typical oxine mode of action. This action can be defined as the rapid killing of bacteria and fungi at high dilution, requiring the presence of either iron or copper, and preventable by a trace of cobalt (but no other metal).[25]

What was happening at the molecular level was revealed in 1959 when Sijpesteijn and Janssen (Holland) showed that these metal complexes catalyzed the destruction of lipoic acid (**15**), the coenzyme of pyruvic oxidase.[26] This chain reaction of consecutive oxidations, accompanied by reduction of the metal ion, is uniquely blocked by cobalt, as in nonbiological situations. Clearly, lipoic acid has to be recognized as the receptor for this drug, but at the time it caused surprise to find one of such low molecular weight.

We may now sum up all of the foregoing as follows: Drug scientists, after following for too long the seductive clue of 1869, slowly realized that chemical structure in a drug is usually only secondary to the physical properties that the structure can generate and which can be obtained also from distinctly different structures. The relevant physical properties are those that distribute the drug to the receptor and those that bind it when it gets to the receptor. It seems that these properties are three in number: lipophilicity, electron distribution (as evidenced by ionization, chelation, or Hammett σ values), and a steric nature complementary to the receptor. Already in 1900, Overton and Meyer had demonstrated the overriding importance of lipophilicity in the design of general anesthetics and hypnotics. For all other kinds of biological action, we now know that *all three* types of physical properties play a part but in different degree for different drug–receptor combinations.

Corwin Hansch has devised the "multiple regression equation" which, by the use of statistical methods and a computer, helps to predict optimal values for each of these three variables.[27] This approach has given some excellent results and is particularly valuable when time is at a premium. However, the biological situation is more complex than such an equation can accomodate. For example, distribution need not depend on lipophilicity but on the use of facilitated channels that exist for the uptake of natural products, such as sugars, purines, amino acids, and even choline. The steric term in the regression equation presents problems too, because a three-dimensional cavity in a receptor is likely to have *several* relevant dimensions, which cannot be expressed by a single numeral. For these reasons, those who have the time and the interest will continue to examine structure–action relationships in all their fine detail and complexity.

Nor is that the whole of their work! The rules that teach us how to produce *activity* tell us nothing about how to achieve *selectivity*, that very desirable property which enables a drug to act strongly on the designated cells wihout affecting any of the others. How is selectivity to be achieved? In my opinion, and I have been publishing along these lines for a quarter of a century,[28] there are three important approaches for differentiating between the economic and the uneconomic cell, namely, by the use of selective distribution, selective biochemistry, and selective cytological structure. There is no doubt in my mind that attention to these three variables will lead us to more powerful, yet much safer, drugs than have yet been at our command.

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Articles

Synthesis and Pharmacological Evaluation of Conformationally Restricted Phenothiazine Analogues

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The synthesis of 3-(dimethylamino)-2,3-dihydro-4-chloro-1*H*-pyrido[3,2,1-*kl*]phenothiazine, its 10-chloro analogue, and two chloro isomers of 2-[(dimethylamino)methyl]-2,3-dihydro-1*H*-pyrido[3,2,1-*kl*]phenothiazine is described. In these compounds the side chain of chlorpromazine is fixed into a certain position in order to study the dopamine-overlap theory and the role of the substituents in the phenothiazine neuroleptics. The compounds were tested for their influence on dopamine metabolism, while for the second set their ability to displace [^{3}H]spiroperidol from dopamine receptors was assessed. No neuroleptic activity was found from the pharmacological data. The results are discussed on the basis of conformational requirements for dopamine antagonistic activity.

