(*R*)- and (*S*)-5,6,7,8-Tetrahydro-1-hydroxy-*N*,*N*-dipropyl-9*H*-benzocyclohepten-8-ylamine. Stereoselective Interactions with 5-HT_{1A} Receptors in the Brain

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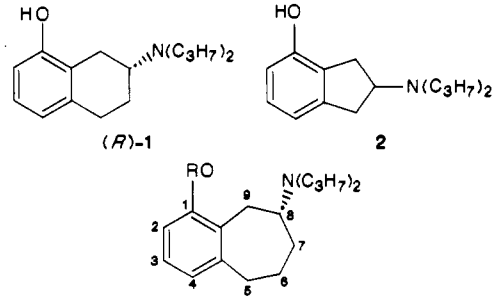
The enantiomers of 5,6,7,8-tetrahydro-1-hydroxy-*N*,*N*-dipropyl-9*H*-benzocyclohepten-8-ylamine (**3**) have been synthesized and evaluated for central 5-hydroxytryptamine (5-HT) and dopamine (DA) receptor activity by use of behavioral and biochemical tests in rats. In addition, the ability of the compounds to displace [^{3}H]-8-OH-DPAT from 5-HT_{1A} binding sites was evaluated. The absolute configuration of the enantiomers of **3** was determined indirectly by X-ray diffraction of (+)-(8*R*,α*R*)-5,6,7,8-tetrahydro-1-methoxy-*N*-(α-phenethyl)-9*H*-benzocyclohepten-8-ylamine hydrochloride (**9**·HCl), a resolved synthetic precursor. The stereoselectivity of the interaction of **3** with 5-HT_{1A} receptors was more pronounced than that of 8-hydroxy-2-(dipropylamino)tetralin (**1**; 8-OH-DPAT); only (*R*)-**3** displayed 5-HT activity. However, (*R*)-**3** was of lower potency than any of the enantiomers of **1**. The enantiomer (*S*)-**3**, which was found to be inactive as a 5-HT-receptor agonist, appeared to be a weakly potent DA-receptor agonist whereas (*R*)-**3** seemed to be devoid of dopaminergic activity. The conformational preferences of **3** were studied by use of NMR spectroscopy and molecular mechanics calculations. Preferred conformations of (*R*)-**3** are similar in shape to those of the stereoselective 5-HT_{1A}-receptor agonist (2*R*,3*S*)-8-hydroxy-3-methyl-2-(dipropylamino)tetralin.

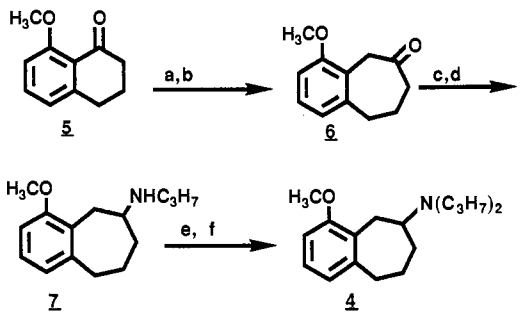
Recently, several putative subtypes of 5-hydroxytryptamine (5-HT) receptors have been identified.[1] These include 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, 5-HT_2, and 5-HT_3 receptors.[2] The possibility that the anxiolytic profiles of buspirone and isapirone may be related to 5-HT_{1A} receptor mediated mechanisms[3] has spurred synthetic efforts aiming toward novel 5-HT_{1A}-receptor ligands.[4] However, rather few studies on the structural modifications of 8-hydroxy-2-(dipropylamino)tetralin (**1**, 8-OH-DPAT),[5] the



first 5-HT_{1A}-receptor agonist,[6] can be found in the literature.[7] We and others have studied the pharmacological effects of the following modifications of **1**: (a) The phenol function has been moved to the 5-, 6-, or 7-positions,[5a] (b) additional hydroxyl groups have been introduced in the ortho and para positions,[5b,8] (c) the size of the *N*-alkyl groups has been varied,[5b,9] (d) methyl groups have been introduced in the C1, C2, and C3 positions,[10-12] and (e) the C4 methylene group has been exchanged for an oxygen.[13]

Additional information might be obtained by changing the size of the nonaromatic ring in **1**; ring-contracted and ring-expanded analogues can be expected to possess other

Scheme I[a]



[a] Reagents: (a) CH_2=PPh_3; (b) $Tl(NO_3)_3$, MeOH; (c) *n*-$C_3H_7NH_2$, C_6H_6; (d) Pd(C)/H_2, MeOH; (e) C_2H_5COCl, $N(C_2H_5)_3$; (f) $LiAlH_4$.

conformational characteristics and different distances between the plane/center of the aromatic ring and the

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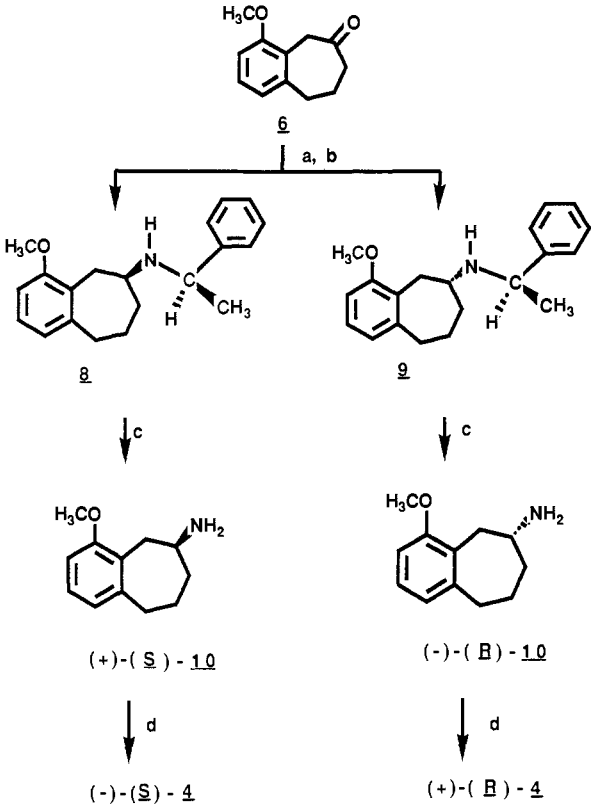
Scheme II[a]



[a] Reagents: (a) (+)-(*R*)-$C_6H_5CH(CH_3)NH_2$, MeOH, $NaBH_3CN$; (b) flash chromatography; (c) Pd(C)/H_2, MeOH; (d) C_3H_7I, K_2CO_3.

nitrogen. In addition, the shapes of preferred conformations of such compounds should be different from those of 1. The ring-contracted analogue 4-hydroxy-2-(dipropylamino)indan (2),[14] is a potent dopamine-receptor agonist that appears to lack ability to stimulate 5-HT receptors.[15] In the present investigation, we have synthesized and tested pharmacologically the enantiomers of the ring-expanded analogue 5,6,7,8-tetrahydro-1-hydroxy-*N*,*N*-dipropyl-9*H*-benzocyclohepten-8-ylamine (3). (*R*)-3 is able to stimulate 5-HT receptors in vivo and binds

