Stereoisomeric Ligands as Opioid Receptor Probes

PHILIP S. PORTOGHESE

Department of Medicinal Chemistry, College of Pharmacy, University of Minnesota, Minneapolis, Minnesota 55455

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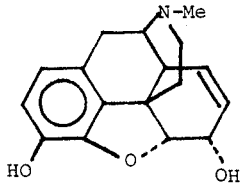
Opioids[1] are substances whose actions are similar to those of the prototype narcotic analgetic, morphine.



Morphine

Their principal therapeutic use is in the relief of pain. The interaction of opioids with biological systems has been the subject of extensive investigation over the past decade in an effort to acquire a better understanding of how these agents exert their effects.[2-12] Interest in this area of research has been heightened by the recent development of new opioid receptor binding procedures[13,14] and by the identification of endogenous opioid peptides known as endorphins.[15]

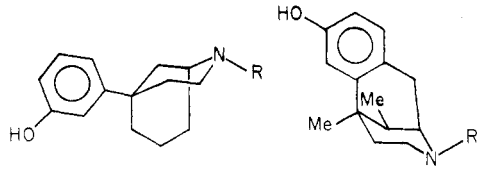
Early stereochemical investigations of synthetic opioids arose primarily as an outgrowth of structure–activity relationship studies, but the role of steric factors was largely unexplored and not well understood. The classical studies of Beckett and Casy[16] first drew attention to the use of stereochemically defined chiral ligands to study the geometry of opioid receptors, and this work stimulated a host of subsequent investigations in this area.[2,5] This Account reviews our use of stereoisomeric ligands as probes to explore opioid receptor topography. The results of such studies suggest that conformation and chirality play major and inseparable roles in determining enantiomeric stereoselectivity[17] of opioid receptors.

Multiple Modes of Interaction between Ligands and Opioid Receptors

A remarkable feature of the opioids is their diverse chemical constitution; yet they are highly selective in their effects, and they are known to interact with opioid receptors in a highly stereoselective fashion. This apparent paradox was resolved by the multiple modality model for the interaction of ligands with opioid receptors.[18] The model envisages the interaction of ligands with either a single type of opioid receptor or a group of related but not identical opioid receptors. In either case, multiple modes of interaction arise from the association of different ligands with different recognition loci on the receptors.

There are two criteria which have been employed to distinguish between multiple and identical binding modes. First, different modes of ligand–receptor interaction often lead to divergent stereochemical requirements for different opioids since a segment of these molecules resides in different receptor environments. The second criterion is based on the reasonable assumption that ligands involved in different modes of

Table I
Pharmacologic Activities of N-Substituted Phenylmorphan and Benzomorphan Derivatives



R	Equatorial-phenyl series: ED_{50}, μmol/kg[a,b]	Equatorial-phenyl series: Relative[c] potency	Axial-phenyl series: ED_{50}, μmol/kg[a,b]	Axial-phenyl series: Relative[d] potency
Me	8.7	1	11.3	1
CH_2CH_2Ph	12.5	0.7	0.32	35
$CH_2CH{=}CH_2$	20.1[e]	0.4	Antagonist	. . .

[a] All compounds in this table were tested subcutaneously in mice by the hot-plate method [N. B. Eddy and D. Leimbach, *J. Pharmacol. Exp. Therap.*, **107**, 385 (1953)]. [b] Data obtained from N. B. Eddy, *Chem. Ind. (London)*, 1462 (1959), unless otherwise specified. [c] Relative to R = Me in the equatorial series. [d] Relative to R = Me in the axial series. [e] Only a hint of antagonist activity was observed at the ED_{50} dose [H. H. Ong, T. Oh-ishi, and E. May, *J. Med. Chem.*, **17**, 133 (1974)].

Philip S. Portoghese is Director of Graduate Studies and Head of Medicinal Chemistry in the College of Pharmacy, University of Minnesota. He was born in New York City in 1931, studied at Columbia University for both the B.S. and M.S. degrees, and then went to the University of Wisconsin, where he received the Ph.D. in 1961. Among his research interests are structure–activity relationships of strong analgetics and narcotic antagonists, conformational and configurational analysis of drugs, and the design and synthesis of medicinal agents. Professor Portoghese is Editor of the *Journal of Medicinal Chemistry*.

