Comparison of (-)-Eseroline with (+)-Eseroline and Dihydroseco Analogues in Antinociceptive Assays: Confirmation of Rubreserine Structure by X-ray Analysis

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The enantiomers of eseroline bind to opiate receptors of rat brain membranes with equal affinities and show opiate agonist properties as inhibitors of adenylate cyclase in vitro. However, only (-)-eseroline shows potent narcotic agonist activity in vivo, similar to that of morphine. Neither (-)-noreseroline, (+)-eseroline, nor the open dihydroseco analogue (-)-8 shows analgetic effects in vivo. The structure of rubreserine being a resonance hybrid of an o-quinone with its zwitterionic mesomer is confirmed by solid-state X-ray diffraction analysis.

(-)-Physostigmine, an alkaloid from Calabar beans,[1] is medically used as an anticholinesterase agent[2] and by hydrolysis of the *N*-methylcarbamoyl function affords (-)-eseroline ((-)-**4**), probably a major metabolite of the drug.[3] It was recently reported that (-)-**4** possessed morphine-like analgetic properties, since analgesia was suppressed by treatment of animals with the narcotic antagonist naloxone.[4] The best known alkaloid with narcotic analgetic properties is morphine, a member of the morphinan family of alkaloids.[5] In our opinion, the report that (-)-**4**, bearing an indoline moiety and being a representative of the indole family of alkaloids, has similar pharmacological properties to morphine deserved special attention. The analgetic effect of *N*-methylmorphinan analgetics is known to be enantiospecific and to be largely abolished by N-demethylation to nor compounds or by conversion into open analogues by Hoffman degradation.[5] A comparison of (-)-**4** with its (+) antipode previously prepared,[6] and with (-)-noreseroline ((-)-**2**) and open dihydroseco analogue (-)-**8**, was therefore indicated and was believed to provide a qualitative structure–activity profile for the eseroline group of analgetics.

The investigations reported here were greatly facilitated by the development of a novel and better synthesis of (-)- and (+)-N^1-noreseroline *O*-methyl ether ((-)- and (+)-**1**),[8] followed later by chemical reactions applied earlier in the natural alkaloid series.[1] Eserolines in solution are extremely sensitive to autoxidation, forming red dyes, with rubreserine (**9**) as the major constituent.[9] The proposed resonance structures of **9**, that is, *o*-quinone **9A** and its zwitterionic mesomer **9B**,[10] have now been confirmed by X-ray diffraction analysis of a single crystal. Analgetic activities of compounds reported here were measured by classical screening and supplemented by data on binding to μ-opiate receptor preparations and inhibition of adenylate cyclase.

Chemistry

Optical resolution of (±)-**1** prepared by a variant of the total synthesis of (-)-physostigmine according to Julian and Pikl[11] was achieved by the methylbenzyl isocyanate (MBIC) method.[8,12] Both optical antipodes (+)-**1** and (-)-**1**, used for the subsequent reactions and shown in Chart I, were of optical purity that was >90% (HPLC analysis after reaction with (-)-MBIC). Reactions accomplished in the (+) series are summarized as follows: Reductive N-methylation of (+)-**1** with formaldehyde and sodium cyanoborohydride in the presence of triethylamine, conditions that avoid opening of ring C, afforded (+)-eseroline *O*-methyl ether ((+)-**3**) and (+)-eseroline ((+)-**4**) after reaction of (+)-**3** with BBr_3 in $CHCl_3$ at room temperature. O-Demethylation of (+)-**3** with 48% HBr afforded deeply colored materials that could not be purified. (-)-Eseroline ((-)-**4**) prepared from natural physostigmine[14] and (+)-eseroline ((+)-**4**) made here for the first time were characterized and used as sulfates, prepared from free bases in acetone solution by the addition of 1 equiv of sulfuric acid. (-)-N^1-Noreseroline ((-)-**2**) was obtained from (-)-**1** by reaction with BBr_3 and isolated as a crystalline sulfate, which in neutral solution immediately turned red. Formation of red color, which was suppressed by the addition of ascorbic acid, also was observed with solutions of sulfate salts of **4**, which slowly oxidized to rubreserine. Therefore, pharmacological studies were performed with freshly prepared solutions of sulfate salts. Dihydroseco compound (+)-**7** was easily obtained from (-)-**3**, prepared from physostigmine,[15,16] by simultaneous