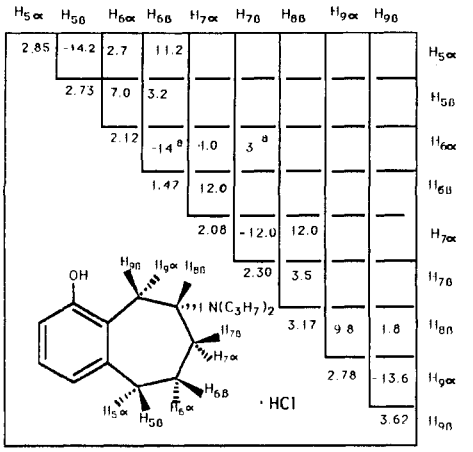


Figure 1. Selected ^1H NMR spectroscopic data of 3·HCl in methanol-d_4. Chemical shifts (in ppm) are shown on the diagonal. Proton–proton coupling constants are in Hz. (a) Approximate value due to the complex splitting pattern.

to [^3H]-8-OH-DPAT-labeled sites whereas (*S*)-3 seems to lack ability to interact with 5-HT_{1A} receptors.

Chemistry

Syntheses. Treatment of 8-methoxy-1-tetralone (5)[11,16] with methylenetriphenylphosphorane, followed by a thallium(III)-promoted ring expansion,[17] afforded 5,6,7,8-tetrahydro-1-methoxy-9*H*-benzocyclohepten-8-one (6),[18] which served as starting material for the benzocycloheptenylamine derivatives. The synthesis of the racemic benzocycloheptenylamine derivatives, which followed standard procedures, is outlined in Scheme I. The secondary amine 7 was obtained by reductive amination of 6. Acylation of 7 followed by hydride reduction produced the dipropylamino derivative 4.

Attempts to resolve any of the racemic amino derivatives (or the benzylamino derivative), by fractional crystallization of various diastereomeric salts, were unsuccessful. However, reductive amination of 6 by use of (+)-(*R*)-1-phenethylamine as the amine component produced a separable 1:1 mixture of diastereomers 8 and 9 (Scheme II). The diastereomers were separated by use of flash chromatography.[19] The *N*-(α-phenylethyl) group of 8 was removed by hydrogenolysis and the resulting primary amine [(+)-(10)] was N,N-dipropylated with 1-iodopropane to afford (−)-4. Compounds (−)-10 and (+)-4 were obtained from 9 by the same procedure.

The phenols (±)-3, (−)-3, and (+)-3 were prepared from the corresponding methoxy-substituted derivatives by use of 48% aqueous hydrogen bromide.

NMR Spectroscopy. High-resolution ^1H NMR spectral data for the seven-membered-ring protons of 3·HCl in methanol-d_4 are shown in Figure 1. Use of 400-MHz spectroscopy allowed analysis of most resonances by first-order approximations although some signals were complicated due to higher order couplings or overlapping resonances. However, C–H correlation, COSY, and "spin–spin" decoupling experiments allowed assignment of the signals and spin–spin simulations verified the ob-

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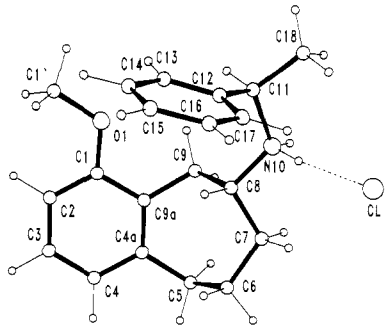
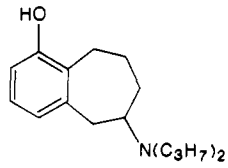


Figure 2. Perspective view of 9·HCl with atoms labeled as in the text. The solid and dashed lines represent covalent and hydrogen bonds, respectively.

served coupling constants. In addition to the resonances given in Figure 1, signals from the aromatic hydrogens were observed at δ 6.96 (H3), 6.72 (H4), and 6.62 (H2), and signals from the *N*-propyl groups appeared at δ 3.25 (Hα), 1.80 (Hβ), and 1.04 (Hγ). The ^{13}C NMR spectrum of 3·HCl showed signals at δ 11.5 (Cγ), 19.9 (Cβ), 26.7 (C9), 27.2 (C6), 33.6 (C7), 35.4 (C5), 54.4 (Cα), 64.3 (C8), 114.7 (C2), 121.5 (C4), 123.4 (C9a), 129.0 (C3), 145.9 (C4a), and 155.6 (C1). The assignments were verified by use of heteronuclear 2D chemical shift correlation spectroscopy.

The presence of the large coupling constants $J_{6\beta,7\alpha}$ and $J_{5\alpha,6\beta}$ as well as the 7-Hz coupling constant $J_{5\beta,6\alpha}$ in the ^1H NMR spectrum of 3·HCl in methanol-d_4 agrees with a predominance of a chair conformation; an anti relationship among $H_{7\alpha}$, $H_{6\beta}$, and $H_{5\alpha}$ is indicated and the angle between $H_{6\alpha}$ and $H_{5\beta}$ seems to be smaller than 60°. The large coupling constants $J_{8\beta,9\alpha}$ and $J_{7\alpha,8\beta}$ suggest that the dipropylammonium group assumes a pseudoequatorial disposition. Consequently, the ^1H NMR data suggest that 3·HCl preferentially adopts a chair conformation with a pseudoequatorial dipropylammonium substituent in methanol-d_4 solution.

The hydroxyl group strongly influences the chemical shift values of the signals from the neighboring aliphatic protons. A comparison of the ^1H NMR chemical shifts of 3·HCl and the reported data for the regioisomer 6,7,8,9-tetrahydro-1-hydroxy-*N*,*N*-dipropyl-5*H*-benzocyclohepten-6-ylamine hydrobromide (11·HBr),[20] in which the



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dipropylammonium group is located at C6, shows that the C1 hydroxyl group induces a downfield shift of ~0.6 ppm for the pseudoequatorial proton H9β positioned peri to the phenol-bearing carbon and an upfield shift of ~0.5 ppm for the pseudoaxial proton on the closest aliphatic carbon. The hydroxyl group also induces a chemical shift change of the C9 signal approximately 10 ppm upfield.

X-ray Crystallography. The absolute configuration of the synthetic precursor (+)-9·HCl was determined by X-ray crystallography to be 8*R*,αR. The absolute configuration at C8 was unambiguously deduced from the molecular geometry since the absolute configuration of the *N*-α-phenethyl moiety was known to be αR. The final atomic coordinates of the correct enantiomer are listed in Table I, and the structure is depicted in Figure 2.

