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Quantum Chemical Studies of Meperidine and Prodine

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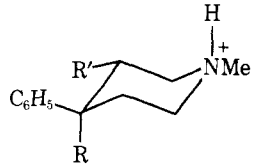
Extensive quantum chemical calculations have been made of the electronic distribution and conformational behavior of meperidine and desmethyl-, (+)-α-, and (+)-β-prodine using PCILO, a semiempirical molecular orbital method. For this series of opiates, a phenyl equatorial conformation was preferred over a phenyl axial one, with the equatorial conformer most favored in the most potent compounds. Using the low-energy equatorial conformer obtained for each compound, together with calculated net atomic charges, their observed potency variation could successfully be explained. From the results, these compounds appear to act at the morphine receptor with an identical piperidine rather than phenyl ring site.

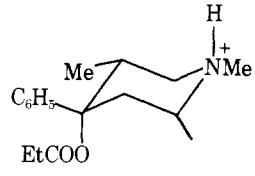
The relationship between the absolute stereochemistry of conformationally mobile opiate narcotics of the 4-phenylpiperidine class and their analgesic activity has been the subject of recent study.[1] The four optical isomers of prodine have been separated and their analgesic activity determined by standard methods along with desmethylprodine and meperidine.[2] In vivo potencies[3,4] and potencies from model guinea pig ileum studies[5] are known. Meperidine is one-tenth as potent as morphine when administered subcutaneously to mice and tested by a modified hot-plate method.[3] However, recent intraventricular data,[4] determined by a tooth pulp test in rabbits, indicate that it is only 1/60 as potent as morphine when transport and distribution factors are eliminated. Evidence for meperidine type opiates acting at the morphine receptor is given by the fact that both are antagonized by nalorphine and naloxone.[6] The differences in potency among the prodines have been shown[7] to be due solely to receptor interactions and not different brain level concentrations. This result is confirmed by the similarity of the (+)-α:(+)-β potency ratio in vivo[3] and in guinea pig ileum studies.[5]

There are only small structural differences between prodine (**1a**), desmethylprodine (**1b**), and meperidine (**1c**). The reversal of the ester chain from desmethylprodine to meperidine gives a tenfold potency difference. A difference in potency by a factor of almost 100 is seen in the optical isomers of prodine.



1a, R = OOCEt; R′ = CH_3
b, R = OOCEt; R′ = H
c, R = COOEt; R′ = H

The significant conformational aspects of this series that could have direct bearing on receptor site interaction are (1) the conformation of the piperidine ring (chair, boat, or skew boat); (2) the relationship between the phenyl and piperidine rings, phenyl axial (**2a**) or phenyl equatorial (**2e**);



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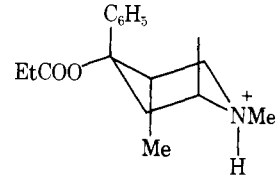
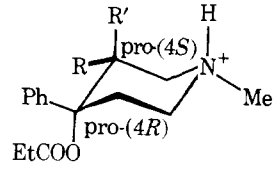
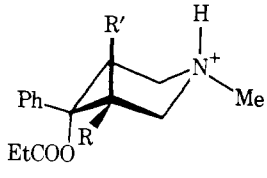


2a

(3) the torsion angle (τ_1) between the phenyl and piperidine rings; (4) the torsion angle (τ_2) relating the ester chain and the piperidine ring; and (5) the mirror conformations of the diasteriomers (**3a,b** and **4a,b**). Differences in electronic structure, especially between meperidine and prodine, could also contribute to potency differences.



3a [(+)-α], R = Me; R′ = H
b [(+)-β], R = H; R′ = Me



4a [(−)-α], R = Me; R′ = H
b [(−)-β], R = H; R′ = Me

By analogy to the morphine structure (Figure 1), it was proposed that an axial phenyl conformation would be better at the receptor site.[8] On this basis it was thought that the enhanced potency of the β isomer was due to its greater propensity to exist in the axial conformation even though subsequent X-ray structures of α- and β-prodine[9-11] and meperidine[12] all show a phenyl equatorial conformation.

