Resolved *cis*-10-Hydroxy-4-*n*-propyl-1,2,3,4,4a,5,6,10b-octahydrobenzo[*f*]quinoline: Central Serotonin Stimulating Properties

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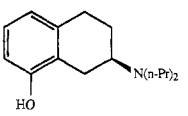
Received November 10, 1986

cis-10-Hydroxy-4-*n*-propyl-1,2,3,4,4a,5,6,10b-octadhydrobenzo[*f*]quinoline (4) is a centrally acting serotonin (5-HT) receptor agonist of moderate potency. Due to its semirigid character and the obvious similarity between (4a*R*,10b*S*)-4 and more potent, centrally acting 5-HT receptor agonist *cis*-(1*S*,2*R*)-8-hydroxy-1-methyl-2-(di-*n*-propylamino)tetralin (**2**), we carried out the preparation (via resolution of **6**, a precursor of **4**) and the pharmacological testing of the enantiomers of 4. We were able to show that the active enantiomers of 4 and **2** coincide in terms of stereochemistry, i.e., that it is the 4a*R*,10b*S* enantiomer of 4 that is the more active one. The absolute configuration was assigned on the basis of single-crystal X-ray analysis of the precursor (+)-**6** of the active enantiomer (-)-4. Conformational analysis with molecular mechanics (MM2) calculations were performed on the *N*-methyl analogues of compounds *cis*-(1*S*,2*R*)-**2** (*cis*-(1*S*,2*R*)-**3**) and *cis*-(4a*R*,10b*S*)-4 (*cis*-(4a*R*,10b*S*)-**7**). Both ammonium and free amine forms were subjected to these calculations. The results show a preference for the N-equatorial conformation, which is corroborated by the X-ray structure of (+)-**6**·HCl. The relatively low potency of compound *cis*-(4a*R*,10b*S*)-4 might be explained by unfavorable direction of the N-lone pair (or ammonium hydrogen) bond in this compound as compared to *cis*-(1*S*,2*R*)-**2** and *trans*-(4a*R*,10b*R*)-**5**, which can be predicted to be the more active enantiomer of compound **5**.

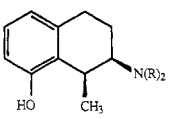
Arvidsson et al. have recently reported a series of compounds (i.a. **1**, **2**) with serotonin (5-HT) receptor stimulating properties in the central nervous system (CNS).[1-3] The most thoroughly studied of these new pharmacological probes is 8-hydroxy-2-(di-*n*-propylamino)tetralin (**1**; 8-OH-DPAT), which has contributed to the renewed interest in the 5-HT research field seen today.[4,5] All of these new compounds have been tested in their pure enantiomeric forms and, therefore, furnish useful pieces of information for mapping the central 5-HT receptor(s). It is now established, from recently reported receptor binding studies, that compound **1** has a high affinity for 5-HT_{1A} receptor sites.[6] It is likely that the compounds discussed in this paper act at this receptor site.

The semirigid 1,2,3,4,4a,5,6,10b-octahydrobenzo[*f*]-quinolines (OHBQ) constitute a structural class of compounds that has recently been investigated, mainly for their central dopaminergic effects.[7-10] However, the racemic compound *cis*-10-hydroxy-4-*n*-propyl-OHBQ (4) is a 5-HT agonist that is 70 times less potent than 1 and 14 times less potent than its racemic trans analogue **5**.[10] A structural comparison between the active enantiomer *cis*-(1*S*,2*R*)-8-hydroxy-1-methyl-DPAT (*cis*-(1*S*,2*R*)-**2**) and compound 4 leads to the prediction that the 5-HT receptor stimulating properties of 4 reside in its 4a*R*,10b*S* enantiomer. In order to investigate this problem we undertook the preparation of the enantiomer of 4, via resolution of the precursor *cis*-10-methoxy-OHBQ (**6**). The (-)-4 enantiomer was assigned the 4a*R*,10b*S* absolute configuration, on the basis of single-crystal X-ray analysis of the precursor (+)-**6**.

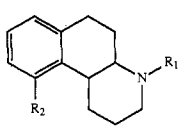
Even though compounds **2** and 4 might sterically be very similar, there is a possibility that different preferences for the N-lone pair (or N-ammonium hydrogen) directions might explain the potency difference between these two compounds in their actions on central 5-HT receptors. Conformational analysis of the *N*,*N*-dimethylammonium analogue of *cis*-(1*S*,2*R*)-**2** (*cis*-(1*S*,2*R*)-**3**), by driving the C2–N bond 0–360° with 20° increment and with nitrogen



R-1



compd	R
cis-1S,2R-2	n-Pr
cis-1S,2R-3	Me



compd	R_1	R_2
cis-4	n-Pr	OH
trans-5	n-Pr	OH
cis-6	H	OMe
cis-7	Me	OH
trans-8	Me	OH

in both equatorial (N-eq) and axial (N-ax) conformation, were included in order to study these directional preferences.

Conformational Analyses

Molecular mechanics (MM2)[11] conformational calcula-

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Tabl I. Physical Data

compd	yield,[a] %	mp,[b] °C	$[\alpha]_D^{22}$, deg (MeOH)	optical purity, %	formula
(4a*R*,10b*S*)-**6**	53	239–242	+1.5 (*c* 2.0)	>99	$C_{14}H_{19}NO\cdot HCl$
(4a*S*,10b*R*)-**6**	31	239–241	−1.0 (*c* 1.7)	>99	$C_{14}H_{19}NO\cdot HCl$
(4a*R*,10b*S*)-**4**	44	oil	−11.7 (*c* 2.0)		$C_{16}H_{23}NO\cdot HCl$
(4a*S*,10b*R*)-**4**	50	oil	+10.9 (*c* 2.8)		$C_{16}H_{23}NO\cdot HCl$

[a] The yield in the last step. [b] Recrystallized from CH_3CN/ether.