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Chart I

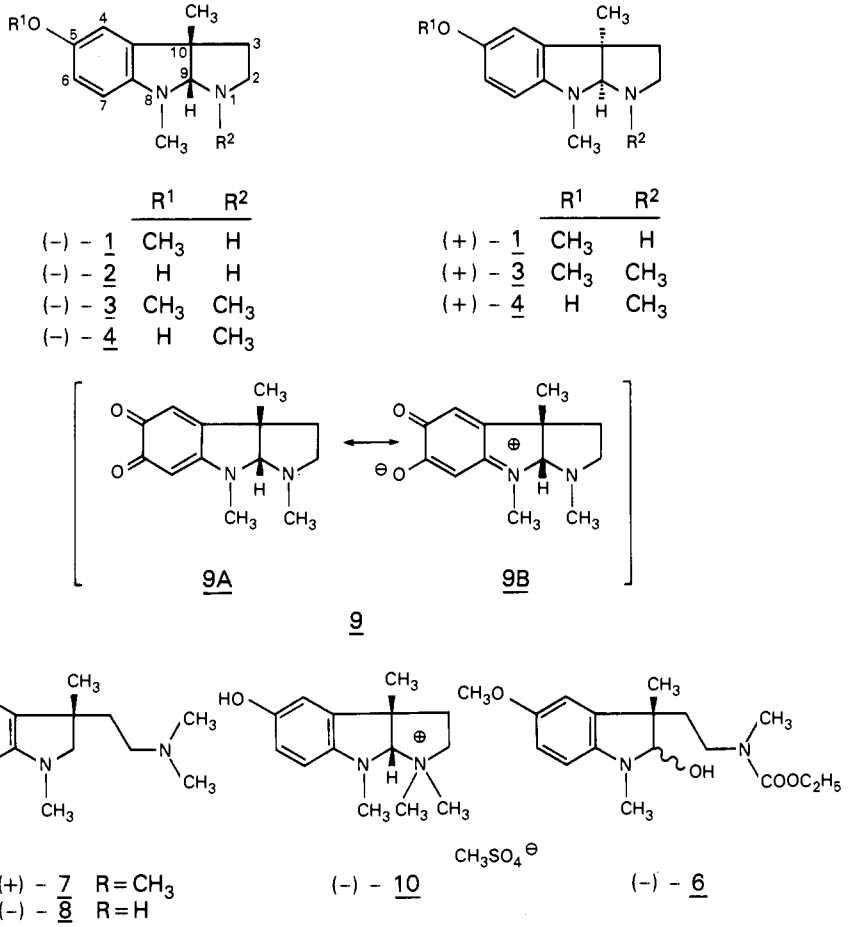

Table I. Assays for Narcotic Agonist and Antagonist Activity in Mice

compound	HP[a]	TF[b]	PPQ[c]	TFA[d]
(-)-4	1.6 (1.1-2.2)	2.3 (1.0-5.3)	0.7 (0.3-1.7)	I
(+)-4	I	I	2.3 (0.5-9.6)	I
morphine	1.3 (0.9-1.6)	5.8 (5.7-5.9)	0.23 (0.20-0.26)	I
naloxone	I	I	I	0.03 (0.01-0.09)
(-)-2	I			

[a] Hot plate assay, sc, in mg/kg (Atwell, L.; Jacobson, A. E. *Lab. Anim.* **1978**, *7*, 42). Numbers in parentheses indicate 95% confidence limits. I = inactive at 5 and 20 mg/kg. [b] Tail-flick assay, sc mg/kg (Dewey, D. L.; Harris, L. S.; Howes, J. F.; Nuite, J. A. *J. Pharmacol. Exp. Ther.* **1970**, *175*, 435. Dewey, D. L.; Harris, L. S. *J. Pharmacol. Exp. Ther.* **1971**, *179*, 652). Numbers in parentheses indicate 95% confidence limits. I = inactive at 1, 10, and 30 mg/kg. [c] Phenylquinone assay, sc, mg/kg (see footnote *b* for references). [d] Tail-flick antagonism assay vs. morphine, sc, mg/kg (see footnote *b* for references). Numbers in parentheses indicate 95% confidence limits. I = inactive at 1, 10, and 30 mg/kg.

reductive ring opening and N-methylation. Alternatively, (+)-**7** was also obtained from (-)-**3** by reaction with ethyl chloroformate, resulting in ring opening and affording the carbinolamine (-)-**6** showing an OH signal in the IR spectrum at 3400 cm^{-1}, and reduction to (+)-**7** with LAH in refluxing ether. Cleavage of the methoxy group in (+)-**7** with BBr_3 afforded the phenolic compound (-)-**8**, characterized as the hydrochloride. A better way to prepare (-)-**8** was by quaternization of (-)-**4** with dimethyl sulfate, to give the methosulfate (-)-**10** of structure confirmed by X-ray analysis,[17] and reductive ring opening of the latter by $NaBH_4$. A standard sample of rubreserine (**9**), identical by TLC with the red pigment obtained in the oxidation of (-)-**4**, was obtained by the published procedure.[10] X-ray diffraction analysis of a single crystal showed that the proposed structure of rubreserine was correct,[18] with the qualification that the solid state favors the zwitterion **9B** over the *o*-quinone mesomer **9A**.

Results

In Vivo Experiments. In assays for narcotic agonist activity (hot-plate, tail-flick, and phenylquinone assays, sc injection, in mice), only (-)-eseroline clearly showed potency comparable to morphine (Table I). The activity of (-)-eseroline as an antinociceptive agent has been noted previously.[4] None of the other compounds displayed any activity in the hot-plate or tail-flick assays. In the phenylquinone assay, which is not as specific an indicator for narcotic agonist activity as the other two agonist assays, (+)-eseroline showed about 10% of the activity of morphine. None of the compounds, including (+)-eseroline, appeared to display any narcotic antagonist activity in the tail-flick antagonism assay vs. morphine (Table I).

