4a,9b-*trans*-8-Fluoro-5-(4-fluorophenyl)-2-[4-(4-fluorophenyl)-4-hydroxybutyl]-2,3,4,4a,5,9b-hexahydro-1*H*-pyrido[4,3-*b*]indole Hydrochloride, a New Potent Neuroleptic

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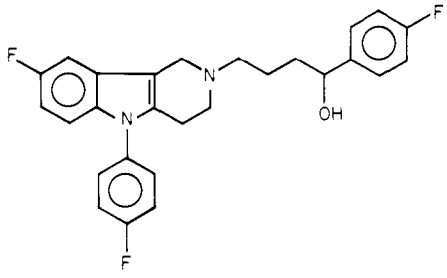
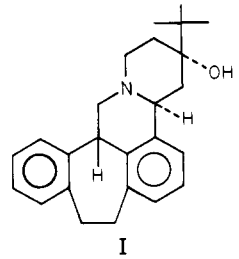
The preparation and testing of the two racemic diastereoisomers and the four optically active enantiomers of the title compound in in vitro and in vivo models for determining potential antipsychotic activity are described. Both racemic diastereoisomers and two of the four chiral enantiomers are potent and long-acting neuroleptic compounds.

We have previously reported that 8-fluoro-5-(4-fluorophenyl)-2-[4-(4-fluorophenyl)-4-hydroxybutyl]-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole[1] [1, CP-36,584 (flutroline)] displays potent and long-acting activity in in vivo



1

and in vitro models of neuroleptic activity.[2] This clinically active agent, like other neuroleptic compounds, exerts its action by blocking the action of dopamine (DA) at receptors in striatal and limbic structures of the central nervous system, a mechanism often postulated to explain the clinical antipsychotic effects of neuroleptic drugs.[3] The activity of 1 is believed to result from two factors: first, its lipophilicity which permits ready access to brain, and second, a semirigid structure that positions the extended phenethylamine moiety in a spatial arrangement that is eminently suitable for interaction with the DA receptor. That the DA receptor has well-defined stereospecific requirements for such interactions is illustrated by the results of DA receptor binding experiments in which (+)-butaclamol (I), a neuroleptic DA receptor antagonist,[4] binds



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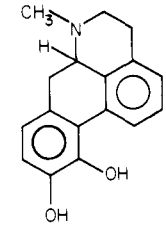

II

to isolated rat striatal membranes with high affinity, the respective (-) isomer being much less potent,[5] and by the in vivo pharmacological activity of the DA agonist (-)-apomorphine (II) whose emetic activity is not shared by its (+) isomer.[6] Comparison of X-ray data for 1,[7] for (+)-butaclamol,[8] and for (-)-apomorphine[9] show that the distances between the aryl ring and the basic nitrogen are virtually the same in all cases. Furthermore, in one inversional conformer of 1 the distance of the basic nitrogen out of the plane defined by the aromatic ring is consistent with those of the active forms of butaclamol and apomorphine.[2]

Comparison of minimized energy conformations of the two inversional conformers of the tricyclic portion of the tetrahydro nucleus Tα and Tβ with models of the *d* and *l* enantiomers of the tricyclic moiety of *trans*-hexahydro-γ-carboline Hα and Hβ[10] (Figure 1) reveals a remarkably good overlap between each of the inversional conformers Tα and Tβ and the corresponding *d* or *l* isomer of the relatively more rigid *trans*-hexahydro structures Hα and Hβ. Furthermore, because of this rigidity, the spatial positioning of the basic nitrogen is fixed above or below the plane of the indole ring in the hexahydro structures as it is not in the tetrahydro analogues. Since the evidence presented above suggests that the dopamine receptor has very strict stereospecific requirements, one such hexahydro enantiomer would be expected to display neuroleptic properties, whereas the other should be much less active or inert.

Reductions of carbolines and analogous compounds with borane in THF to generate a trans ring juncture selectively have been reported recently.[11,12] A modification of this procedure was applied to obtain the 5-arylhexahydro-γ-carbolines described herein.