(1) The term "opioid" is synonymous with narcotic analgetic and is defined as any substance that has morphine-like pharmacologic effects; see "Pharmacological Basis of Therapeutics", 5th ed, Louis S. Goodman and Alfred Gilman, Ed., Macmillan, New York, N.Y., 1975, p 245.
(2) P. S. Portoghese, *J. Pharm. Sci.*, **55**, 865 (1966).
(3) W. R. Martin, *Pharmacol. Rev.*, **19**, 463 (1967).
(4) H. R. Fraser and L. S. Harris, *Annu. Rev. Pharmacol.*, **6**, 277 (1967).
(5) P. S. Portoghese, *Annu. Rev. Pharmacol.*, **10**, 51 (1970).
(6) J. W. Lewis, K. W. Bentley, and A. Cowan, *Annu. Rev. Pharmacol.*, **11**, 241 (1971).
(7) N. B. Eddy and E. L. May, *Science*, **181**, 407 (1973).
(8) A. E. Takemori, *Annu. Rev. Biochem.*, **43**, 15 (1974).
(9) A. Goldstein, *Life Sci.*, **14**, 615 (1974).
(10) S. H. Snyder, C. B. Pert, and G. W. Pasternak, *Ann. Intern. Med.*, **81**, 534 (1974).
(11) H. W. Kosterlitz and A. A. Waterfield, *Annu. Rev. Pharmacol.*, **15**, 29 (1975).
(12) See *Annu. Reports Med. Chem.*, **11**, chapters 3 and 4 (1976), and previous volumes.
(13) E. J. Simon, J. M. Hiller, I. Edelman, *Proc. Natl. Acad. Sci. U.S.A.*, **70**, 1947 (1973).
(14) C. B. Pert and S. H. Snyder, *Science*, **179**, 1011 (1973).
(15) A. Goldstein, *Science*, **193**, 1081 (1976).
(16) A. H. Beckett and A. F. Casy, *J. Pharm. Pharmacol.*, **6**, 986 (1954).
(17) The term "stereoselectivity" rather than "stereospecificity" is employed where pharmacologic activity is found predominantly in one isomer, though not exclusively. The latter term implies that activity resides only in one isomer. Since the former situation is more widely observed among opioid ligands, this term is employed throughout this Account.
(18) P. S. Portoghese, *J. Med. Chem.*, **8**, 609 (1965).

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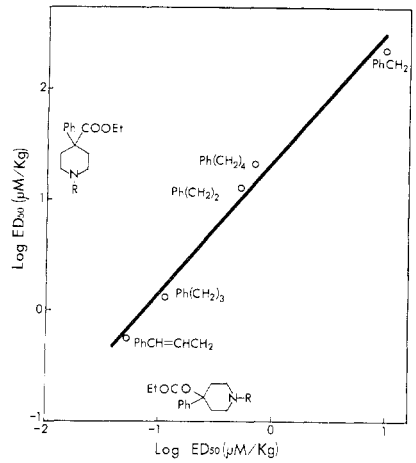


Figure 1. Correlation (r = 0.99) between the analgetic log ED_{50} dose in the normeperidine series (ordinate) vs. that in the corresponding reversed esters (abscissa) containing identical changes in the R group.

interaction should exhibit dissimilar structure–activity profiles. Thus, different modes of interaction between ligands and opioid receptors are characterized by a difference in the rank order potencies among congeners, in two or more series, whose N substituent is varied in an identical manner.[19] This is exemplified in Table I. Inasmuch as the phenylmorphans and benzomorphans have their aromatic groups fixed in equatorial and axial conformations, respectively, it is apparent that the overall geometry of the ligand is an important factor which determines the orientation of the N substituent in a specific receptor environment.

In contrast, ligands whose mode of interaction with opioid receptors is identical exhibit similar incremental changes in potency when identically N-substituted congeners in two series are compared. This is due to the identically modified N substituent in each series contributing to the pharmacologic effect by the same mechanism because it is situated in an identical physicochemical environment on the receptor. If, in fact, the biological data are sufficiently reliable, a plot of the potencies of the congeners in one series vs. those of a second series should afford a linear regression with a slope of unity. This would suggest that the incremental potency changes brought about by an identical modification of the N substituent in both series are the same. Such a relationship[19] is exemplified in Figure 1 and strongly suggests that the meperidine-type congeners and the corresponding reversed esters[20] possess very similar modes of interaction with opioid receptors.

$EtCOCPh_2CHRCHR^1NMe_2$

1a erythro R = R^1 = Me

1b threo R = R^1 = Me

2 R = R^1 = H

Both in vivo and in vitro studies[21,22] of meperidine congeners suggest that the observed potency differences are due to receptor-related events rather than to differential access to the receptors.