Table II. Biochemical Data

	5-HTP accumulation:[a] ED_{50}, μmol/kg			Dopa accumulation: ED_{50}, μmol/kg		
compd	limbic	striatum	hemisph	limbic	striatum	hemisph
4[b]	3.40	3.40	4.50	I[c]	I	I
(4a*R*,10b*S*)-**4**	2.57 (0.660–9.92)	3.63 (1.22–10.8)	3.47 (1.00–11.8)	I	I	I
(4a*S*,10b*R*)-**4**	*d*	*d*	*d*	*d*	*d*	*d*
(1*S*,2*R*)-**2**[e]	0.090 (0.050–0.160)	0.040 (0.010–0.070)	0.090 (0.060–0.170)	I	I	I

[a] The 95% confidence limits given in parentheses were calculated with a modified probit analysis previously used.[3] [b] Data taken from ref 10. [c] I means inactive. No significant effects were seen on Dopa accumulation at doses up to 50.0 μmol/kg in any of the three brain regions studied. [d] A partial agonist response (60–80% of control) was monitored for both Dopa and 5-HTP accumulation in all three brain regions studied at the doses 12.5, 25.0, and 50.0 μmol/kg. [e] Data taken from ref 3, paper IV.

tions were performed on the model compounds *cis*-(1*S*,2*R*)-**3** and (4a*R*,10b*S*)-**7** in both N-eq and N-ax conformations. The two conformations (N-eq and N-ax) were compared with respect to their steric energy difference, in both the amine and ammonium forms, in order to establish which conformation is more stable.

The X-ray structure of *cis*-(4a*R*,10b*S*)-**6**·HCl was used as input in another MM2 calculation and the X-ray and the minimized structures were compared. The X-ray structure has the N-eq conformation. For comparison, the N-ax conformation was also calculated. Both free base and ammonium forms were considered.

The starting geometries for all OHBQs with N-eq conformation were choosen to have the X-ray-derived geometry (half-chair/chair).[12] The N-ax conformations (half-chair/chair) were created by the Model program.[13] The same program was used to build the model compound *cis*-(1*S*,2*R*)-**3** in both N-eq and N-ax conformation. Locally developed software was used for molecular display and data extraction.[14] The possible conformational influence of solvents (e.g., water solvation) was not considered in this study. Conformational data are presented in Table III.

Chemistry

The racemate of compound **6** was synthesized as previously described.[10] Resolution of **6** was performed by acylation with (*R*)-(−)-α-methoxyphenylacetyl chloride, yielding the two diastereomeric amides, which were separated by chromatography on silica gel.[15,16]

Amide cleavage was achieved with potassium *tert*-butoxide in tetrahydrofuran (THF)[15,16] and the resolved secondary amines were converted to their *n*-propyl analogues by known methods. The phenols were obtained by hydrolyzing the corresponding methyl ethers in 48% aqueous HBr. Physical data are presented in Table I.

Pharmacology

The compounds were tested in reserpinized rats according to the in vivo biochemical method previously described.[17] Behavioral observations were made throughout this procedure.

The in vivo biochemical test method utilizes the well-established phenomenon of receptor-mediated feedback inhibition of the presynaptic neuron.[18–20] Thus, the synthesis rate of 5-HT is inhibited by 5-HT-receptor agonists and the synthesis rate of the catecholamines dopamine (DA) and norepinephrine (NE) is inhibited by agonists activating (and stimulated by antagonists blocking) dopaminergic and adrenergic receptors, respectively. The 5-HTP accumulation following decarboxylase inhibition by means of (3-hydroxybenzyl)hydrazine (NSD 1015) was used as an indicator of the rate of 5-HT synthesis in the three brain regions examined (limbic system, corpus striatum, and the remaining portions of the hemispheres, i.e., mainly cortex). In the same assay Dopa was monitored, thus giving an indication of the potential dopaminergic and adrenergic (the value from the NE-rich hemi-

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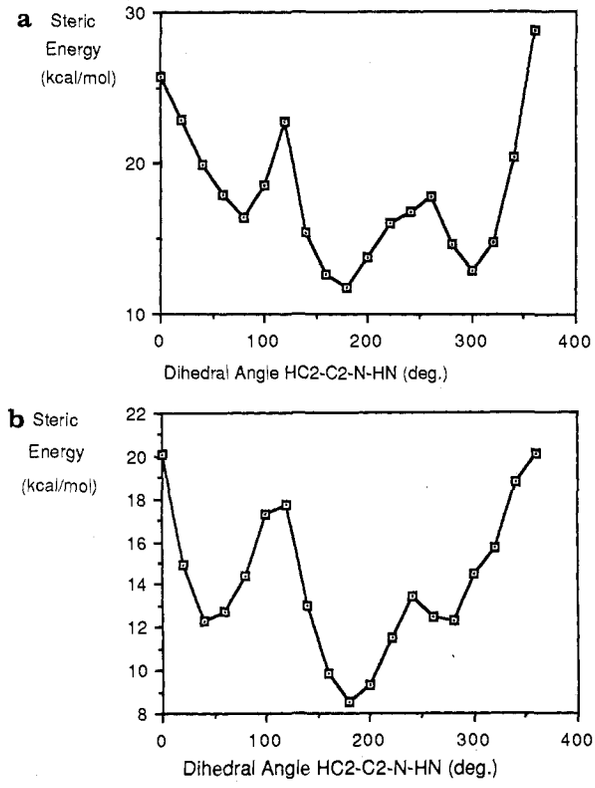

Figure 1. Energy plot of the dihedral driver (τ = H_{C2}–C2–N–H_N; 0–360°, 20° increment) for compound *cis*-(1*S*,2*R*)-**3** in N-ax (a) and N-eq (b) conformations.

spheres represents the adrenergic effect) agonistic effects of the compounds tested.