Chemistry. The synthesis of compound 1 by an eight-step process from *p*-fluorophenylhydrazine has been described.[13] The tertiary amine-borane complex III was

(1) The common name for the pyrido[4,3-*b*]indole ring system is γ-carboline, which is used subsequently.
(2) C. A. Harbert, J. J. Plattner, W. M. Welch, A. Weissman, and B. K. Koe, *Mol. Pharmacol.*, 17, 38 (1980).
(3) A. Carlsson and M. Lindqvist, *Acta Pharmacol. Toxicol.*, **20**, 140 (1963).
(4) D. H. Mielke, D. M. Gallant, T. Oelsner, C. M. Kessler, W. K. Tomlinson, and G. H. Cohen, *Dis. Nerv. Syst.*, **36**, 7 (1975).
(5) D. R. Burt, I. Creese, and S. H. Snyder, *Mol. Pharmacol.*, **12**, 800 (1976).
(6) W. S. Saari and S. W. King, *J. Med. Chem.*, **16**, 171 (1973).
(7) J. Bordner, J. J. Plattner, and W. M. Welch, *Cryst. Struct. Commun.*, submitted.
(8) F. T. Bruderlein, L. G. Humber, and K. Voith, *J. Med. Chem.*, 18, 185 (1975).
(9) J. Giesecke, *Acta Crystallogr., Sect. B*, **29**, 1785 (1973).
(10) The calculations were made using program number 318 obtained from the Quantum Chemistry Program Exchange (QCPE), University of Indiana, Bloomington.
(11) J. G. Berger, S. R. Teller, C. D. Adams, and L. G. Guggenberger, *Tetrahedron Lett.*, 1807 (1975).
(12) J. G. Berger (Endo Laboratories Inc.), Dutch Patent 7 703 900.
(13) C. A. Harbert, J. J. Plattner, W. M. Welch, A. Weissman, and B. K. Koe, *J. Med. Chem.*, in press.

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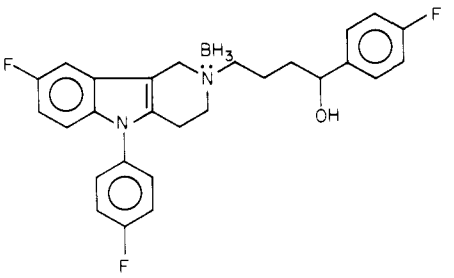
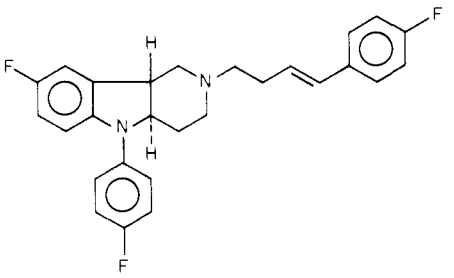


III



IV

obtained in essentially quantitative yield by treatment of 1 in THF with excess borane–THF complex in THF solution. Excess reagent was removed by evaporation, and the residual amine–borane complex was decomposed in acid according to the published procedure.[12] Following chromatographic separation of the reaction products, three components were identified: a 1:1 mixture of the desired racemic diastereoisomers 2 and 5 (46% yield), the corresponding dehydrated compound IV (6%), and recovered starting material (2–3%).

Dehydration of the benzylic alcohol was anticipated, since 1 has been observed to undergo a similar acid-catalyzed dehydration under virtually identical conditions.[13] The recovery of starting material suggests competition between acid-catalyzed hydrolysis of the borane adduct, yielding 1, and protonation of the indole double bond followed by hydride transfer from the borane complex to give the desired hexahydro derivatives 2 and 5.

The mixture of racemic diastereoisomers 2 and 5 was separated by crystallization of the free base from mixtures of ethyl acetate and hexane. Two further crystallizations gave diastereoisomer 2 in ≥99% purity by high-performance LC analysis. The second diastereoisomer 5 was obtained in approximately 95% purity after three recrystallizations from acetonitrile–methanol of hydrochloride salts obtained from the mother liquors from the first crystallization of 2 above.

