Conformational Analysis of Methylphenidate and Its Structural Relationship to Other Dopamine Reuptake Blockers Such as CFT

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Purpose. This work was performed 1) to determine the conformational preferences of the *threo* and *erythro* isomers of the dopamine reuptake blocker methylphenidate, 2) to determine the crystal conformation of the *threo* isomer, 3) to confirm the absolute configuration of the more active *threo* enantiomer, and 4) to incorporate the compound into a previously determined pharmacophore for dopamine reuptake blockers.

Methods. A conformational analysis was performed with the MM2-87 program, a crystal of the (−)-*threo* HCl salt was analyzed by x-ray crystallography, and the global minima of the (+)-*threo* isomer and the potent dopamine reuptake blocker CFT were superimposed.

Results. In the global minimum of the *threo* isomer, the carbonyl oxygen of the ester group is oriented toward the ammonium group as was also found in the crystal state. In the *erythro* isomer, the ester group prefers an extended conformation relative to the piperidine group. The absolute configuration of the biologically active (+)-*threo* enantiomer was confirmed to be R,R. The atomic sequence from the amine group through the ester group is identical in the active enantiomers of methylphenidate and CFT.

Conclusions. The dopamine reuptake protein requires a precise orientation of the ammonium and ester groups but allows considerable leeway in the position of the phenyl ring. The pKa of the *threo* isomer is predicted to be higher than that of the *erythro* isomer.

KEY WORDS: methylphenidate; molecular mechanics; x-ray crystallography; dopamine reuptake blockers; absolute configuration; pharmacophore.

INTRODUCTION

Methylphenidate (Scheme I) is a central nervous system stimulant that is the drug of choice for the treatment of attention deficit disorder in children. The mode of action of the compound is believed to be its ability to block the reuptake of the neurotransmitter dopamine into presynaptic neuronal stores resulting in the enhanced activation of dopaminergic neurons. The compound is highly potent in receptor binding assays using tritiated cocaine (1), in schedule-controlled behavioral experiments (2), and in self-administration studies (3). This high potency exists despite the compound being tested as a racemate, which implies that the active enantiomer has twice the affinity, and despite the lack of phenyl substituents, which generally enhance the potency of dopamine reuptake blockers (4,5).

In a previous study of dopamine reuptake blockers (4), conformational analyses were performed on a number of compounds than contain two phenyl rings (3-phenyl-1-indanamines such as LU 19-005, 1-amino-4-phenyltetralins, hexahydropyrrolo[2,1-a]isoquinolines, a nomifensine analog, and hexahydro[1,2-b]pyridines) and a common, energetically favored three dimensional structure was found which could explain their common activity (4). The pharmacophore was also able to explain why secondary amines were more potent than tertiary amines in some classes of compounds. That is, an added N-alkyl group in these compounds was found to prefer the position required for the ammonium hydrogen. Conformational analyses were also performed for cocaine and CFT (Scheme I) which contain a single phenyl ring and either one or two ester groups. It was found that the methyl ester groups in cocaine and CFT appear to correspond to the second phenyl ring of the other compounds in that they occupy similar three dimensional positions. Structurally, methylphenidate appears to be similar to cocaine and CFT (Scheme I) with which it shares an ester group and this work was initiated to incorporate the compound into the pharmacophore. It should be noted that while the axial methyl ester group is required for significant blockade of neuronal dopamine reuptake in cocaine and related compounds (6,7), recent work has shown that replacement of the ester by other groups, such as vinyl, can also produce potent dopamine reuptake blockers (8,9).

Methylphenidate contains two asymmetric carbon atoms and, therefore, will exist as two pairs of diastereomeric enantiomers. While the two racemates exhibit similar pressor effects and lethality (10), most other pharmacological activities appear to be primarily associated with the *threo* isomer whose (+)-enantiomer has been found to contain the stimulant properties (10–17). By conversion to a known compound, the absolute configuration of the active enantiomer was determined to be R,R (18).