Results and Discussion

As seen from the biochemical results in Table II, the prediction that the 4a*R*,10b*S* enantiomer of **4** is the more active one is confirmed in this study. The other enantiomer is a partial agonist at both 5-HT and DA receptors. This is consistent with the 5-HT receptor stereoselectivity observed for *cis*-(1*S*,2*R*)-**2**.[3] Figure 2a–e shows stereo pictures of the global minima of the ammonium forms of compounds *cis*-(1*S*,2*R*)-**3** and *cis*-(4a*R*,10b*S*)-**7** in both N-ax and N-eq conformations, and compound *trans*-(4a*R*,10b*R*)-**8**. A previous study with MM2 on 2-aminoindans showed that there is a relatively strong H-bond interaction in the N-ax conformation between the ammonium hydrogen closest to the aromatic ring and the two closest carbon atoms in that ring.[21] Obviously, this situation is mimicked also by the cis compounds in their ammonium forms with the N-ax conformation. As seen from Table III, the two conformations (N-ax and N-eq) of *cis*-(4a*R*,10b*S*)-**6** in the ammonium form are equistable according to these calculations, and when analyzing the details of the results, one sees that the difference in the van der Waals (VdW) interactions (other interactions than 1,4) is about 1.5 kcal/mol between the two conformations, in favor of the N-ax conformation. This difference is fully accounted for by the H-bonding mentioned above.[11] However, this stabilizing factor for the N-ax, in rellation to the N-eq, conformation is outweighted by VdW repulsions (1,4 interactions; 0.6 kcal/mol) and torsional energy (0.8 kcal/mol), which are more pronounced in the N-ax than in the N-eq conformation. Possibly, the presence of the Cl counterion and other crystal forces, not accounted for here, could make a difference in favor of the N-eq conformation in the X-ray crystal (see Figures 3 and 4).

Table III. Conformational Data

structure	steric energy difference: (N-ax) – (N-eq), kcal/mol	distance, Å N–O	distance, Å N–A[a]
cis-(1*S*,2*R*)-**3**-N-ax-amm[b,c]		4.50	2.21
	3.4		
cis-(1*S*,2*R*)-**3**-N-eq-amm[b,c]		5.24	−0.32
cis-(4a*R*,10b*S*)-**6**-N-ax-amm		4.82	1.71
	0.0		
cis-(4a*R*,10b*S*)-**6**-N-eq-amm		5.14	−0.28
cis-(4a*R*,10b*S*)-**6**-N-ax-base		4.96	1.73
	2.5		
cis-(4a*R*,10b*S*)-**6**-N-eq-base		5.15	−0.34
cis-(4a*R*,10b*S*)-**6**-X-ray		5.14	−0.30
cis-(4a*R*,10b*S*)-**7**-N-ax-amm		4.82	1.88
	0.6		
cis-(4a*R*,10b*S*)-**7**-N-eq-amm		5.18	−0.37
cis-(4a*R*,10b*S*)-**7**-N-ax-base		4.92	1.87
	2.3		
cis-(4a*R*,10b*S*)-**7**-N-eq-base		5.19	−0.40
trans-(4a*R*,10b*R*)-**8**-amm		5.25	0.30

[a] N–A means the distance N–(aromatic ring plane). [b] amm means ammonium form. Free amine calculations were not preformed on compound **3**. [c] The rotamers used as input to these calculations were those found to have the lowest energies according to the driver plots in Figure 1a (τ = 180°) and Figure 1b (τ = 180°), respectively.

The introduction of an N-eq methyl substituent in the ammonium compounds discussed above leads to the relative stabilization of the N-eq conformation by 0.6 kcal/mol. The calculations indicate that this is a result of increased compression (0.4 kcal/mol), bending (0.5 kcal/mol), and VdW interactions (1,4 interactions; 0.5 kcal/mol), while the N-ax stabilizing H-bonds have decreased in importance (0.7 kcal/mol). If the N-ax stabilizing ammonium hydrogen is substituted for by a lone pair, as is the case for the free amines in Table III, the N-eq conformations become the more stable ones. The two major interactions in the free amines that destabilize the N-ax conformations are compression and bending. However, we believe that it is the ammonium form that is important at physiological pH at the drug–receptor interaction.

Another reason for selecting the N-eq conformation as the biologically active one is based on the N–O and N–(aromatic ring) distances. In order to approach the distance in the more potent analogue *trans*-**5** (N–O = 5.25 Å and N close to the aromatic plane, cf. *trans*-**8** in Table III), the *cis* analogues will have to have the the N-eq conformation (Table III). The trans analogues can only exist in the N-eq conformation in the half-chair/chair ring conformation. Since all potent, centrally acting 5-HT receptor agonists are fairly planar, the central 5-HT receptor can probably be viewed as a slot. This idea was previously presented by Nichols[22] for 5-HT agonsits, and is very similar to contemporary DA-receptor conepts.[16] On the basis of these data and the conformational data discussed above, we anticipate that it is the N-eq conformation of compound *cis*-(4a*R*,10b*S*)-**4** that is the one interacting with the receptor.