Based on ^1H NMR studies, a different hypothesis was advanced that the enhanced potency of the β isomer was due to the presence of a skew boat conformer of the piperidine ring.[13] However, more recent work seems to favor the phenyl equatorial, piperidine chair conformer.[14] Further

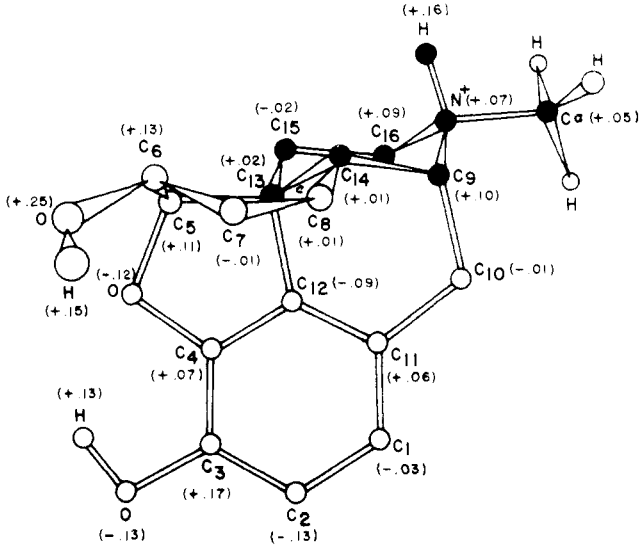


Figure 1. Minimum energy conformation of morphine from X-ray structure[31] and previous calculations.[26]

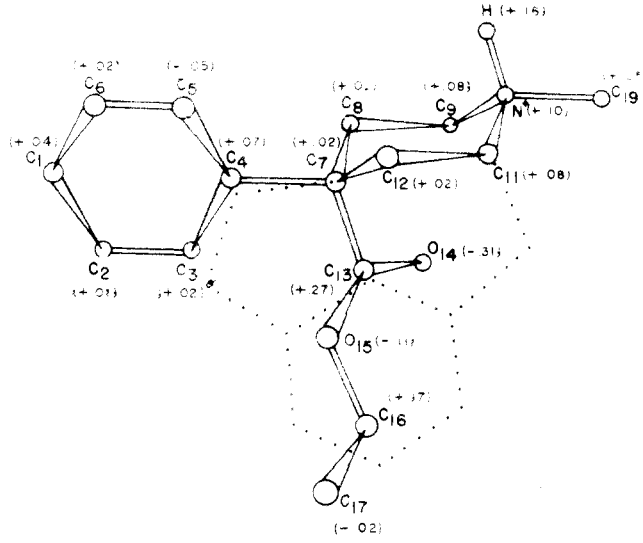


Figure 2. Minimum energy conformer of meperidine with piperidine ring superimposed on that of morphine (pharmacophore I) and net atomic charges.

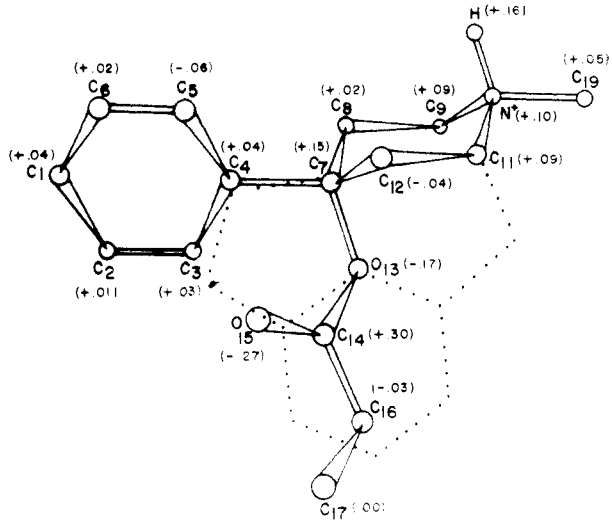


Figure 3. Desmethylprodine with piperidine ring superimposed on that of morphine (pharmacophore I) and net atomic charges (minimum energy conformer).

experiments with conformationally restricted prodine analogs have now definitively demonstrated that both equatorial and axial phenyl conformations can be equally efficacious.[15] Additional evidence against the requirement for a phenyl axial conformation has been presented by showing that the α:β potency ratio is reversed in the 3-allyl-, propyl-, and ethylprodine analogs.[16-18]

More recently it has been theorized[3,19] that the 3-substituent on the piperidine ring affects the receptor interaction in two ways. First, the receptor can discern enantiomeric edges and therefore the chirality of the 3-substituent positions is such that it either enhances or hinders the receptor interaction. Secondly, the placing of the 3-substituent on the different chiral edges affects the conformation of the molecule (τ_1), producing more and less favorable conformations. No definitive conformational studies have been done, however, to test these effects.

