ruthenocene: TLC (*n*-BuOH/H_2O/HOAc, 4:1:1) R_f 0.46 with positive color response when sprayed with ninhydrin; HPLC, LiChrosorb RF-18 (10 μm), mobile phase 0.01 M phosphate buffer, pH 3.0/MeOH (60:40), flow rate 1 mL/min, detection; UV max 245 nm; t_R = 9.92 min; IR (KBr) 3415 (br), 2050 (br), 1600 (s), 1510 (m), 1440 (w), 1415 (m), 1355 (m), 1190 (w), 1155 (w), 1110 (m), 1030 (m), 1045 (m), 1010 (m), 865 (m), 820 (s); ^1H NMR (D_2O, NaOD) δ 2.51 (t, 2 H, CH_2), 3.24 (dd, 1 H, CH), 4.51 (s, 2 H, α-H), 4.59 (s, 5 H, Cp), 4.6 (s, 2 H, β-H); the mass spectrum of the bis(trimethylsilyl) derivative contained groups of ions characteristic of an intact ruthenocene nucleus with a parent peak at *m/z* 463 and a base peak at *m/z* 245. Anal. ($C_{13}H_{15}N_2Ru \cdot H_2O$) C, H, N.

β-[^{103,106}Ru]Ruthenocenylalanine (5b). The EtOH solution of the crude alkylation product from the previous reaction was evaporated to 0.5 mL under a stream of argon, and 0.5 mL of 1 M NaOH was then added. The solution was refluxed for 2 h, cooled, acidified to pH 1–3 with H_3PO_4, and passed over a strong cation-exchange resin (Amberlite CG-120, 100–200 mesh). The first 5 mL of eluate contained the product, 106 μCi (40% theoretical yield), which chromatographed with authentic **5a** with *n*-BuOH/HOAc/H_2O (4:1:1) or *n*-BuOH/HOAc/pyridine/H_2O (4:1:1) on silica gel G.

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Registry No. 1 (^{103}Ru derivative), 12093-76-4; 1 (^{106}Ru derivative), 89462-67-9; **2**, 33293-45-7; **2** (^{103}Ru derivative), 89462-68-0; **2** (^{106}Ru derivative), 89462-69-1; **3**, 33292-36-3; **3** (^{103}Ru derivative), 89462-70-4; **3** (^{106}Ru derivative), 89462-71-5; **4**, 89462-72-6; **4** (^{103}Ru derivative), 89462-73-7; **4** (^{106}Ru derivative), 89462-74-8; **5**, 89462-75-9; **5** (^{103}Ru derivative), 89462-76-0; **5** (^{106}Ru derivative), 89462-77-1; $^{103}RuCl_3$, 65234-97-1; $^{106}RuCl_3$, 63767-78-2; cyclopentadienylsodium, 63936-05-0; *N,N'*-bis(dimethylamino)methane, 51-80-9; ethyl 3-ruthenocenyl-2-formamidopropionate, 89462-78-2; diethyl formamidomalonate, 6326-44-9.

Aporphines. 58. *N*-(2-Chloroethyl)[8,9-^{2}H]norapomorphine, an Irreversible Ligand for Dopamine Receptors: Synthesis and Application[1]

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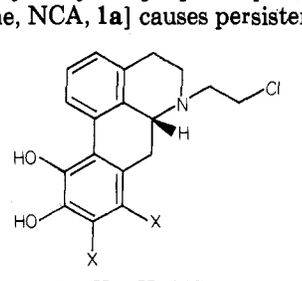
The synthesis of the title compounds (**1c** and its ^{2}H isomer **1b**) from *N*-(2-hydroxyethyl)norapomorphine was carried out by ring bromination, followed by chlorination to the 2-chloroethyl compound **6**. Further reduction with $^2H_2$ or $^3H_2$ and Pd/C gave **1b** or **1c**. Radiochemically pure (97%) **1c** was obtained with a specific activity of 16.3 Ci/mmol. The purity of **1c** was determined by LC, HPLC, UV, and NMR. [^{3}H]NCA was shown to label the D_2 receptor; however, the D_2 signal appears to be only a small portion of the total signal, which may include binding to other dopamine receptor subtypes (D_1 and D_3).