The stereoselectivities exhibited by *cis*-(1*S*,2*R*)-**2** and *cis*-(4a*R*,10b*S*)-**4** are most likely due to steric effects imposed by the 1-methyl grup and the piperidine ring, respectively, since compound (*R*)-**1**, lacking such a substit-

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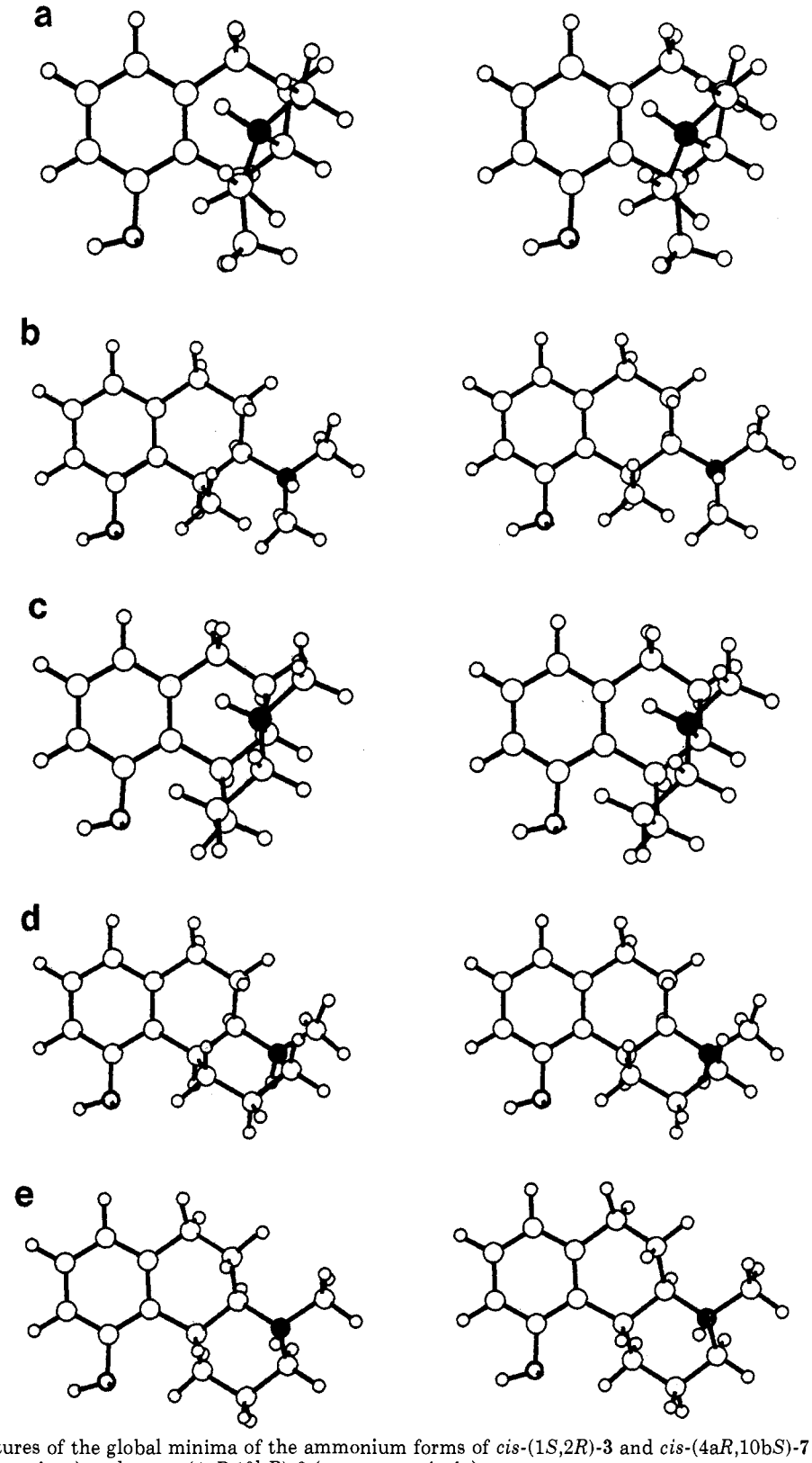

Figure 2. Stereo pictures of the global minima of the ammonium forms of *cis*-(1*S*,2*R*)-**3** and *cis*-(4a*R*,10b*S*)-**7** (both compounds in N-ax and N-eq conformations) and *trans*-(4a*R*,10b*R*)-**8** (a–e, respectively).

uent in that region of its structure, shows only a moderate (a factor 2) stereoselectively.[1,2]

As seen from the driver plot for the N-eq conformer of compound *cis*-(1*S*,2*R*)-**3** (Figure 1b), it has its global minimum around 180° for the torsion angle τ = H_{C2}–C_2–N–H_N. This gives the same conformation as the one exhibited in the single-crystal X-ray analysis of the methoxy analogue of *cis*-(1*S*,2*R*)-**2**, as presented by Arvidsson (cf. ref 3, paper IV). The torsion angle C_1–C_2–N–H_N for this conformation is −57.0°, and the corresponding torsion angles in *cis*-(4a*R*,10b*S*)-**7**-N-eq-amm and *trans*-(4a*R*,10b*R*)-**8** amm (C_{10b}–C_{4a}–N–H_N) are 58.5° and −50.8°, respectively. Obviously, the N-lone pair (or N-ammonium hydrogen) directions in compounds *cis*-(1*S*,2*R*)-**2** and *cis*-(4a*R*,10b*S*)-**4**, in their preferred conformations, are divergent when a molecular fit including the nitrogens, the aromatic rings and the phenolic OH groups is considered (cf. Figure 2, parts b, d, and e). It is thus quite possible that the relatively low potency of compound *cis*-(4a*R*,10b*S*)-**4**, as compared to the very potent compounds

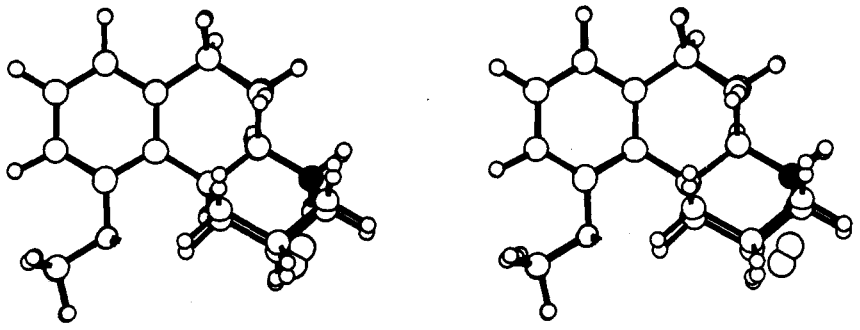

Figure 3. Stereo picture of the overlay of the X-ray and the minimized structures of *cis*-(4a*R*,10b*S*)-**6**.