The techniques now available to quantum chemistry allow the detailed investigations of the electronic distribution and energy–conformation behavior of molecules. We have used a semiempirical molecular orbital method to study the behavior of the four optical isomers of prodine, desmethylprodine, and meperidine. The goal of the work was to identify the preferred conformations of each molecule, to relate these conformational aspects and their electronic structure to their potencies, and identify a favorable pharmacophoric orientation for the 4-phenylpiperidine narcotics at the receptor site.

Experimental Section

The method utilized in this work is a refined all-valence-electrons procedure, designated the Perturbative Configuration Interaction using Localized Orbitals (PCILO) method,[20] obtained from the laboratory of B. Pullman. This method has been used successfully in the study of conformations of a large number of biomolecules.[21-24] The original geometries were taken from the crystal structures for protonated α- and β-prodine[9-11] and for meperidine.[12] For the purpose of this study nitrogen was protonated because the pK_a's show these compounds to be >90% protonated at physiological pH's.[25] Torsion angles τ(AB–CD) are defined as rotation of A into D clockwise about axis BC. τ_1 is defined by atoms C_3C_4–C_7C_8 and τ_2 is defined by atoms C_4C_7–$C_{13}O_{15}$ for meperidine (Figure 2) and by atoms C_4C_7–$O_{13}C_{14}$ for prodines (Figure 3). It should be noted that the enantiomeric pairs of prodines will have equivalent energy–conformation behavior as long as their torsion angles are reflected through the mirror plane defined by atoms 4,7,13.

In their crystal structures, α- and β-prodine and meperidine were all found to be in a phenyl equatorial form with the torsion angles τ_1 (C_3C_4–C_7C_8) = 58°, τ_2 (C_4C_7–$O_{13}C_{14}$) = 294° for (+)-α; τ_1 = 158°, τ_2 = 62° for (−)-β; and τ_1 = 0°, τ_2 = 90° for meperidine. In all three structures the NCH_3 group was equatorial and the ester chain was an extended chain to within 10°. The positions of the hydrogen atoms were placed only in the crystal structure of the β isomer, and the 1- and 3-methyl groups were both staggered. There were small bond angle and bond length differences between the two isomers with a maximum of 3° in angles and 0.2 Å in length. Energy calculations of the crystal structures yielded the α isomer 30 kcal/mol more stable than the β isomer. The geometries of each isomer were then regularized to eliminate small discrepancies between the crystal structures and to assure perfect mirroring of the enantiomorphic pairs. The phenyl group was made into a perfect benzene ring with standard bond lengths and angles; the piperidine ring was similarly regularized into a perfect chair and the ester chain was made into a perfect extended chain. By regularization, the energy of the α and β isomers became respectively 2 and 28 kcal/mol more stable. The energy difference between them was reduced to 2.5 kcal/mol (in favor of the α isomer), a much more reasonable energy difference between isomers. These regularized structures were then used to calculate the energy–conformation behavior of α- and β-prodine. They also formed the basis of the geometries used for desmethylprodine and meperidine, since the crystal structure geometry for meperidine was very close to that of the prodines.

Table I. Salient Energy-Conformation Characteristics of Six Compounds Studied

Compound	X-Ray[f] structure		Low-energy conformers						ΔE (eq–ax), kcal/mol
			ϕ equatorial			ϕ axial			
	τ_1[a]	τ_2[a]	τ_1[a]	τ_2[a]	ΔE[b]	τ_1[a]	τ_2[a]	ΔE[b]	
Meperidine	0	90	60 (0–90)[c]	0	0	30	±45	0	5.3
			120 (60–150)	±90	0	30	All values	2	
			60	0 ± 90	4				
			150	±45	4				
Desmethylprodine			60	0	0	30	±45, 180	0	6.6
			150	±45	0				
			30 (0–30)	±45	3	30	All values	3	
			120 (60–120)	±90	4				
α-(+)-Prodine	58	294	105 (75–135)	300	0	45 (30–45)	0 (300–60)	0	8.6
α-(−)-Prodine	(182)[d]	(66)	135 (105–165)	60	0	15 (15–30)	0 (300–60)	0	8.6
β-(+)-Prodine	(82)[d]	(248)	90 (45–105)	300	0	0	180[e]	0	21
			45	0 (300–60)	2.5	120 (105–120)	180 (180–300)	1	
β-(−)-Prodine	158	62	150 (135–195)	60	0	0	180[e]	0	21
			195	0 (300–60)	2.5	120 (120–135)	180 (60–180)	1	

[a] τ_1, τ_2 in degrees as defined in the Experimental Section. [b] ΔE relative to minimum energy conformer in kcal/mol. [c] Values of angles in parentheses indicate range of broad minima. [d] Inferred values from X-ray structure of isomer. [e] In these conformers the 3-CH_3 group was eclipsed rather than staggered. [f] See ref 9–12.

