the 6,7-bis(cyclopropylcarbonyl) derivative **6a** as a light-yellow syrup. This was refluxed with $LiAlH_4$ (0.3 g, 7.9 mmol) in THF (50 mL) for 3.5 h. The cooled mixture was treated with saturated Rochelle salt solution, extracted with $CHCl_3$, and dried. Evaporation of the solvent gave 110 mg (92.6%) of **7a** as colorless crystals. Recrystallization from Me_2CO–Et_2O gave pure **7a**, mp 170–172 °C. Anal. ($C_{21}H_{29}NO$) C, H, N.

Compound **7b** was obtained from **4b** by similar acylation and the subsequent reduction in 90% yield: mp 140–141.5 °C (from Me_2CO–Et_2O). Anal. ($C_{21}H_{29}NO$) C, H, N.

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Agonist Effects of β-Phenethylamines on the Noradrenergic Cyclic Adenosine 3′,5′-Monophosphate Generating System in Rat Limbic Forebrain. Stereoisomers of *p*-Hydroxynorephedrine

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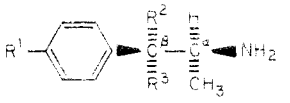
Significant agonist activity for the specific noradrenergic cyclic adenosine 3′,5′-monophosphate (cAMP) generating system in rat limbic forebrain requires a β-3,4-dihydroxyphenethylamine with a β-hydroxyl group in the *R* configuration. Thus, neither of the enantiomers of *p*-hydroxynorephedrine nor of *p*-hydroxynorpseudoephedrine mimics the agonist activity of (*R*)-norepinephrine. It has yet to be established whether or not one of the *p*-hydroxynorephedrines exhibits antagonist activity in this same system.

In rats, (*S*)-amphetamine (*d*-amphetamine) [(*S*)-**1**] is converted predominantly in the liver to (*S*)-*p*-hydroxyamphetamine [(*S*)-**2**]. The latter is then transported to

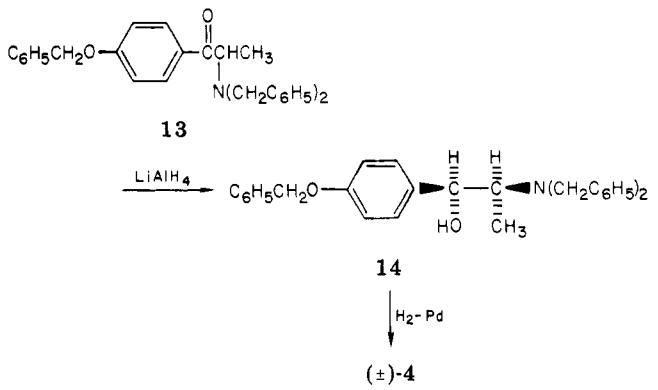


(*S*)-**1**, $R^1 = R^2 = R^3 = H$
(*S*)-**2**, $R^1 = OH$; $R^2 = R^3 = H$
(α*S*,β*R*)-**3**, $R^1 = R^2 = OH$; $R^3 = H$
(α*S*,β*S*)-**4**, $R^1 = OH$; $R^2 = H$; $R^3 = OH$

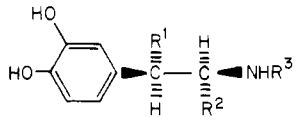
the brain and converted in noradrenergic neurons by dopamine β-hydroxylase to a metabolite identified as *p*-hydroxynorephedrine.[1-3] The absolute configuration of (*S*)-**2** follows from the known absolute configuration of (*S*)-**1**,[4] and the major metabolite of (*S*)-**2** is most likely (α*S*)-*p*-hydroxynorephedrine [(α*S*,β*R*)-**3**].[5] It has not been established conclusively, however, whether the major metabolite of (*S*)-**2** is (α*S*,β*R*)-**3** or (α*S*)-*p*-hydroxynorpseudoephedrine [(α*S*,β*S*)-**4**] or whether both stereoisomers are produced together. The metabolite of (*S*)-**2**, however, can displace endogenous norepinephrine in nerve terminals and can be released upon nerve stimulation,[6] and it thus can serve as a false neurotransmitter.[7] The metabolite of (*S*)-**2** has also been implicated in the persistent reduction of brain norepinephrine after administration of (*S*)-**1** and in the development of tolerance to certain pharmacologic actions of (*S*)-**1**.[7-9]