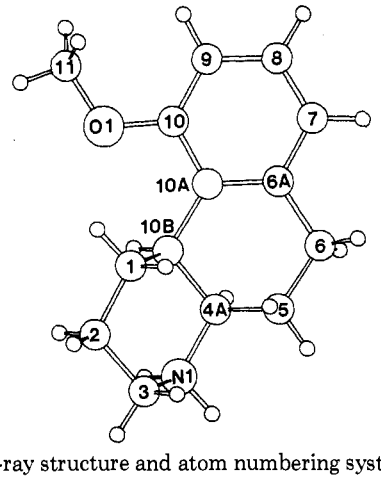

Figure 4. X-ray structure and atom numbering system for (+)-**6**.

1 and *cis*-(1*S*,2*R*)-**2**, could be explained by the inability of *cis*-(4a*R*,10b*S*)-**4** to achieve an optimal N-lone pair (or N-ammonium hydrogen) bond direction at the drug–receptor interaction.

In Figure 3, in stereo, is shown the overlay of the X-ray and the minimized structures of *cis*-(4a*R*,10b*S*)-**6**. In the relaxation from the X-ray geometry, the chloride ion was fixed at its starting position for computational reasons. Obviously, the minimized structure is very close to the X-ray structure, and an option in our software package[14] confirms this by giving the following root mean square values for the heavy atoms in this comparison: stretching 0.0134 Å, bending 1.43°, torsion 3.35°. Even though these discrepancies are small, they may be largely artifacts of the experiment. The experimental bond lengths are too short, because they have not been corrected for the thermal motion in the crystal. The angles and torsion angles are expected to be deformed slightly by the crystal packing forces.

Experimental Section

Chemistry. Melting points (uncorrected) were determined with a melting poit microscope (Reichert Thermovar). ^{1}H NMR spectra were recorded with a Bruker 500 MHz and a Varian EM-360 MHz instrument (Me_4Si). GC was performed with a HP 5830A instrument with a flame-ionization detector. A fused silica column (11 m, 0.22 mm i.d.) coated with cross-linked SE-54 (film thickness 0.3 μm, gas H_2, gas velocity 40 cm/s) was used throughout.

GC/MS spectra were recorded on a HP 5970A Mass Selective Detector working at 70 eV and interfaced with a HP 5700A gas chromatograph with a fused silica column (see above). High-resolution MS spectra were recorded on a ZAB high-resolution mass spectrometer (VG-Analytical) with direct inlet, working in the EI mode (60 eV). All spectra were in accordance with assigned structures.

HPLC was performed on a Water 5 Si 10 column using hexane/EtOAc/EtOH (91:7.5:1.5) as the mobile phase, working in the pressure range 1000–3000 psi and with a flow rate of 2 mL/min. Detection was made by Waters Model 440 UV monitor. Optical purity was estimated by comparing peak areas.

The elemental analyses (C, H, N) for the new substances were within 0.4% of the theoretical values. For purity tests, TLC was performed on fluorescent silica gel plates developed in at least two different systems. For all the compounds, only one spot (visualized by UV light and I_2 vapor) was obtained.

***cis*-(4a*S*,10b*R*)-10-Methoxy-1,2,3,4,4a,5,6,10b-octahydrobenzo[*f*]quinoline ((4a*S*,10b*R*)-6).** (*R*)-(−)-*O*-methylmandelic acid (210 mg, 1.3 mmol) was dissolved in CH_2Cl_2 (10 mL), $SOCl_2$ (1.0 mL) was added, and the mixture was refluxed for 30 min. Solvent and excess $SOCl_2$ were evaporated in vacuo, and the residual acid chloride was dissolved in CH_2Cl_2 (5 mL) and was added at room temperature to *cis*-10-methoxy-1,2,3,4,4a,5,6,10b-octahydrobenzo[*f*]quinoline hydrochloride (**6**·HCl) (280 mg, 1.1 mmol) suspended in CH_2Cl_2 (5 mL). Dropwise addition of Et_3N (0.15 mL) led to the formation of the two diastereomeric amides. After 1 h 10% HCl (10 mL) was added and the product was extracted with ether. The ether phase was separated, dried (Na_2SO_4), filtered, and evaporated to give an oil (480 mg, 101%), which was chromatographed on SiO_2 (0.040–0.063 mm) with petroleumether/ether (1:1) as eluant.

The fractions containing the diastereomer that eluted first in at least 98% optical purity (according to HPLC) were combined, and the solvent was evaporated, giving 86 mg (18%) of the desired diastereomer with an optical purity exceeding 99%. This oil (86 mg, 0.24 mmol) was dissolved in dry THF (10 mL), potassium *tert*-butoxide (300 mg, 2.7 mmol) was added, and the mixture was stirred at room temperature. After 23 h only 55% (according to GC analysis) of the desired amine had formed. The other 45% of the mixture consisted of the *N*-formyl derivative according to GC/MS analysis (cf. also ref 15 and 16). An additional amount of potassium *tert*-butoxide (100 mg, 0.89 mmol) was added and stirring was continued for another 48 h. At this point only 6% of the *N*-formyl derivative remained and 10% HCl was added and the product was washed with ether. The acidic water layer was basified with Na_2CO_3(s) and extracted with EtOAc. The organic phase was separated, dried (Na_2CO_3), and filtered and the solvent was evaporated to give an oil, which was converted to the hydrochloride with HCl-saturated EtOH. Evaporation and recrystallization (CH_3CN/ether) gave crystals (32 mg, 53%) of the desired product (4a*S*,10b*R*)-**6**·HCl. The optical purity of this compound was determined to be greater than 99% by recoupling a small sample with (*R*)-(−)-*O*-methylmandeloyl chloride and performing HPLC analysis. GC/MS (HP5970A) showed M^+ as the base peak at *m/e* 217.