In Vitro Experiments. The enantiomers of eseroline bind to the opiate receptors of rat brain membranes with equal affinities. Displacement curves, as illustrated in Figure 1, show that (+)- and (-)-eseroline compete with

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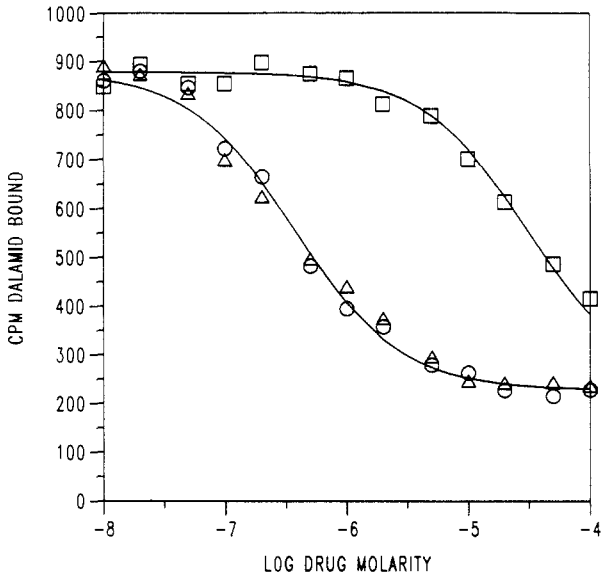

Figure 1. Displacement of [^{3}H]dalamid from rat brain membranes by (−)-eseroline (△), (+)-eseroline (○), and physostigmine (□) at the concentrations shown. Binding assays were performed with rat brain P_2 membranes[1] with use of 2 nM dalamid, a concentration equal to its K_d. Assays were carried out at 37 °C in 10 mM Tris·HCl, pH 7.5, and unbound dalamid was removed by gel filtration in the cold as previously described.[3]

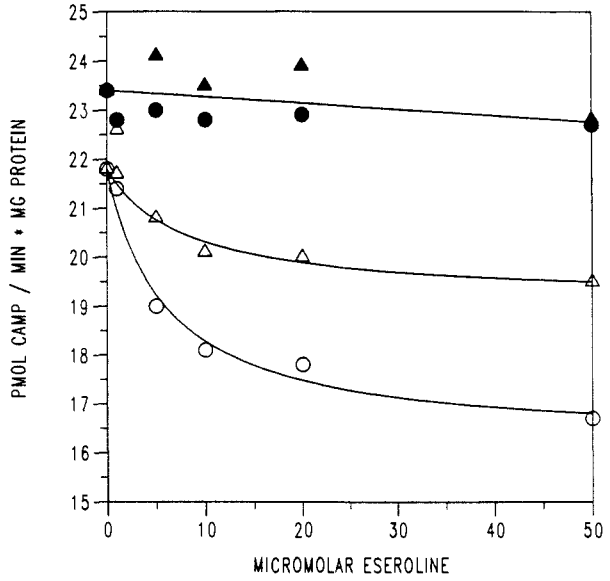

Figure 2. Effects of eseroline enantiomers on the adenylate cyclase activity of NG108-15 cell membranes in the presence and absence of naloxone. The concentrations of (−)-eseroline (○, ●) and (+)-eseroline (△, ▲) used are as shown. The open symbols refer to data obtained in the absence of naloxone, and the filled symbols correspond to data obtained in the presence of 10 μM naloxone. Each point is the average of three determinations whose standard errors are less than 3% of the mean. The experiment was performed twice with identical results.

the opioid peptide dalamid for binding to the μ- and δ-receptors of rat brain membranes with a concentration dependence showing a single class site and a K_d of 0.7 μM. The related compound, physostigmine, binds to the membranes with a 100-fold-lower affinity as shown in Figure 1. Weak binding of physostigmine to brain opioid receptors has been observed previously.[19]

Both (−)- and (+)-eseroline are opiate agonists when tested as inhibitors of adenylate cyclase in neuroblastoma

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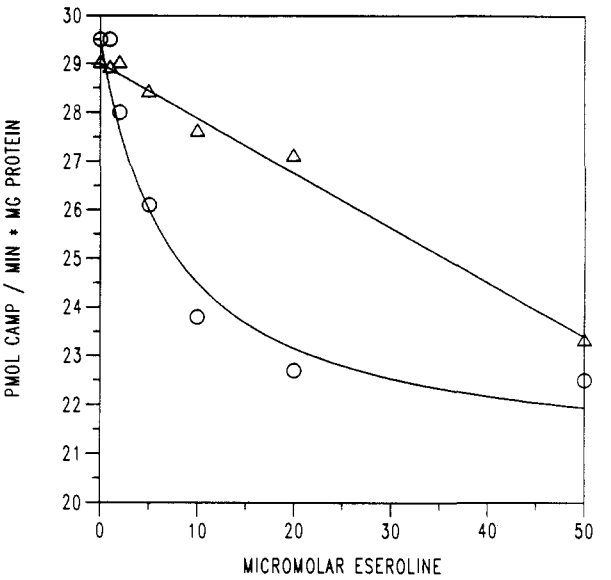

Figure 3. Inhibition of adenylate cyclase activity of NG108-15 membranes by (−)-eseroline at the concentrations shown, in the absence (○) and presence (△) of 100 μM (+)-eseroline.