In related studies in our laboratories, we have demonstrated that slices of the rat limbic forebrain contain a cyclic adenosine 3′,5′-monophosphate (cAMP) generating system which displays properties of a norepinephrine receptor with α and β characteristics.[10,11] Since stereoselectivity is observed for pharmacologic α- and β-receptors,[12] and since the norepinephrine receptor coupled adenylate cyclase system in the limbic forebrain shows stereoselective blockade by (+)-butaclamol,[13] it was of interest to prepare the enantiomers of *p*-hydroxynorephedrine (**3**) and *p*-hydroxynorpseudoephedrine (**4**) and to examine their effect on this receptor system in comparison with that elicited by the enantiomers of norepinephrine[14] (**5**). For a more extensive assessment of the

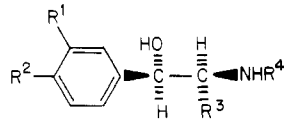
Scheme I



structural and steric requirements of the receptor, other β-phenethylamines were also examined. Included in this



(*R*)-**5**, R¹ = OH; R² = R³ = H
(*R*)-**6**, R¹ = OH; R² = H; R³ = CH_3
(*R*)-**7**, R¹ = OH; R² = H; R³ = $CH(CH_3)_2$
(α*S*,β*R*)-**8**, R¹ = OH; R² = CH_3; R³ = H
9, R¹ = R² = R³ = H



(*R*)-**10**, R¹ = OH; R² = R³ = H; R⁴ = CH_3
(*R*)-**11**, R¹ = H; R² = OH; R³ = R⁴ = H
(α*S*,β*R*)-**12**, R¹ = OH; R² = H; R³ = CH_3; R⁴ = H

latter group were the enantiomers of amphetamine[4] (**1**), *p*-hydroxyamphetamine[4] (**2**), and epinephrine[15] (**6**) as well as (*R*)-isoproterenol[16] [(*R*)-**7**], (±)-3,4-dihydroxynorephedrine[17] [(±)-**8**], dopamine (**9**), (±)-phenylephrine[18] [(±)-**10**], (±)-octopamine[19] [(±)-**11**], and (α*S*,β*R*)-metaraminol[20] [(α*S*,β*R*)-**12**].

Results and Discussion

Preparation of *p*-Hydroxynorephedrines. Racemic *p*-hydroxynorephedrine [(±)-**3**] was purchased while racemic *p*-hydroxynorpseudoephedrine [(±)-**4**] was prepared by a procedure outlined earlier.[21] In this sequence the lithium aluminum hydride reduction of 4′-benzyloxy-2-(dibenzylamino)propiophenone (**13**) (Scheme I) yielding *threo*-1-(4-benzyloxyphenyl)-2-dibenzylamino-1-propanol (**14**) is the crucial stereoselective step.

The proton magnetic resonance (^{1}H NMR) of the respective racemic hydrochlorides of (±)-**3** and (±)-**4** now confirms the relative configurational assignments (erythro and threo, respectively) made earlier.[21] Amino alcohols with structures such as **3** and with the erythro (ephedrine) configuration produce a coupling constant ($J \sim 4$ Hz) for the interaction of the α and β tertiary protons about half as great as those ($J \sim 9$ Hz) for similar compounds with the threo (pseudoephedrine) configuration.[22]

The enantiomers of **3** were obtained by recrystallization of the (+)- and (−)-tartaric acid salts, followed by treatment of the latter with 2 equiv of aqueous sodium hydroxide and continuous ether extraction to recover the free bases, with physical properties somewhat different than those previously reported.[23] The enantiomers of **4** were obtained using (+)- and (−)-10-camphorsulfonic acid[24] after it was found that the commonly used carboxylic acid resolving agents (e.g., tartaric acid and *N*-acetylleucine) were too weak to form stable salts.