***cis*-(4a*R*,10b*S*)-10-Methoxy-1,2,3,4,4a,5,6,10b-octahydrobenzo[*f*]quinoline ((4a*R*,10b*S*)-6).** The fractions from the preparation of (4a*S*,10b*R*)-**6** above, containing the diastereomer that was eluted last, with an optical purity exceeding 98%, were collected, and the solvent was evaporated to give the desired diastereomer as an oil (160 mg, 0.44 mmol). The optical purity of this oil was determined (cf. above) to be greater than 99%. The amide (160 mg, 0.44 mmol) was cleaved and worked up as described above to yield 34 mg (31%) of (4a*R*,10b*S*)-**6**·HCl after recrystallization (CH_3CN/ether). The optical purity of the crystals was determined to be greater than 99% (cf. above). GC/MS (HP5970A) showed M^+ as the base peak at *m/e* 217.

***cis*-(4a*S*,10b*R*)-10-Hydroxy-4-*n*-propyl-1,2,3,4,4a,5,6,10b-octahydrobenzo[*f*]quinoline ((4a*S*,10b*R*)-5).** Compound (4a*S*,10b*R*)-**6**·HCl (135 mg, 0.53 mmol) was suspended in CH_2Cl_2

(10 mL) and Et_3N (0.5 mL) was added. Propionyl chloride (0.3 mL) was added dropwise with stirring at room temperature. After 1 h water was added and ether extraction was performed. The organic layer was separated, dried (Na_2So_4), and filtered and the solvent was evaporated to yield an oil (140 mg, 0.51 mmol, 98%), which was dissolved in dry ether (10 mL). $LiAlH_4$ (500 mg, 13 mmol) was added with stirring at room temperature. Workup after 1 h in the usual way (0.5 mL of water, 0.5 mL of 15% NaOH, 1.5 mL of water, filtration, and extraction with ether/water) gave the desired *cis*-(4a*S*,10b*R*)-10-methoxy-4-*n*-propyl-1,2,3,4,4a,5,6,10b-octahydrobenzo[*f*]quinoline as an oil (78 mg, 0.30 mmol; 50%). GC/MS (HP5970A) showed M^+ at *m/e* 259 (11%) and the base peak at *m/e* 230.

The methyl ether was converted to the phenol by heating (120 °C) in 48% aqueous HBr (3 mL) under N_2(g) for 2 h. The hydrobromic acid was evaporated and the residue was basified with 10% Na_2CO_3 and extracted with ether. The ether layer was separated, dried (Na_2CO_3), and filtered and the solvent was evaporated, yielding an oil, which was chromatographed on basic Al_2O_3 with CH_2Cl_2/MeOH (30:1) as eluant. The fractions containing pure product according to GC and TLC were pooled, and the solvent was evaporated. The oily residue (38 mg; 50% was converted to its hydrochloride with HCl-saturated EtOH and evaporated. This product was isolated as an oil. High-resolution MS was performed and showed M^+ at *m/e* 245 (7.7%) ($C_{16}H_{23}NO$·HCl; calcd 245.178, found 245.174 ± 3) and the base peak at *m/e* 216. No impurities were detected by GC or TLC.

***cis*-(4a*R*,10b*S*)-10-Hydroxy-4-*n*-propyl-1,2,3,4,4a,5,6,10b-octahydrobenzo[*f*]quinoline ((4a*R*,10b*S*)-4).** Compound (4a*R*,10b*S*)-6·HCl (70 mg, 0.28 mmol) was converted to the propionamide (75 mg, 0.27 mmol; 96%), which was then reduced to the 4-*n*-propylamine derivative (50 mg, 0.19 mmol; 71%). This amine was analyzed by GC/MS (HP5970A) and showed M^+ at *m/e* 259 (12%) and the base peak at *m/e* 230. It was subsequently converted to the phenol and worked up to yield the desired (4a*R*,10b*S*)-4 (25 mg, 44%), which was converted to its hydrochloride with HCl-saturated EtOH. All the synthetic steps were performed analogously to those in the preparation of (4a*S*,10b*R*)-4·HCl above. The product was isolated as an oil. High-resolution MS was performed and showed M^+ at *m/e* 245 (7.7%) ($C_{16}H_{23}NO$·HCl; calcd 245.178, found 245.176 ± 3) and base peak at *m/e* 216. No impurities were detected by GC or TLC. High-resolution 1 H NMR (Bruker 500 MHz): δ 0.9–1.2 (t, 3 H), 1.5–1.6 (m, 1 H), 1.7–2.1 (m, 5 H), 2.2–2.4 (d, 2 H), 2.8–3.6 (m, 7 H), 2.7–2.8 (d, 1 H), 6.5–6.7 (d, 2 H), 6.9–7.0 (t, 1 H).

Determination of Absolute Configuration of (+)-6·HCl by Single-Crystal X-ray Analysis. Crystals of (+)-6·HCl were grown from a warm CH_3CN solution with the addition of a small amount of ether, and a crystal with the dimensions 0.58 × 0.36 × 0.22 mm was used for data collection with an Enraf-Nonius CAD 4F-11 diffractometer. The angular settings of 25 reflections ($4 < \theta < 40°$) were measured to calculate the lattice parameters. Intensity data for reflections within one hemisphere and with $0 < \theta < 60°$ were collected by the $\theta/2\theta$ scan method using monochromated Cu K_α radiation. Three intensity control reflections, which were measured every 2 h, indicated no crystal decay. A total of 2357 reflections were recorded, and of these 1920 reflections with $I > 3\sigma(I)$ were considered observed. All intensities were corrected for Lorentz and polarization effects but not for absorption or extinction.