Enantiomeric separation was accomplished in each case by preparing the diastereoisomeric esters with *t*-Boc-protected L-phenylalanine under DCC catalysis, cleaving of the *t*-Boc protecting group at 0 °C with trifluoroacetic acid, and careful column chromatography (Scheme I). High-performance LC analysis of the separated derivatives revealed them to be ≥98% pure. Finally, brief treatment of the amino esters with methanolic NaOH regenerated the respective enantiomers 3 and 4 and 6 and 7 (Table I).

Discussion

Given the spatial and stereochemical requirements of the DA receptor and, assuming from experience with 1, that the activity of these compounds derives primarily from the carboline nucleus,[2] we predicted that the neuroleptic potency of 2 and 5 should be approximately equal and that two of the four enantiomers should be much less active than the remaining pair. The testing data confirm these predictions (Table I). The stereotypy induced in rats by *d*-amphetamine[14] was antagonized by compounds 2, 3, 5, and 6 at very low doses as compared with active doses of standards. Enantiomers 4 and 7 were much less active in this test. Likewise, the racemic mixtures and enantiomers 3 and 6, unlike 4 and 7, displaced ^{3}H-labeled spiroperidol[5] from striatal DA receptors at very low concentrations. The equivalence of 3 and 6 in these tests suggests that chirality in the side-chain alcohol has little

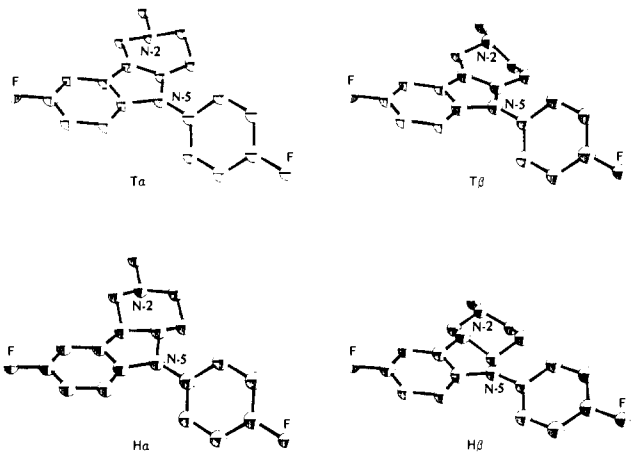
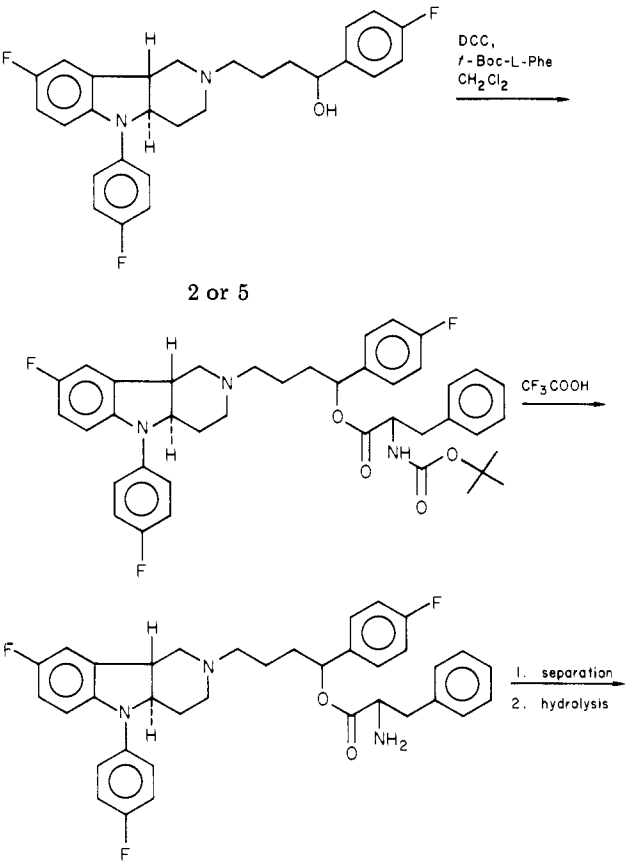


Figure 1. Lowest energy conformations of inversional conformers of N^2-methyl-substituted tetrahydro-γ-carboline nucleus (Tα and Tβ) and of *d* and *l* enantiomers of *trans*-hexahydro-γ-carboline nucleus (Hα and Hβ). Hydrogen atoms are absent to facilitate depiction of features of molecular overlap.