Due to the presence of single bonds about which rotation can readily occur, methylphenidate is conformationally flexible and it was of interest to determine the conformation that is responsible for its biological activities. Despite being known for over fifty years (19), we were unable to find any quantitative studies of the conformational properties of the compound. In addition, there have been no crystallographic studies published for the compound. Therefore, a conformational analysis of the compound has been performed for the *threo* and *erythro* isomers using the MM2-87 program. The availability of the resolved *threo* enantiomers (16) allowed us to attempt to solve their structure by x-ray crystallography. This was successfully done and a crystallographic determination of the HCl salt of one of the *threo* enantiomers has been performed to provide additional conformational information and to confirm the absolute configuration of the active enantiomer.

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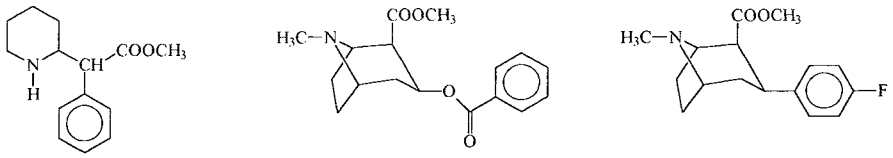

methylphenidate cocaine CFT

Scheme I.

METHODS

Conformational energy calculations were performed with the MM2-87 program and parameter set (20,21). All calculations were performed on the protonated form of the molecule. For the *threo* isomer, the calculations were performed on the active stereoisomer. To prevent electrostatic and hydrogen bond terms from dominating the results of the calculations in the absence of explicit solvent molecules (4,22), the calculations were performed with a dielectric constant of 80 and the hydrogen bonding terms set to zero. There were no missing parameters. The numbering system is shown in Figure 1. Initial Cartesian coordinates for the energy minimizations were generated by a previously described program (23), by the PCMODEL program (24), or by the DRIVER option of the MM2-87 program. The convergence criterion for the energy minimizations was set to ⅛ of its default value to ensure complete convergence.

The only dihedral angles in methylphenidate that require variation are τ1(C3-C2-C7-C8) and τ2(C2-C7-C8-O9) (Figure 1). The final, energy minimized dihedral angles of the conformational searches are listed as [τ1,τ2]. The conformation of the terminal methyl group was not varied since it would be expected to have a strong tendency to eclipse the carbonyl oxygen. The conformation of the phenyl ring was also not varied due to its symmetry.

A suitable crystal of (−)-*threo*-methylphenidate HCl was found in the sample that was received (16). The monoclinic crystal was in the $P2_1$ space group with cell dimensions a = 9.401(3) Å, b = 7.3053(7) Å, c = 11.113(3) Å, β = 109.260(2)°, V = 720.5(3) Å^3, Z = 2, computed ρ = 1.243 g/cm^3, extinction coefficient μ(Cu-Kα) = 23.0/cm. A Nonius CAD4 automated diffractometer was used to collect the crystallographic data with T = 22°C and Cu Kα λ = 1.54178 Å. The crystals were stable and showed no deterioration. Data were corrected for Lorentz, polarization, and extinction effects, but not for absorption. The structure was solved using the direct methods program teXsan (25). Anomalous scattering curves were taken from the International Tables (26). There were 1797 reflections, of which 1234 had I>3σ. The R factor for the S,S enantiomer was 0.070 as compared with 0.073 for the R,R enantiomer.

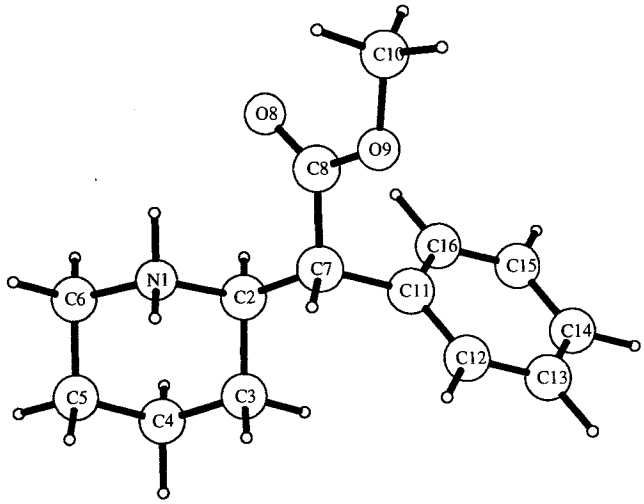

Fig. 1. Global minimum [172°,73°] of *threo*-methylphenidate as computed by the MM2-87 program.