Crystal Data. Molecular formula $C_{14}H_{19}NO$·HCl, space group $P2_12_12_1$, unit cell $a = 6.348$ (2) Å, $b = 14.731$ (2) Å, $c = 14.960$ (5) Å, orthorhombic, $V = 1399$ Å^3, $Z = 4$, M_r 253.77 $D_c = 1.205$ g cm^{-3}, μ (Cu K_α) = 39.7 cm^{-1}.

The chlorine positions, obtained from a Patterson synthesis, were used as input to the direct methods program DIRDIF,[23] which provided the non-hydrogen atom positions. All hydrogen atom positions were obtained from Fourier difference synthesis maps. Refinement was carried out by the full-matrix least-squares method using anisotropic temperature factors for the non-hydrogen atoms. The hydrogen atoms were assigned a common temperature factor $B = 5.0$ Å^2. The hydrogen atom parameters were not refined. In order to determine the absolute configuration of (+)-6·HCl, anomalous dispersion factors[24] were introduced for the non-hydrogen atoms. The atomic parameters of the non-hydrogen atoms for both enantiomers were then refined. Two sets of unique reflections (*hkl*,*h*-*kl*) were used in the refinement and nonobserved reflections were allowed to contribute when $F_c > F_o$. When the refinement was finished, the residuals for the 4a*R*,10b*S* and 4a*S*,10b*R* enantiomer were $R = 0.052$ and $R = 0.062$ ($R_w = 0.069$ and $R_w = 0.080$), respectively. With Hamilton's test[25] the ratio R_w(4a*S*,10b*R*)/R_w(4a*R*,10b*S*) = 1.16 is sufficiently great to reject the 4a*S*,10b*R* enantiomer at the 0.005 significance level. Furthermore, for the 4a*R*,10b*S* enantiomer, among the 16 Bijvoet pairs for which $|F_c(hkl) - F_c(h-kl)| > 1.5$, 15 of the F_c differences had the same sign as the corresponding F_o differences. The weighting scheme used in the later part of the refinement was $w = 1/(1 + ((|F_o| - 8)/12)^2)$.[26] The form factors used were those given by Cromer and Mann.[27] All calculations have been performed on a DEC-system-10 computer using mainly the X-ray 72 program system.[28]

The molecular conformation and the atom numbering scheme is shown in Figure 4.

Pharmacology. Animals used in the biochemical experiments were male rats of Sprague–Dawley strain (ALAB, Sollentuna, Sweden), weighing 200–300 g. All substances to be tested were dissolved in saline immediately before use, occasionally with the addition of a few drops of glacial acetic acid and/or moderate heating in order to obtain complete dissolution. Reserpine was dissolved in a few drops of glacial acetic acid and made up to volume with 5.5% glucose. Injection volumes were 5 or 10 mL/kg and all solutions had neutral pH (except for solutions of reserpine).

Biochemistry. The biochemical experiments and the determinations of 5-HTP and Dopa by means of HPLC with electrochemical detection were performed as previously described.[15-17] Separate dose–response curves based on four to six dose levels of each substance (sc administration) and each brain area were constructed. From these curves the dose of the drug yielding a half-maximal decrease (ED_{50} value) of the 5-HTP level was calculated with a modified probit analysis technique (Table II).[3]

Acknowledgment. We thank Ingrid Bergh, Boel Göransson, and Lucia Gaete for their skillful work in the pharmacological testing and Maria Lindbäck for skillful synthetic work. Max Lundmark and Lars Ahlqvist are gratefully acknowledged for X-ray work and for drawing the X-ray structure, respectively. Karin Sabel is gratefully acknoledged for plotting the stereopictures from the CHEMX system with the Calcomp plotter and Dr. Tor Magnusson for photographing these pictures. High-resolution MS was performed by Dr. Weston Pimlott at the Department of Medical Biochemistry, University of Göteborg. The financial support from Astra Läkemedel AB and The Swedish Board of Technical Development is gratefully acknowledged. Håkan Wikström was the recipient of a Fogarty International Research Fellowship (NIH 1 F05 TW 03628-01 B15 (AHR)) to study molecular mechanics with Dr. Allinger at the Department of Chemistry, University of Georgia. He was additionally funded by the Swedish Medical Research Council (MFR), Göteborgs Kungliga Vetenskaps- och Vitterhets-Samhälle,

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Magnus Bergvalls Stiftelse, Stiftelsen Lars Hiertas Minne, Adlebertska Forskningsfonden, Kungliga Vetenskapsakademien, AB Hässle, and Sverige-Amerika Stiftelsen, and all these grants are gratefully acknowledged.