In our study of conformational behavior, nested (i.e., all combinations of) rotations about τ_1 and τ_2 in both the equatorial and axial positions of the phenyl ring in (+)-α-, (+)-β-, and desmethylprodine and meperidine were performed. The ester chain was kept in an extended chain, staggered position. The rotational positional of the 1- and 3-methyl groups was varied from staggered only in selected conditions in which their position appeared to be significant in determining optimum energies. Trial calculations were performed with the *N*-methyl group axial for both phenyl equatorial and phenyl axial positions. In all cases the energy of the equatorially placed group was at least 3 kcal more favorable and, hence, the equatorial position of the *N*-methyl group was used in the rotational calculations.

Because of the symmetry of the phenyl ring, τ_1 was only varied through 180° in 30° intervals, calculating additional points when necessary to locate minima. For unsubstituted piperidines, τ_2 also was only varied through 180°, while for α- and β-prodine τ_2 was varied through 360° in 45–60° intervals.

Figures 8–15, which give the complete energy curves of the axial and equatorial forms of all compound studies, will appear in microfilm (see paragraph at end of paper regarding supplementary material).

Results

Table I summarizes the salient energy conformation behavior for all the protonated molecules. The net atomic charges for meperidine and prodine are included in Figures 2 and 3. These did not vary significantly as a function of conformation nor were they significantly different for any of the prodines. For comparison, the net charges calculated for protonated morphine from PCILO[26] are given in Figure 1.

Meperidine exhibited a very flat energy curve in the phenyl equatorial position. Two very broad absolute minima were found at $\tau_1 = 60°$, $\tau_2 = 0°$ and $\tau_1 = 120°$, $\tau_2 = 90°$. In addition, two broad local minima ($\Delta E = 4$ kcal/mol) at $\tau_1 = 60°$, $\tau_2 = 45°$ and $\tau_1 = 150°$, $\tau_2 = 45°$ were obtained. The axial conformer was much less flexible with steep local minima at $\tau_1 = 30°$ for all values of τ_2. The absolute minima was at $\tau_1 = 30°$, $\tau_2 = 45°$ with all other $\tau_1 = 30°$ values within 2 kcal of it. The best equatorial conformer was 5.3 kcal/mol more stable than the best axial conformer.

Desmethylprodine differs from meperidine only in the reversal of the ester chain. In the equatorial form two absolute minima were found, both corresponding to very low energy forms of meperidine at $\tau_1 = 60°$, $\tau_2 = 0°$ and $\tau_1 = 150°$, $\tau_2 = 45°$. Unlike meperidine, there are only two relative minima less than 10 kcal at $\tau_1 = 30°$, $\tau_2 = 45°$ and $\tau_1 = 120°$, $\tau_2 = 90°$. The axial behavior is much like meperidine with all values of τ_2 at $\tau_1 = 30°$ local minima and the absolute minima at $\tau_1 = 30°$, $\tau_2 = 45°$ and $\tau_1 = 30°$, $\tau_2 = 180°$. The best equatorial conformer was 6.6 kcal/mol more stable than the best axial.

(+)-α-Prodine has a 3-methyl group trans to the phenyl ring. The equatorial conformer exhibits very conformationally restricted behavior with a single broad absolute minimum at $\tau_1 = 150°$, $\tau_2 = 300°$ [$\tau_1 = 135°$, $\tau_2 = 60°$ for (−)-α]. There are no local minima within 18 kcal/mol. The axial conformer has behavior similar to the previous two axial conformers with the absolute minimum at $\tau_1 = 45°$, $\tau_2 = 0°$ [$\tau_1 = 15°$, $\tau_2 = 0°$ for (−)-α]. There are accessible minima in the range $\tau_1 = 30$–$45°$, $\tau_2 = 300$–$60°$ [$\tau_1 = 15$–$30°$, $\tau_2 = 300$–$60°$ for (−)-α]. The equatorial conformer is 8.6 kcal/mol more stable.