Table II
Enantiomeric Stereoselectivity of Opioid Receptors toward Ligands with a Chiral Center in Common with Methadone

$$R{-}CH_2\underset{\displaystyle Me}{\underset{|}{C}}H{-}B$$

Compd	R	B	Configuration[a]
1	Ph_2C-COEt (methadone)	NMe_2	R[b]
2	Ph_2C-COEt	NC_4H_8O	R[c]
3	Ph_2C-SO_2Et	NMe_2	R[b]
4	Ph_2C-COOEt	NM_2	S[b]
5	Ph_2C-CH(OH)Et	NMe_2	$3S,6S$[d,e]
6	Ph_2C-CH(OH)Et	NMe_2	$3S,6R$[d,e]
7	Ph_2C-CH(OAc)Et	NMe_2	$3R,6R$[d,e]
8	Ph_2C-CH(OAc)Et	NMe_2	$3S,6R$[d,e]
9	Ph_2C-CH(OAc)Et	NHMe	$3R,6R$[d,e]
10	PhN-COEt	$N(Me)CH_2Ph$	S[f,g]
11	PhN-COEt	$N(Me)CH_2C_6H_4$-p-Me	S[h]
12	PhN-COEt	$N(Me)CH_2CH_2Ph$	S[f,g]
13	PhN-COEt	$N(Me)CH_2CH_2$-CH_2Ph	S[h,i]
14	$(C_4H_3S)_2C{=}CHCH(Me)NMe_2$		R[b]
15	$(C_4H_3S)_2C{=}CHCH(Me)NEt_2$		R[b]

[a] Configuration of the more potent enantiomer. [b] A. H. Beckett and A. F. Casy, *J. Chem. Soc.*, 900 (1955). [c] A. H. Beckett and A. F. Casy, *ibid.*, 3076 (1957). [d] Reference 33. [e] E. L. May and N. B. Eddy, *J. Org. Chem.*, 17, 1210 (1952). [f] P. S. Portoghese and D. L. Larson, *J. Pharm. Sci.*, **53**, 302 (1964). [g] W. B. Wright and R. A. Hardy, Jr., *J. Med. Chem.*, **6**, 128 (1963). [h] P. S. Portoghese and T. N. Riley, *J. Pharm. Sci.*, **54**, 1831 (1965). [i] The S isomer is slightly more potent than R, though this may not be statistically significant.

Recent biochemical investigations[23-28] lend additional support to the multiple modality concept of binding. It is, however, not known whether different binding modes arise from the interaction of opioids with identical receptors or with a family of related receptors.

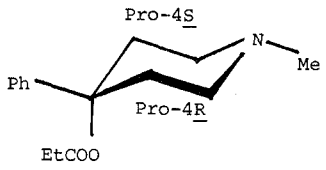
Chiral Ligands as Opioid Receptor Probes

Enantiomers have been used as opioid receptor probes because they complement structure–activity studies that utilize racemic or achiral congeners. The fact that enantiomeric ligands possess identical partition coefficients makes them more likely to achieve identical or similar brain levels as compared to congeners having constitutional differences. As this has been demonstrated[29-32] for a variety of enantiomeric pairs, it is reasonable to correlate potency differences between enantiomers with receptor-related events when differential metabolism is not an overriding factor.

(19) P. S. Portoghese, *J. Pharm. Sci.*, **54**, 1077 (1965).
(20) P. A. Janssen and N. B. Eddy, *J. Med. Pharm. Chem.*, **2**, 31 (1960).
(21) D. L. Larson and P. S. Portoghese, *J. Med. Chem.*, **19**, 16 (1976).
(22) C. Pert, S. Snyder, and P. S. Portoghese, *J. Med. Chem.*, **19**, 1248 (1976).
(23) C. B. Pert and S. H. Snyder, *Mol. Pharmacol.*, **10**, 868 (1974).
(24) E. J. Simon, J. M. Hiller, J. Groth, and I. Edelman, *J. Pharmacol. Exp. Ther.*, **192**, 531 (1975).
(25) G. W. Pasternak, H. A. Wilson, and S. H. Snyder, *Mol. Pharmacol.*, 11, 340 (1975).
(26) G. W. Pasternak and S. H. Snyder, *Mol. Pharmacol.*, 11, 478 (1975).
(27) I. Creese, G. W. Pasternak, C. B. Pert, and S. Snyder, *Life Sci.*, 16, 1837 (1975).
(28) T. Akera, C.-Y. Lee, and T. M. Brody, *Life Sci.*, **16**, 1801 (1975).
(29) N. A. Ingoglia and V. P. Dole, *J. Pharmacol. Exp. Ther.*, **175**, 84 (1970).
(30) B. A. Berkowitz and E. L. Way, *J. Pharmacol. Exp. Ther.*, **177**, 500 (1971).
(31) M. M. Abdel-Monem, D. L. Larson, H. J. Kupferberg, and P. S. Portoghese, *J. Med. Chem.*, **15**, 494 (1972).
(32) H. R. Sullivan, S. L. Due, and R. E. McMahon, *J. Pharm. Pharmacol.*, **27**, 728 (1975).