Tritiated catechol aporphines, such as apomorphine (APO) and *N*-*n*-propylnorapomorphine (NPA), are known to bind selectively at sites believed to represent dopamine (DA) receptors in the central nervous system (CNS).[2,3] The synthesis[4] and demonstration that (−)-*N*-(2-chloroethyl)-10,11-dihydroxynoraporphine [*N*-(2-chloroethyl)-norapomorphine, NCA, **1a**] causes persistent DA receptor



1a, X = H (NCA)
b, X = ^{2}H
c, X = ^{3}H

blockage suggested that this inhibition of DA receptor function may involve covalent bonding of a receptor binding site.[4-8] NCA blocked DA-sensitive adenylate cyclase activity in a noncompetitive and apparently irreversible manner.[5] This effect was prevented by coincubation with DA or APO but not with norepinephrine. Analogues of NCA with low affinity for DA receptor sites defined by binding of 1 nM [^{3}H]APO[3] also had much weaker effects against the DA-sensitive cyclase activity. These included the 10-O-methylated derivative (analogue of apocodeine) and a 10,11-diester derivative of NCA.

Additional pharmacological actions of NCA have been described.[9-14] Mustafa et al.[9] reported that NCA (57 μmol) locally injected into the nucleus accumbens of rat brain can block the behavioral excitation produced by similarly

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(1) Abstracted in part from the thesis of J.H.G., which was submitted in June, 1982, to Northeastern University in partial fulfillment of the requirements for the M.S. degree in Medicinal Chemistry.
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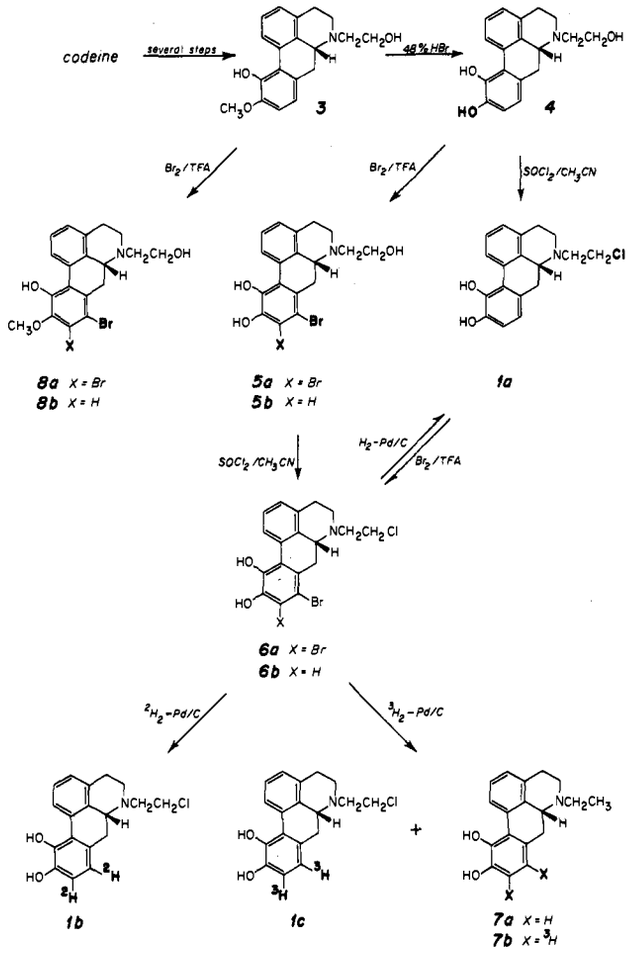
Scheme I. Synthesis of [8,9-^{2}H]NCA and [8,9-^{3}H]NCA



locally administered DA agonists, such as ADTN, for nearly a week. Evidence has also been presented by Lehman and Langer[10] that NCA may have reversible, DA agonist-like actions at DA autoreceptors, since it can inhibit [^{3}H]DA release from rat striatal slices in vitro and decrease levels of the DA-metabolite DOPAC in mouse striatum in vivo (both effects presumably indicating autoreceptor-mediated decreases in release or turnover of DA).[10] NCA inhibited in a concentration-dependent manner the electrically evoked [^{3}H]acetylcholine release in slices of cat caudate.[11] Goosey and Doggett reported[12] that NCA appears to selectively inhibit the binding of [^{3}H]APO to domperidone-sensitive binding sites, which probably represent D_2 receptors, in rat striatal tissue. The peripheral DA antagonistic effects of NCA in DNA-induced renal vasodilation in the isolated perfused rat kidney[13] and in aggravating cysteamine-induced duodenal ulcer in the rat[14] have recently been observed.

