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

Håkan Wikström,*† Bengt Andersson,† Thomas Elebring,† John Jacyno,‡ Norman L. Allinger,‡ Kjell Svensson,§ Arvid Carlsson,§ and Staffan Sundell[¶]

Organic Chemistry Unit, Department of Pharmacology, University of Göteborg, S-400 33 Göteborg, Sweden, Department of Chemistry, University of Georgia, Athens, Georgia 30602, Department of Pharmacology, University of Göteborg, S-400 33 Göteborg, Sweden, and Department of Structural Chemistry, University of Göteborg, S-400 33 Göteborg, Sweden. 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 (4aR,10bS)-4 and more potent, centrally acting 5-HT receptor agonist cis-(1S,2R)-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 4aR,10bS 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-(1S,2R)-2 (cis-(1S,2R)-3) and cis-(4aR,10bS)-4 (cis-(4aR,10bS)-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-(4aR,10bS)-4 might be explained by unfavorable direction of the N-lone pair (or ammonium hydrogen) bond in this compound as compared to cis-(1S,2R)-2 and trans-(4aR,10bR)-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.⁴⁵ 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-(1S,2R)-8-hydroxy-1-methyl-DPAT (cis-(1S,2R)-2) and compound 4 leads to the prediction that the 5-HT receptor stimulating properties of 4 reside in its 4aR,10bS 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 4aR,10bS 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-(1S,2R)-2 (cis-(1S,2R)-3), by driving the C2–N bond 0–360° with 20° increment and with nitrogen



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-

- Arvidsson, L.-E.; Hacksell, U.; Nilsson, J. L. G.; Hjorth, S.; Carlsson, A.; Lindberg, P.; Sanchez, D.; Wikström, H. J. Med. Chem. 1981, 24, 921.
- (2) Arvidsson, L.-E.; Hacksell, U.; Johansson, A. M.; Nilsson, J. L. G.; Lindberg, P.; Sanchez, D.; Wikström, H.; Svensson, K.; Hjorth, S.; Carlsson, A. J. Med. Chem. 1984, 27, 45.
- (3) Arvidsson, L.-E. Thesis, Acta Universitatis Upsaliensis, Abstracts of Uppsala Dissertations from the Faculty of Pharmacy 109, 1985, ISSN 0346-6353, ISBN 91-554-1720-5.
- (4) Hjorth, S.; Carlsson, A.; Lindberg, P.; Sanchez, D.; Wikström, H.; Arvidsson, L.-E.; Hacksell, U.; Nilsson, J. L. G. J. Neural Transm. 1982, 55, 169.
- (5) Tricklebank, M. D.; Middlemiss, D. N.; Fozard, J. R. Trends Pharmacol. Sci. 1984, 5, 415.
- (6) Middlemiss, D. N.; Fozard, J. R. Eur. J. Pharmacol. 1983, 90, 151.
- (7) Cannon, J. G.; Hatheway, G. J.; Long, J. P.; Sharabi, F. M. J. Med. Chem. 1976, 19, 987.
- (8) Cannon, J. G.; Suarez-Gutierrez, C.; Lee, T.; Long, J. P.; Costall, B.; Fortune, D. F.; Naylor, R. J. J. Med. Chem. 1979, 22, 341.
- (9) Cannon, J. G.; Lee, T.; Goldman, H. D.; Long, J. P.; Flynn, J. R.; Vermier, T.; Costall, B.; Naylor, R. J. J. Med. Chem. 1980, 23, 1.
- (10) Wikström, H.; Sanchez, D.; Lindberg, P.; Arvidsson, L.-E.; Hacksell, U.; Johansson, A.; Nilsson, J. L. G.; Hjorth, S.; Carlsson, A. J. Med. Chem. 1982, 25, 925.

[†]Orangic Chemistry Unit, Department of Pharmacology, University of Göteborg.

[‡]Department of Chemistry, University of Georgia.

[§]Department of Pharmacology, University of Göteborg.

Department of Structural Chemistry, University of Göteborg.