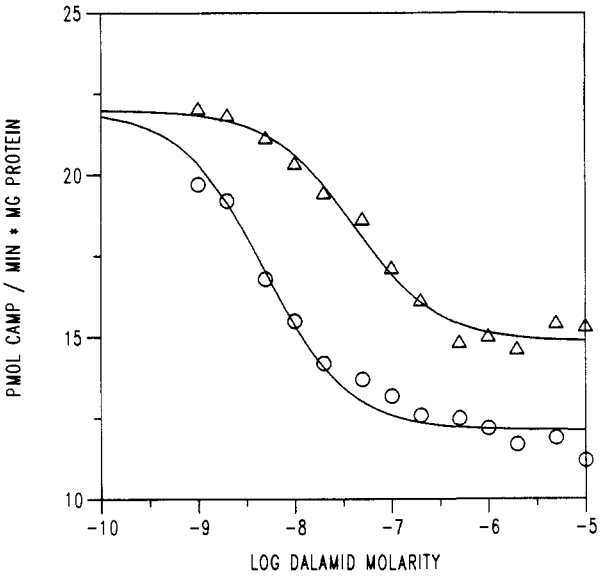

Figure 4. Inhibition of adenylate cyclase activity of NG108-15 membranes by dalamid at the concentrations shown, in the absence (○) and presence (△) of 100 μM (+)-eseroline.

× glioma, NG108-15, hybrid cell membranes. Interestingly, although the two enantiomers display approximately equal potencies as agonists (K_i = 6–8 μM), they show different efficacies as seen in Figure 2. It is clear from the figure that the extent of inhibition seen at saturating concentrations of eseroline is twice as great with (−)- as with (+)-eseroline. Inhibition produced by either enantiomer is reversed by naloxone, showing that these agents are acting as true opiates. The data of Figure 2 suggest that (+)-eseroline should act as an antagonist of (−)-eseroline, a prediction that is confirmed by the data shown in Figure 3. Thus, the extent of inhibition of adenylate cyclase produced by (−)-eseroline is diminished by (+)-eseroline at all concentrations of the (−) enantiomer tested. Furthermore, (+)-eseroline is also an effective antagonist of the opioid peptide dalamid, as shown by the data in Figure 4.

X-ray Analysis of "Natural" Rubreserine (9) Prepared from (−)-Eseroline ((−)-4). X-ray crystallographic data for rubreserine hydrate, $C_{13}H_{15}N_2O_2 \cdot H_2O$, M_r 249.29,

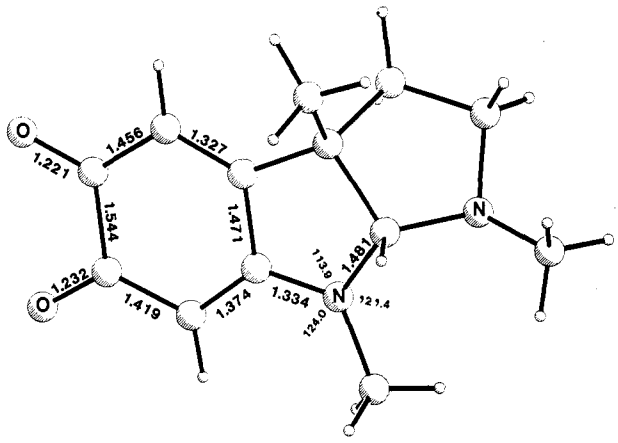

Figure 5. X-ray structure of rubreserin prepared from (−)-eseroline. The figure is drawn with use of experimentally determined data.

clear ruby color crystal (size = 0.47 × 0.13 × 0.12 mm), orthorhombic, space group $P2_12_12_1$, a = 6.532 (2) Å, b = 13.829 (2) Å, c = 14.605 (2) Å, V = 1319.2 (3) Å^3, Z = 4, D_{calcd} = 1.25 g cm^{-3}.

A total of 1959 independent reflections were measured to $2\theta_{max}$ = 120° with a computer-controlled diffractometer (Nicolet R3M), Cu Kα radiation (λ = 1.541 78 Å), with an incident-beam graphite monochromator. The structure was solved by using direct method.[22] Refinement was by blocked-cascade least squares on coordinates and thermal parameters for the non-hydrogen atoms. Hydrogen atoms were allowed to ride on covalently bonded atoms. The final R factor for 1912 observed reflections ($|F_o| > 3\sigma|F_o|$) was 3.1% (R_w = 4.2%). The results of the X-ray analysis on rubreserine are shown in Figure 5. The structure is actually a resonance hybrid of the mesomers indicated in Chart I, and the X-ray results do not clearly indicate which form contributes more to the hybrid. The zwitterionic contribution is indicated by the short C–N bond, 1.334 Å, and the planar geometry of the N atom; a purely single bond would lie in the 1.4–1.5-Å range and be accompanied by pyramidal geometry of the three single bonds involving the nitrogen atom. On the other hand, the C–O bond length of 1.232 Å is certainly in the double-bond range, which indicates a strong contribution from **9A**. In the O–C–C–C–N moiety, the C–C bond distances differ markedly from those located symmetrically across the ring. Without the zwitterionic resonance, these distances would be expected to be quite similar. The X-ray results indicate that the N and O atoms are at least partially charged by this resonance interaction; however, the exact degree is impossible to estimate and, indeed, it may vary considerably in solution depending on the surrounding molecular environment. The terminal five-membered ring has a normal envelope conformation while the six-membered ring is coplanar with the central five-membered ring. The two five-membered rings are cis with respect to one another (i.e., the methyl group and hydrogen atom substituents on the common C–C bond are both on the same side of the ring). Two water molecules were also found in the crystal, one at a partial occupancy (10%), and both act as donors in hydrogen bonds. One water molecule is a donor in a three-centered hydrogen bond to both carbonyl oxygens and in a second hydrogen bond to the nitrogen atom in the terminal five-membered ring. The second water molecule is a donor to the first water molecule and to one of the carbonyl oxygens. The absolute configuration was not determined by the X-ray work. The "hand" of the molecule was chosen to agree with what had already been determined by the "natural" isomer. The figure is drawn with use of experimentally determined coordinates. The estimated standard deviations are 0.002 Å for the labeled bond lengths and 0.1° for the labeled angles. (Tables of coordinates and bond lengths and angles have been deposited with the Crystallographic Data Center, Cambridge University, University Chemical Lab, Cambridge CB2 1EW, England.)