These pharmacological effects of NCA may be attributed to a covalent interaction of the 2-chloroethylamine mediated through the aziridium ion intermediate.[8]

The availability of [8,9-^{3}H]NCA made it possible for Seeman and his group using isoelectric focusing to label

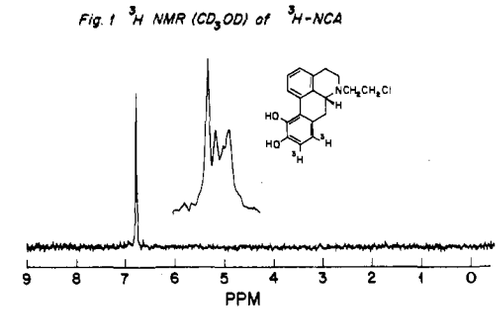


Figure 1. ^{3}H NMR (CD_3OD) of [^{3}H]NCA.

macromolecular species associated with DA receptors.[15] Baldessarini and Kula[16] have carried out preliminary characterizations of the interactions of [^{3}H]NCA with a tissue preparation of the DA receptor rich mammalian basal ganglia. Jenner et al.[17] established that [^{3}H]NCA was irreversibly bound to a saturable catecholamine binding site in rat striatal membranes. In light of these findings, we report the details of the syntheses of both 8,9-^{2}H- and -^{3}H-labeled *N*-(2-chloroethyl)norapomorphine (NCA) and present more recent evidence that indicates that [^{3}H]NCA labels the D_2 receptor.

Chemistry. The synthesis of **1b** and **1c** is shown in Scheme I. The synthesis of (−)-*N*-(2-hydroxyethyl)norapocodeine (**3**) and *N*-(2-hydroxyethyl)norapomorphine (**4**) were carried out in several steps from codeine (**2**) by modifications of previously described methods (Scheme I).[4] Treatment of **4** with bromine in trifluoroacetic acid gave a crystalline product in 97% yield, which consisted of 95 parts of the dibromo derivative **5a** and 5 parts of a monobromo compound **5b**. Further conversion of the bromination product **5** with thionyl chloride in acetonitrile gave (−)-*N*-(2-chloroethyl)-8,9-dibromonorapomorphine hydrochloride (**6a**) containing a small quantity (12% by HPLC analysis) of the 8-bromo product (**6b**). Alternatively, bromination of NCA (**1a**) gave **6a** and **6b**, which were identical with the products obtained from **5**. The reduction of **6a** and **6b** with 10% hydrogen and Pd/C in ethanol led to **1a**, which was identical in all respects with an authentic sample.[4] Similarly, **6a** and **6b** could be reduced with $^3H_2$ or $^2H_2$ to yield **1b** or **1c**. Purification of **1c** was accomplished by HPLC. Because crude TLC indicated the presence of some (−)-*N*-ethyl[^{3}H]norapomorphine (**7b**), the trailing two-thirds of the (−)-[^{3}H]NCA (**1c**) peak alone was collected in the dark at 4 °C under argon. In this way, 41 mCi (6.8% radiochemical yield based on cold precursor) of **1c** was collected and found to be 97% radiochemically pure by TLC and HPLC.[18] The specific activity of **1c** was determined to be 16.3 Ci/mmol by UV, where the UV spectrum of the radiolabeled substance was superimposable on that of the cold standard. A ^{3}H NMR of **1c** gave a multiplet at δ 6.85, consonant with exclusive aromatic labeling (Figures 1). For long-term storage and to circumvent the hydrolysis of **1c** to (−)-[^{3}H]**4**,

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(17) Jenner, P.; Kilpatrick, G. J.; Marsden, C. D. *Br. J. Pharmacol.* **1983**, *80*, 547P.
(18) The (−)-[^{3}H]NCA was found to contain by HPLC about 1% solvent front impurities and 2% **7b**. Before HPLC is performed, it is necessary to demonstrate in the aforementioned HPLC system a separation of (−)-NHEA from (−)-NCA *before* a (−)-[^{3}H]NCA shot is made. Although (−)-NEA can be separated as a faster eluting peak from (−)-NCA, a base-line separation was rarely seen.

it was found advisable to lower the pH of the 0.01 N KH_2PO_4/EtOH (95:5) to 1.60 by the addition of concentrated phosphoric acid. The **1c** was found to contain, by HPLC, about 1% solvent-front impurities and 2% **7b**.