Tabl I.	Physical	Data
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compd	yield,ª %	mp, ^b ⁰C	$[\alpha]_{\rm D}^{22}$, deg (MeOH)	optical purity, %	formula
(4aR,10bS)-6	53	239-242	+1.5 (c 2.0)	>99	C14H10NO.HCl
(4aS, 10bR)-6	31	239 - 241	-1.0 (c 1.7)	>99	C ₁₄ H ₁₀ NO·HCl
(4aR, 10bS)-4	44	oil	-11.7 (c 2.0)		C ₁₄ H ₀₀ NO·HCl
(4aS, 10bR)-4	50	oil	+10.9 (c 2.8)		$C_{16}H_{23}NO \cdot HCl$

^a The yield in the last step. ^bRecrystallized from CH₃CN/ether.

Table II. Biochemical Data

	5-HTP accumulation: ^a ED_{50} , $\mu mol/kg$			$\mathrm{Dopa}\; accumulation: \; \mathrm{ED}_{50}, \ \mu\mathrm{mol/kg}$		
compd	limbic	striatum	hemisph	limbic	striatum	hemisph
4 ^b	3.40	3.40	4.50	Ic	I	I
(4aR, 10bS)-4	2.57 (0.660-9.92)	3.63(1.22-10.8)	3.47 (1.00-11.8)	I	I	I
(4aS, 10bR)-4	d	d	d	d	d	d
(1S,2R)-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. °I means inactive. No significant effects were seen on Dopa accumulation at doses up to 50.0 μ mol/kg in any of the three brain regions studied. ^dA 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 μ mol/kg. ^eData taken from ref 3, paper IV.

tions were performed on the model compounds cis-(1S,2R)-3 and (4aR,10bS)-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-(4aR,10bS)-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-(1S,2R)-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

- (11) Allinger, N. L.; Yuh, Y. Quantum Chemistry Program Exchange 1980, program 13, 395. The MM2 program (MM2-85, available from the Quantum Chemistry Program Exchange and from Molecular Design, Ltd.) has been modified (R. Kok, unpublished) to calculate hydrogen bonds. This hydrogen bond term has the same functional form as a van der Waals interaction, and we have adjusted these parameters to reproduce the energies obtained from several ab initio calculations and electron-diffraction results on compounds that hydrogen bond either with another molecule or internally. Of particular interest here are the ammonium hydrogen (type 28) to phenol oxygen (type 6) or aromatic carbon (type 2) interactions, which have the hydrogen bond parameters: VdW constant $\epsilon = 2.200$ kcal and sum of VdW radii = 2.080 Å, and VdW constant ϵ = 1.000 kcal and sum of VdW radii = 2.180 Å, respectively. Of course, the dipole-dipole interactions inherent in MM2 are still included in the calculations, separate from the new H-bond parameters.
- (12) Kocjan, D.; Hadzi, D. J. Pharm. Pharmacol. 1983, 35, 780. (13) Still, W. C. Department of Chemistry, Columbia University, New York, NY 10027.
- (14) Lii, J.-H.; Yuh, Y., unpublished graphics software from the Department of Chemistry, University of Georgia, Athens, GA 30602, 1986.

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 wellestablished 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-

- (15) Wikström H.; Sanchez, D.; Lindberg, P.; Hacksell, U.; Arvidsson, L.-E.; Johansson, A. M.; Thorberg, S.-O.; Nilsson, J. L. G.; Svensson, K.; Hjorth, S.; Clark, D.; Carlsson, A. J. Med. Chem. 1984, 27, 1030.
- Wikström, H.; Andersson, B.; Sanchez, D.; Lindberg, P.; Ar-(16)vidsson, L.-E.; Johansson, A. M.; Nilsson, J. L. G.; Svensson, K.; Hjorth, S.; Carlsson, A. J. Med. Chem. 1985, 28, 215.
- (17) Hacksell, U.; Svensson, U.; Nilsson, J. L. G.; Hjorth, S.; Carlsson, A.; Wikström, H.; Lindberg, P.; Sanchez, D. J. Med. Chem. 1979, 22, 1469.
- (18) Ande'n, N.-E.; Carlsson, A.; Häggendal, J. Ann. Rev. Pharmacol. Toxicol. 1969, 9, 119.
- (19) Aghajanian, G. K.; Bunney, B. S.; Kuhar, M. J. In New Concepts in Neurotransmitter Regulation, Mandell, A. J., Ed.; Plenum: New York, 1973; p 119. Neckers, L. M.; Neff, N. H.; Wyatt, R. J. Nauyn-Schmiede-
- (20)berg's Arch. Pharmacol. 1979, 306, 173.