Discussion

It is apparent that the in vitro determination of narcotic antagonist activity for (+)-eseroline is not reflected in the in vivo assay. In vitro and in vivo data for analgesic compounds are not necessarily directly correlatable; the in vivo data are highly dependent on various biological processes (metabolism, transport, etc.), as well as enzymic interactions with substrates in situ. Although enantiomeric drugs might be, a priori, considered subject to similar, if not identical, biological processes (metabolism, transport, etc.), it is conceivable that enzymes can differentially interact with them. A similar difference between the in vitro and in vivo activity of enantiomers has been noted in the 6,7-benzomorphan series.[23]

Like with the morphinans, N-demethylation of (−)-4 abolishes the in vitro analgetic effect. Eserolines and morphinans both have a phenolic hydroxy group and a tertiary amine function (N^1-CH_3 in 4), which may well be the points of interaction with the opioid receptors. The inactivity of the dihydro seco analogue (−)-**8**, with a tertiary amino group that can freely rotate, supports the view that the analgesic activity of (−)-4 is most likely due to the presence of a tricyclic structure and not due to the presence of a bicyclic indolium species.[7] Sensitivity of eserolines to air-oxidation, although suppressed by antioxidants, such as ascorbic acid, is in our opinion a serious drawback to further development of compounds of this series as pharmaceutically useful drugs. Reports on the analgesic activity observed after medication with (−)-physostigmine[19] may be related to its breakdown into highly potent (−)-eseroline and its metabolites.

The enantiomers of eseroline bind with equal affinities to the mixture of μ- and δ-opiate receptors present in rat forebrain membranes as well as to the pure population of δ-receptors present in neuroblastoma × glioma, NG108-15, membranes. The mode of binding of the (−) and (+) enantiomers must differ somewhat, however, since the two display different efficacies as opiate agonists. Whereas (−)-eseroline is an effective inhibitor of adenylate cyclase activity in NG108-15 membranes, the (+) enantiomer is much less so and is a strong antagonist of both (−)-eseroline and the opioid peptide dalamid. Even (−)-eseroline is not a pure agonist since its efficacy is only 60% of that of dalamid in the adenylate cyclase assay (data not shown). In this test, morphine displays an efficacy of 79% and nalorphine of 50%.[20] Thus (−)-eseroline is a partial agonist in a potentially useful range of efficacy and with a potency

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in the adenylate cyclase assay similar to that of morphine.[21]

Experimental Section

Chemistry. Melting points were determined on a Fisher-Johns melting point apparatus, and optical rotations were taken on a Perkin-Elmer 241 MC polarimeter. IR spectra were determined on a Beckman IR 4230 instrument, ^{1}H NMR spectra were measured on a Varian XL 300 (300 MHz) or a Varian HR 220 (220 MHz) spectrometer, and mass spectra were taken on a Hitachi Perkin-Elmer RMU-6E (EI) or a Finnigan 1015D instrument (CI). Silica gel GHLF plates from Analtech, Inc., were used for TLC, and column chromatography was done with Kieselgel 60 (Merck), 40–63 μm (flash chromatography). All $CHCl_3$ used was freshly filtered through activated, basic Alox; reaction mixtures containing BBr_3 were kept under Ar; and BBr_3 and solutions thereof were transferred with dried glass syringes. Extraction of an aqueous solution with an organic solvent includes drying of the combined organic extracts with $MgSO_4$, concentration under reduced pressure, and drying under high vacuum to constant weight.

(+)-Eseroline *O*-Methyl Ether ((+)-3). A solution of (+)-1 (421 mg, 1.93 mmol) in CH_3OH (10 mL), Et_3N (0.7 mL), and 37% aqueous formaldehyde (1 mL) was stirred at room temperature for 1 h and then cooled to 0 °C, and then $NaBH_4$ (300 mg) was added in portions. After $^1/_2$ h the reaction mixture was concentrated in vacuo (Vigreux column), and the residue was mixed with enough 2 M HCl to dissolve the solid borates (6 mL). This acidic solution was extracted with Et_2O (10 mL), basified with saturated aqueous Na_2CO_3, and extracted with Et_2O (5 × 10 mL). The residue was distilled (Kugelrohr, 0.8 torr, 150 °C) to give TLC-pure (+)-3 (399 mg, 89%) as a colorless oil: $[\alpha]_D$ +85.3° (*c* 1.2, $CHCl_3$) ((−)-3, derived from natural physostigmine,[15,16] $[\alpha]_D$ −89.9° (*c* 1.3, $CHCl_3$)); IR and NMR spectra are identical with those of (−)-3.