Table I. Fractional Atomic Coordinates and Equivalent Isotropic Temperature Factors of the Non-Hydrogen atoms of 9·HCl[a]

atom	x/a	y/b	z/c	U_{aq},[b] Å^2
Cl	0.11867 (7)	0.40154	0.05137 (6)	0.0525 (2)
C(1)	−0.3514 (3)	0.0627 (4)	0.3706 (3)	0.052 (1)
C(2)	−0.3695 (4)	0.0545 (5)	0.4889 (4)	0.069 (2)
C(3)	−0.2526 (5)	0.0153 (5)	0.5995 (4)	0.072 (2)
C(4)	−0.1215 (4)	−0.0146 (4)	0.5902 (3)	0.066 (2)
C(4a)	−0.1034 (3)	−0.0088 (4)	0.4697 (3)	0.051 (1)
C(5)	0.0428 (4)	−0.0422 (4)	0.4625 (4)	0.063 (2)
C(6)	0.1133 (3)	0.0868 (5)	0.4242 (3)	0.060 (2)
C(7)	0.0574 (3)	0.1202 (4)	0.2788 (3)	0.050 (1)
C(8)	−0.1023 (3)	0.1592 (3)	0.2189 (3)	0.037 (1)
C(9)	−0.2047 (3)	0.0399 (3)	0.2260 (3)	0.043 (1)
C(9a)	−0.2184 (3)	0.0306 (3)	0.3592 (3)	0.043 (1)
O(1)	−0.4616 (2)	0.1002 (3)	0.2567 (2)	0.066 (1)
C(1′)	−0.5899 (4)	0.1574 (6)	0.2681 (4)	0.082 (2)
N(10)	−0.1304 (3)	0.1978 (3)	0.0789 (2)	0.040 (1)
C(11)	−0.2752 (3)	0.2659 (4)	0.0013 (3)	0.047 (1)
C(12)	−0.3020 (3)	0.3952 (4)	0.0713 (3)	0.049 (1)
C(13)	−0.4174 (4)	0.3958 (5)	0.1140 (4)	0.073 (2)
C(14)	−0.4392 (5)	0.5139 (6)	0.1835 (5)	0.094 (2)
C(15)	−0.3500 (5)	0.6311 (5)	0.2053 (4)	0.088 (2)
C(16)	−0.2391 (5)	0.6318 (5)	0.1596 (5)	0.090 (2)
C(17)	−0.2140 (4)	0.5141 (4)	0.0947 (4)	0.071 (2)
C(18)	−0.2761 (4)	0.2971 (4)	−0.1343 (3)	0.064 (2)

[a] Esd's are given in parentheses. [b] $U_{eq} = {}^1/_3\sum_i\sum_j U_{ij}\mathbf{a}^*_i\mathbf{a}^*_j\mathbf{a}_i\mathbf{a}_j$.

In the crystal, (8*R*,αR)-9·HCl adopts a folded conformation with the cycloheptene ring in a chair conformation. In this conformation the cycloheptene ring system has an approximate plane of symmetry through C7. The ring-puckering parameters, calculated according to Cremer and Pople,[21] are Q = 0.788 (4) Å, θ = 34.3 (3), Φ_2 = 154.7 (4)°, and Φ_3 = −129.1 (3)°. The ammonium group adopts a pseudoequatorial position and the atoms of the fused benzene ring of the bicyclic moiety are coplanar within 0.017 Å. The methoxy substituent is slightly bent out of the ring plane (the C(2)–C(1)–O(1)–C(1′) torsion angle is 11.6 (5)°). The aromatic ring of the phenethyl substituent is flat within 0.027 Å. The angle between the least-squares planes through the two phenyl rings of the molecule is 99.8 (1)°. The observed bond lengths and bond angles generally conform to the expected values (see the supplementary material).

The crystal structure is illustrated in Figure 3. Each chloride ion forms hydrogen bonds with two ammonium hydrogens and each ammonium hydrogen forms hydrogen bonds with two chloride ions [$N(10)_{x,y,z}\cdots Cl$ = 3.221 (2) Å, N(10)–H(101) = 0.89 Å, H(101)···Cl = 2.33 Å, N(10)–H(101)···Cl = 172° and $N(10)_{-x,y-1/2,-z}\cdots Cl$ = 3.138 (2) Å, N(10)–H(102) = 0.83 Å, H(102)···Cl = 2.33 Å, N(10)–H(102)···Cl = 165°].

Molecular Mechanics (MMP2) Calculations. In a recent investigation,[20] we studied the conformational preferences of 11 by molecular mechanics (MMP2) calculations and several other techniques and identified 14 low-energy MMP2 conformations. Relative conformational energies and geometries appear to be unaffected by the positions of the phenolic group in the 2-aminotetralins except when the hydroxyl group and a benzylic substituent adopt a peri relationship.[22] Therefore, after having moved

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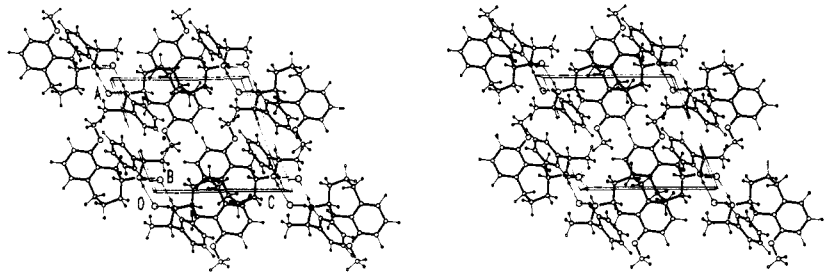

Figure 3. Stereoscopic illustration of the crystal structure of **9**·HCl.

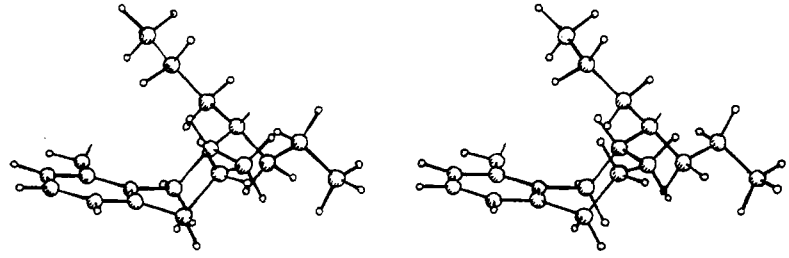

Figure 4. Stereoscopic view of the minimum-energy MMP2 conformation of (*R*)-**3**. Other low-energy conformations are listed in the supplementary material.