An alternative method for the preparation of **5a** by the bromination of **3**, failed to yield the desired 8,9-dibromo product, **8a**. However, we did isolate in 98% yield the 8-bromoapocodeine derivative **8b**. Cold *N*-ethylnorapomorphine (**7a**) was prepared from norcodeine and was identical with the previously reported product obtained from morphine.[19]

Pharmacology. Preparation of Canine Striatal and Porcine Anterior Pituitary Membranes. Frozen canine brains were obtained from Pel Freeze (Arkansas) and prepared as previously described.[20] Striata were dissected out over ice and homogenized in 10 mL of ice-cold 0.25 M sucrose per gram wet weight by using a Teflon–glass homogenizer. The suspension was centrifuged at 1100*g*, and the supernatant was retained. The pellet was resuspended in an additional 10 volumes of sucrose, and the resulting mixture was again centrifuged at 1100*g*. The supernatant of this spin was combined with the first, and the combined fractions were centrifuged at 100000*g* for 60 min. This pellet was then resuspended by homogenization into 10 mL/g original wet weight of TEAN buffer (50 mM Tris, 5 mM EDTA, 0.01% ascorbate, 10 μM nialamide) containing 5 mM $MgCl_2$ and 120 mM NaCl. Protein was subsequently determined to be about 4 mg/mL using the Lowry method. The suspensions were stored at −20 °C until use.

Frozen pituitary glands (porcine) were purchased from a Toronto abattoir. The anterior pituitary portions were removed, and the combined anterior pituitary tissues were prepared in the same way as those for the canine striatal membranes, except that the buffer used contained the following: 50 mM Tris-HCl (pH 7.4), 5 mM KCl, 1.5 mM $CaCl_2$, 4 mM $MgCl_2$, 1 mM EDTA, 0.1% ascorbic acid, and 12 μM nialamide (further details can be found in ref 21 and 22).

Binding Assays. (a) (−)-NCA. The method used was a modification of that described by Hartley and Seeman.[23] Membranes from above were washed 3 times by centrifugation in 25 volumes of TEAN, Mg^{2+}, Na^+ buffer and resuspended at a protein concentration of about 1 mg/mL. The mixture was polytroned for 15 s (machine setting 6 out of 10), and a 0.2-mL aliquot was added to glass culture tubes containing (−)-NCA at final concentration ranging from 1 to 10000 nM. This mixture was incubated for 2 h at 4 °C, and [^{3}H]spiperone was then added (final concentration 1 nM). The incubation was continued for an additional 3 h at 4 °C and then filtered through Whatman GF/B filters. Radioactivity was determined by liquid scintillation spectrometry following extraction of the filter in 10 mL of Scint-A (Packard).

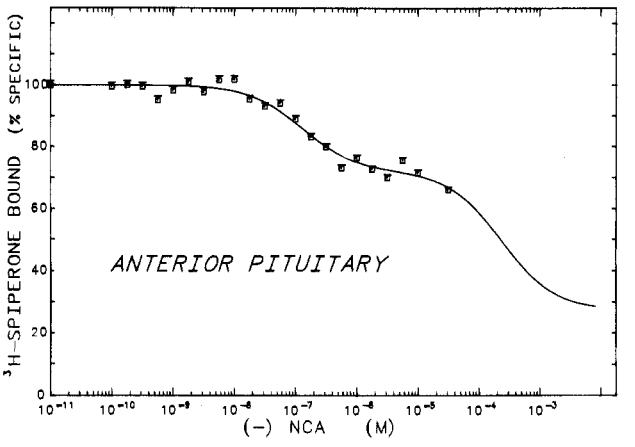

Figure 2. Effect of (−)-NCA on the binding of 184 pM [^{3}H]-spiperone to porcine anterior pituitary membranes. Computer-assisted fitting (LIGAND) indicated that (−)-NCA inhibited [^{3}H]spiperone binding in two phases: a high-affinity phase with a K_D of 53 nM at the D_2^{high} state of the dopamine receptor and a low-affinity phase with a K_D of 98 μM at the D_2^{low} state of the receptor. The base-line level (0% specific binding) was defined by 1 μM (+)-butaclamol.