RESULTS AND DISCUSSION

A conformational search of *threo*- and *erythro*-methylphenidate produced the results shown in Table I. For the *threo* isomer, the global minimum occurred at [172°, −177°] which was preferred by 0.8 kcal/mole or greater. For the *erythro* isomer, the global minimum occurred at [−160°,66°] which was preferred by 0.7 kcal/mole or greater. The preferred conformers are shown in Figures 1 and 2. In the *threo* isomer, the carbonyl oxygen of the ester group is in close proximity to the amine group whereas the ester group is in an extended conformation relative to the piperidine ring in the *erythro* isomer. In both isomers, the global minimum is the conformation in which the two edges of the piperidine ring are trans to the ester group and phenyl ring. This was the basis for previous qualitative proposals concerning the preferred conformations of the isomers of methylphenidate (12,13,16). The most important dihedral angles that describe the global minimum for the *threo*-isomer are shown in Table II.

It should be noted that, for the global minimum of the *threo* isomer, the preferred position of the carbonyl oxygen is to be near to and oriented toward the ammonium nitrogen, even with the electrostatic and hydrogen-bonding terms in the calculations turned off. Electrostatic and hydrogen-bonding interactions will further stabilize this conformation and should stabilize the protonated form of the molecule

Table I. Steric Energies of Various Minimized Conformers [τ1,τ2] of *Threo*- and *Erythro*-Methylphenidate as Computed by MM2-87

Threo-isomer	Steric energy (kcal/mole)	*Erythro*-isomer	Steric energy (kcal/mole)
[6°,74°]	10.4	[49°,62°]	8.7
—	—	[21°,171°]	9.9
[110°,−60°]	10.6	[96°,−51°]	11.4
[177°,73°]	9.3	[−160°,66°]	8.1
[172°,−177°]	8.1	[−174°,178°]	8.8
[165°,−61°]	9.3	[−175°,−66°]	9.7
[−98°,46°]	12.5	—	—
[−32°,−173°]	8.9	—	—
[−84°,−56°]	10.7	[−92°,−48°]	11.7

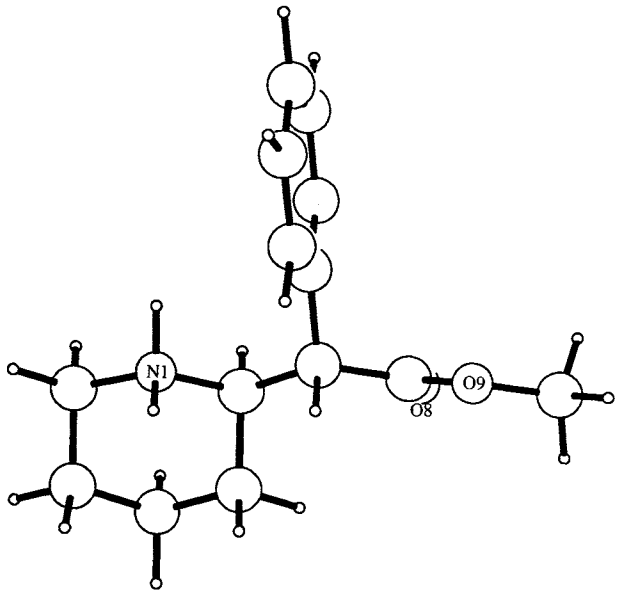

Fig. 2. Global minimum [−160°,66°] of *erythro*-methylphenidate as computed by the MM2-87 program.

relative to the unprotonated form. That suggests that *threo*-methylphenidate should have a higher pKa than the *erythro* isomer in which the ester group is trans to the ammonium group (Figure 2) (27). This also occurs in cocaine where the diastereomer with an axial ester group has a higher pKa than the one with an equatorial ester group since an intramolecular hydrogen bond is only possible in the former (4).