In a previous paper[1] we described our study concerning the role of the 2-substituent in the phenothiazine neuroleptics with flexible side chains. From the measurement of brain concentrations of promazine, chlorpromazine, and triflupromazine and their potential to increase the level of the dopamine metabolite homovanillic acid (HVA), it was evident that the relative potencies based on brain

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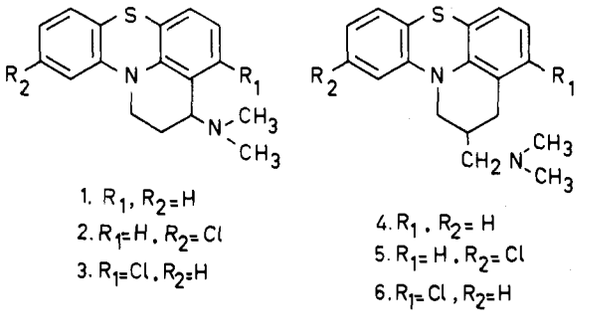
0022-2623/82/1825-0005$01.25/0

Chart I



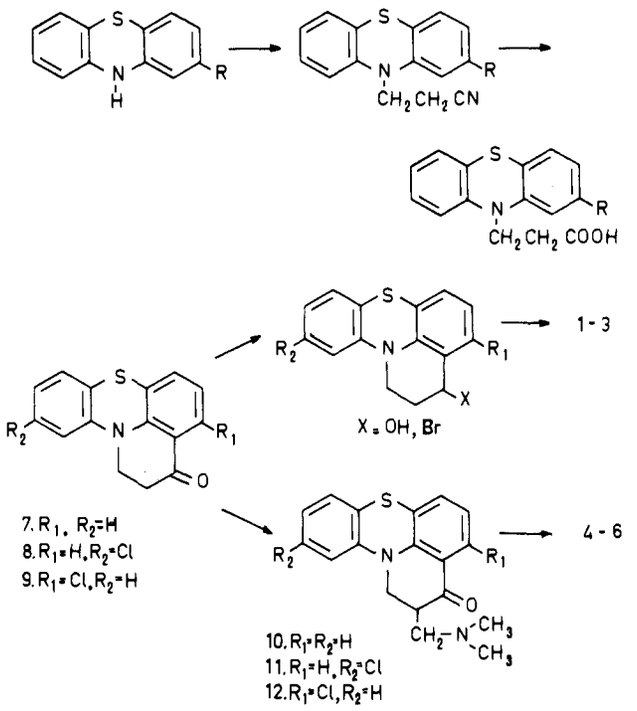
concentrations and the relative potencies from ip doses were virtually equal. This indicates that merely increasing lipophilicity by introducing a substituent does not improve the penetration into the brain.

We further investigated the role of the substituent on the conformation of the side chain by theoretical MO calculations. A variation of the substituent from H to Cl and CF_3 showed an increased probability of that conformation in which the side chain was folded toward the benzene nucleus bearing the substituent. This directive influence of the substituent is in agreement with other calculations[2] and the X-ray structure of chlorpromazine[3] and is supported by the existence of active compounds like butaclamol, in which the side chain is fixed already in a certain position[4] by the rigidity of the molecule. On the other hand, there are semirigid compounds wherein the side chain is already in the favorable asymmetric conformation and where the introduction of a substituent leads to *more* active compounds, indicating a direct interaction of the substituent with the receptor.[5]

To obtain a better insight into the role of the substituent on the conformation of the phenothiazines, we designed some structures with a partly fixed side chain (Chart I). In these compounds the side chain is connected to the aromatic nucleus, still fulfilling the criterion of a three-carbon side chain between the ring nitrogen and the tertiary amine nitrogen, necessary for neuroleptic activity of the phenothiazines. These pyridophenothiazines should provide one with the opportunity to study two possible effects. When the only influence of the substituent is a directive one, then the compounds with and without a substituent should have the same activity because the side chain is already fixed. If there is a direct interaction between the substituent and the receptor with the side chain still in its asymmetric position, the compounds with a substituent should be more active.