Scheme I



3 and 4 or 6 and 7

(14) A. Weissman, B. K. Koe, and S. S. Tenen, *J. Pharmacol. Exp. Ther.*, 151, 339 (1966).
(15) C. S. Weil, *Biometrics*, 8, 249 (1952).

Table I. In Vivo and in Vitro Neuroleptic Activities

compd	$[\alpha]_D$[a]	mp, °C	antagonism of amphetamine (rat): ED_{50} (95% CL), mg/kg sc[b] 1 h	5 h	24 h	inhibn of [^{3}H]-spiroperidol binding:[c] IC_{50}, nM
1		233-235	1.0 (0.6-1.8)	0.15 (0.07-0.31)	2.2 (0.6-4.6)	14
2		259-260	0.72 (0.5-1.2)	0.09 (0.05-0.16)	0.02 (0.01-0.04)	23
3	+32.2°	266-267	0.05 (0.03-0.09)	0.02 (0.01-0.03)	0.02 (0.02-0.04)	22
4	-33.0°	266-267	>10	18.1	>10	1800
5		237-239	1.1 (0.8-1.4)	0.13 (0.10-0.18)	0.06 (0.03-0.13)	23
6	+3.1°	260-261	0.21 (0.2-0.3)	0.05 (0.03-0.07)	0.02 (0.01-0.03)	25
7	-2.7°	260-261	>10	5.7	>10	350
chlorpromazine			5.3 (4.4-6.4)	8.5 (7.3-9.9)	>32	51
(+)-butaclamol			0.38 (0.22-0.56)	0.37 (0.14-1.0)	>3.2	13

[a] Determined in CH_3OH (*c* 1.67). [b] The effect of each compound on prominent amphetamine-elicited symptoms were studied in rats using the rating scale and method reported by Weissman et al.[14] Groups of five rats were treated subcutaneously with compounds at doses separated by 0.5 log unit (i.e., . . ., 0.32, 1.0, 3.2, 10, . . ., mg/kg) and were then treated with *d*-amphetamine sulfate, 5 mg/kg ip, 1, 5, and 24 h later. ED_{50} values and 95% confidence limits were determined using the tables of Weil.[15] [c] [^{3}H]Spiroperidol binding to rat striatal membranes using 0.5 nM ligand was performed by the method of Burt et al.[5] IC_{50} values were determined graphically using four drug concentrations separated by 0.5 log unit. Entries are means of two to three determinations.

or no effect on in vivo activity or on binding affinity in vitro. This is consistent with our earlier observations in the tetrahydro series,[2,13] which suggested that the principal determinant of activity of compound **1** and analogues derives from the tricyclic moiety.

In vivo, the potencies of **2**, **3**, **5**, and **6** exceed that of **1** by a factor of about 3 at the 5-h point and by a factor of 30 to 100 at the 24-h testing period. In vitro, however, **1** is somewhat more potent, indicating that the stereochemical parameters of the active hexahydro species and of the rotational conformer of **1** responsible for interaction with the DA receptor are similar. The hexahydro derivatives may be distributed to relevant brain areas more effectively than the tetrahydro compounds, accounting for their greater in vivo potency. The apparently longer duration of action of the hexahydro compounds may result from the saturation of the potentially metabolically labile indole double bond.

Parameters referred to above which are pertinent to dopamine receptor interaction are the aromatic ring–basic nitrogen distance and the distance of the basic nitrogen above the plane of the aromatic ring.[2] These distances are 5.12 and 0.60 Å for **1** (from X-ray data[7]) and 5.2 and 0.55 Å for **2** and **5** (from Dreiding models). Comparison of the tricyclic portion of **2** and **5** with the published absolute configurations of (+)-butaclamol[8] and apomorphine[9] suggests further that the absolute configuration of the tricyclic moiety of the active enantiomers **3** and **6** is 4a*R*, 9b*R*. Experiments to determine the absolute configuration of the active species by X-ray analysis are in progress.