The crystal structure of the less active (−)-enantiomer of the *threo* isomer is shown in Figure 3. The most important dihedral angles that describe the crystal structure have been listed in Table II. The absolute configuration of the (−)-enantiomer is found to be S,S. Therefore, the absolute configuration of the active (+)-enantiomer is R,R, confirming

Table II. Dihedral Angles Found in the Crystal Structure and in the Computed Global Minimum of *Threo*-Methylphenidate

Dihedral angle	x-ray[a]	MM2-87
N1-C2-C3-C4	56°	58°
C2-C3-C4-C5	−53°	−58°
C3-C4-C5-C6	50°	57°
C4-C5-C6-N1	−53°	−57°
C5-C6-N1-C2	56°	62°
C6-N1-C2-C3	−56°	−62°
C7-C2-N1-C6	178°	177°
C7-C2-C3-C4	178°	174°
C8-C7-C2-N1	−68°	−61°
C8-C7-C2-C3	169°	−177°
O9-C8-C7-C2	139°	171°
C10-O9-C8-C7	−179°	179°
C11-C7-C2-N1	169°	175°
C11-C7-C2-C3	46°	59°
C12-C11-C7-C2	60°	68°
C16-C11-C7-C2	−125°	−112°
O8-C8-C7-C2	−42°	−8°
O8-C8-O9-C10	2°	−3°

[a] The dihedral angles for the crystal structure have been converted to the more active (+)-enantiomer to simplify comparisons.

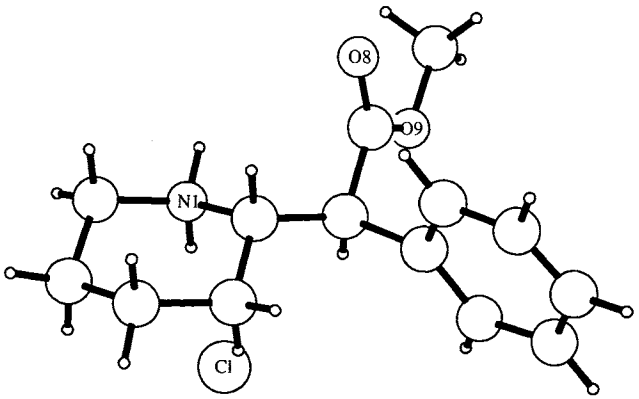

Fig. 3. Crystal structure of the inactive (−)-*threo* enantiomer of methylphenidate HCl showing the absolute configuration to be S,S. Therefore, the active enantiomer has the R,R configuration.

the absolute configuration found by chemical conversion to known compounds (18).

The protonated piperidine nitrogen forms an ionic bond with the chloride counterion (N−H . . . Cl 3.20Å). The nitrogen also forms a contact with a carbonyl oxygen of a neighboring molecule (N−H . . . O=C 3.32Å). The molecules pack in the crystal lattice such that the phenyl rings are parallel. However, the contact distances (3.6Å) are outside the range of intermolecular stacking interactions.

There is good agreement between the global minimum found by calculation and the structure found in the crystal. The piperidine rings of the two structures have been superimposed in Figure 4. The major difference is a slightly different orientation of the ester group due to the close contact between the carbonyl oxygen and the ammonium nitrogen of a neighboring molecule (see previous paragraph).

As indicated in the introduction, one of the goals of this work is the determination of the structural factors that are important for the common pharmacological properties of methylphenidate, cocaine, and CFT. The structural elements that are likely to be important for the properties of the compounds are the protonated amine, the ester group, and the aromatic ring. Superposition of these elements was attempted for the global minima of methylphenidate and CFT which has somewhat less flexibility than cocaine (Scheme I) (4). However, it was not possible to superimpose all of these elements simultaneously. There was a near perfect fit, however, for the six atom sequence from the ammonium group through the methyl ester (Figure 5). This is a significant result in that this sequence encompasses two asymmetric cen-

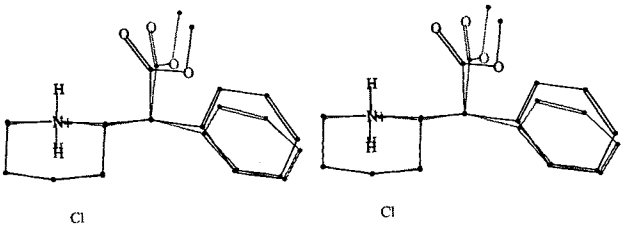

Fig. 4. Superposition of the piperidine rings of the computed global minimum (light line) of *threo*-methylphenidate with the crystal structure. The crystal structure has been converted to the active (+)-R,R-enantiomer by a reflection.