Chemistry. Although the compounds 1–3 are known,[6] there is no report of their pharmacological activity. The compounds were made as outlined in Scheme I. After a Michael addition of acrylonitrile to phenothiazine or 2-chlorophenothiazine, the formed nitrile was converted into the methyl ester, followed by hydrolysis. The ring closure was accomplished with $ZnCl_2$ in HOAc.[7] The chloro isomers were obtained in a ratio of 1:4 (4-Cl/10-Cl) and separated by repeated fractional crystallization. The

Scheme I



structures were confirmed by NMR spectroscopy where the hydrogen atom at position 4 in compound **8** shifted downfield by the magnetic anisotropic effect of the carbonyl group. This downfield shift was absent in the spectrum of compound **9** and disappeared after the reduction of the carbonyl group.

The pyridophenothiazine **1** was made by reduction of the ketone **7** with $LiAlH_4$, conversion to the bromide with PBr_3 and substitution with dimethylamine according to the method of Hromatka.[7] The chloro isomers were made in the same way and have been isolated as picrates by Fujii,[6] who made these compounds by a Leuckart reaction of the ketones with formamide, followed by a $LiAlH_4$ reduction.

The amino ketone **10**, originally made by a classical Mannich reaction,[6] was synthesized by us with a variant of this reaction[8] using *N,N*-dimethyl(methylene)ammonium chloride in acetonitrile to give a 56% yield. The chloroamino ketones **11** and **12** were obtained in 73 and 78% yield, respectively. In order to reduce the amino ketone, we tried a number of reactions, including catalytic hydrogenation, Clemmensen reduction, and dissolving metal reductions, but either the starting material was obtained or the reaction mixture turned dark red, probably due to the formation of thiazinium salts. Success was finally achieved with BH_3–THF. In an intermediate step, a borate was isolated that gave, after hydrolysis, the aminopyridothiazines **4–6**, which were converted into their succinic acid salts.

Pharmacology. Neuroleptic drugs are known to increase DA turnover in the basal ganglia[9] and the mesolimbic system, an effect interpreted as being a result of blockade of dopamine receptors.[10] More direct evidence

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Table I. Pharmacological Data of Pyridophenothiazines 1-6

compd	HVA[a] dose, μg/g	HVA SEM	HVA % control	DOPAC dose, μg/g	DOPAC SEM	DOPAC % control	n[b]	[³H]spiroperidol binding:[a] IC_{50}, M
control	0.44	0.04	100	0.63	0.06	100	3	
1[c]	0.41	0.02	93	0.60	0.03	95	3	
2	0.39	0.03	88	0.62	0.02	98	3	
3	0.44	0.08	100	0.73	0.07	115	3	
CPZ[d]	1.62	0.23	368	2.70	0.18	428	3	
control	0.70	0.04	100	1.07	0.03	100	6	
4[e]	0.63	0.06	90	1.15	0.11	107	5	1.4×10^{-6}
5	0.54	0.07	70	0.95	0.16	89	5	1.3×10^{-5}
6	1.10	0.03	157**	1.26	0.04	118*	5	1.2×10^{-6}
CPZ[e]	2.50	0.09	357	2.62	0.06	245	5	2.5×10^{-8}

[a] See Experimental Section. [b] Number of rats. [c] 70 mg/kg orally. [d] Chlorpromazine, 17 mg/kg orally. [e] 50 μmol/kg ip. ** = $p < 0.001$; * = $p < 0.005$.

of dopamine receptor blockade is provided by the finding that neuroleptic drugs bind specifically to dopaminergic regions of the brain.[12] With these techniques it is thus possible to test the activity of potential neuroleptic compounds.

The pyridothiazines 1–3 were tested for their effect on the concentrations of the DA metabolites HVA and DOPAC in the corpus striatum of the rat.[13] Due to their low solubility, the compounds were administered orally and the brains were removed 3 h thereafter. The metabolites of dopamine, HVA and DOPAC, were measured and found not to be significantly different from control values.