Experimental Section

Melting points were determined using a Dupont Model 900 differential thermal analyzer and are corrected. NMR spectra were recorded on a Varian XL-100 spectrometer with Me_4Si as an internal standard. IR spectra were determined with a Perkin-Elmer Model 21 spectrometer. UV spectra were recorded on a Cary Model 14 spectrophotometer. Mass spectra were obtained with a Perkin-Elmer RMU-6E mass spectrometer. High-performance LC assays were obtained using a modified Waters Associates (Milford, MA) apparatus with a commercial μPorasil analytical column. Flow rates were adjusted to 2 mL/min and compounds were detected by means of a variable wavelength detector set at 272 nm. Compounds were quantitated by measurement of relative peak heights. Optical rotations were measured with a Perkin-Elmer Model 141 polarimeter. Microanalyses were performed by the Pfizer Analytical Department.

Preparation of the Mixture of Racemic Diastereoisomers 2 and 5. A solution of 25.0 g (0.055 M) of **1** in 295 mL of THF was added dropwise to a chilled borane solution (177 mL of 0.94 M borane in THF) in a 1000-mL three-necked round-bottom flask equipped with magnetic stirrer, dropping funnel, and N_2 atmosphere. The resulting solution was allowed to stir at room temperature for 30 min and was then refluxed for 30 min. The solution was cooled, and excess reagent and THF was removed in vacuo on a rotary evaporator. A 100-mL portion of a 1:1 mixture of glacial acetic acid and 6 N HCl was added cautiously to the residual colorless oil. Initially, this addition was accompanied by vigorous gas evolution. The resulting mixture was heated at reflux for 1 h and was then cooled in an ice bath while being basified with 50% aqueous NaOH. The mixture of products was extracted into chloroform, which was dried with $MgSO_4$ and evaporated to a pale yellow foam. This material was chromatographed on 400 g of EM Reagents 70–230 mesh silica gel using 1:1 EtOAc–hexane as eluent. Compound IV and a mixture of **2** and **5** were thus separated and were then converted to their respective salts with HCl gas in ether. The diastereoisomeric ratio of **2** and **5** was determined to be 1:1 by analytical high-performance LC using the mobile phase hexane/$CHCl_3$/EtOAc/1-butanol/Et_3N in the ratio 63:15:20:2:0.1 (v/v). The presence of 2–3% of unreacted **1** in the crude reaction product mixture was confirmed in the same analysis.

Compound IV: 6% yield; mp 270–273 °C; NMR ($CDCl_3$) δ 1.86–2.80 (8 H, m), 3.00–3.24 (3 H, m), 3.58 (1 H, d, J = 10 Hz); MS *m/e* 434, 299, 256 (100%), 229, 192, 148, 135; IR (KBr) 3.10, 4.10, 6.18, 6.58, 6.76, 8.20, 8.40, 12.35 μm; UV (CH_3OH) λ_{max} 248 nm (log ε 4.383). Anal. ($C_{27}H_{25}N_2F_3$·HCl) C, H, N, Cl, F.

Separation of Racemic Diastereoisomers 2 and 5. A 5-g quantity of the hydrochloride salt of a 1:1 mixture of racemic diastereoisomers **2** and **5** was converted to the free base by partitioning between CH_2Cl_2 and 10% aqueous NaOH. The organic phase was dried ($MgSO_4$) and evaporated to a foam, which was dissolved in 12.5 mL of EtOAc and 45 mL of hexane at the boiling point. The product which separated after scratching and cooling overnight weighed 2.24 g and melted at 126–129 °C. This material was recrystallized twice from mixtures of EtOAc and hexane, giving, ultimately, 1.22 g of diastereoisomer **2**, mp 132–134 °C. This base was converted to its HCl salt by addition of an ethereal solution of HCl gas to a solution of the base in CH_3OH: yield 1.30 g; mp 259–260 °C. High-performance LC analysis of this mixture using the above mobile phase indicated that it was ≥99% pure **2**.