(+)-β-Prodine has the 3-methyl group cis to the phenyl ring. The equatorial conformer has a broad absolute minimum at $\tau_1 = 90°$, $\tau_2 = 300°$ [$\tau_1 = 150°$, $\tau_2 = 60°$ for (−)-β]. One other accessible minimum exists ($\Delta E = 2.5$ kcal/mol) at $\tau_1 = 45°$, $\tau_2 = 30°$ [$\tau_1 = 95°$, $\tau_2 = 60°$ for (−)-β]. The axial conformer is quite different from the other three axial conformers; $\tau_1 = 30°$ is no longer a local minimum. An absolute minimum is found at $\tau_1 = 0°$, $\tau_2 = 180°$ [$\tau_1 = 60°$, $\tau_2 = 180°$ for (−)-β] with the 3-methyl group eclipsed. Other accessible minima occur at $\tau_1 = 120°$, $\tau_2 = 180$–$300°$ [$\tau_1 = 120°$, $\tau_2 = 60$–$180°$ for (−)-β]. The equatorial conformer is 21 kcal/mol more stable than the axial.

Discussion

In the interpretation of our results, not only absolute minima but all local minimum with $\Delta E \leq 15$ kcal/mol are considered. While it is not totally impossible, it is highly unlikely that perturbations at the receptor site could provide more than this amount of energy.

For each compound, the calculated low-energy conformers are consistent with the X-ray data, but our results show additional low-energy conformers in τ_1 and τ_2 (Table I). In each case the energy of the best phenyl equatorial conform-

Table II. Relationship between Potency and Relative Axial Energies in Meperidine and Prodines

Drug	(±)-β-Prodine	(±)-α-Prodine	Desmethylprodine	Meperidine
ΔE (eq–ax)[a]	21.0	8.6	6.6	5.3
Potency (ED_{50})	0.32	1.7	1.3	13.1

[a] ΔE in kcal/mol.

er was significantly lower than the best phenyl axial one. As noted in Table II, the higher the energy of the axial form, the more potent the drug. These results confirm previous objections[15,27] to the idea that a phenyl axial conformation per se is more efficacious at the receptor.

Even the axial forms (with the exception of β-prodine) do not resemble those of rigid opiates. The phenyl rings of these more flexible opiates are approximately perpendicular (τ_1 = 30°) to their fixed position in rigid opiates (τ_1 = 145°). While the β-prodine does have a minimum energy axial conformer which mimics rigid opiates, it is 21 kcal/mol less stable than the equatorial form.

Our results strongly indicate that these 4-phenylpiperidine compounds act in a phenyl equatorial form unless there is a large amount of energy available for conformation changes at the active site. Since complete superposition of a phenyl equatorial form of these compounds on rigid opiates is impossible, it is evident that these two classes of opiates occupy only partially overlapping receptor sites. This result is consistent with a similar suggestion made on the basis of observed differences in some of their physiological effects and in the nonparallel behavior between phenylpiperidine derivatives and derivatives of morphine-like compounds, with at least the three fused rings of the 6,7-benzomorphan nucleus.[1,27] The question remains as to the actual orientation of the phenylpiperidine compounds at the receptor.

We have considered two likely orientations these compounds could take at the opiate receptor. Relative to the rigid opiates these orientations would differ in either their phenyl or nitrogen group contact.

In one orientation (I) (Figures 2 and 3) the piperidine ring of these compounds is directly superimposed on the piperidine ring of morphine, used as the prototype rigid opiate, implying a fixed cationic receptor for both types of compounds. The phenyl ring is then displaced to a position identical with that proposed for the phenyl substituent of 5-phenylbenzomorphan (GPA 1657).[28] In the other orientation (II) (Figures 4 and 5) the phenyl rings of the two types of compounds are assumed to make the same receptor contact, displacing the piperidine rings. An optimum correlation between observed potencies and calculated properties is obtained using the first orientation, i.e., assuming a fixed cationic receptor for the two types of opiates with a displaced phenyl ring contact as shown schematically by Portoghese.[1,27]