The second class of compounds (4–6) was tested with the same model, and for the 4-chloro isomer a significant increase in HVA and DOPAC levels was found. Although the increase was much weaker than for chlorpromazine, these compounds were also screened for their ability to displace [³H]spiroperidol. The specific binding was defined as that displacable by 1 μM (+)-butaclamol and was about 80% of the total binding. The IC_{50} values were found to be in the micromolar range, indicating a low affinity for dopamine receptors. The results of the tests are summarized in Table I.

Discussion

In the search for an explanation of the activity of neuroleptics as antagonists of the DA receptors, considerable attention has been paid to the DA-overlap theory in which common features between dopamine and neuroleptics has been sought. In this model the sulfur atom of the phenothiazines is thought to be comparable with the meta-oxygen atom in dopamine and wherein the amino nitrogens of both the agonists and antagonists interact with the same place on the receptor.[15] In our publication[16] about the conformation of dopamine we defined a topography of a DA receptor based upon MO calculations and a comparison with rigid DA analogues. In this topography, an area was suggested, defined by the meta-oxygen atom, at a distance 6.3–7.2 Å from the nitrogen-interaction location. It was shown that many conformations of chlorpromazine could fulfill this criterion. Another approach to the topography of the DA receptor was followed by Humber[17] and is based upon the activity of (+)-butaclamol and isobutaclamol as DA antagonists. A primary binding site was suggested to be a region on the DA receptor which could be occupied by at least two different benzene rings, in

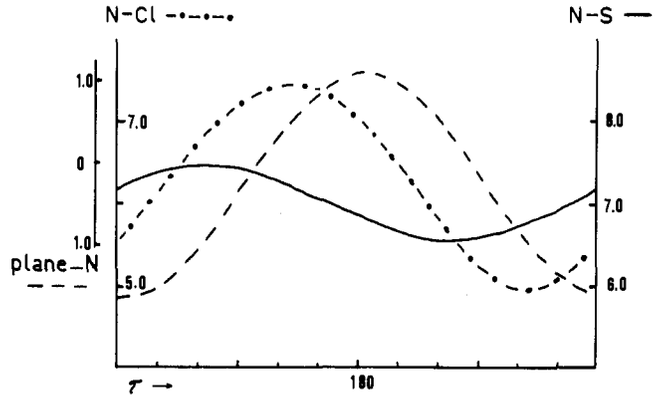

Figure 1. Intramolecular distances from the amine nitrogen in compound **6** to the sulfur atom (—), to the chlorine atom (●-●), and to the plane through ring A (---), as a function of the torsion angle.

Chart II

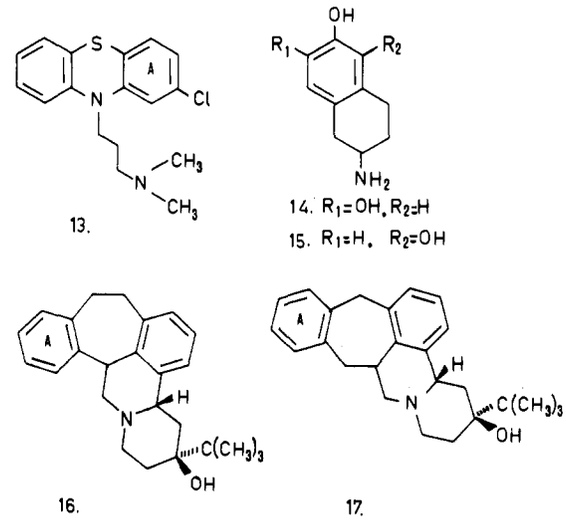

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Table II. Intramolecular Distances

compd	distance,[a] A			
	N-S(O_m)	NA	N-Cl	N-plane
6,7-ADTN (14)[b]	7.32	5.05		0.05
6,7-ADTN·HBr[c]	7.32	5.15		0.001
5,6-ADTN (15)	6.21	5.05		0.05
(+)-butaclamol·HCl (16)[d]		5.1		0.9
(+)-isobutaclamol·HBr (17)[d]		6.4		0.9
chlorpromazine (13)[e]	6.94	5.10	4.81	1.6
2[b]	6.3	3.65	7.65	−0.9
3	6.3	3.65	2.60	−0.9
5	6.5-7.4	5.3-6.0	5.0-7.4	−1.6-1.1
6	6.5-7.4	5.3-6.0	4.9-6.7	−1.6-1.1