Table I. [^{3}H]NCA Binding to Canine Striatal Membranes

drug	% total binding inhibited	apparent IC_{50}[b]
(±)-sulpiride	15	200 nM
haloperidol	15	5 nM
(+)-butaclamol	18	50 nM
dopamine	50	15 μM
apomorphine	35	25 μM
legend		

[a] Specificity of binding of [^{3}H]NCA to canine striatal membranes. [b] IC_{50} values are reported as "apparent", since [^{3}H]NCA is purported to be irreversible, thus making it impossible to realiably determine these parameters. Results are the mean of two separate experiments, each performed in triplicate. Binding of [^{1}H]NCA to filters has been subtracted in all cases.

The effect of (−)-NCA on the binding of [^{3}H]spiperone to anterior pituitary membranes was done in the same way as that for canine striatal membranes, except for the different buffer used (mentioned previously), and the final [^{3}H]spiperone concentration was 184 nM.

(b) [^{3}H]NCA. To determine the specificity of [^{3}H]NCA binding, we used the membranes prepared in (a). Competition-style assays were used, since other experiments indicated that the association of [^{3}H]NCA with membranes at 4 °C was reversible during the first several hours. Experiments where membranes were preincubated with competing drug prior to exposure to [^{3}H]NCA showed no difference from the competition-style assays. Membranes were added to test tubes containing [^{3}H]NCA (final concentration 2.5 nM) and competing drug. Following incubation for 30 min at 37 °C, the mixture was filtered through Whatman GF/B filters, and radioactivity was measured as described.

Results

(−)-NCA competed with [^{3}H]spiperone binding, resulting in curves with shallow slopes. In all experiments (n = 7), (−)-NCA had an apparent IC_{50} of 8 μM vs. [^{3}H]-spiperone binding for canine striated membranes. In some instances (n = 3), there was some indication of competition in the 1–10 nM range, but this was not a consistent finding. At 100 μM, (−)-NCA displaced up to 70% of the total [^{3}H]spiperone binding.

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Figure 2 illustrates a typical experiment for the inhibition of [^{3}H]spiperone binding to the anterior pituitary tissue by (–)-NCA. Computerized analysis (using LIGAND from Drs. P. Munson and D. Rodbard of Bethesda, MD; see ref 21) revealed that (–)-NCA inhibited the binding of [^{3}H]spiperone in two phases: the high-affinity component of [^{3}H]spiperone binding to the D_2^{high} state was inhibited by (–)-NCA with a K_D of 53 nM, while the (–)-NCA inhibited [^{3}H]spiperone binding with a K_D of 98 μM at the low-affinity site (D_2^{low}).

[^{3}H]NCA. D_2 anatagonists [sulpiride, haloperidol, and (+)-butaclamol] were only able to displace about 15% of the total [^{3}H]NCA binding, making the estimation of IC_{50} values difficult (see Table I). However, these drugs do have a consistent effect on the [^{3}H]NCA binding. Agonists (dopamine, apomorphine) displaced up to 50% of the total binding (filter background subtracted), with IC_{50} values in the micromolar range (see Table I).

Discussion

It is clear that(–)-NCA influences the D_2 receptor, since it has a potency of 8 μM in displacing [^{3}H]spiperone binding. This was consistent with the classification of (–)-NCA as a D_2 dopamine agonist, like its congener, (–)-NPA. This is further supported by the occasional appearance of a high-affinity phase; however, further experiments using an optimized assay system would be required to establish the size of the higher affinity component. The high-affinity component (D_2^{high}) was more readily resolved by using the anterior pituitary tissue.[21,22] (–)-NCA inhibited [^{3}H]-spiperone binding to the D_2^{high} and D_2^{low} sites with K_Ds of 53 nM and 98 μM, respectively. This compares with D_2^{high} K_D values of approximately 0.3 nM for (±)-*N*-propylnorapomorphine, 0.5 nM for (–)-apomorphine, 2 nM for (±)-6,7-dihydroxy-2-aminotetralin, and 10 nM for dopamine (S. George et al., to be published).