Meperidine (Figure 2) and desmethylprodine (Figure 3) have similar low-energy conformers. While meperidine has many possible low-energy values of τ_1 and τ_2, desmethylprodine is much more rigid with high-energy barriers between the local minima noted in Table II. Thus meperidine could assume any conformation that the more potent desmethyl conformer could at the receptor site. The differences in apparent potency between them do not then appear to lie in different conformations relative to τ_1 and τ_2 but rather to differences in the relative position of the polar atoms of the ester chain. It is possible that the reversal of the ester chain affects properties related to transport and distribution in which case intraventricular data for the two compounds would decrease the apparent tenfold difference in potency between them. Alternatively, the desmethylprodine ester chain might make more efficacious contact with the receptor site. The receptor site for the ester chain is not known. While tentative, inferences about its nature can be made by complementarity to those portions of rigid opiates which bind there.

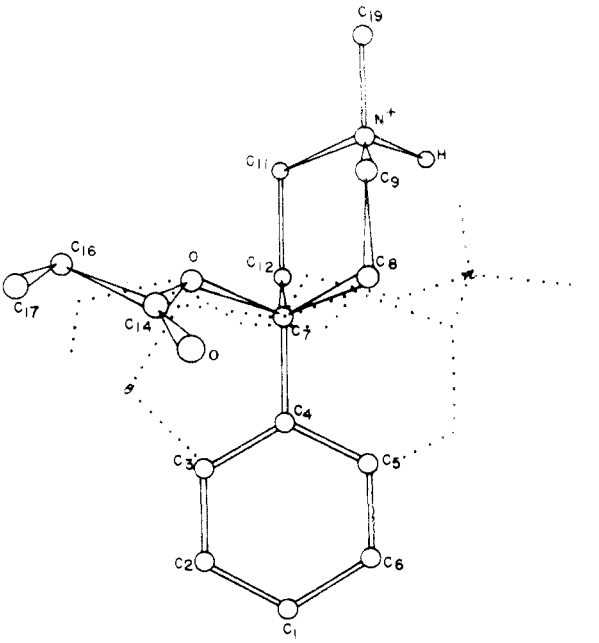


Figure 4. Pharmacophore II. Minimum energy conformer of meperidine with phenyl ring superimposed on that of morphine.

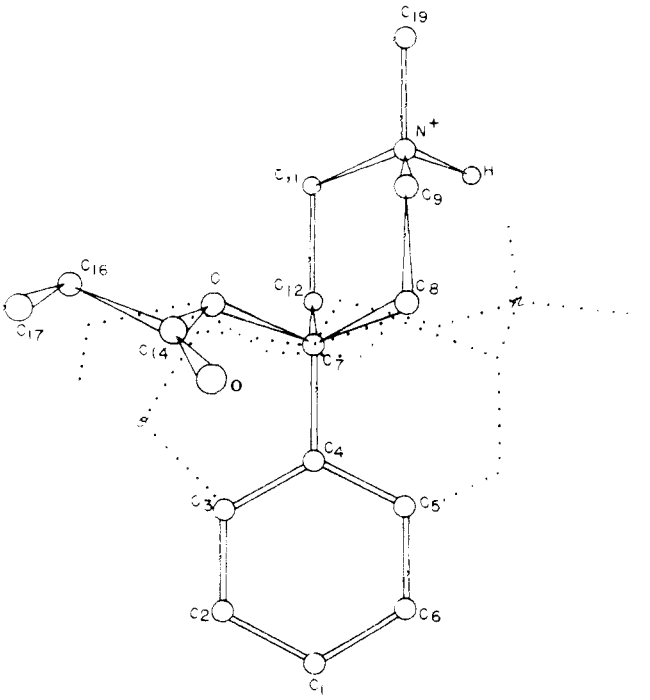


Figure 5. Pharmacophore II. Minimum energy conformer of prodine with phenyl ring superimposed on that of morphine.

In orientation I of desmethylprodine (Figure 3), the highly negative carbonyl oxygen (O_{15}) is very close to the negative furan oxygen of morphine (Figure 1); the positive carbonyl carbon (C_{14}) is near the positive C_4 of morphine and the negative ester oxygen (O_{13}) is nearly superimposed

on the negative C_{12} of morphine. By contrast, the reversal of the ester group to meperidine (Figure 2) superimposes the polar O=C—O group on a relatively neutral part of the phenyl ring in morphine far from either the negative furan oxygen, the positive C_3, or the phenolic oxygen. Thus, if the ability of the 4-phenylpiperidines to mimic the phenyl and furan contact made by morphine is important, the more potent desmethylprodine accomplishes this more completely than meperidine.