The earlier assignment of the absolute configuration of (−)-*p*-hydroxynorephedrine [(−)-**3**] as α*S* by comparison of its rotatory power using sodium D light with that of (*S*)-(−)-ephedrine[23] was supported by comparison of the optical rotatory dispersion curves of (−)-**3**, its hydrochloride, and its dithiocarbamate and *N*-nitroso derivatives with model compounds of established absolute configuration[25] and is here firmly established by examination of the circular dichroism (CD) of the *N*-5-bromosalicylidene derivatives of (+)-**3**. The *N*-5-bromosalicylidene derivatives of both (+)-**3** and (−)-**4** show strong negative Cotton effects at about 255 and 325 nm and, on application of the salicylidenimino chirality rule,[26] are both assigned the α*R* configuration. The α*S* configuration follows for the enantiomers (−)-**3** and (+)-**4**.

Biological Activity. In agreement with earlier reports[10,27] (*R*)-norepinephrine[28] [(*R*)-**5**] is active in the noradrenergic cAMP generating system and produces a significant effect at a concentration of 1 μM. The concentration necessary for half-maximal stimulation (EC_{50}) was approximately 5 μM, and the maximum effect was produced by a concentration of 30–50 μM. The system, however, was not stimulated by (*S*)-**5**[29] in concentrations up to 10^{-3} M. Significantly, the enantiomers of *p*-hydroxynorephedrine[28] (**3**) and *p*-hydroxynorpseudoephedrine[28] (**4**) were also inactive up to 10^{-4} M. Activity was observed, however, for (*R*)- and (*S*)-epinephrine[29] (**6**) and the *N*-isopropyl analogue of (*R*)-**6**[28] [(*R*)-**7**]. Both (*R*)-**6** and (*R*)-**7** have EC_{50} values of 5 μM, similar to that of (*R*)-**5**, but that of (*S*)-**6** is greater than 100 μM. (±)-3,4-Dihydroxynorephedrine[28] [(±)-**8**] had an EC_{50} value of 10–15 μM, slightly less than half the activity of (*R*)-**5**, most of the activity presumably residing in the enantiomer having the α*S*,β*R* configuration. The enantiomers of amphetamine[30] (**1**) and of *p*-hydroxyamphetamine[28] (**2**) were inactive up to 10^{-4} M. Dopamine[28] (**9**), (±)-phenylephrine[28] [(±)-**10**], (±)-octopamine[28] [(±)-**11**], and (α*S*,β*R*)-metaraminol[29] [(α*S*,β*R*)-**12**] were also devoid of activity up to this same concentration.

The foregoing demonstrates that for the specific noradrenergic receptor coupled adenylate cyclase system in rat limbic forebrain, significant agonist activity requires a β-3,4-dihydroxyphenethylamine with a β-hydroxyl group in the *R* configuration. Neither of the β-hydroxylated phenethylamines derived from (*S*)-*p*-hydroxyamphetamine [(*S*)-**2**], (α*S*)-*p*-hydroxynorephedrine [(α*S*,β*R*)-**3**] and (α*S*)-*p*-hydroxynorpseudoephedrine [(α*S*,β*S*)-**4**], mimics the agonist activity of (*R*)-norepinephrine [(*R*)-**5**]. It has yet to be established, however, whether or not an enantiomer of **3** or **4** will exhibit antagonist activity in the same system.

Experimental Section

Melting points were taken in open capillary tubes and are corrected. Optical rotations at the sodium D line were measured using a visual polarimeter and a 1-dm tube. Proton magnetic resonance (^{1}H NMR) spectra were obtained in deuterium oxide using a JOEL MH-100 spectrometer with tetramethylsilane as an external standard. Isotropic electronic absorption (EA) spectra were measured with a Cary Model 14 spectrometer with the normal variable slit. Circular dichroism (CD) spectra were measured at 25–28 °C with a Cary Model 60 spectropolarimeter with a CD Model 6001 accessory, and the slit was programmed for a spectral band width of 1.5 nm. The microanalysis was done by Galbraith Laboratories, Inc., Knoxville, Tenn.

Except for the enantiomers of *p*-hydroxynorephedrine (**3**) and *p*-hydroxynorpseudoephedrine(**4**), the β-phenethylamines were obtained from outside sources as appropriate salts.[31] The salts were used without further purification.