[^{3}H]NCA can also be shown to label the D_2 receptor,[2] since its binding is displaceable with D_2 receptor blockers and agonists. However, the D_2 signal appears to be only a small portion of the total signal, which may include ligand binding to other dopamine receptor subtypes (D_1, D_3). Thus, the usefulness of this agent will depend on its being able to separate the various subpopulations of dopamine sites labeled to facilitate isolation of any one.

Experimental Section

All melting points were determined on a Thomas-Hoover apparatus and are uncorrected. Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN. Thin-layer chromatography (TLC) used precoated silica gel 13181 polyethyleneterphthalate sheets (Eastman Kodak, Rochester, NY). HPLC used Ramen short-column C-18, mobile phase 0.025 M NaH_2PO_4 (pH 3)/CH_3CN, 75:25.

***N*-(2-Hydroxyethyl)-8,9-dibromonorapomorphine Hydrobromide (5a).** To a solution of 500 mg (1.32 mmol) of *N*-(2-hydroxyethyl)norapomorphine hydrochloride (4) in 120 mL of trifluoroacetic acid (TFA) was added dropwise, at room temperature over 30 min, 140 μL (2.7 mmol) of bromine in 28 mL of TFA with rapid stirring in the dark. After the mixture was stirred for an additional 1.5 h, excess solvent was evaporated, and the crude product was recrystallized in methanol/ether to yield 215 mg of a white solid, identified as the hydrobromide salt. Evaporation of the filtrate yielded an additional 476.5 mg (97.4% yield) by HPLC: The first product of crystallization contained 94.75% dibromo and 5.25% monobromo compound. The second product of crystallization contained 92.08% the dibromo compound **5a** and 7.92% of the monobromo compound **5b**: MS, *m/e* (M^+) 454. The mixture of **5a** and **5b** was converted to **6a** and **6b**.

***N*-(2-Chloroethyl)-8,9-dibromonorapomorphine Hydrochloride (6a). Method A.** *N*-(2-Hydroxyethyl)-8,9-dibromonorapomorphine hydrobromide (**5**) (containing ~8% **5b**; 300 mg, 0.56 mmol) was dissolved in 50% methanol in chloroform, and 10 mL of 3% sodium bicarbonate solution was added. The solution was extracted three times with 30-mL portions of 10% methanol in chloroform, the combined organic layer was washed with distlled water, dried over magnesium sulfate, and filtered, and the filtrate was evaporated to dryness to yield 220 mg of **6a** free base.

Acetonitrile (4 mL) was added to the free base of **6a**, and then 0.4 mL (5.5 mmol) of thionyl chloride was added. The mixture was stirred at room temperature for 24 h, and the solvent was removed under vacuum. the residue was dissolved in a minimum amount of methanol. Ethereal hydrochloride was added, the solution was evaporated to dryness, and the crude product was recrystallized in methanol/ether to yield 197.3 mg (69.3%) of **6a** as an off-white crystal; mp 245–248 °C. HPLC showed 8-Br-NCA (12%) eluting faster than the 8,9-Br_2-NCA (μCN column, 0.01 N KH_2PO_4/ethanol, 9:1).

Compound **6a** (7.5 mg) was reduced for 3 h at 24 °C with 15 mg of 10% Pd/C in 1.5 mL of methanol to give 5.2 mg (70%) of NCA (**1**), identical with an authentic sample.

Method B. To a solution of 115.8 mg (0.33 mmol) of **1a** in 30 mL of TFA was added dropwise, at room temperature over 15 min, 35 μL (0.678 mmol) of bromine in 7 mL of TFA with rapid stirring in the dark. after the solution was stirred for an additional 1 1/2 h, a crystalline precipitate formed. The precipitate was filtered, washed with a few milliliters of cold TFA, and dried under vacuum to yield 82.1 mg (49%) of **6a**: mp 245–248 °C; HPLC: contained 92.45% of Br_2-NCA and confirmed that this compound is the same as that obtained by method A. This compound was also reduced with hydrogen to NCA (**1a**).