Registry No. (1*S*,2*R*)-**2**, 109062-25-1; t(1*S*,2*R*)-**2**-N-amm, 108970-00-9; (1*S*,2*R*)-**3**, 108969-99-9; (1*S*,2*R*)-**3**-N-amm, 108969-95-5; (4a*R*,10b*S*)-**4**, 109062-23-9; (4a*R*,10b*S*)-4, 109119-95-1; (4a*S*,10b*R*)-**5**, 109062-21-7; (4a*S*,10b*R*)-**5**-HCl, 109119-94-0; *cis*-**6**·HCl, 82171-88-8; (4a*S*,10b*R*)-**6**, 109062-18-2; (4a*S*,10b*R*)-**6**·HCl, 109214-33-7; (4a*R*,10b*S*)-**6**, 109062-19-3; (4a*R*,10b*S*)-**6**·HCl, 109119-93-9; (4a*R*,10b*S*)-**6**-N-amm, 108969-96-6; (4a*R*,10b*S*)-**7**, 108969-98-8; (4a*R*,10b*S*)-**7**-N-amm, 108969-97-7; (4a*R*,10b*S*)-**8**, 109062-26-2; (4a*R*,10b*S*)-**8**-N-amm, 109062-24-0; (*R*)-(−)-*O*-methylmandelic acid, 3966-32-3; (4a*S*,10b*R*)-4-[(*R*)-2-methoxy-2-phenyl-1-oxoethyl]-10-methoxy-1,2,3,4,4a,5,6,10b-octahydrobenzo[*f*]quinoline, 108969-93-3; (4a*R*,10b*S*)-4-[(*R*)-2-methoxy-2-phenyl-1-oxoethyl]-10-methoxy-1,2,3,4,4a,5,6,10b-octahydrobenzo[*f*]quinoline, 109062-17-1; (4a*S*,10b*R*)-10-methoxy-4-(1-oxopropyl)-1,2,3,4,4a,5,6,10b-octahydrobenzo[*f*]quinoline, 108969-94-4; 5,6,10b-octahydrobenzo[*f*]quinoline(4a*S*,10b*R*)-10-methoxy-4-*n*-propyl-1,2,3,4,4a,5,6,10b-octahydrobenzo[*f*]quinoline, 109062-20-6; (4a*R*,10b*S*)-10-methoxy-4-(1-oxopropyl)-1,2,3,4,4a,5,6,10b-octahydrobenzo[*f*]quinoline, 109062-27-3; (4a*R*,10b*S*)-10-methoxy-4-*n*-propyl-1,2,3,4,4a,5,6,10b-octahydrobenzo[*f*]quinoline, 109062-22-8.

Supplementary Material Available: X-ray data of (4a*R*,10b*S*)-**6**·HCl, including positional and thermal parameters, bond lengths, and bond angles (2 pages); tables of observed and calculated structure factors (19 pages). Ordering information is given on any current masthead page.

γ-Aminobutyric Acid Esters. 3. Synthesis, Brain Uptake, and Pharmacological Properties of C-18 Glyceryl Lipid Esters of GABA with Varying Degree of Unsaturation

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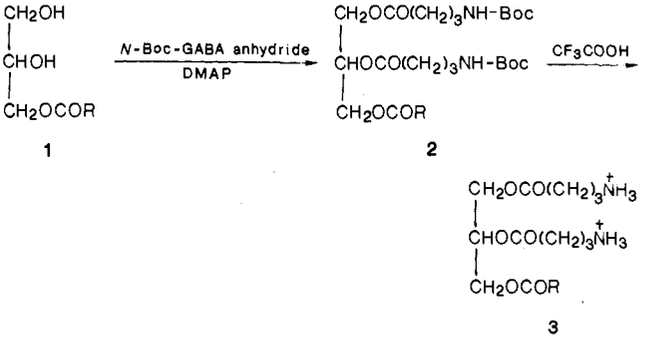
Received December 8, 1986

A series of ^{14}C-labeled and unlabeled di-γ-aminobutyric acid esters of glyceryl lipids having zero to three double bonds (stearoyl, oleoyl, linoleoyl, and linolenoyl) were synthesized. Measurements of the octanol/water partition coefficients of the compounds showed an increase with decreasing number of double bonds (i.e., from linolenoyl to stearoyl). The brain-uptake index went up from 31.5 (linolenoyl) to 45.1 (stearoyl) and similarly the brain-penetration index went up from 15 (linolenoyl) to 28 (stearoyl). Intraperitoneal injections of these di-GABA lipid esters produced a substantial inhibition of the general motor activity in mice at a dose of 30 mg/kg; the most active molecules were those containing two and three double bonds, i.e., the linoleoyl and linolenoyl derivatives. This is in reverse order to that predicted by brain-uptake and lipid-solubility properties, suggesting that the structure of the fatty acid side chain may be an additional factor in influencing biological activity.

Several neurological and neuropsychiatric disorders such as epilepsy and Huntington's disease have been reported[1-3] to be associated with a decrease in γ-aminobutyric acid (GABA) levels in the central nervous system (CNS). This has led to the search for GABA agonists that might improve the poor uptake of GABA through the blood–brain diffusion barrier.[4] In previous studies we demonstrated that certain cholesteryl[5] and glyceryl[6] esters can easily penetrate the blood–brain diffusion barrier to function as prodrugs which release GABA into the CNS after hydrolysis by esterases. Thus 1-linolenoyl-2,3-bis(4-aminobutyryl)propane-1,2,3-triol (LG_2) and 1,2-dilinolenoyl-3-(4-aminobutyryl)propane-1,2,3-triol were found to have a 75- and 127-fold greater uptake than that of GABA and to have electrophysiological[7] and pharmacological activity.[6]

Scheme I[a]



a: R = $(CH_2)_{16}CH_3$ [stearoyl (SG_2)]
b: R = $(CH_2)_7(CH{=}CHCH_2)(CH_2)_6CH_3$ [oleoyl (OG_2)]
c: R = $(CH_2)_7(CH{=}CHCH_2)_2(CH_2)_3CH_3$ [linoleoyl (L^2G_2)]
d: R = $(CH_2)_7(CH{=}CHCH_2)_3CH_3$ [linolenoyl (LG_2)]

[a] DMAP, 4-(dimethylamino)pyridine.

In this paper we have extended this study to investigate the effect of varying the degree of unsaturation in the C-18 lipid moiety of the glyceride molecule on the neuropharmacological and brain-uptake properties of the molecule. Thus, we have synthesized linoleoyl, oleoyl, and stearoyl glyceryl lipids containing two GABA ester groups in each

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