Indeed, this feature has been central to the development of methods[15] for the biochemical and pharmacologic characterization of opioid receptors.

Methadone and Related Structures. As it is apparent that structural differences between ligands can



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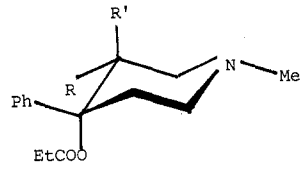
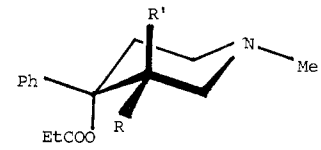
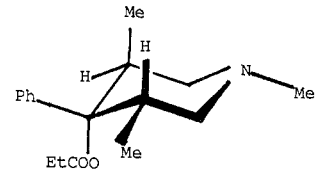
give rise to divergent modes of interaction with opioid receptors, the model suggests that this may be manifested by an inversion of receptor stereoselectivity. This could arise as a consequence of the interaction of a common chiral unit in the ligands with different topographic environments on the receptor. Thus, if the more potent enantiomers of two racemates each possess a common chiral center of opposite configuration, this might suggest different modes of ligand-receptor binding. The lack of consistency in the relationship between chirality and potency (Table II) among ligands having a chiral center in common with methadone illustrates this point. However, if these isomers are categorized into groups which have similar modes of interaction with opioid receptors, the stereostructure–activity relationship becomes clarified. Such analysis is possible when the potencies of a sufficient number of N-substituted analogues are known so that the rank order potencies in different series can be compared as a function of N substitution.[2] Using this approach we have concluded[18] that the modes of interaction of **1–3**, **7–9**, **14**, and **15** (Table II) with opioid receptors are similar. Note that the configurations of these ligands are identical. The anilides **10–13** fall into a distinctly different category by these standards; this is corroborated by the fact that the more potent enantiomers in this series have a chiral center whose configuration is opposite to that of the aforementioned group. Although these anilides and the methadone analogue **4** are of identical configuration, their rank order potencies arising from N-substituent variation are different and thus suggest different modes of binding at opioid receptors.

An interesting example of inverted stereoselectivity is seen among ligands having the same carbon skeleton as methadone. The 3*S*,6*S* (**5**) and 3*S*,6*R* (**6**) diastereomers of methadol[33,34] (Table II) are more potent than either of their corresponding enantiomers or (6*S*)-methadone (less active enantiomer). This minor modification therefore results in the inversion of receptor stereoselectivity from 6*R* in methadone to 6*S* in **5**. As the 3*S* configuration also is present in α-isomethadol (**2**, Table III) and 6-demethylmethadol,[35] it appears that the hydroxyl function in the more potent enantiomers plays an important role in aligning the ligands on the opioid receptor. We suggest that the hydroxyl function has a recognition locus on the receptor which is different from that which binds a carbonyl group. Consistent with this view is the fact that acetylation relegates the C(3) chiral center to a relatively minor stereochemical role in that both methadol esters (**6** and **7**, Table II) have the 6*R* configuration. The diminished importance rendered to C(3)





4a	R = Me, R′ = H	**5a**
4b	R = H, R′ = Me	**5b**
10a	R = Et, R′ = H	**11a**
12a	R = Pr, R′ = H	**13a**
12b	R = H, R′ = Pr	**13b**
14a	R = $CH_2CH{=}CH_2$, R′ = H	**15a**
14b	R = H, R′ = $CH_2CH{=}CH_2$	**15b**



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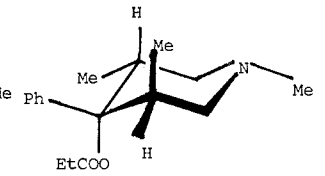