Table II. (*R*)- and (*S*)-5,6,7,8-Tetrahydro-1-hydroxy-*N*,*N*-dipropyl-9*H*-benzocyclohepten-8-ylamine: Effects on in Vivo Accumulations of 5-HTP and DOPA in Rat Brain[a]

compd	dose, μmol/kg	reserpine pretreatment	5-HTP,[b] ng/g		DOPA[b], ng/g	
			striatum	limbic	striatum	limbic
(*R*)-**3**	3.2	no	85 ± 7.3*	132 ± 1.7**	1068 ± 5.6	408 ± 30
	10	no	60 ± 0.7**	122 ± 4.1**	919 ± 27	314 ± 17*
	32	no	54 ± 7.6**	76 ± 7.7**	1035 ± 34	437 ± 14
(*S*)-**3**	10	no	110 ± 4.6	194 ± 7.2	1003 ± 42	331 ± 7.5*
	32	no	98 ± 7.7	170 ± 21	830 ± 92*	381 ± 25
	32[c]	yes	74 ± 6.1*	154 ± 9.9	1934 ± 179**	606 ± 4.5**
control		no	107 ± 4.1	186 ± 6.7	1152 ± 45	432 ± 15
control		yes	117 ± 12	207 ± 19	3558 ± 213	1024 ± 74

[a] Nonreserpinized or reserpinized (8 μmol/kg, 4 h before) animals were injected with test drug and NSD 1015 (573 μmol/kg, ip) 30 min before death. [b] The values are means ± SEM (n = 17–19 and 3–7 in the control and experimental groups, respectively). Statistics: one-way ANOVA followed by Dunnett's t test: (**) $p < 0.01$, (*) $p < 0.05$ vs control. [c] The following DOPA-levels were obtained after this dose: hemispheres, 138 ± 2.8 ng/g (control, 172 ± 13 ng/g); brain stem, 315 ± 24 ng/g (control, 337 ± 7.5 ng/g). These DOPA-levels were not significantly different ($p > 0.05$) from the control values.

the hydroxyl group to the proper position, we used the 14 identified low-energy conformations of **11** as starting geometries in MMP2 minimizations of **3**. The resulting 14 energy-minimized conformations of **3** had almost identical relative energies and geometries as those of **11**. In the lowest energy conformation, the cycloheptene ring of **3** adopts a half-chair conformation and the dipropylamino substituent is pseudoequatorially dispositioned (Figure 4). These results agree well with those from the NMR spectroscopic investigation.

Pharmacological Results

Behavior. Stimulation of postsynaptic 5-HT and dopamine (DA) receptors was studied in rats in which the presynaptic monoamine stores had been depleted by reserpine pretreatment. Behavioral observations were made, particularly with regard to flat body posture and forepaw treading (5-HT motor syndrome).[23,24]

In reserpine-treated rats, (*R*)-**3** (32 μmol/kg, sc), produced a clear 5-HT motor syndrome. In contrast, (*S*)-**3** (32 μmol/kg, sc) induced sudden jumps or an increase in locomotor activity in reserpine-pretreated rats. This behavior was not seen if the rats received haloperiodol (2 mg/kg, i.e., 376 μmol/kg, ip) 1 h before (*S*)-**3** was administered. Instead, they exhibited stereotypical movements with the head and forelegs. In nonpretreated rats, (*S*)-**3** did not produce any behavior changes.

Biosynthesis of 5-HT and DA. Agonists at 5-HT, dopamine and norepinephrine (NE) receptors inhibit the synthesis of the corresponding monoamine.[25] Thus, the monoamine synthesis can be used as an indicator of pre- and postsynaptic receptor activation. In the present study, the synthesis was measured indirectly by determining the accumulation of 5-hydroxytryptophan (5-HTP) in the various brain parts and of 3,4-dihydroxyphenylalanine (DOPA) in the DA predominant (corpus striatum, limbic system) and NE predominant (brain stem, hemispheres) rat brain regions following aromatic L-amino acid decarboxylase inhibition with (3-hydroxybenzyl)hydrazine (NSD 1015).[26] The rats were not pretreated with reserpine.

The accumulation of 5-HTP following NSD 1015 was decreased after all tested doses of (*R*)-**3** in corpus striatum and the limbic system (Table II). A similar decrease in 5-HTP levels was also observed in the brain stem and the hemispheres (data not shown). The DOPA accumulation was not affected by (*R*)-**3**. In nonpretreated rats, the *S*

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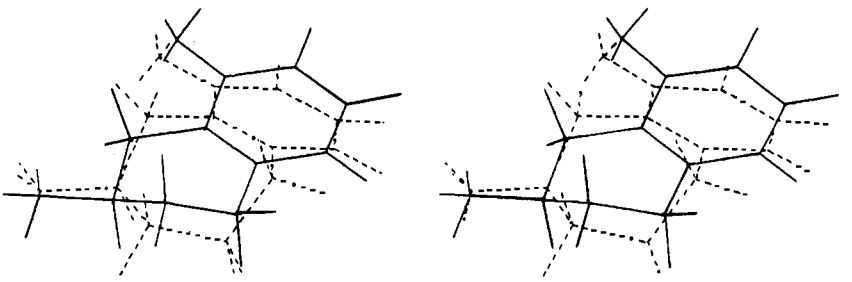


Figure 5. Stereoscopic representation of a computer-aided structural fit of the 2-aminotetralin moieties of the minimum-energy conformations of (*R*)-1 (ref 27; solid lines) and (*R*)-3 (dashed lines). This structural fit, in which C8, C4, and N of (*R*)-1 have been fitted with Cl, C4, and N of (*R*)-3, gives an average distance between fitted atoms of 0.23 Å, and the angle between the planes of the aromatic rings is 25°.

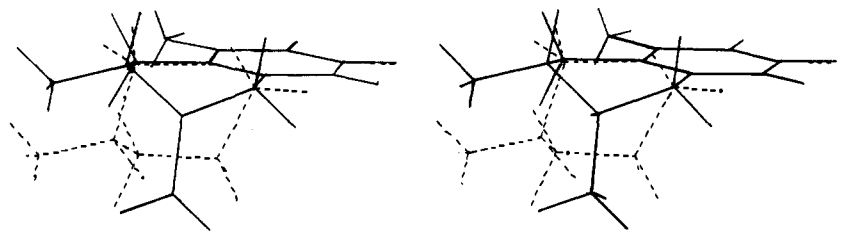


Figure 6. Stereoscopic representation of a computer-aided structural comparison of the 2-aminotetralin moieties of the minimum-energy conformations of (2*R*,3*S*)-12 (ref 12a; solid lines) and (*R*)-3 (dashed lines) in which only the phenolic moieties have been included in the fitting procedure (thus, the average distance between fitted atoms is 0 Å).

Table III. Affinities of the Enantiomers of 3 at 5-HT$_{1A}$ Receptor Sites

compd	K_i, nM	N_H
(*R*)-3	29.7, 46.3	−0.75, −0.71
(*S*)-3	>300, >300	
(±)-1[a]	1.0	−0.92

[a] Included for comparison.

enantiomer (10 and 32 μmol/kg, sc) decreased the accumulation of DOPA without significantly affecting the 5-HTP accumulation. However, in reserpine-pretreated rats, (*S*)-3 (32 μmol/kg) produced decreases in both the DOPA and the 5-HTP levels, in the corpus striatum and the limbic system. In the brain stem and the hemispheres, there was no statistically significant decrease in DOPA levels. The latter results indicate that the effects were unrelated to NE-receptor activation.

Affinity for 5-HT$_{1A}$ Binding Sites in Vivo. The enantiomers of 3 were evaluated for their affinities to the 5-HT$_{1A}$-receptor sites (Table III). In agreement with the in vivo studies, (*R*)-3 was found to be the more potent enantiomer. The affinity of (*R*)-3 for 5-HT$_{1A}$ sites was much smaller (almost 40 times) than that of racemic 1. Thus, the in vitro and in vivo studies indicate that (*R*)-3 is a stereoselective 5-HT$_{1A}$-receptor agonist of moderate potency.

