nuclear magnetic resonance spectra were recorded on a Bruker WM500 spectrometer using tetramethylsilane as an internal standard. Mass spectra were performed on a VG instrument with glycerol matrix. All chromatographic separations were performed on Merck silica gel (Kieselgel 60, 230–400 mesh, ASTM). *N*-Dansylcadaverine, N^{ϵ}-dansyl-L-lysine, and human ceruloplasmin (5% solution in 0.25 M sodium chloride and 0.05 M sodium acetate) were purchased from Sigma. Bufotenine was prepared by demethylation[16] of 5-methoxy-3-[2-(dimethylamino)ethyl]indole.[17]

2-[4-(Dansylamino)butyl]-7-[2-(dimethylamino)ethyl]-5*H*-pyrrolo[2,3-*f*]benzoxazole (4): Oxidation of Bufotenine (2) with MnO_2. To a suspension of bufotenine (2) (100 mg, 0.48 mmol) in water (500 μL) was added a solution of dansylcadaverine (400 mg, 1.19 mmol) in dichloromethane (5 mL) and methanol (5 mL). After addition of MnO_2 (167 mg, 1.91 mmol), the reaction mixture was stirred at room temperature for 24 h and then filtered through Celite. The filtrate was concentrated in vacuo and the residue was purified by two columns [first, eluent, gradient from CH_2Cl_2 to CH_2Cl_2/MeOH (85/15); second, eluent, CH_2Cl_2/MeOH/NH_4OH (25/5/3) organic phase] and then by HPLC (Lichrosorb NH_2 7-μm Merck column, 250 × 10 mm) with a linear gradient, MeOH/$CHCl_3$, 0.1 to 10 in 20 min, flow 5.5 mL/min, to give a yellow oil: yield 18 mg (7%); NMR (Me_2SO + D_2O) δ 8.43 (d, 1 H), 8.28 (d, 1 H), 8.07 (d, 1 H), 7.55 (m, 2 H), 7.31 (s, 2 H), 7.20 (s, 1 H), 7.22 (d, 1 H), 3.05 (t, 2 H), 2.85 (m, 2 H), 2.75 (m, 8 H), 2.26 (s, 6 H), 1.71 (m, 2 H), 1.45 (m, 2 H); MS (FAB$^+$), 534.55 (M^+); UV (EtOH, 96%) λ_{max} 335, nm. 305, 296 ; HPLC (Lichrosorb NH_2 5-μm Merck column, 250 × 4 mm) with the linear gradient MeOH/$CHCl_3$, 0 to 10 in 20 min, t_R = 11.72 min, flow 1 mL/min.

To a solution of bufotenine (2) (2 mg, 0.010 mmol) in water (100 μL) was added a solution of N^{ϵ}-dansyl-L-lysine (11 mg, 0.03 mmol) in dichloromethane (10 mL). After addition of MnO_2 (2.6 mg, 0.03 mmol), the reaction mixture was stirred at room temperture for 48 h. After usual purifications, we obtained a yellow oil, which exhibited the same retention time (HPLC) and gave one single peak when mixed with compound 4 isolated from dansylcadaverine.

Compound 4: Oxidation of Bufotenine with Ceruloplasmin. To a solution of bufotenine (2) (100 mg, 0.48 mmol) in 0.2 M acetate buffer, pH 5.40 (5 mL), and methanol (2 mL) was added a solution of dansylcadaverine (400 mg, 1.19 mmol) in dichloromethane (5 mL) and methanol (5 mL). The reaction mixture was vigorously stirred and human ceruloplasmin (2 mL), 4900 units/mL was added, by portion of 100 μL over 8 h. The suspension was stirred overnight and concentrated in vacuo. The residue was extracted four times with dichloromethane (30 mL). After drying (Na_2SO_4) and evaporation, the residue was purified as previously described for the oxidation by MnO_2: yield 1.5 mg (0.6%); MS (FAB$^+$), 534.16 (M^+); UV (EtOH, 96%) λ_{max} 335 nm, 305, 296; HPLC, Lichrosorb NH_2 5-μm Merck column, 250 × 4 mm) with a linear gradient, MeOH/$CHCl_3$, 0 to 10 in 20 min, t_R = 11.85 min, flow 1 mL/min, the mixture with the compound obtained from oxidation with MnO_2 gave one single peak.

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Radioiodinated *p*-Iodoclonidine: A High-Affinity Probe for the α_2-Adrenergic Receptor

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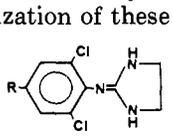
Received August 25, 1986

The chemical synthesis of 2-[(2,6-dichloro-4-iodophenyl)imino]imidazolidine (PIC) and its radioiodinated analogue [^{125}I]PIC is described. PIC was synthesized from 2,6-dichloroaniline in five synthetic steps. This agent displayed a high affinity for the α_2-adrenergic receptor (IC_{50} = 1.5 nM) in competitive binding assays conducted with purified human platelet plasma membrane fractions. For the synthesis of radioiodinated PIC the triazene intermediate 11 was synthesized from 2,6-dichloro-4-nitroaniline in five synthetic steps. Acid-catalyzed decomposition of 11 with no-carrier-added Na^{125}I afforded high specific activity [^{125}I]PIC. In view of its high affinity for the α_2-adrenergic receptor, [^{125}I]PIC is a potentially useful probe for studies in adrenergic pharmacology.

Clonidine (1) is a potent antihypertensive drug whose mechanism of action is believed to be via stimulation of centrally located α_2-adrenergic receptors.[1] The commercial availability of tritiated α_2-adrenergic probes, such as [^{3}H]clonidine and [^{3}H]*p*-aminoclonidine ([^{3}H]PAC), has proved to be extremely valuable for the identification and characterization of these receptors in various tissues.[2]



1 R = H ; Clonidine

2 R = I ; PIC

As part of our studies in the area of receptor-specific ligands, we were interested in the development of an ^{125}I-labeled clonidine analogue as a suitable α_2-adrenergic receptor probe. In contrast to tritiated tracers, a radioiodinated probe would have several advantages such as a capability to achieve higher specific activity, an increased counting efficiency, and the opportunity to perform in vivo scintigraphic analyses. Moreover, the ability to achieve high specific activity with an ^{125}I-labeled probe would make it possible to analyze those tissues having very low densities of α_2-adrenergic receptors. A recent paper reports the synthesis and characterization of a radioiodinated analogue of rauwolscine, an α_2 antagonist.[3] We report the first

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