(α*S*)-(−)-*p*-Hydroxynorephedrine [(α*S*,β*R*)-3]. (±)-*p*-Hydroxynorephedrine (12.0 g, 71.8 mmol), obtained by treatment of the hydrochloride (14.6 g, 71.8 mmol) (Aldrich Chemical Co.) with an equimolar amount of 2.0 N sodium hydroxide, was added to a solution of (−)-tartaric acid (10.8 g, 72.0 mmol) in water (10

mL). The mixture was heated until homogeneous on a steam bath and then refrigerated. The resulting solid was collected and recrystallized twice from 90% ethanol to yield the optically pure acid tartrate (5.58 g, 49%), $[\alpha]^{25}_D$ −34° (c 1.93, H_2O) [lit.[23] $[\alpha]^{22}_D$ + 32° (10% in H_2O) for the enantiomeric salt]. The salt (5.58 g, 17.6 mmol) was treated with 2.0 N sodium hydroxide (17.6 mL), and the resulting solution was extracted continuously with ether for 7 days to yield crystalline (αS,βR)-**3** (2.31 g, 79%): mp 161–162 °C dec; $[\alpha]^{25}_D$ −18° (c 2.00, CH_3OH) (lit.[23] mp 165–166 °C). Treatment of (αS,βR)-**3** with 6 N hydrochloric acid followed by evaporation to dryness gave the hydrochloride: mp 146–147 °C; $[\alpha]^{25}_D$ −18° (c 4.07, H_2O) [lit.[23] mp 172–172.5 °C; $[\alpha]^{22}_D$ −33° (10% in H_2O)]. The racemic amine hydrochloride had ^1H NMR δ 4.76 (d, 1, J = 4 Hz, β-C-H), 3.50 (m, 1, α-C-H), and 1.10 ppm (d, 3, J = 6 Hz, CH_3).

(αR)-(+)-p-Hydroxynorephedrine [(αR,βS)-3]. Using the procedure outlined above, resolution of (±)-**3** with (+)-tartaric acid gave (αR,βS)-**3**: mp 161–162 °C dec; $[\alpha]^{25}_D$ +17° (c 1.90, CH_3OH). The hydrochloride had mp 145–147 °C and $[\alpha]^{25}_D$ +17° (c 2.95, H_2O) [lit.[23] mp 171.5–172 °C; $[\alpha]^{22}_D$ +33° (10% in H_2O)].

(αR)-(−)-N-(5-Bromosalicylidene)-p-hydroxynorephedrine. The derivative was prepared from (αR,βS)-**3** and 5-bromosalicylaldehyde[32] and was recrystallized from benzene: mp 98–100 °C; $[\alpha]^{25}_D$ −80° (c 1.18, CH_3OH); EA max (CH_3OH) 417 nm (ε 1800), 328 (2300), 275 (4500) (sh), 261 (8600) (sh), 254 (10 000), 249 (11 000), and 224 (36 000); CD (CH_3OH) (c 0.0272) $[\theta]_{500}$ ±0, $[\theta]_{415}$ −2200 (max), $[\theta]_{370}$ −1200 (min), $[\theta]_{345}$ −4800; CD (c 0.00272) $[\theta]_{345}$ −4100; $[\theta]_{327}$ −9300 (max), $[\theta]_{298}$ ±0, $[\theta]_{276}$ +3100 (max), $[\theta]_{269}$ ±0, $[\theta]_{257}$ −15 000 (max), $[\theta]_{240}$ ±0, $[\theta]_{235}$ +13 000. Anal. ($C_{16}H_{16}BrNO_3$) C, H.