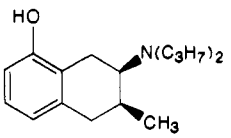
Discussion

The pharmacological in vivo data indicate that (*R*)-3 is a selective 5-HT-receptor agonist of moderate potency whereas the profile of (*S*)-3 involves a dominating dopaminergic component. These conclusions are supported by the results from the receptor binding assay; (*R*)-3 is able to compete fairly potently with [^{3}H]-8-OH-DPAT for 5-HT$_{1A}$ sites whereas the affinity of (*S*)-3 is at least 10 times smaller. Thus, in contrast to 1, which is a weakly stereoselectively 5-HT$_{1A}$-receptor agonist,[5a,9] the ring-expanded analogue 3 exhibits considerable stereoselectivity in its interaction with 5-HT$_{1A}$ receptors.

The observation that (*S*)-3 decreases the DOPA levels more powerfully in reserpine-pretreated than in nonpretreated animals indicates that it is a weak DA-receptor agonist since DA receptors become more sensitive to agonists after reserpine-pretreatment. In addition, the behavior induced by (*S*)-3 was effectively counteracted by pretreatment with the DA-receptor antagonist haloperidol.

The "5-HT receptor activating pharmacophore" in for example the potent 5-HT$_{1A}$-receptor agonists (*R*)- and (*S*)-1 may be described by the relative positions of the aromatic substituent, the aromatic ring, and the nitrogen atom (for a thorough discussion, see ref 27). Low-energy conformations of 1 have short distances (about 0.6 Å) between the nitrogen and the plane of the aromatic ring (N–ArP distances).[27] In contrast, preferred conformations of (*R*)-3 have N–ArP distances of around 2 Å. Consequently, a computer-aided fit of the oxygens, the aromatic rings, and the nitrogens of low-energy conformations of (*R*)-3 and (*R*)- or (*S*)-1 produce superpositions in which the planes of the aromatic rings are tilted at a 20–25° angle (compare Figure 5). It should be noted that conformations of (*R*)-3 with short N–ArP distances can be expected to have relative steric energies well above 6 kcal/mol.[20]

The 2-aminotetralin derivative (2*R*,3*S*)-8-hydroxy-3-methyl-2-(dipropylamino)tetralin [(2*R*,3*S*)-12] is a 5-HT$_{1A}$-receptor agonist, similar in potency to (*R*)-3.[12] In



(2*R*,3*S*)-12

addition, (2*R*,3*S*)-12 exhibits a similar degree of stereoselectivity, the 2*S*,3*R* enantiomer being the less potent antipode.[12] It is therefore informative to compare the topographies of energetically accessible conformations of (*R*)-3 and (2*R*,3*S*)-12. In the most interesting superposition (see Figure 6), only the phenolic moieties were fitted. This structural comparison puts the C3-methyl of (2*R*,3*S*)-12 and the C7-methylene of (*R*)-3 in similar relative spatial positions. However, the nitrogens are separated by 2.1 Å.

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In conclusion, the present results demonstrate that the N-ArP distance of 5-HT_{1A}-receptor agonists may vary between wider limits than previously believed.[27]

Experimental Section

Chemistry. General Comments. Routine ^1H and ^{13}C NMR spectra were recorded at 90 MHz and 22.5 MHz, respectively, on a JEOL FX 90Q spectrometer and were referenced to internal tetramethylsilane. For the conformational analysis, ^1H and ^{13}C NMR spectra were recorded on a JEOL GX-400 spectrometer using 0.04 M (^1H) and 0.2 M (^{13}C) methanol-d_4 solutions of **3**·HCl at 40 °C. Apparent proton–proton coupling constants were obtained from expanded (2 Hz/cm) spectra and refined by use of the spin simulation program INMGX-COMIC-2, available in the GX software. Standard pulse sequences were used for the 2D experiments (homo- and heteronuclear COSY, long-range COSY) with 45° or 90° mixing pulses. IR spectra (recorded on a Perkin-Elmer 157 G spectrometer), and mass spectra (recorded at 70 eV on a LKB 9000 spectrometer using a direct insertion probe) were all in accordance with the assigned structures. Melting points (uncorrected) were determined in open glass capillaries on a Thomas-Hoover apparatus. Optical rotations were obtained on a Perkin-Elmer 241 polarimeter. The elemental analyses (C, H, and N), which were performed by Micro Kemi AB, Uppsala, Sweden, were within ±0.4% of the theoretical values. For the determination of percent diastereomeric excess and for purity tests, capillary GC was performed on a Carlo Erba 4200 instrument equipped with an SE 54 fused-silica capillary column (10 m).

5,6,7,8-Tetrahydro-1-methoxy-9*H*-benzocyclohepten-8-one (6). A slurry of 80% sodium hydride (3.4 g, 113 mmol) in dry dimethyl sulfoxide (20 mL) was heated under nitrogen at 80 °C for 1 h. After addition of dry dimethyl sulfoxide (20 mL), methyltriphenylphosphonium bromide (41 g, 113 mmol) was added in portions. The mixture was stirred at 70 °C for 30 min and a solution of 8-methoxy-1-tetralone (**5**)[12,16] (10.0 g, 57 mmol) in dry dimethyl sulfoxide (20 mL) was added over 15 min. The reaction temperature was kept at 70 °C overnight. The mixture was poured into ice–water (100 mL) and hexane (100 mL) was added. Precipitated triphenylphosphine oxide was filtered off and the organic layer was washed with water, dried (magnesium sulfate), filtered, and concentrated to give crude 8-methoxy-1-methylenetetralin (9.0 g). A solution of this Wittig product dissolved in methanol (60 mL) was added in one portion to a freshly prepared solution of thallium(III) nitrate trihydrate (21.75 g, 48 mmol) in methanol (200 mL). The mixture was stirred vigorously for 1 min and chloroform (200 mL) was added. The resulting suspension was filtered and the organic layer was washed with saturated aqueous sodium bicarbonate and concentrated. The residue was purified by flash chromatography with ether–light petroleum 1:4 as eluant, yielding 8.0 g (74%) of pure **6**: R_f = 0.29 (SiO_2, ether–light petroleum 1:4), ^1H NMR (chloroform-*d*) δ 7.30–7.05 (m, 1 H), 6.90–6.70 (m, 2 H), 3.95–3.65 (m, 2 H), 3.82 (s, 3 H), 2.98–2.75 (m, 2 H), 2.65–2.35 (m, 2 H), 2.34–1.75 (m, 2 H); mass spectrum, *m/z* (relative intensity) 190 (100), 162 (62), 134 (88). Anal. ($C_{12}H_{14}O_2$) C, H.