(–)-*N*-(2-Chloroethyl)[8,9-^{3}H]norapomorphine (1c). A solution of 20 mg (0.037 mmol) of (–)-8,9-Br_2-NCA·HCl (6a) in 15 mL of EtOH with 45 mg of 10% Pd/C was reduced with 100 Ci of tritium gas at atmospheric pressure, 24 °C, for 1 h in the dark with rapid stirring. After this time period, labile compounds were removed with EtOH, and following catalyst filtration, the crude product was packaged in 10 mL of EtOH (total activity 190 mCi). Purification of the crude (–)-[^{3}H]NCA was accomplished by HPLC [μCN column, 0.01 N KH_2PO_4 (pH 3)/EtOH, 95:5] at 1 mL/min, 254 nm). In this HPLC system, the following retention times were observed: (–)-*N*-(2-hydroxyethyl)norapomorphine (**4**), 9 min; (–)-*N*-ethylnorapomorphine (**7a**), 12 min; (–)-**1a**, 13 min; (–)-8,9-Br_2-NCA (**6a**), >20 min. Because crude TLC (silica gel; EtOH/HOAc, 6:1) indicated the presence of some (–)-[^{3}H]**7a**, the trailing two-thirds of the (–)-[^{3}H]NCA peak alone was collected in the dark at 4 °C under argon. In this way, 41 mCi (6.8% radiochemical yield based on cold precursor) of (–)-[^{3}H]NCA was collected and found to be 97%[18] radiochemically pure by TLC (silica gel; EtOH/HoAc, (6:1) and HPLC [μCN column, 0.01 N KH_2PO_4 (pH 3)/EtOH, 95:5]. In these chromatographic systems, the (–)-[^{3}H]NCA cochromatographed with authentic cold standard. The specific activity of (–)-[^{3}H]NCA was determined to be 16.3 Ci/mmol by UV [0.01 N KH_2PO_4 (pH 3)/EtOH, 95:5, where ϵ_{273} is 19 880 for cold (–)-NCA], and the UV spectrum of the radiolabeled substance was superimposable on that of the cold standard. A ^{3}H NMR (CD_3OD) of (–)-[^{3}H]NCA gave a multiplet at δ 6.85, consonant with exclusive aromatic labeling (Figure 1). For long-term storage and to circumvent the hydrolysis of (–)-[^{3}H]NCA to (–)-[^{3}H]4, it was found advisable to lower the pH of the 0.01 N KH_2PO_4/EtOH (95:5) to 1.60 by the addition of concentrated phosphoric acid.

***N*-(2-Chloroethyl)[8,9-^{2}H]norapomorphine Hydrochloride (1b).** A solution of 31 mg (0.06 mmol) of **6a**·HCl in 25 mL of ethanol with 62 mg 10% Pd/C was reduced with deuterium gas at 3 lb/in^2 at room temperature in the dark for 4 h with shaking. After removal of the catalyst, excess solvent was evaporated, the residue was taken up a minimum of methanol, ethereal hydrochloride was added, and the crude hydrochloride salt of **10** was recrystallized in methanol/ether: yield 12.8 mg (60%).

The ^{1}H NMR (CD_3OD) and integration of the aromatic region for **16** is 87%.

N-(2-Hydroxyethyl)-8-bromonorapocodeine Trifluoroacetate (5b). To a solution of N-(2-hydroxyethyl)norapocodeine **3**; 1.7 g, 5.5 mmol) in 450 mL of TFA was added dropwise, at room temperature over 2 h, 556 μL of bromine in 150 mL of TFA with rapid stirring in the dark. After 2 h, the solvent was evaporated under vacuum to yield a white solid. Recrystallization from MeOH/ether gave **8a**: yield 2.73 g (98%); mp 230 °C dec. HPLC, one peak. Anal. ($C_{19}H_{20}BrNO_3 \cdot CF_3COOH$) C, H, N; Br: calcd, 15.9; found, 16.25.

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Topical Carbonic Anhydrase Inhibitors

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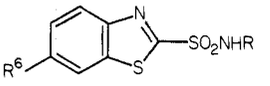
Ethoxzolamide and several derivatives (1–6) were synthesized and evaluated for carbonic anhydrase inhibition (CAI), solubility, pK_a, distribution, and corneal permeability. The 6-hydroxy (**5**) and, particularly, the 6-chloro (**6**) analogues have the best combination of properties for penetrating the site of action and reducing intraocular pressure. Both **5** and **6** exhibited topical effectiveness in the normal rabbit, with **6** showing greater potency.