(±)-p-Hydroxynorpseudoephedrine [(±)-4]. 4′-Benzyloxy-2-(dibenzylamino)propiophenone[21] (**13**) (52.8 g, 0.121 mol) was added in portions to a stirred suspension of lithium aluminum hydride (7 g, 0.2 mol) in ether (600 mL). Stirring was continued overnight and water (25 mL) was then added slowly with continued stirring until the complex and excess lithium aluminum hydride had reacted completely. The mixture was filtered and the filter cake extracted with boiling benzene. Evaporation of the extract yielded *threo*-1-(4-benzyloxyphenyl)-2-dibenzylamino-1-propanol (**14**) (47.6 g, 90%): mp 135–137 °C (lit.[21] 141–142 °C). An additional amount of **14** (0.6 g, 1%) was obtained on evaporation of the filtrate and recrystallization of the residue from 95% ethanol. A solution of **14** (20.7 g, 47.3 mmol) in absolute ethanol (400 mL) and 2 N hydrochloric acid (25 mL) was hydrogenated over 10% palladium on charcoal until uptake ceased (20 h). The catalyst was removed by filtration and the filtrate evaporated to dryness. The residue was treated with 2 N sodium hydroxide (24 mL) and the resulting free base was collected, washed with water, and dried, yielding (±)-**4** (7.9 g, 100%): decomposition without melting at ~155 °C (lit.[21] mp 143–144 °C). The hydrochloride had ^1H NMR δ 4.46 (d, 1, J = 9 Hz, β-C-H), 3.50 (m, 1, α-C-H), and 1.04 ppm (d, 3, J = 6 Hz, CH_3).

(αS)-(+)-p-Hydroxynorpseudoephedrine [(αS,βS)-4]. A solution of (±)-**4** (7.8 g, 47 mmol) and (−)-10-camphorsulfonic acid[24] (10.9 g, 46.9 mmol) in 95% ethanol was evaporated to dryness and the residue was dissolved in hot water (40 mL). The salt which crystallized on cooling was recrystallized three times from water to yield optically pure camphorsulfonate (2.14 g, 23%): $[\alpha]^{25}_D$ +23° (c 1.85, CH_3OH). It was stirred 1 h with excess 4 N ammonium hydroxide and the resulting free base was collected, washed with water, and dried. The amine [(αS,βS)-**4**] (0.88 g, 98%) had mp 193–195 °C dec and $[\alpha]^{25}_D$ +88° (c 0.50, CH_3OH). Treatment of (αS,βS)-**4** with 6 N hydrochloric acid followed by evaporation to dryness gave the hydrochloride: mp 167–169 °C dec; $[\alpha]^{25}_D$ +76.1° (c 2.01, H_2O).

(αR)-(−)-p-Hydroxynorpseudoephedrine [(αR,βR)-4]. Using the procedure outlined above, resolution of (±)-**4** with (+)-10-camphorsulfonic acid[24] gave (αR,βR)-**4**: $[\alpha]^{25}_D$ −88° (c 0.41, CH_3OH). The hydrochloride had mp 167–169 °C dec and $[\alpha]^{25}_D$ −75.5° (c 1.84, H_2O).

(αR)-(−)-N-(5-Bromosalicylidene)-p-hydroxynorpseudoephedrine. The derivative was prepared[32] from (αR,βR)-**4** and was an amorphous solid: $[\alpha]^{25}_D$ −226° (c 1.02, CH_3OH); EA max (CH_3OH) 413 nm (ε 2200), 328 (2900), 276 (5100) (sh), 248 (12 000) (sh), 223 (35 000); CD (CH_3OH) (c 0.0681) $[\theta]_{500}$ ±0, $[\theta]_{416}$ −4700 (max), $[\theta]_{355}$ −1900 (min), $[\theta]_{327}$ −5600 (max), $[\theta]_{293}$ −800 (min), $[\theta]_{277}$ −2900 (sh), $[\theta]_{270}$ −5600; CD (c 0.0272) $[\theta]_{270}$ −4000, $[\theta]_{256}$ −16,000 (max), $[\theta]_{245}$ −9800.

Biological Testing. Male Sprague–Dawley rats (Sprague–Dawley Co., Madison, Wis.) weighing 180–250 g were used. The animals had free access to water and standard laboratory diet and were maintained under standard laboratory conditions with a controlled 12-h light–dark cycle. The animals were decapitated, and their brains were quickly removed and placed on an ice-chilled porcelain plate and dissected.[10] Slices from individual halves of the brain part referred to as the limbic forebrain area were prepared and incubated in Krebs–Ringer bicarbonate buffer (pH 7.4, 95% O_2–5% CO_2).[10] Dose–response curves for the β-phenethylamines were established by exposing tissue slices for 10 min to various concentrations of the potential agonists. The slices were homogenized using a polytron homogenizer in 3.5 mL of 0.3 N perchloric acid. Proteins were assayed[33] in a 0.5-mL aliquot, and cAMP in the remaining 3 mL was isolated by ion-exchange chromatography (Dowex G50-W-X8, 100–200 mesh, acidic form) and determined by a protein binding assay.[34] Basal control values varied between 18 and 28 pmol of cAMP/mg of protein (N = 6–12).