(±)-5,6,7,8-Tetrahydro-1-methoxy-*N*-propyl-9*H*-benzocycloheptenyl-8-amine (7). A solution of **6** (1.0 g, 5.3 mmol) and *n*-propylamine (1.07 g, 10.6 mmol) in benzene (60 mL) was heated to reflux under nitrogen in a Dean–Stark apparatus. Additional *n*-propylamine (1.07 g, 10.6 mmol) was added after 20 h and the heating was continued for 24 h. The mixture was concentrated and the residue was dissolved in methanol (30 mL) and hydrogenated over palladium (10%) on carbon at atmospheric pressure. The catalyst was filtered off (Celite) and the solution was concentrated. Ethereal hydrogen chloride was added to an ethereal solution of the residue and the resulting hydrochloride was recrystallized from ethanol–ether, affording 830 mg (58%) of **7**·HCl: mp 192–194 °C; ^1H NMR (methanol-d_4) δ 7.25–7.00 (m, 1 H), 6.92–6.67 (m, 2 H), 3.83 (s, 3 H), 3.71–3.47 (m, 1 H), 3.19–2.65 (m, 6 H), 2.44–1.44 (m, 6 H), 0.95 (t, 3 H, *J* = 7.3 Hz); mass spectrum, *m/z* (relative intensity) 233 (5), 98 (100). Anal. ($C_{15}H_{23}NO$·HCl) C, H, N.

(±)-5,6,7,8-Tetrahydro-1-methoxy-*N*,*N*-dipropyl-9*H*-benzocycloheptenyl-8-amine [(±)-(4)]. A solution of *n*-propionyl chloride (240 mg, 2.6 mmol) in dry ether (5 mL) was added to a cold (0 °C) solution of **7** (700 mg, 2.6 mmol), and triethylamine (0.26 g, 2.6 mmol) in dry ether (15 mL). The mixture was stirred at room temperature for 2 h, chloroform (100 mL) was added, and the organic layer was washed with 1 M hydrochloric acid and 1 M aqueous sodium hydroxide. The organic layer was dried (magnesium sulfate), filtered, and concentrated. The residue was dissolved in dry tetrahydrofuran (15 mL) and added to a slurry of lithium tetrahydridoaluminate (0.6 g, 15 mmol) in tetrahydrofuran (15 mL). The mixture was heated to reflux overnight under nitrogen and the reaction was quenched by addition of water and aqueous sodium hydroxide. The resulting precipitate was filtered off and the filtrate was dried (potassium carbonate), filtered, and concentrated. The residue was converted into the hydrochloride and recrystallized from ethanol–ether to afford 580 mg (72%) of pure (±)-**4**·HCl: mp 201–203 °C; ^1H NMR (methanol-d_4) δ 7.26–7.02 (m, 1 H), 6.94–6.68 (m, 2 H), 3.84 (s, 3 H), 3.81–3.52 (m, 1 H), 3.46–2.63 (m, 8 H), 2.40–1.50 (m, 8 H), 1.03 (t, 6 H); mass spectrum, *m/z* (relative intensity) 275 (4), 175 (13), 140 (100). Anal. ($C_{18}H_{29}NO$·HCl) C, H, N.

(+)-(8*S*,α*R*)- and (+)-(8*R*,α*R*)-5,6,7,8-Tetrahydro-1-methoxy-*N*-(α-phenylethyl)-9*H*-benzocycloheptenyl-8-amine (8 and 9). To a solution of (+)-(*R*)-1-phenylethylamine (5.4 mL, 42 mmol) and **6** (4 g, 21 mmol) in methanol (60 mL), which had been colored by a small amount of methyl red, was added methanolic hydrogen chloride until a red color was obtained. Sodium cyanoborohydride (1.4 g, 19 mmol) was added in portions. The red color of the solution was maintained during the reaction by addition of more methanolic hydrogen chloride when necessary. The mixture was stirred at room temperature for 2 days, acidified to pH 2 by addition of concentrated hydrochloric acid, and concentrated. The residue was dissolved in water and washed with ether. The water layer was alkalinized to pH 10 with sodium hydroxide and extracted with ether. The ether layer was dried (potassium carbonate), filtered, and concentrated. The residue was purified by flash chromatography by use of ammonia-saturated ether–light petroleum 1:1 as eluant to afford 2.6 g (42%) of a 1:1 mixture of diastereomers **8** and **9**. Repetitive flash chromatography by use of ammonia-saturated ether–light petroleum 1:4 as eluant afforded pure **8**. Compound **8** was converted into the hydrochloride and recrystallized from ethanol–ether: yield, 840 mg (24%); R_f = 0.12 SiO_2, ammonia-saturated ether–light petroleum 1:4): mp 219–222 °C; $[\alpha]^{22}_D$ +49.5° (methanol, *c* 1.0); ^1H NMR (methanol-d_4) δ 7.64–7.33 (m, 5 H), 7.21–6.98 (m, 1 H), 6.90–6.61 (m, 2 H), 4.62 (q, 1 H, *J* = 6.8 Hz), 3.82 (s, 3 H), 3.70–3.48 (m, 1 H), 2.94–2.62 (m, 4 H), 2.60–1.80 (m, 2 H), 1.71 (d, 3 H, *J* = 6.8 Hz), 1.52–1.07 (m, 2 H). Anal. ($C_{20}H_{25}NO$·HCl) C, H, N.

The remaining fractions from the columns were concentrated and the residue was converted into the hydrochloride and recrystallized twice from ethanol–ether, affording 450 mg (13%) of pure **9**·HCl: R_f = 0.08 (SiO_2, ammonia-saturated ether–light petroleum 1:4); mp 259–261 °C; $[\alpha]^{22}D$ +144.9° (methanol, *c* 1.0); ^1H NMR (methanol-d_4) δ 7.65–7.29 (m, 5 H), 7.21–6.97 (m, 1 H), 6.91–6.59 (m, 2 H), 4.75 (q, 1 H, *J* = 6.9 Hz), 3.92 (s, 3 H), 3.86–3.64 (m, 1 H), 3.00–2.40 (m, 4 H), 2.35–1.80 (m, 2 H), 1.73 (d, 3 H, *J* = 6.9 Hz), 1.51–0.98 (m, 2 H). Anal. ($C_{20}H_{25}NO$·HCl) C, H, N.

The percent diastereomeric excess in **8** and **9** was determined by capillary GC to be larger than 98% for each.

(+)-(*S*)-5,6,7,8-Tetrahydro-1-methoxy-9*H*-benzocyclohepten-8-ylamine [(+)-(*S*)-10]. To a solution of **8**·HCl (1.5 g, 4.55 mmol) in methanol (100 mL) was added palladium on carbon and the mixture was hydrogenated at 3 atm. The catalyst was filtered off (Celite) and the filtrate was concentrated. The residue was converted into the hydrochloride and recrystallized from ethanol–ether to afford 940 mg (91%) of (+)-(*S*)-**10**·HCl: mp 247–249 °C; $[\alpha]^{22}D$ +12.6° (methanol, *c* 1.0); ^1H NMR (methanol-d_4) δ 7.23–6.98 (m, 1 H), 6.91–6.60 (m, 2 H), 3.82 (s, 3 H), 3.68–3.41 (m, 1 H), 3.13–2.63 (m, 4 H), 2.37–1.12 (m, 4 H). ($C_{12}H_{15}NO$·HCl) C, H, N.