[a] Distances from the amine nitrogen to the sulfur atom of the phenothiazines or to the meta-oxygen of the tetralines, to the center of the aromatic rings (N-A), to the substituent, and to the plane formed by ring A. [b] Distances were calculated by a computer program and are based upon standard bond lengths and angles. [c] Values determined by X-ray crystallography.[18] [d] References 17 and 19. [e] Reference 3.

which the distances from the amine to the center of these rings were 5.1 or 6.4 Å. As is clear from our pharmacological data, the pyridophenothiazines are not active as DA antagonists. The lack of activity of the compounds (1–3) might be rationalized because the conformation is quite different from other semirigid compounds, in that the side chain is inclined too close to the aromatic nucleus. We find a distance of 6.29 Å to the sulfur atom and a distance of 3.65 Å to the center of the aromatic ring from the amine nitrogen.

In the second group of pyridophenothiazines, the dimethylaminomethylene group has rotational freedom in the sense that the distance from the amine to the sulfur atom can vary between 6.5 and 7.4 Å (Figure 1), while the distance between the amine nitrogen and the plane of the benzene nucleus might vary between 1.6 Å below and 1.1 Å above this plane. From these data it is clear that we modified the phenothiazine molecule with a free rotating side chain into molecules in which crucial distances are restricted within certain values. These values are comparable with those found in active, rigid dopamine antagonists like butaclamol and agonists like the aminotetralines (Table II, Chart II).

According to the dopamine-overlap theory some activity could therefore be expected. Although compound **6** elevated HVA levels to 157% (Table I), it was only weakly active in the [^{3}H]spiroperidol test, and taking these two facts together we do not feel that these findings indicate significant neuroleptic activity. In our opinion this finding suggests that the explanation for the dopaminergic receptor blocking action of the antagonists, solely based on the superimposability of the trans nearly coplanar dopamine molecule and a dopamine like part of the antagonists, is probably too simple. It might be that the conformational requirements are much more critical and are not met by our compounds or that there are other factors besides the conformation that determine whether a compound is a neuroleptic or not.

Experimental Section

Melting points were determined on a Büchi-Tottoli melting apparatus and are uncorrected. Infrared spectra were obtained on a Beckman IR-33 spectrophotometer in KBr disks. Proton NMR spectra were recorded on a Perkin-Elmer R-24 spectrometer in deuteriochloroform with tetramethylsilane as internal standard. Microanalyses were performed by the microanalytical laboratories in our department of chemistry and by Galbraith Laboratories, Knoxville, TN. Analytical results were withing ±0.4% of the theoretical values, unless otherwise indicated.

2,3-Dihydro-3-oxo-1*H*-pyrido[3,2,1-*kl*]phenothiazines (7–9). These compounds were prepared by a cyclization reaction of the appropriate acids with $ZnCl_2$.[7] A mixture of the 4-Cl and 10-Cl ketones was obtained from the 2-Cl acid in a proportion of 1:4. The isomers were separated by repeated fractional crystallization from ethanol or with preparative HPLC (EtOAc–hexane).