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Differences in Antischistosomal and Mutagenic Properties between an Isothiocyano- and an Isocyanonitrodiphenylamine

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While 4-isothiocyano-4′-nitrodiphenylamine has high schistosomicidal activity in vivo and is devoid of mutagenic properties in vitro, the reverse is true for the isocyano analogue of this compound; i.e., replacement of the sulfur by oxygen results in a compound that has no demonstrable antischistosomal effects and exhibits significant mutagenic activity.

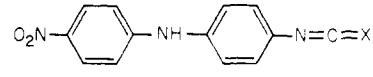
Recently it has been reported that 4-isothiocyano-4′-nitrodiphenylamine (1) (CGP 4540) has high chemotherapeutic activity when administered to animals experimentally infected with schistosomes including *Schistosoma japonicum*.[1,2] It is noteworthy that this compound, in contrast to other schistosomicidal agents, exhibited no mutagenic effects on the sensitive bacterial tester strains of Ames et al.[3] In order to obtain information about the structural characteristics conferring antischistosomal activity, the effect of replacing sulfur by oxygen in the side chain, i.e., the biological activity of the corresponding isocyanate derivative **2**, was determined.

Results and Discussion

Antischistosomal Activity. The antischistosomal effectiveness of **1** is greatly increased when the particle size is reduced to an average diameter of 0.5 μ by ball milling.[2] Alternatively, suspension of larger particle size of the compound in Emulphor EL was nearly as effective. When administered as a single oral dose in the same vehicle, up to 50 times higher doses of the isocyanate analogue **2** had no demonstrable schistosomicidal activity (Table I). In fact, even at the highest dose tested, there was no temporary shift of the worms from the mesenteric veins to the liver sinuses. This phenomenon is considered as a manifestation of minimal antischistosomal activity.

In contrast to the isothiocyanate **1**, the oxygen analogue **2** exhibited mutagenic activity; the latter was fairly substantial with one tester strain (TA 100) but low with the other (TA 98). With neither strain was there any activation by the microsomal preparation (see Table I).

The lack of schistosomicidal activity of **2** may be due to its more rapid rate of hydrolysis when compared to the hydrolysis rate of **1**,[4] thus rendering the former inactive in in vivo studies. In any case, it is evident that the sulfur atom is critical for conferring both schistosomicidal activity and a lack of mutagenic activity. This is reversed when the sulfur atom is replaced by oxygen. This results in a loss of schistosomicidal activity and confers mutagenic properties. Previous studies have shown that antischistosomal and mutagenic activities are not necessarily associated with each other. In fact, investigations with hycanthone analogues have demonstrated that structural modifications can bring about dissociation of these two activities.[5,6] The properties of the two nitrodiphenylamines provide further support for the lack of obligatory association between antischistosomal and mutagenic properties.



1, X = S
2, X = O

Table I. Antischistosomal and Mutagenic Activities of 4-Isothiocyano- and 4-Isocyano-4′-nitrodiphenylamine

	Antischistosomal activity				Mutagenic activity				
						No. of mutants in excess of controls			
						TA-100		TA-98	
Compd	Single oral dose, mg/kg	No. of mice	% redn in no. of worms	% mice with parasital cures	nmol per plate	(−) S_9	(+) S_9	(−) S_9	(+) S_9
1	5	7	68	14	5	0	0	0	0
	7	8	82	25	16	0	0	0	0
	10	12	99	92	53	0	0	0	0
	20	8	100	100	158	0	0	0	0
2	50	6	0	0	17	42 (2.47)	42 (2.42)	6 (0.35)	6 (0.35)
	125	7	0	0	60	100 (1.80)	104 (1.73)	20 (0.35)	18 (0.30)
	250	6	0	0					
	500	12	0	0					

[a] Numbers in parentheses indicate the number of revertants per nanomole.