(+)-Eseroline Sulfate ((+)-4·H_2SO_4). A solution of (+)-3 (388 mg, 1.67 mmol) in $CHCl_3$ (10 mL) at 0 °C was mixed dropwise and with stirring with a solution of BBr_3 (1 mL, 10.5 mmol) in $CHCl_3$ (10 mL). The resulting suspension, containing a gummy precipitate, was stirred for 68 h at room temperature. Water (1 mL) was added, the mixture was vigorously stirred for $^1/_2$ h and then poured on saturated aqueous $NaHCO_3$ (20 mL), and enough saturated aqueous Na_2CO_3 was added to beginning alkalinity. This two-phase system was extracted with $CHCl_3$/2-propanol (4:1, 4 × 10 mL), the brown residue was taken up in acetone (5 mL), and a diluted solution of H_2SO_4 in acetone was added dropwise and with shaking until the solution was just acidic and lost its color. The gummy precipitate, after decantation of the mother liquor and washing with acetone, was dried in high vacuum to yield a pale brown powder (412 mg, 78%) almost pure by TLC analysis. Recrystallization from EtOH/AcOEt gave (+)-4·H_2SO_4 as crystalline powder identical with (−)-4 from natural physostigmine[14] on TLC: mp 206–210 °C; $[\alpha]_D$ +116.0° (*c* 1.1, CH_3OH) ((−)-4·H_2SO_4, derived from natural physostigmine,[14] $[\alpha]_D$ −118.4°). Anal. ($C_{13}H_{20}N_2O_5S$) C, H, N.

(−)-N^1-Noreseroline Sulfate ((−)-2·H_2SO_4). A solution of (−)-1 (297 mg, 1.36 mmol) in $CHCl_3$ (4 mL) at 0 °C was mixed dropwise and with stirring with a solution of BBr_3 (400 μL, 4.2 mmol) in $CHCl_3$ (2 mL). The resulting suspension, containing a gummy precipitate, was stirred for 72 h at room temperature. At 0 °C, H_2O (2 mL) was added dropwise, the mixture was stirred vigorously for $^1/_2$ h and then poured on saturated aqueous $NaHCO_3$ (10 mL), and enough saturated aqueous Na_2CO_3 was added to beginning alkalinity. This two-phase system was extracted with $CHCl_3$/2-propanol (3:1, 4 × 8 mL), the brown, foamy residue was taken up in acetone, and the sulfate was precipitated by addition of 1 equiv of H_2SO_4 in acetone. The solid, after washing with acetone, was dried in high vacuum to give (−)-2·H_2SO_4 as a pinkish hygroscopic and amorphous salt (242 mg, 59%), almost pure by TLC analysis:MS (EI, on a sample of the free base), *m/e* 204 (M^+, 100), 174 (50), 160 (50).

(3*S*)-1,3-Dimethyl-3-[2′-(dimethylamino)ethyl]-5-methoxyindoline ((+)-7). To a stirred solution of (−)-3 (1.19 g, 5.11 mmol) in CH_3CN (20 mL) were added aqueous, 37% CH_2O (2 mL), $NaBH_3CN$ (900 mg), and then dropwise HCl in CH_3OH until the reaction mixture was slightly acidic. The reaction mixture was kept acidic by successive addition of HCl in CH_3OH. After $^3/_4$ h, the reaction mixture was concentrated in vacuo and the residue mixed with saturated aqueous $NaHCO_3$ (50 mL) and extracted with Et_2O (4 × 30 mL). The residue was chromatographed on a silica gel column (CH_2Cl_2/CH_3OH, 9:1, 1% concentrated aqueous NH_3) to give, after drying in high vacuum, (+)-7 as a yellowish oil (600 mg, 47%): $[\alpha]_D$ +1.3° (*c* 3.9, $CHCl_3$);[24] IR ($CHCl_3$) 2940 (s), 2850 (s), 2820 (s), 2790 (m), 2765 (w), 1587 (m), 1483 (s), 1455 (s), 1371 (m), 1390 (w), 1320 (w), 1295 (w), 1270 (s), 1140 (w), 1100 (m), 1055 (s), 1020 (m), 970 (w), 944 (w) cm^{-1}; ^{1}H NMR (220 MHz, $CDCl_3$) δ 6.72–6.58 (m, H-C(4) and H-C(6)), 6.28 (d, *J*(6,7) = 8.2, H-C(7)), 3.74 (s, OCH_3), 3.14 (d, *J*(gem.) = 8.8, H-C(2)), 2.92 (d, *J*(gem.) = 8.8, H′-C(2)), 2.67 (s, CH_3-N(1)), 2.41 (ddd, *J*(gem.) = *J*(1′,2′) = 12, *J*(1′,2$^{1(1)}$) = 6, H-C(1′)), 2.29–2.13 (m, H′-C(1′)), 2.20 (s, $(CH_3)_2$-N′), 1.85–1.70 (m, H- and H′-C(2′)), 1.27 (s, CH_3-C(3)); MS (CI, NH_3), *m/e* 249 (M^+ + 1, 90), 176 (100).