3-(Dimethylamino)-2,3-dihydro-1*H*-pyrido[3,2,1-*kl*]-phenothiazine (1) and Its 4-Chloro (3) and 10-Chloro Isomers (2). These compounds were prepared according to the method developed by Hromatka[7] and are described as their picrates by Fujii.[6]

2-[(Dimethylamino)methyl]-1*H*-pyrido[3,2,1-*kl*]phenothiazin-3(2*H*)-one Hydrochloride (10) and Its 10-Chloro (11) and 4-Chloro (12) Analogues. In 30 mL of dry acetonitrile, 2 g of freshly prepared *N,N*-dimethyl(methylene)ammonium chloride was suspended. To this suspension under N_2, a solution of 2 g (8 mmol) of ketone **1** in 20 mL of dry THF was added. The mixture was stirred for 14 h and poured into 5% HCl. The yellow solution was washed with ether, basified, extracted with dichloromethane, washed (NaCl), and dried ($MgSO_4$). After evaporation of the solvent, the base was, if necessary, converted to its HCl salts with ether–HCl. **7**·HCl salt: mp 168–170 °C (lit.[6] mp 161–162 °C). **11**·base: mp 95–96 °C (from EtOH). Anal. ($C_{18}H_{17}N_2ClSO$) C, H, N, S, Cl. **12**·HCl salt: mp 171–173 °C (from EtOAc). Anal. ($C_{18}H_{18}N_2Cl_2SO$) C, H, N, S, Cl.

2-[(Dimethylamino)methyl]-2,3-dihydro-1*H*-pyrido[3,2,1-*kl*]phenothiazine (4) and Its 10-Chloro (5) and 4-Chloro Analogues (6). To 1 g (3.2 mmol) of the amino ketone **10** in 8 mL of dry THF was added 6 mL of borane–THF; the mixture was refluxed for 1 h and, after cooling, 1 mL of MeOH was added to the clear solution. The mixture was then poured into H_2O, extracted with ether, washed with NaCl, and dried ($MgSO_4$). After evaporation of the solvent, a foamy solid was obtained. This borate (complex, salt) was dissolved in 10 mL of MeOH and 25 mL of a mixture of MeOH–concentrated HCl (1:1) was added. After the mixture was left standing for 6 h, water was added and the solution was washed with ether. Base was added to the acid layer, and the solution was then extracted twice with ether. The organic layer was washed (NaCl), dried ($MgSO_4$), and evaporated, leaving an oil, which was chromatographed on SiO_2 with MeOH–$CHCl_3$ (1:9) to give 0.8 g (85%) of an oil. The base was converted to its succinate by the addition of succinic acid in MeOH. The solid was recrystallized from acetone–ether, mp 131–132 °C. Anal. ($C_{22}H_{26}N_2O_4S$) C, H, N, S. 4-chloro isomer: yield 72%; mp 116–117 °C. Anal. ($C_{22}H_{25}N_2O_4SCl·1H_2O$) C, H, S, Cl; N: calcd, 6.0; found, 5.5. 10-chloro isomer: yield 66%; mp 138–139 °C. Anal. ($C_{22}H_{25}N_2O_4SCl·1H_2O$) C, H, N, Cl; S: calcd, 6.87; found, 6.4.

Effects on Dopamine Turnover. Compounds **2** and **3** were administered orally, due to their poor solubility, to male Wistar rats (180–200 g), while compounds **4–6** were dissolved in saline and injected ip. The rats were decapitated 3 h after addition of the compounds, and the corpus striata were dissected and frozen on dry ice. The levels of HVA and DOPAC were assayed after isolation on small Sephadex G columns by automated fluorimetric analysis.[13]

[^{3}H]Spiperone Binding. Binding experiments were carried out in twice-washed 5% homogenates obtained from frozen calf caudate. The brain tissue was weighed and then homogenized

in ice-cold 0.05 M sodium-potassium phosphate buffer (pH 7.4). After centrifugation at 48000g for 20 min (Sorvall RC2-B), the supernatant was discarded, the pellet was resuspended in distilled water, and the process was repeated. The final pellet was resuspended in 50 volumes of ice-cold 0.05 M Tris buffer (pH 7.4). Aliquots of brain tissue (final concentration 1.25 mg/mL), [^{3}H]spiperone (100 pM), and drugs (freshly prepared in distilled water) were incubated in buffer (final assay volume 2 mL) for 30 min at 37 °C. The binding reaction was terminated by filtration in vacuo over Whatman GF/B filters, and radioactivity was extracted overnight in 6 mL of scintillation fluid [1 L of toluene (Baker), 1 L of Triton X-100 (NEN), 169 of Omnifluor (NEN)] and measured in a Searle Mark II liquid scintillation counter (45% efficiency). Specific [^{3}H]spiperone binding was defined as the difference between binding in the absence and in the presence of 1 μM (+)-butaclamol. The negative logarithm of the concentration of drug producing a 50% inhibition of specific binding (pIC_{50}) values were estimated graphically from logarithmic Hill plots and converted to IC_{50} values.