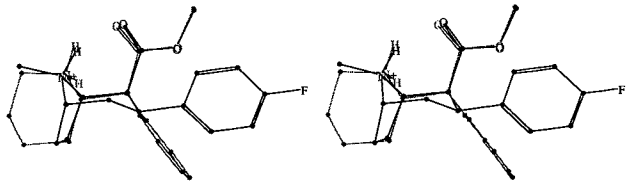

Fig. 5. Superposition of the sequence of atoms from the amine group through the ester group of the global minimum of (+)-R,R-*threo*-methylphenidate (light line) and CFT (dark line) showing their three dimensional similarity.

ters in methylphenidate and one asymmetric center in CFT. Of course, this means that the location of the phenyl ring varies between the compounds. However, based on structural differences between cocaine and CFT (4) and CFT and methylphenidate in the present work, it appears that the dopamine reuptake protein can accommodate a variety of positions for the aromatic ring. These appear to be real differences in the positions of the phenyl ring in the three compounds since there is an extra ester group in cocaine relative to CFT and the phenyl ring in methylphenidate is one carbon atom closer to the ester group than in CFT (Scheme I).

The superposition in Figure 5 is for the global minima of *erythro*-methylphenidate and CFT. These conformers are global minima even in the absence of electrostatic and hydrogen bonding interactions which would preferentially stabilize these conformations in both compounds (4). However, even if these conformers are not, in fact, the biologically active forms, these portions of the molecules would appear to correspond closely. It should also be noted that the piperidine ring of methylphenidate lines up with the ethyl bridge rather than the piperidine portion of the tropane structure in CFT. Thus, the N-methyl group in CFT aligns with the piperidine ring of methylphenidate, and one would expect different structure-activity relationships with regard to N-substitution.

Table III. Final Atomic Coordinates and Isotropic Thermal Parameters for Nonhydrogen Atoms in the Crystal Structure of (−)-*Threo*-Methylphenidate

Atom	x	y	z	B_{eq}
N1	−0.8852 (9)	0.120 (1)	−1.0300 (8)	3.1 (2)
C2	−0.739 (1)	0.049 (2)	−0.940 (1)	3.9 (2)
C3	−0.614 (1)	0.092 (1)	−0.988 (1)	5.1 (3)
C4	−0.640 (1)	0.021 (2)	−1.122 (1)	5.0 (3)
C5	−0.792 (2)	0.086 (1)	−1.213 (1)	5.3 (3)
C6	−0.915 (1)	0.049 (2)	−1.160 (1)	4.3 (2)
C7	−0.721 (1)	0.124 (1)	−0.809 (1)	3.3 (2)
C8	−0.834 (1)	0.051 (2)	−0.7527 (9)	3.8 (2)
O9	−0.889 (1)	0.172 (1)	−0.6908 (9)	4.9 (2)
C10	−0.998 (2)	0.113 (2)	−0.634 (1)	5.8 (4)
C11	−0.563 (1)	0.093 (1)	−0.709 (1)	3.9 (3)
C12	−0.509 (2)	−0.091 (2)	−0.681 (1)	5.6 (3)
C13	−0.376 (2)	−0.114 (3)	−0.590 (2)	7.2 (4)
C14	−0.290 (2)	0.009 (6)	−0.522 (2)	11.3 (8)
C15	−0.343 (2)	0.204 (4)	−0.548 (2)	9.0 (6)
C16	−0.479 (2)	0.242 (2)	−0.643 (1)	5.9 (3)
O3	−0.871 (1)	−0.1124 (9)	−0.7590 (9)	5.7 (2)
Cl	−0.8105 (3)	0.5484	−1.0089 (3)	4.72 (6)