A. (3*S*)-1,3-Dimethyl-3-[2′-(dimethylamino)ethyl]-5-hydroxyindoline ((−)-8) from (+)-7. A stirred solution of the methoxy compound (+)-7 (315 mg, 1.27 mmol) in $CHCl_3$ (9 mL) at room temperature was slowly mixed with a solution of BBr_3 (480 μL, 5.1 mmol) in $CHCl_3$ (12 mL), resulting in a gummy precipitate, which solidified after 2 h. Ice was then added to the reaction mixture with vigorous stirring and enough saturated aqueous Na_2CO_3 to render the suspension just basic. Extraction with $CHCl_3$/2-propanol (3:1, 6 × 7 mL) gave a brown oil that was taken up in absolute EtOH. Addition of 1 equiv of HCl in EtOH and acetone gave the crystalline and hygroscopic hydrochloride (−)-8·HCl (124 mg, 36%): mp 215–220 °C dec; $[\alpha]_D$ −1.6° (*c* 1.1, CH_3OH), −4.2° (*c* 1.0, 0.02 M KOH in CH_3OH).[24] The air-sensitive base (−)-8 was freed for analytical purposes: IR ($CHCl_3$) 3650 (m), 3620 (w), 2970 (s), 2880 (m), 2845 (m), 2820 (w), 1605 (m), 1495 (s), 1466 (s), 1385 (w), 1340 (w), 1290 (w), 1117 (m), 1066 (w), 1017 (s), 988 (w), 914 (w), 870 (m); ^{1}H NMR (220 MHz, $CDCl_3$) δ 6.49 (br d, *J*(6,7) = 8, H-C(6)), 6.33 (br s, H-C(4)), 6.30 (d, *J*(6,7) = 8, H-C(7)), 3.02 (d, *J*(gem.) = 9, H-C(2)), 2.87 (d, *J*(gem.) = 9, H′-C(2)), 2.61 (s, CH_3-N(1)), 2.47–2.32 and 2.26–2.08 (2 m, H- and H′-C(1′)), 2.18 (s, $(CH_3)_2$-N′), 1.78–1.65 (m, H- and H′-C(2′)), 1.18 (s, CH_3-C(3)); MS (CI, NH_3), *m/e* 235 (M^+ + 1, 45), 162 (100).

B. Via (−)-10. To a stirred solution of the methosulfate (−)-10 (535 mg, 1.55 mmol) in CH_3OH (10 mL) at 0 °C was added an excess of $NaBH_4$ in portions. After $^1/_2$ h, 2 M HCl (10 mL) was added cautiously, and the mixture was stirred for $^1/_2$ h and then concentrated in vacuo to remove most of the CH_3OH. Addition of enough saturated aqueous Na_2CO_3 to beginning alkalinity and extraction with $CHCl_3$/2-propanol (3:1, 6 × 10 mL) gave a quantitative yield of (−)-8 as brown oil, which was converted at once into its hydrochloride as described above.

(−)-Eseroline Methosulfate ((−)-10). A stirred solution of (−)-eseroline ((−)-4) (580 mg, 2.66 mmol) in acetone (15 mL) at room temperature was treated dropwise with $(CH_3)_2SO_4$ (251 μL, 2.66 mmol). After 12 h the resulting suspension was filtered to give, after drying in high vacuum, crystalline (−)-10 (535 mg, 58%) as a brownish powder. Recrystallization from EtOH/acetone gave platelets: mp 175–176 °C dec; $[\alpha]_D$ −151.1° (*c* 0.9, CH_3OH); ^{1}H NMR (300 MHz, Me_2SO-d_6) 6.65 (d, *J*(4,6) = 2.3, H-C(4)), 6.60 (dd, *J*(6,7) = 8.3, *J*(4,6) = 2.3, H-C(6)), 6.52 (d, *J*(6,7) = 8.3, H-C(7)), 4.94 (s, H-C(9)), 3.,48 (ddd, *J*(gem.) = 11, *J*(2,3) = 6, *J*(2,3′) = 4, H-C(2)), 3.37, 3.11, 3.08, and 2.79 (4 s, 3 CH_3-N and $CH_3SO_4^-$), 2.93 (ddd, *J*(gem.) = *J*(2′,3) = 11, *J*(2′,3′) = 6.5, H′-C′(2)), 2.41 (ddd, *J*(gem.) = 14, *J*(2′,3) = 11, *J*(2,3) = 6, H-C(3)), 2.28 (ddd, *J*(gem.) = 14, *J*(2′,3′) = 6.5, *J*(2,3′) = 4, H′-C(3)), 1.45(s, CH_3-C(10)). Anal. ($C_{15}H_{24}N_2O_5S$) C, H, N.

Biological Testing. Membranes were prepared from rat forebrains, homogenized in 0.32 M sucrose, as described previously,[18] as were membranes of neuroblastoma × glioma, NG108-15, hybrid cells.[25] Dalamid was purchased from Calbiochem, San Diego, CA.

Binding assays[26] were performed by measuring the competition between unlabeled drug and [^{3}H]-D-Ala^2-Met^5-enkephalinamide

(24) The small optical rotations found for (+)-7 and (−)-8 of the *S* series suggest that the solvent and the presence of the compound as a free base or a salt determine the magnitude of optical rotation.

(25) Koski, G.; Klee, W. A. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 4185–4189.

(26) Simonds, W. F.; Koski, G.; Streaty, R. A.; Hjelmeland, L. M.; Klee, W. A. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 4623–4627.

(dalamid) for sites on brain membranes. Membranes were incubated for 10 in at 37 °C with [^{3}H]dalamid in the presence (or absence) of competing compoundin 10 mM Tris·HCl, pH 7.5. At the end of the incubation, the samples (0.5 mL) were cooled to 4 °C and passed through columns of Sephadex G-25 (PD-10, Pharmacia). The columns were eluted with four aliquots (1 mL) of Tris buffer directly into scintillation vials. After addition of 12 mL of Aquasolve (New England Nuclear Co.), the radioactivity was monitored by scintillation counting. Unbound radioactivity is retained by the columns under these conditions. After use, columns were washed with 6 M urea and regenerated with Tris buffer.