In orientation II (Figures 4 and 5) with the phenyl rings placed as they are in rigid opiates, a different consequence of ester chain reversal is seen. In both compounds, the O=C—O group is near the furan oxygen and C-ring region of morphine making it more difficult to account for the higher potency of desmethylprodine, an argument in favor of fixed nitrogen receptor contact (orientation I).

From brain level studies,[7] the difference in potency between desmethyl and the four isomers of prodine appears to be due to receptor events. Our results further suggest that conformational differences rather than differences in electronic structure are important. The charge on C_8 and C_{12} remains the same in desmethyl and all the prodines, and all other atoms have similar net charges in these compounds. Differences in τ_1 and τ_2 could both affect relative potency. The most potent isomers [(+)-α, (+)-β] have lowest energy values around τ_1 = 90–105°, while the least potent isomers [(−)-α, (−)-β] are most favored for τ_1 = 135–150°. Meperidine and desmethylprodine are symmetrical about the mirror plane and therefore are equally favored at each set of values. This torsion angle difference in the (+)/(−) isomers confirms previous speculation based on the X-ray structures and absolute configuration.[3,19]

In orientation I the cationic group is positioned as in morphine, i.e., behind the ϕ plane, in the more potent (+) isomers, and in front of this plane in the (−) isomers. The reverse is the case for orientation II. While it is uncertain that the cationic group need be on the same side of the ϕ group as it is found to be in rigid opiates, this correlation is another argument in favor of orientation I and helps to understand differences in (+)/(−) potencies.

Differences in τ_2 appear to be most directly related to the enhanced potency of the (+) isomers, particularly if orientation I is assumed. In this orientation, the minimum energy conformers of the least potent isomers (−) have τ_2 = 60° (Figure 6), causing the ester chain to protrude into the presumably planar phenyl receptor site of rigid opiates on the same side as the piperidine ridge atoms. This position could hinder receptor interaction with the phenyl and piperidine rings. A value of τ_2 = 300° in the most potent (+) isomers (Figure 7) places the ester chain on the opposite side as the piperidine ridge atoms but still close enough to the plane of the postulated phenyl receptor site for rigid opiates to interact with the receptor. It is interesting to note that the potent isomer (−)-α is "locked" into the unfavorable value of τ_2 = 60°, whereas (−)-β, though favored at τ_2 = 60°, has an accessible energy minima at 300° as well. Thus on the basis of τ_2 alone, the predicted order of potency would be (+)-α = (+)-β = (+)-β > (−)-α in good agreement with potencies. On the contrary, using orientation II with a fixed phenyl receptor site, the predicted order of potencies would be reversed since τ_2 = 300° is hindering and τ_2 = 60° is not, further argument against such an orientation.

As a final agreement for a fixed cationic receptor site, the net atomic charges on the nitrogen and its surrounding atoms in prodine and meperidine match perfectly with morphine. Thus these molecules could have the same delocalized interaction with a diffuse anionic receptor site as has been previously proposed for morphine.[26,29] In addition, the importance of the orientation of the nitrogen lone pair (proton) has recently been linked to analgesic potency.[30] Orientation I places the lone pair in exactly the same position as morphine, whereas orientation II actually points the lone pair in the opposite direction.

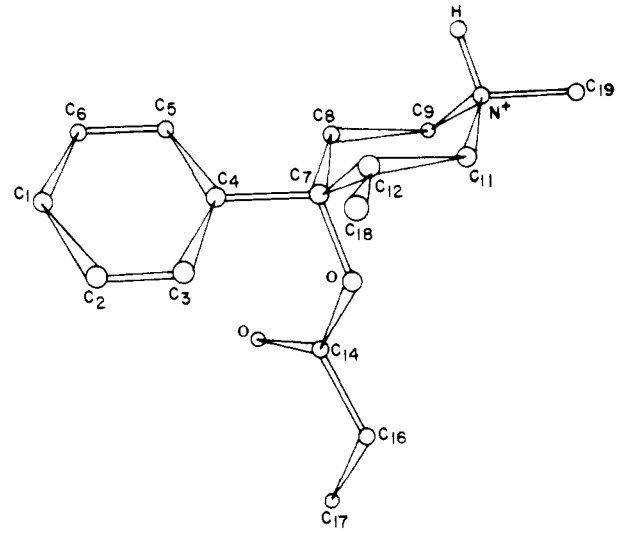


Figure 6. Minimum energy conformer for (−)-α- and (−)-β-prodine in pharmacophore I.