Figure 1. Energy plot of the dihedral driver ($\tau = H_{C2}$ -C2-N-H_N; $0-360^{\circ}$, 20° increment) for compound cis-(1S,2R)-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 4aR,10bS 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-(1S,2R)-2.³ Figure 2a-e shows stereo pictures of the global minima of the ammonium forms of compounds cis-(1S,2R)-3 and cis-(4aR,10bS)-7 in both N-ax and N-eq conformations, and compound trans-(4aR, 10bR)-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-(4aR,10bS)-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

Table III. Conformational Data

	steric energy difference: (N-ax) - (N-eq)	distance, Å	
structure	kcal/mol	N-0	N-A ^a
cis-(1S,2R)-3-N-ax-amm ^{b,c}		4.50	2.21
	3.4		
cis-(1S,2R)-3-N-eg-amm ^{b,c}		5.24	-0.32
cis-(4aR, 10bS)-6-N-ax-amm		4.82	1.71
	0.0		
cis-(4aR,10bS)-6-N-eq-amm		5.14	-0.28
cis-(4aR,10bS)-6-N-ax-base		4.96	1.73
	2.5		
cis-(4aR,10bS)-6-N-eq-base		5.15	-0.34
cis-(4aR,10bS)-6-X-ray		5.14	-0.30
cis-(4aR,10bS)-7-N-ax-amm		4.82	1.88
	0.6		
cis-(4aR,10bS)-7-N-eq-amm		5.18	-0.37
cis-(4aR,10bS)-7-N-ax-base		4.92	1.87
	2.3		
cis-(4aR,10bS)-7-N-eq-base		5.19	-0.40
trans-(4aR,10bR)-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. ^cThe rotamers used as input to these calculations were those found to have the lowest energies according to the driver plots in Figure 1a ($\tau = 180^\circ$) and Figure 1b ($\tau =$ 180°), respectively.

accounted for here, could make a difference in favor of the N-eq conformation in the X-ray crystal (see Figures 3 and 4).

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 = 5.25Å 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 Nichols²² 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-(4aR,10bS)-4 that is the one interacting with the receptor.

The stereoselectivities exhibited by $cis \cdot (1S, 2R) \cdot 2$ and cis-(4aR,10bS)-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-

⁽²¹⁾ Wikström, H.; Lii, J.-H.; Allinger, N. L., submitted for publication.

⁽²²⁾ Nichols, D. E. Acta Pharm. Suec. Suppl. II 1985, 139.

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Figure 2. Stereo pictures of the global minima of the ammonium forms of cis-(1S,2R)-3 and cis-(4aR,10bS)-7 (both compounds in N-ax and N-eq conformations) and trans-(4aR,10bR)-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 $\tau =$ $H_{C2}-C_2-N-H_N$. 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 C₁-C₂-N- H_N for this conformation is -57.0°, and the corresponding torsion angles in cis-(4aR,10bS)-7-N-eq-amm and trans-(4aR,10bR)-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-(1S,2R)-2 and cis-(4aR,10bS)-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-(4aR,10bS)-4, as compared to the very potent compounds cis-10-Hydroxy-4-n-propyloctahydrobenzo[f]quinoline



Figure 3. Stereo picture of the overlay of the X-ray and the minimized structures of cis-(4aR,10bS)-6.