Adenylate cyclase activity of NG108-15 membranes was measured by assaying the conversion of [α-^{32}P]ATP to cyclic AMP during a 10-min incubation at 37 °C, as previously described.[20]

All experiments with eseroline were performed with freshly prepared solutions that were used within 1 h of preparation. Control experiments showed that, under these conditions, the addition of an equivalent concentration of ascorbic acid had no effect on the potency of eseroline in binding assays.

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Registry No. (+)-**1**, 104069-10-5; (−)-**1**, 104069-11-6; (−)-**2**, 6785-93-9; (−)-**2**·H_2SO_4, 104015-27-2; (+)-**3**, 104069-12-7; (−)-**3**, 65166-97-4; (+)-**4**, 29347-15-7; (+)-**4**·H_2SO_4, 104015-28-3; (−)-**4**, 469-22-7; (−)-**4**·H_2SO_4, 104015-29-4; (+)-**7**, 104015-30-7; (−)-**8**, 104015-32-9; (−)-**8**·HCl, 104015-31-8; **9**, 18455-27-1; (−)-**10**, 104034-13-1; adenylate cyclase, 9012-42-4.

Substituted 1,3,4-Thiadiazoles with Anticonvulsant Activity. 1. Hydrazines

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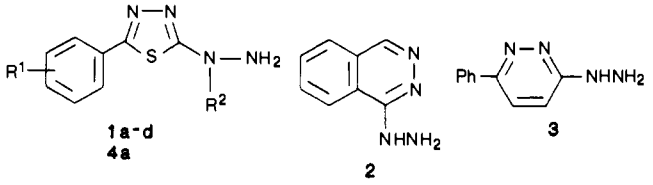
The synthesis and anticonvulsant activity of a series of 2-aryl-5-hydrazino-1,3,4-thiadiazoles are described. The combination of preferred aromatic substituents in the 2-position coupled with alkyl substitution on the hydrazine moiety led to a number of potent compounds lacking sedation, ataxia, or lethality. 5-(2-Biphenylyl)-2-(1-methylhydrazino)-1,3,4-thiadiazole (4m) represents a new class of anticonvulsant agent and compares favorably with the standard drugs phenytoin, phenobarbital, and carbamazepine.

A series of 2-aryl-5-hydrazino-1,3,4-thiadiazoles exemplified by the general structure (1, R^2 = H)[1] were designed as analogues of the known vasodilators hydralazine (**2**)[2] and the pyridazinylhydrazine **3**.[3] Subsequent evaluation of



this series 1 showed that some analogues possessed both antihypertensive activity (vasodilation) and anticonvulsant activity[4] (Table I). Furthermore, it was found that particular substitution in the 2-position of the aromatic ring (i.e., R^1 = 2-Cl, 2-Ph, and 2-hexyloxy) produced compounds with reduced antihypertensive activity but with a desirable anticonvulsant profile.[4] It was also found that methylation on the α-nitrogen (N^1) of the hydrazine group in the *o*-tolyl series (**1a** → **4a**) decreased the vasodilator activity without concurrent decrease in anticonvulsant activity (Table I). Thus a combination of the preferred aromatic substituents in the 2-position coupled with various alkyl and aryl substitution on the hydrazine moiety was a prime objective in this work.

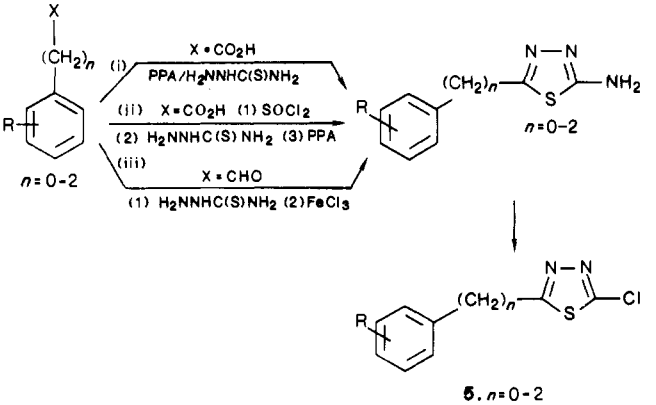
We report here the synthesis and pharmacological evaluation of a series of substituted thiadiazole hydrazines.

Table I

compd	R^1	R^2	DOCA test:[a] % fall in BP at 100 mg/kg, po	ED_{50}, mg/kg, po MMS[b]	ED_{50}, mg/kg, po MES[c]
1a	2-CH_3	H	63	30	>100
1b	2-Cl	H	21	25	70
1c	2-Ph	H	18	33	28
1d	2-$C_6H_{13}O$	H	10	33	20
4a	2-CH_3	CH_3	29	41	75

[a] Screening for antihypertensive activity (maximum percentage fall in mean arterial blood pressure recorded) was carried out with DOCA hypertensive rats (minimum of five) according to the procedures previously reported.[5] [b] MMS = maximum metrazol seizures[6] (mouse). [c] MES = maximum electroshock test[7] (mouse).

Scheme I



In particular, structure–activity relationships were examined in the 2-CH_3, 2-Cl, 2-Ph and 2-hexyloxy series of compounds (Tables II–VII).

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(4) Turner, S.; Myers, M. (Reckitt and Colman), unpublished results. See also ref 1.

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