Table IV. Atomic Coordinates for Hydrogen Atoms in the Crystal Structure of (−)-*Threo*-Methylphenidate

Atom	x	y	z
H1	−0.965	0.085	−1.001
H1	−0.880	0.250	−1.033
H2	−0.746	−0.080	−0.936
H3	−0.525	0.038	−0.933
H3	−0.603	0.221	−0.990
H4	−0.639	−0.110	−1.119
H4	−0.561	0.064	−1.150
H5	−0.786	0.215	−1.225
H5	−0.812	0.025	−1.291
H6	−1.004	0.105	−1.214
H6	−0.928	−0.080	−1.159
H7	−0.737	0.252	−0.817
H10	−1.028	0.215	−0.594
H10	−0.956	0.021	−0.572
H10	−1.085	0.065	−0.698
H12	−0.566	−0.192	−0.726
H13	−0.343	−0.238	−0.572
H14	−0.1960	−0.018	−0.460
H15	−0.284	0.302	−0.501
H16	−0.515	0.364	−0.664

CONCLUSIONS

A conformational analysis of *threo*-methylphenidate indicated that the carbonyl oxygen is oriented toward and makes a good intramolecular hydrogen bond with the ammonium group. A similar conformation was observed in the crystal structure of the (−)-enantiomer of *threo*-methylphenidate. Superposition of the global minimum with that of the potent dopamine reuptake blocker CFT indicates that the sequence of atoms from the ammonium nitrogen through the ester group is identical in the two compounds. However, the positions of the phenyl rings vary in the two compounds and between CFT and cocaine indicating that the dopamine reuptake protein can accommodate a variety of positions of the phenyl ring. The crystallographic study also confirmed the R,R absolute configuration of the active (+)-enantiomer of *threo*-methylphenidate.

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REFERENCES

1. B. K. Madras, M. A. Fahey, J. Bergman, D. R. Canfield, and R. D. Spealman. Effects of cocaine and related drugs in nonhuman primates. I. [^{3}H]Cocaine binding sites in caudate-putamen. *J. Pharm. Exp. Ther.* 251:131–141 (1989).
2. R. D. Spealman, B. K. Madras, and J. Bergman. Effects of cocaine and related drugs in nonhuman primates. II. Stimulant effects on schedule-controlled behavior. *J. Pharm. Exp. Ther.* 251:142–149 (1989).
3. J. Bergman, B. K. Madras, S. E. Johnson, and R. D. Spealman. Effects of cocaine and related drugs in nonhuman primates. III. Self-administration by squirrel monkeys. *J. Pharm. Exp. Ther.* 251:150–155 (1989).