(−)-(*R*)-5,6,7,8-Tetrahydro-1-methoxy-9*H*-benzocyclohepten-8-ylamine [(−)-(*R*)-10]. Debenzylation of compound **9**·HCl (1.0 g, 3.0 mmol) was accomplished as described above for the preparation of (+)-(*S*)-**10**. Recrystallization from ethanol–ether afforded 600 mg (88%) of (−)-(*R*)-**10**·HCl: mp 248–249 °C; $[\alpha]^{22}D$ −12.7° (methanol, *c* 1.0). Anal. ($C_{12}H_{15}NO$·HCl) C, H, N.

(−)-(*S*)-5,6,7,8-Tetrahydro-1-methoxy-*N*,*N*-dipropyl-9*H*-benzocyclohepten-8-ylamine [(−)-(*S*)-4]. A mixture of (+)-

(*S*)-**10**·HCl (300 g, 1.3 mmol), 1-iodopropane (520 mg, 3.06 mmol), and potassium carbonate (1.3 g, 9.4 mmol) in acetonitrile (20 mL) was stirred at 50 °C under nitrogen. Additional portions of 1-iodopropane (230 mg and 120 mg) were added during the course of 2 days. Ether (100 mL) was added and the reaction mixture was filtered and concentrated. The residue was chromatographed on an alumina column with ether–light petroleum 1:4 as eluant. The amine was converted into the hydrochloride and recrystallized from ethanol–ether, yielding 350 mg (84%) of (–)-(*S*)-**4**·HCl: R_f = 0.67 (Al_2O_3, ether–light petroleum 1:4); mp 193–195 °C; $[\alpha]^{22}_D$ –9.5° (methanol, *c* 1.0); ^{1}H NMR (methanol-d_4) δ 7.22–7.00 (m, 1 H), 6.92–6.65 (m, 2 H), 3.84 (s, 3 H), 3.79–3.53 (m, 1 H), 3.47–2.59 (m, 8 H), 2.42–1.20 (m, 8 H), 1.17–0.88 (t, 6 H). Anal. ($C_{18}H_{29}NO$·HCl) C, H, N.

(+)-(*R*)-5,6,7,8-Tetrahydro-1-methoxy-*N*,*N*-dipropyl-9*H*-benzocyclohepten-8-ylamine [(+)-(*R*)-4]. This compound was prepared from (–)-(*R*)-**10**·HCl (460 mg, 2.0 mmol) according to the procedure for the synthesis of (–)-(*S*)-**4**. The yield of (+)-(*R*)-**4**·HCl after recrystallization was 525 mg (83%): mp 195–196 °C; $[\alpha]^{22}D$ + 9.5° (methanol, *c* 1.0). Anal. ($C_{18}H_{29}NO$·HCl) C, H, N.

(±)-5,6,7,8-Tetrahydro-1-hydroxy-*N*,*N*-dipropyl-9*H*-benzocyclohepten-8-ylamine [(±)-3]. A solution of (±)-**4**·HCl (450 mg, 1.44 mmol) in freshly distilled aqueous 48% HBr was heated at 120 °C under nitrogen for 3 h. The volatiles were evaporated in vacuo and the residue was partitioned between dichloromethane and saturated aqueous sodium bicarbonate. The organic layer was dried (sodium sulfate), filtered, and concentrated. The residue was converted into the hydrochloride and recrystallized from ethanol–ether to afford 350 mg (81%) of (±)-**3**·HCl: mp 239–241 °C; ^{1}H NMR (methanol-d_4) δ 7.07–6.82 (m, 1 H), 6.75–6.51 (m, 2 H), 3.75–3.51 (m, 1 H), 3.39–2.64 (m, 8 H), 2.30–1.40 (m, 8 H), 1.02 (t, 6 H, *J* = 7.1 Hz); mass spectrum, *m*/*z* (relative intensity) 261 (3), 161 (15), 140 (100). Anal. ($C_{17}H_{27}NO$·HCl·$^1/_4H_2O$) C, H, N.

(–)-(*S*)-5,6,7,8-Tetrahydro-1-hydroxy-*N*,*N*-dipropyl-9*H*-benzocyclohepten-8-ylamine [(–)-(*S*)-3]. Demethylation of compound (–)-(*S*)-**4**·HCl (250 mg, 0.8 mmol) was accomplished as described above for the preparation of (±)-**4**. The resulting hydrochloride was recrystallized from ethanol–ether to give 190 mg (80%) of pure (–)-(*S*)-**3**·HCl: mp 226.5–228.5 °C; $[\alpha]^{22}_D$ –10.7° (methanol, *c* 1.0). Anal. ($C_{17}H_{27}NO$·HCl) C, H, N.

(+)-(*R*)-5,6,7,8-Tetrahydro-1-hydroxy-*N*,*N*-dipropyl-9*H*-benzocyclohepten-8-ylamine [(+)-(*R*)-3]. This compound was prepared from (+)-(*R*)-**4**·HCl (200 mg, 0.64 mmol) as described above for the preparation of (±)-**3**. The yield of (+)-(*R*)-**3**·HCl after recrystallization was 150 mg (79%): mp 227–229 °C; $[\alpha]^{22}_D$ +9.5° (methanol, *c* 1.0). Anal. ($C_{17}H_{27}NO$·HCl) C, H, N.

Determination of Absolute Configuration of 9·HCl by Single-Crystal X-ray Analysis. A colorless crystal of **9**·HCl ($C_{20}H_{26}ONCl$, M_w = 331.88, $D_{c,X\text{-}ray}$ = 1.16 g cm^{-3}) with the approximate dimensions 0.32 × 0.34 × 0.32 mm was selected and sealed in epoxy glue for the x-ray study. The intensity data was obtained at room temperature on a Siemens STOE/AED 2 diffractometer using graphite-monochromated Mo Kα (λ = 0.7107 Å) radiation. A total of 3780 reflections (θ_{max} = 32.5°) were collected and corrected for background, Lorentz, polarization, and decay effects. The relatively low absorption effect ($\mu_{X\text{-}ray}$ = 2.03 cm^{-1}) was, however, neglected. The crystal symmetry is monoclinic ($P2_1$) with two molecules per unit cell. The cell dimensions, *a* = 9.9313 (4) Å, *b* = 9.3393 (3) Å, *c* = 10.9970 (4) Å, and β = 111.20 (3)°, were refined by a least-squares method using the angular settings of 60 reflections (11.8° < θ < 16.8°), accurately centered on the diffractometer. The structure was solved by direct methods[28] and refined by full-matrix least-squares treatment based upon *F*.[29] The non-hydrogen atoms were refined anisotropically and three group isotropic temperature factors were refined for the hydrogen atoms. The position of the H atoms, bonded to carbon atoms, were calculated after each cycle of the refinement using geometric evidence (C–H = 1.08 Å). The two nitrogen-bonded hydrogens were located from difference electron density calculations and held riding on the N atom during the subsequent calculations. The methyl groups were treated as rigid groups. *R* values[29] for 2103 reflections, all with $f > 5\ \sigma(F)$, became *R* = 0.036, R_w = 0.039, and R_G = 0.048. The weights of the structure factors were calculated as $w = 1.694/(\sigma^2(F) + 0.000385\ F^2)$.