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

1 and cis-(1S,2R)-2, could be explained by the inability of cis-(4aR,10bS)-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-(4aR,10bS)-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 package¹⁴ 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). ¹H NMR spectra were recorded with a Bruker 500 MHz and a Varian EM-360 MHz instrument (Me₄Si). 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 μ m, gas H₂, 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 -(4aS,10bR)-10-Methoxy-1,2,3,4,4a,5,6,10b-octahydrobenzo[f]quinoline ((4aS,10bR)-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 (Na₂SO₄), filtered, and evaporated to give an oil (480 mg, 101%), which was chromatographed on SiO₂ (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 Na₂CO₃(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 (CH₃CN/ether) gave crystals (32 mg, 53%) of the desired product (4aS, 10bR)-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-(4aR,10bS)-10-Methoxy-1,2,3,4,4a,5,6,10b-octahydrobenzo[f]quinoline ((4aR,10bS)-6). The fractions from the preparation of (4aS,10bR)-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 (4aR,10bS)-6-HCl after recrystallization (CH₃CN/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-(4aS,10bR)-10-Hydroxy-4-n-propyl-1,2,3,4,4a,5,6,10boctahydrobenzo[f]quinoline ((4aS,10bR)-5). Compound (4aS,10bR)-6-HCl (135 mg, 0.53 mmol) was suspended in CH₂Cl₂ (10 mL) and Et₃N (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 (Na₂So₄), 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). LiAlH₄ (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 N₂(g) for 2 h. The hydrobromic acid was evaporated and the residue was basified with 10% Na₂CO₃ and extracted with ether. The ether layer was separated, dried (Na₂CO₃), and filtered and the solvent was evaporated, yielding an oil, which was chromatographed on basic Al₂O₃ with CH₂Cl₂/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%) (C1₆H₂₃NO·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-(4aR,10bS)-10-Hydroxy-4-n-propyl-1,2,3,4,4a,5,6,10boctahydrobenzo[f]quinoline ((4aR,10bS)-4). Compound (4aR,10bS)-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 (4aR,10bS)-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 (4aS,10bR)-4.HCl above. The product was isolated as an oil. High-resolution MS was performed and showed M⁺ at m/e 245 (7.7%) (C₁₆H₂₃NO·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 CH₃CN solution with the addition of a small amount of ether, and a crystal with the dimensions $0.58 \times 0.36 \times 0.22$ mm was used for data collection with an Enraf-Nonius CAD 4F-11 diffractometer. The angular settings of 25 reflections (4 $< \theta < 40^{\circ}$) were measured to calculate the lattice parameters. Intensity data for reflections within one hemisphere and with 0 $< \theta < 60^{\circ}$ were collected by the $\theta/2\theta$ scan method using monochromated Cu K_a 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 Å³, Z = 4, M_r 253.77 $D_c = 1.205$ g cm⁻³, μ (Cu K_c) = 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 $B = 5.0 \text{ Å}^2$. The hydrogen atom parameters were not refined. In order to determine the absolute configuration of (+)-6-HCl, anomalous dispersion factors²⁴ were introduced for the non-hydrogen atoms. The atomic parameters of the nonhydrogen 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_{e} $>F_{\rm o}.$ When the refinement was finished, the residuals for the 4aR,10bS and 4aS,10bR enantiomer were R = 0.052 and R = 0.062 $(R_w = 0.069 \text{ and } R_w = 0.080)$, respectively. With Hamilton's test²⁵ the ratio $R_w(4aS,10bR)/R_w(4aR,10bS) = 1.16$ is sufficiently great to reject the 4aS,10bR enantiomer at the 0.005 significance level. Furthermore, for the 4aR,10bS 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_0 differences. The weighting scheme used in the later part of the refinement was $w = 1/(1 + ((|F_0| - 8)/12)^2)^{.26}$ 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 (ED₅₀ value) of the 5-HTP level was calculated with a modified probit analysis technique (Table II).³

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- (25) Hamilton, W. C. Acta Crystallogr. 1965, 18, 502.
- (26) Mills, O. S.; Rollet, J. S. Computing Methods and the Phase Problem in X-ray Crystal Analysis; Pergamon: London, 1961; p 107.
- (27) Cromer, D. T.; Mann, J. B. Acta Crystallogr., Sect. A 1968, 24, 321.
- (28) Stewart, J. M.; Kruger, G. J.; Ammon, H. L.; Dickinson, C.; Hall, S. R. The X-ray System—version of June 1972; Technical Report TR-192, Computer Science Center, University of Maryland, College Park, MD, 1972.