Compound 2: NMR ($CDCl_3$; free base) δ 1.48–2.44 (9 H, m), 2.44–2.68 (2 H, m), 2.94–3.36 (3 H, m), 3.48 (1 H, d, J = 10 Hz), 4.67 (1 H, broadened t), 6.41–7.50 (11 H, m); MS *m/e* 452, 299, 285, 256, 243, 229, 210 (100%), 178, 165, 149; IR (KBr) 2.99, 3.88, 3.96, 6.64, 6.80, 6.91, 8.00, 8.20, 8.50, 8.71, 11.98, 12.27 μm; UV (CH_3OH) λ_{max} 243 nm (s) (log ε 5.980), 264 (8.484), 270 (8.016), 298 (s) (3.500). Anal. ($C_{27}H_{27}ON_2F_3$·HCl) C, H, N, Cl, F.

The mother liquors from the first crystallization above were evaporated to a foam, dissolved in ether, and converted to the HCl salts with ethereal HCl solution. The resulting crystalline solid was recrystallized three times from CH_3CN–CH_3OH giving,

ultimately, 1.03 g of diastereoisomer **5**, mp 237–239 °C. High-performance LC analysis of the product using the above mobile phase indicated that it was about 95% pure **5** contaminated with about 5% of **2**. Additional recrystallizations did not change this ratio.

Compound 5: NMR ($CDCl_3$; free base) δ 1.48–2.42 (9 H, m), 2.44–2.66 (2 H, m), 2.90–3.22 (3 H, m), 3.64 (1 H, d, J = 10 Hz), 4.68 (1 H, broadened t), 6.36–7.50 (11 H, m); MS m/e 452, 299, 285, 256, 243, 229, 210 (100%), 178, 165, 149; IR (KBr) 2.95, 3.76, 6.60, 6.76, 6.99, 7.95, 8.14, 8.46, 12.04, 12.16 μm; UV (CH_3OH) λ_{max} 243 nm (s) (log ε 6.029), 264 (8.582), 270 (8.115), 298 (s) (3.525). Anal. ($C_{27}H_{27}ON_2F_3$·HCl) C, H, N, Cl, F.

Synthesis of Diastereoisomeric L-Phenylalanine Esters. A solution of 2.40 g (5.3 mM) of racemic diastereoisomer **5** and 2.0 g (7.5 mM) of *N*-(*tert*-butoxycarbonyl)-L-phenylalanine in 80 mL of CH_2Cl_2 under an N_2 atmosphere was cooled in an ice bath. To the stirred solution was added 1.55 g (7.5 mM) of dicyclohexylcarbodiimide. The reaction mixture was then stirred for 1 h at 0–5 °C and for 1 h at room temperature. The resulting white solid was separated by filtration and washed with CH_2Cl_2. After removal of solvent from the filtrate, the residues were chromatographed on 40 g of EM Reagents 70–230 mesh silica gel, eluting with 5:1 CH_2Cl_2–ethyl acetate. The resulting product was 2.51 g of a white amorphous foam.

To this foam was added 30 mL of cold anhydrous trifluoroacetic acid (TFA). The reaction mixture was stirred in an ice bath for 30 min, at which time solution had occurred. The TFA was removed on a rotary evaporator without external warming of the flask. The residues were dissolved in cold CH_2Cl_2 and washed to neutrality with cold 1% aqueous $NaHCO_3$. The organic layer was dried with $MgSO_4$ and the solution was evaporated to a pale yellow gum (1.6 g), which was chromatographed on 40 g of EM Reagents 230–400 mesh silica gel using 35:1 ethyl acetate–CH_3OH as eluent. High-performance LC analysis of the two isolated one-spot diastereoisomeric esters utilizing a mobile phase consisting of EtOAc/CH_3OH in the ratio 97.5:2.5 (v/v) indicated that each was ≥98% pure. These samples weighed 636 and 474 mg, respectively, the disparity arising from discard of several overlapping fractions. These esters were not further characterized but were used directly in the next step.