Molecular Mechanics Calculations. The structural modeling was performed by use of the interactive computer graphics program MIMIC[30] (Methods for Interactive Modeling In Chemistry) and Chem-X.[31] MMP2 calculations were performed by using Allingers MMP2 1980-force field[32] to which had been added parameters for the phenol[33] and amino groups.[34] Geometries were obtained without restrictions in the minimization process. Calculations were done on a Microvax II computer. Computational times ranged from 1 to 25 min.

Pharmacology. Materials and Methods. Male Sprague–Dawley rats (Alab, Stockholm) weighing 180–220 g were used. Reserpine was dissolved in a minimal quantity of glacial acetic acid and made up to volume with 5.5% glucose solution. The other substances were dissolved in 0.9% NaCl. Throughout, injection volumes were 5 mL/kg. Substances to be tested were given to rats as the hydrochlorides, subcutaneously in the neck region.

Behavior. The rats were pretreated with reserpine (5 mg/kg, i.e. 8.2 μmol/kg sc). The experimental drugs were given 6 h later. The rats were placed in circular plastic cages and observed for behavioral changes. Proper dose levels were identified in preliminary experiments in which the observer did not know which dose or compound that had been administered. Throughout, attention was particularly paid at flat body posture and forepaw treading since these signs are considered as typical elements for the so-called 5-HT motor syndrome.[23,24]

Biosynthesis of 5-HT and DA. The tryptophan and tyrosine hydroxylase activities were determined from the accumulation of 5-HTP and DOPA following inhibition of the aromatic L-amino acid decarboxylase by (3-hydroxybenzyl)hydrazine (NSD 1015; 60 mg/kg, i.e. 287 μmol/kg sc, 30 min before killing).[26] The experimental drugs were given sc 30 min prior to NSD 1015.

The rats were killed by decapitation. The brain was directly taken out and placed on an ice-cooled Petri dish. The corpus striatum, the limbic system, the brain stem (thalamus, hypothalamus, mesencephalon, pons, medulla oblongata), and the hemispheres (cerebral neocortex and cerebellum) were separated. The rhinal fissures were used as a landmark in the dissection of the limbic system. The concentrations of 5-HTP and DOPA were determined electrochemically following ion-pair, reversed-phase HPLC (for details, ref 12b).

5-HT$_{1A}$ Binding Assay. Male Sprague–Dawley rats (weighing about 200 g) were decapitated and the cortex and hippocampus were dissected. The tissues (600–900 mg) from each rat were immediately homogenized in 15 mL of ice-cold 50 mM Tris-HCl buffer containing 4.0 mM $CaCl_2$ and 5.7 mM ascorbic acid, pH 7.5, with an Ultra Turrax (Janke & Kunkel, Staufen, FRG) for 10 s. After centrifugation for 12.5 min at 17 000 rpm (39800*g*) in a Beckman centrifuge with a chilled JA-17 rotor (Beckman, Palo Alto, CA), the pellets were resuspended in the same buffer and homogenization and centrifugation were repeated. The pellets from at least six rats were again suspended in the buffer, pooled, homogenized, and stored on ice for 1–4 h. The tissue homogenate was diluted to 10 mg/1.25 mL with the buffer, incubated for 10 min at 37 °C, and supplied with 10 μM pargylin (Sigma, St. Louis, MO) followed by reincubation for 10 min.

Incubation mixtures (2 mL) contained 1–300 nM test compound (diluted in 50 mM Tris-HCl containing 5.7 mM ascorbic acid, pH 7.5), 2 nM [^{3}H]-8-OH-DPAT ([^{3}H]-8-hydroxy-2-(dipropylamino)tetralin hydrobromide, 31.60 Ci/mmol, New England

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Nuclear, Dreieich, FRG and Research Biochemicals, Wayland, MA), 5 mg/mL tissue homogenate in 50 mM Tris-HCl buffer containing 4.0 mM $CaCl_2$ and 5.7 mM ascorbic acid, pH 7.5. Binding experiments were started by the addition of tissue homogenate and followed by incubation at 37 °C for 10 min. The incubation mixtures were filtered through Whatman GF/B glass filteres with a Brandel cell harvester (Gaithersburg, MD). The filters were washed twice with 5 mL of ice-cold 50 mM Tris-HCl buffer, pH 7.5, and counted with 5 mL of Ready-solv HP (Beckman) in a Beckman LS 3801 scintillation counter. Nonspecific binding was measured by the addition of 10 μM 5-HT·HCl to the reaction mixture. The binding data were processed by nonlinear least-squares computer analysis.[35] A K_d value of 1.4 nM for the 8-OH-DPAT binding was obtained from the saturated experiments and was used to calculate the K_i values.

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Supplementary Material Available: Lists of intramolecular bond distances and bond angles, involving the non-hydrogen atoms (Tables I and II), hydrogen atomic coordinates with isotropic temperature factors (Table III), and anisotropic thermal parameters of the non-hydrogen atoms (Table IV) for **9**·HCl, and geometries and steric energies of low-energy MMP2 conformations of **3**·HCl (Table V) (6 pages). Ordering information is given on any current masthead page. The list of observed and calculated structure factors is available directly from the authors on request.

Potential Antiatherosclerotic Agents. 6.[1] Hypocholesterolemic Trisubstituted Urea Analogues

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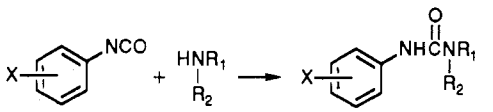
The discovery that a series of *N,N*-dialkyl-*N'*-arylureas were inhibitors of the ACAT enzyme has led to a structure–activity study involving the systematic modification of three sites of the urea backbone. This study culminated in the selection of *N'*-(2,4-dimethylphenyl)-*N*-benzyl-*N*-*n*-butylurea (**115**) for more extensive biological evaluation. ACAT inhibitors are seen as potentially beneficial agents against hypercholesterolemia and atherosclerosis.

The enzyme acyl CoA:cholesterol *O*-acyltransferase (ACAT, EC 2.3.1.26) is responsible for catalyzing the intracellular esterification of cholesterol.[2-4] Studies both in cultured cells[5-9] and in arterial tissue[10-12] have suggested that ACAT activity is increased when cells are exposed to cholesterol-rich lipoproteins. Since the intracellular accumulation of esterified cholesterol is one of the distinctive features of the atherosclerotic plaque, the regulation of ACAT is likely to be of great importance in the progression of atherosclerosis. Furthermore, the ACAT enzyme also plays a crucial role in the intestinal absorption of cholesterol. Despite the fact that free cholesterol is internalized by intestinal mucosal cells,[13] more than 90% of the cholesterol which appears subsequently in the lymph is esterified.[14] Substantial ACAT activity has been observed in intestinal mucosal cells from a variety of animal species[15-18] and man.[19] The site of greatest ACAT activity is the jejunum,[20] which is where the majority of cholesterol absorption occurs. Thus, the inhibition of intestinal ACAT

Scheme I



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