4. M. Froimowitz. Conformational analysis of cocaine, the potent analog 2β-carbomethoxy-3β-(4-fluorophenyl)tropane (CFT), and other dopamine reuptake blockers. *J. Computat. Chem.* **14**:934–943 (1993).
5. P. C. Meltzer, A. Y. Liang, A.-L. Brownell, D. R. Elmaleh, and B. K. Madras. Substituted 3-phenyltropane analogs of cocaine: Synthesis, inhibition of binding at cocaine recognition sites, and positron emission tomography imaging. *J. Med. Chem.* **36**:855–862 (1993).
6. M. E. A. Reith, B. E. Meisler, H. Sershen, and A. Lajtha. Structural requirements for cocaine congeners to interact with dopamine and serotonin uptake sites in mouse brain and to induce stereotyped behavior. *Biochem. Pharmacol.* **35**:1123–1129 (1986).
7. M. C. Ritz, E. J. Cone, and M. J. Kuhar. Cocaine inhibition of ligand binding at dopamine, norepinephrine and serotonin transporters: A structure-activity study. *Life Sci.* **46**:635–645 (1990).
8. A. P. Kozikowski, L. Xiang, J. Tanaka, J. S. Bergmann, and K. M. Johnson. Use of nitrile oxide cycloaddition (NOC) chemistry in the synthesis of cocaine analogues; mazindol binding and dopamine uptake studies. *Med. Chem. Res.* **1**:312–321 (1991).
9. A. P. Kozikowski, M. Roberti, L. Xiang, J. S. Bergmann, P. M. Callahan, K. A. Cunningham, and K. M. Johnson. Structure-activity relationship studies of cocaine: replacement of the C-2 ester group by vinyl argues against H-bonding and provides an esterase-resistant, high affinity cocaine analogue. *J. Med. Chem.* **35**:4764–4766 (1992).
10. L. Szporny and P. Görög. Investigations into the correlations between monoamine oxidase inhibition and other effects due to methylphenidate and its stereoisomers. *Biochem. Pharmacol.* **8**:263–268 (1961).
11. R. Rometsch. Process for the conversion of stereoisomers. U.S. Patent 2,838,519 (1958).
12. C. K. Buckner, P. N. Patil, A. Tye, and L. Malspeis. Steric aspects of adrenergic drugs. XII. Some peripheral effects of (±)-*erythro*- and (±)-*threo*-methylphenidate. *J. Pharm. Exp. Ther.* **166**:308–319 (1969).
13. R. A. Maxwell, E. Chaplin, S. Batmanglidj Eckhardt, J. R. Soares, and G. Hite. Conformational similarities between molecular models of phenethylamine and of potent inhibitors of the uptake of tritiated norepinephrine by adrenergic nerves in rabbit aorta. *J. Pharm. Exp. Ther.* **173**:158–165 (1970).
14. K. S. Patrick, C. D. Kilts, and G. R. Breese. Synthesis and pharmacology of hydroxylated metabolites of methylphenidate. *J. Med. Chem.* **29**:1237–1240 (1981).
15. M. M. Schweri, P. Skolnik, M. F. Rafferty, K. C. Rice, A. J. Janowsky, and S. M. Paul. [^{3}H]*Threo*-(±)-methylphenidate binding to 3,4-dihydroxyphenylethylamine uptake sites in corpus striatum: Correlation with the stimulant properties of ritalinic acid esters. *J. Neurochem.* **45**:1062–1070 (1985).
16. K. S. Patrick, R. W. Caldwell, R. M. Ferris, and G. R. Breese. Pharmacology of the enantiomers of *threo*-methylphenidate. *J. Pharm. Exp. Ther.* **241**:152–158 (1987).
17. D. A. Eckerman, S. S. Moy, A. N. Perkins, K. S. Patrick, and G. R. Breese. Enantioselective behavioral effects of methylphenidate in rats. *Pharmacol. Biochem. Behav.* **40**:875–880 (1991).
18. A. Shafi'ee and G. Hite. The absolute configuration of the pheniramines, methyl phenidates, and pipradrols. *J. Med. Chem.* **12**:266–270 (1969).
19. L. Panizzon. La preparazione di piridil- e piperidil-arilacetonitrili e di alcuni prodotti di transformazione. *Helv. Chim. Acta* **27**:1748–1756 (1944).
20. N. L. Allinger and Y. H. Yuh. Quantum Chem. Program Exch. 12, program 395.
21. Quantum Chemistry Program Exchange, Department of Chemistry, Indiana University, Bloomington, IN 47405.
22. M. Froimowitz and V. Cody. Biologically active conformers of phenothiazines and thioxanthenes. Further evidence for a ligand model of dopamine D2 receptor antagonists. *J. Med. Chem.* **36**:2219–2227 (1993).
23. M. Froimowitz and P. J. Gans. Comparison of semiempirical classical and semiempirical quantum mechanical calculations on acetylcholine. *J. Amer. Chem. Soc.* **94**:8020–8025 (1972).
24. Serena Software, Box 3076, Bloomington, IN 47402-3076.
25. teXsan: Crystal Structure Analysis Package, Molecular Structure Corporation, 1992.
26. D. C. Creagh and J. H. Hubbell. *International Tables for Crystallography,* Volume C, Wilson, A. J. C., ed., Kluwer Academic Publishers, Boston, 1992.
27. I. Weisz and A. Dudás. Über stereoisomere 2-piperidylphenylessigsäure-methylester. Die raumstructur eines 7-phenyl-azabicyclo[0.2.4]octans. *Monatsh. Chem.* **91**:840–849 (1960).