Hydrolysis of Amino Esters to Enantiomers. A stirred solution of 625 mg of the L-phenylalanine ester of enantiomer **3** in 10 mL of CH_3OH at room temperature was treated with 10% aqueous NaOH until the solution turned cloudy and was then stirred for 30 min at room temperature. At this time, CH_3OH was removed by evaporation and 10 mL of water was added. The aqueous suspension was extracted with CH_2Cl_2, and the organic extracts were dried with $MgSO_4$. Evaporation of CH_2Cl_2 gave a pale yellow gum, which was dissolved in 5 mL of acetone and treated with an excess of ethereal HCl. The product crystallized in platelets from this solution: yield 388 mg; mp 266–267 °C; $[\alpha]^{23}_D$ +32.2° (*c* 1.67, CH_3OH). High-performance LC analysis of enantiomers derived from **2** showed diastereioisomeric purities of ≥98%. High-performance LC analysis of enantiomers derived from **5** showed diastereoisomeric purities of 97% for the levorotatory enantiomer and of 95% for the dextrorotatory enantiomer as a result of the 5% contamination of **5** with **2**. These last high-performance LC analyses were accomplished using the mobile phase developed for the mixture of **2** and **5** above.

Acknowledgment. The authors are grateful to Ms. S. Koch and C. Scott and M. Boucher for significant technical assistance, to Dr. B. M. Johnson and Ms. J. D. Gagliardo of the Pfizer Analytical Research Department for the high-performance LC analyses, and to Dr. B. W. Dominy of Pfizer Central Research for conducting the energy calculations.

Synthesis and Anxiolytic Activity of a Series of Pyrazino[1,2-*a*][1,4]benzodiazepine Derivatives

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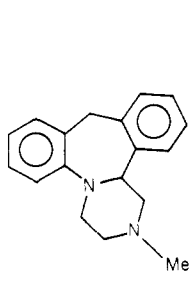
The synthesis and biological evaluation of some derivatives of pyrazino[1,2-*a*][1,4]benzodiazepines for anxiolytic and antidepressant activity are presented. Significant levels of anxiolytic activity were noted for 7-(*o*-chlorophenyl)-9-chloro-1,2,3,4,4a,5-hexahydro-3-methylpyrazino[1,2-*a*][1,4]benzodiazepine (**4b**).

As part of a continuing search for a compound which has both anxiolytic and antidepressant properties, it was of interest to synthesize certain 7-substituted 1,2,3,4,4a,5-hexahydropyrazino[1,2-*a*][1,4]benzodiazepines. These structures combine features of the antidepressant mianserin (I)[1] and the benzodiazepine anxiolytic diazepam (II). It was anticipated that some anxiolytic activity would be retained, since benzodiazepines with additional fused rings, e.g., III, have been claimed to have potent anxiolytic properties.[2]



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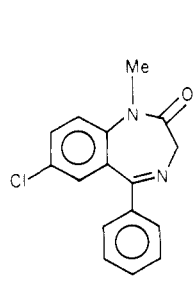

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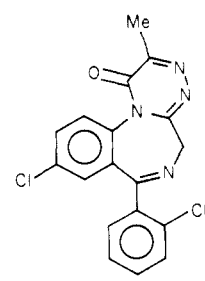

III

Accordingly, some 7-substituted 1,2,3,4,4a,5-hexahydropyrazino[1,2-*a*][1,4]benzodiazepines and further hydrogenated derivatives were synthesized.

Chemistry. The compounds were prepared as depicted in Scheme I. 1-(*p*-Chlorophenyl)-4-methyl-5-cyano-2,3-

(1) (a) A. Coppen, R. Gupta, S. Montgomery, K. Ghose, J. Bailey, B. Burns, and J. J. DeRidder, *Br. J. Psychiatry*, **129**, 342–345 (1976); (b) P. J. Fell, D. C. Quantock, and W. J. van der Burg, *Eur. J. Clin. Pharmacol.*, **5**, 166 (1973); (c) W. J. van der Burg, I. L. Bonta, J. Delobelle, C. Ramon, and B. Vargaftig, *J. Med. Chem.*, **13**, 35 (1970).

(2) R. B. Moffett, G. N. Evenson, and P. F. Von Voigtlander, *J. Heterocycl. Chem.*, 14, 3956 (1977).

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