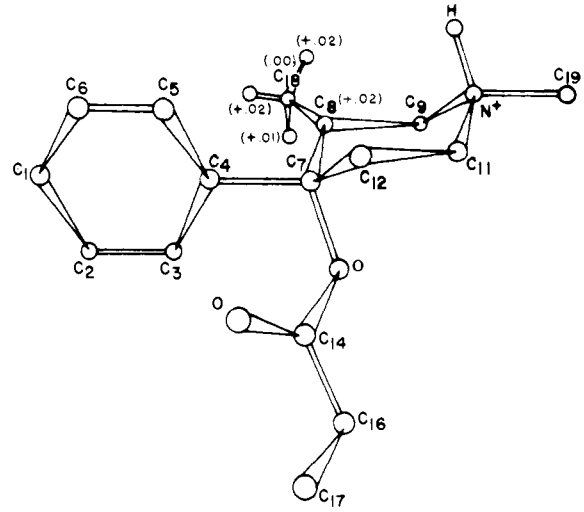


Figure 7. Minimum energy conformer for (+)-α- and (+)-β-prodine in pharmacophore I orientation. Net charges on C_8 carbon and methyl group given. All other net charges as in desmethylprodine.

Thus the sum of the conformational and electronic results presents strong evidence that flexible 4-phenylpiperidine narcotics act in an equatorial conformation with the same cationic receptor site as the rigid opiates. On this basis, the relative potencies of meperidine and desmethylprodine have been accounted for and the potency difference from (+) to (−) isomers is satisfactorally explained.

The reasons for the (+)-α-, (+)-β-, and desmethylprodine potency differences are less clear. They do not appear to be a conformational effect as all three compounds have accessible energy minima at similar values of τ_1 and τ_2 and have the same electron distribution even on C_8. An explanation has been offered,[19] however, that a hydrophobic pocket is present in the receptor which preferentially fits the methyl group in the axial β position. Accommodation for the equatorial α-methyl group must also exist since it is on a piperidine ridge carbon atom. But such accommodation need not add to receptor interactions and hence the

small increase in potency from desmethyl- to (+)-α-prodine.

Recently, data have been obtained[16-18] on the potencies of 3-allyl- and 3-propylprodine derivatives which confirm this idea. The potency of the (+)-α isomers is little affected by chain lengthening but become 40 times more potent by the presence of the allyl group. However, the potency of the (+)-β isomer is severely diminished when allyl or n-propyl is substituted for the methyl. These results are indicative of a "tighter" fit to a methyl group in the β rather than α position. Given the importance and inflexibility of τ_2, changes of the 3-substituent could also easily affect the minimum energy conformations of τ_2 and hence cause variations in the relative potencies, in addition to the effect of changes of τ_1 and of the substituent itself. Work is in progress investigating conformational effects and electronic distribution in these compounds.

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Supplementary Material Available. Figures 8–15 will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 24× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Business Office, Books and Journals Division, American Chemical Society, 1155 16th St., N.W., Washington, D.C. 20036. Remit check or money order for $4.50 for photocopy or $2.50 for microfiche, referring to code number JMED-75-1051.

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A Hydrophobic Binding Site in Acetylcholinesterase

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The dissociation constants have been determined and compared for a series of reversible, noncovalent inhibitors of eel acetylcholinesterase that are structurally related to the very potent inhibitor, 1,2,3,4-tetrahydro-9-aminoacridine (THA). It is concluded that there exists on the enzyme protein, closely adjacent to the anionic subsite, a conformationally flexible, hydrophobic area which tends readily to assume a near planar form. The dimensions of this area are unknown, but it is adequate in size to fully accommodate THA. It is this area, acting conjointly with the adjacent anionic subsite, which provides the attraction for THA and related inhibitors. Uv absorbance maxima and pK_a values are reported for many of the compounds.

To rationally design inhibitors, reactivators, affinity probes, and other perturbing agents for acetylcholinesterase, one requires a knowledge of the surface of the enzyme in the vicinity of its active site. In charting the surface of a macromolecule it is basically assumed that a probing small molecule exhibits a degree of complementarity to its recognition site on the macromolecule. Hence, correlatable changes in small molecule structure with physical or chemi-