Acknowledgment. We gratefully acknowledge the hospitality given by H. I. Yamamura to one of us (C.J.G.) and thank Marla Bliss for technical assistance in performing the [^{3}H] spiperone binding. Research performed at the University of Arizona was supported, in part, by Grant MH 31184 from the United States Public Health Service.

Stereochemical Studies on Medicinal Agents. 25.[1] Absolute Configuration and Analgetic Potency of β-1,2-Dimethyl-4-phenyl-4-(propionyloxy)piperidine Enantiomers

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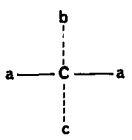
Enantiomers of β-1,2-dimethyl-4-phenyl-4-(propionyloxy)piperidine (4) were employed as probes to demonstrate that opioid receptors are capable of distinguishing between the enantiotopic edges (the Ogston effect) of the piperidine ring. These enantiomers, (−)- and (+)-4·HCl, were prepared by esterification of the corresponding alcohols, (+)- and (−)-**4a**. Single crystal X-ray studies of (−)-**4a**·HCl reveal that it possesses the 2*R*,4*S* absolute configuration. Analgetic testing in mice (hot-plate) and receptor binding studies indicate that (−)-(2*S*,4*R*)-4·HCl is approximately ten times more potent than its enantiomer. The results are consistent with the operation of the Ogston effect in the interaction of achiral 4-phenylpiperidines with opioid receptors. Additionally, it is suggested that the piperidine ring of these and other closely related 4-phenylpiperidines bind within a receptor subsite cleft whose dimensions exclude diequatorial 2,6- and 3,5-dimethyl-substituted ligands.

It is now generally recognized that differentiation of chemically like, paired groups (enantiotopic groups) in a substrate of the Caabc type (1) can be displayed by enzymes.[2] Thus, substrates which contain a plane of symmetry often are found to undergo enzymatic transformation specifically on one of their enantiotopic groups. In his now classical paper, Ogston was the first to point this out in connection with the enzymatic conversion of citrate (2) in the Krebs cycle.[3,4] This type of selectivity is sometimes referred to as the "Ogston effect".[5] Although the Ogston effect can be demonstrated unequivocally in enzyme-catalyzed reactions through radiolabeling experiments, a similar approach cannot be employed to investigate the interaction of ligands with noncatalytic recognition sites.



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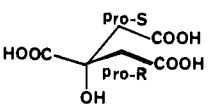

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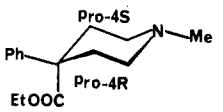

3

In an effort to investigate the Ogston effect in the action of narcotic analgetics, we had employed a methyl group to label the enantiotopic edges of the piperidine ring in 3.[6] The methyl group was selected because it is known that the C-5 and C-6 methyl groups interfere with ligand–receptor association of the less potent enantiomers of methadone and isomethadone, respectively.[7,8] Initially, the C-3 position was the site of attachment of the methyl group to the *pro*-4*R* and *pro*-4*S* enantiotopic edges of 3 in these studies.[6] Later publications discussed related compounds substituted at other centers.[9-14] However, interpretation of the results was complicated by the finding that alkyl substitution vicinal to the C-4 center induced a chiral orientation of the phenyl (Ph) group. In fact, the feature common to all of the analgetically more potent enantiomers is the sign of the torsion angles between the Ph and piperidine ring.[7]

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