Several different classes of drugs are used topically to treat glaucoma through the reduction of intraocular pressure (IOP).[1] Glaucoma is an optic condition in which the increased pressure can constrict capillaries delivering blood to the retina and optic nerve. If the IOP is not controlled, loss of peripheral vision and, eventually, blindness occurs.

Elevated IOP can be controlled by oral administration of carbonic anhydrase inhibitors (CAI), but this therapy produces serious side effects, leading to noncompliance by patients.[2] It may be possible to develop a CAI that would produce a reduced IOP after topical administration. Toward this goal we have selected ethoxzolamide (**1**, 6-ethoxy-2-benzothiazolesulfonamide) as the prototype CAI to be structurally altered in order to improve varous physical properties while retaining CAI activity. Compound **1** has high CAI potency and excellent corneal permeability. However, its aqueous solubility is poor, such that its solubility in tears and the resulting corneal penetration remain low upon topical dosing to the rabbit eye. Nevertheless, with a structure able to be modified, **1** is a promising candidate for optimizing penetration.

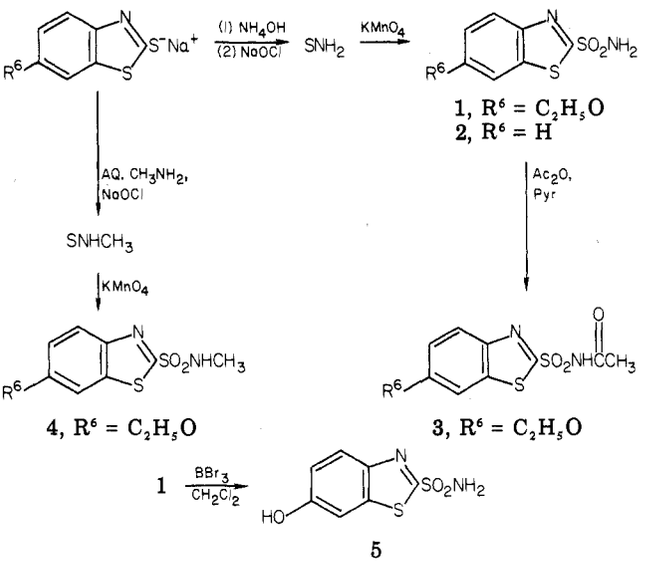


1, R = H; $R^6 = C_2H_5O$
2, R = H; R^6 = H
3, R = $COCH_3$; $R^6 = C_2H_5O$
4, R = CH_3; $R^6 = C_2H_5O$
5, R = H; R^6 = HO

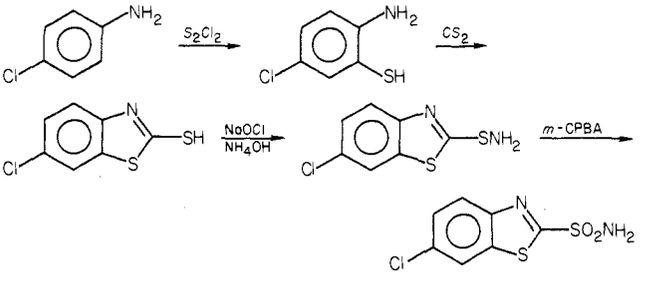
Chemistry. Most of the compounds were synthesized in a straightforward manner. The sulfonamides were prepared from the corresponding 2-mercapto derivative via the sulfenamide and subsequent oxidation. Compound **4** was made from the 2-mercapto compound via the N-methylsulfenamide in order to avoid dimethylation. Compound **5** was synthesized efficiently from **1** by ether cleavage with boron tribromide. Compound **6** was prepared by total synthesis via the Herz reaction from 4-chloroaniline.[3]

Physical Properties. The distribution coefficients, solubilities, pK_a's, and corneal permeability coefficients were determined by standard methods. The data are listed in Table I.

Scheme I



Scheme II



Biological Activity. The in vitro inhibition of carbonic anhydrase activity was determined by Maren's[4] method. The data relative to ethoxzolamide are listed in Table I. Evaluation of the ability of the compounds to reduce IOP on topical dosing to normal rabbits was accomplished.[5]

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