⁽²³⁾ Beurskens, P. T.; Bosman, W. P. J. H.; Doesburg, H. M.; Gould, R. H.; Van der Hark, Th. E. M.; Prick, P. A. J.; Noordik, J. H.; Beurskens, B.; Parthasarthi, V. DIRDIF Direct Methods for Difference Structures; Technical Report 1981/2, Crystallography Laboratory, Toernvooiveld, 6525 ED Nijmegen, The Netherlands, 1981.

⁽²⁴⁾ Cromer, D. T.; Liberman, D. J. Chem. Phys. 1970, 53, 1891.

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. (1S,2R)-2, 109062-25-1; t(1S,2R)-2-N-amm, 108970-00-9; (1S,2R)-3, 108969-99-9; (1S,2R)-3-N-amm, 108969-95-5; (4aR, 10bS)-4, 109062-23-9; (4aR, 10bS)-4, 109119-95-1; (4aS,10bR)-5, 109062-21-7; (4aS,10bR)-5-HCl, 109119-94-0; cis-6-HCl, 82171-88-8; (4aS,10bR)-6, 109062-18-2; (4aS,10bR)-6-HCl,109214-33-7; (4aR,10bS)-6, 109062-19-3; (4aR,10bS)-6·HCl, 109119-93-9; (4aR,10bS)-6-N-amm, 108969-96-6; (4aR,10bS)-7, 108969-98-8; (4aR,10bS)-7-N-amm, 108969-97-7; (4aR,10bS)-8, 109062-26-2; (4aR,10bS)-8-N-amm, 109062-24-0; (R)-(-)-Omethylmandelic acid, 3966-32-3; (4aS,10bR)-4-[(R)-2-methoxy-2-phenyl-1-oxoethyl]-10-methoxy-1,2,3,4,4a,5,6,10b-octahydrobenzo[f]quinoline, 108969-93-3; (4aR,10bS)-4-[(R)-2-methoxy-2phenyl-1-oxoethyl]-10-methoxy-1,2,3,4,4a,5,6,10b-octahydrobenzo[f]quinoline, 109062-17-1; (4aS,10bR)-10-methoxy-4-(1oxopropyl)-1,2,3,4,4a,5,6,10b-octahydrobenzo[f]quinoline, 108969-94-4; 5,6,10b-octahydrobenzo[f]quinoline(4aS,10bR)-10methoxy-4-n-propyl-1,2,3,4,4a,5,6,10b-octahydrobenzo[f]quinoline, 109062-20-6; (4aR,10bS)-10-methoxy-4-(1-oxopropyl)-1.2.3.4.4a.5.6.10b-octahydrobenzo[f]quinoline, 109062-27-3; benzo[f]quinoline, 109062-22-8.

Supplementary Material Available: X-ray data of (4aR,10bS)-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

James N. Jacob, Gary W. Hesse, and Victor E. Shashoua*

Ralph Lowell Laboratories, McLean Hospital, Harvard Medical School, Belmont, Massachusetts 02178. Received December 8, 1986

A series of ¹⁴C-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 reported¹⁻³ 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 cholesteryl⁵ and glyceryl⁶ 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 (LG₂) 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 electrophysiological⁷ and pharmacological activity.⁶

- (1) Tower, D. B. In GABA in Nervous System Function; Roberts, E., Chase, T. N., Tower, D. B., Eds.; Raven: New York, 1976; o 461.
- (2) Bird, E. D.; Mackay, A. V. P.; Rayner, C. N.; Iverson, L. L. Lancet 1973, 1, 1090.
- Van Kammen, D. P.; Sternberg, D. S.; Hare, T.; Ballenger, J.; (3)Marder, J.; Post, R.; Bunney, W. E., Jr. Brain Res. Bull. 1980, 5, 132.
- (4) Krogsgaard-Larsen, P. J. Med. Chem. 1981, 24, 1377.
- (5) Shashoua, V. E.; Jacob, J. N.; Ridge, R.; Campbell, A.; Baldessarini, R. J. J. Med. Chem. 1984, 27, 659.
- (6) Jacob, J. N.; Shashoua, V. E.; Campbell, A.; Baldessarini, R. J. J. Med. Chem. 1985, 28, 106.



^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

Hesse, G. W.; Shashoua, V. E.; Jacob, J. N. Neuropharma-(7)cology 1985, 24, 139.