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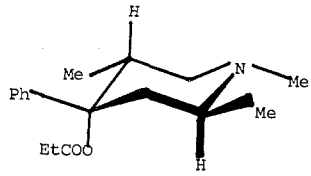

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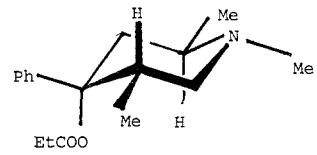

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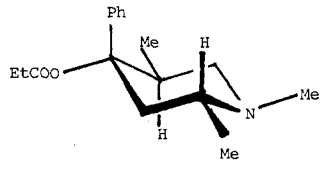

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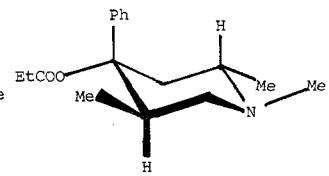

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by acetylation also is observed in the isomethadone series as reflected by the more potent enantiomers (**3** and **4**, Table III) having opposite configuration at this center.

A model which depicts a possible mechanism for the inversion of receptor stereoselectivity for methadone and its derivatives is presented in Figure 2. Proton-acceptor and proton-donor hydrogen-bonding sites situated in different receptor environments would be capable of forming hydrogen bonds with ligands containing hydroxyl and carbonyl functions, respectively.[33,34] The inversion of stereoselectivity at C(3) or C(6) would arise as a result of differences between the orientation of the receptor-bound chiral units in ligands which contain a proton donor group (i.e., hydroxyl) and those which do not (i.e., ketone and ester groups). Although the model depicts the different modes of interaction as occurring on a single type of receptor, the same principles can be used if the ligands bind to a family of receptors having different stereoselectivities.

X-ray crystal structures of salts of methadone,[36] isomethadone,[37] and α-methadol acetate[37] indicate that

(33) P. S. Portoghese and D. A. Williams, *J. Med. Chem.*, **12**, 839 (1969).
(34) P. S. Portoghese and D. A. Williams, *J. Med. Chem.*, **13**, 626 (1970).
(35) A. F. Casy and M. M. A. Hassan, *J. Med. Chem.*, **11**, 601 (1968).
(36) A. W. Hanson and F. R. Ahmed, *Acta Crystallogr.*, **11**, 724 (1958).
(37) E. Shefter, *J. Med. Chem.*, **17**, 1037 (1974).

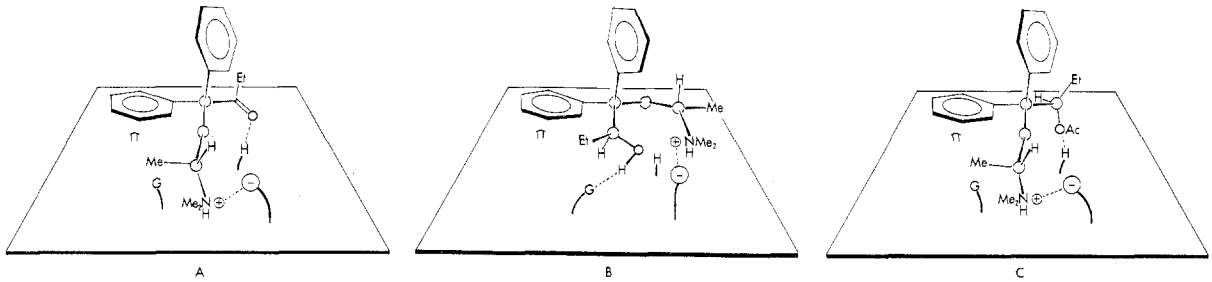
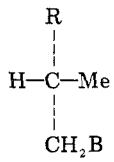


Figure 2. A model rationalizing how modification at C(3) might change the mode of interaction of methadone and related ligands with opioid receptors. Panels A, B, and C illustrate the binding of more potent enantiomers (6*R*)-methadone, (3*S*,6*S*)-methadol, and acetyl-(3*R*,6*R*)-methadol respectively. Hydrogen-bonding proton donor (H) and acceptor (G) sites in different locations on the receptor are postulated to play an important role in the orientation of the ligands. Additional points of interaction are with the anionic site (⊖) and with an aromatic binding site (π).

Table III
Enantiomeric Stereoselectivity of Opioid Receptors toward Ligands with a Chiral Center in Common with Isomethadone



Compd	R	B	Configuration[a]
1	Ph_2CCOEt (isomethadone)	NMe_2	5*S*[b]
2	$Ph_2CCH(OH)Et$	NMe_2	3*S*,5*S*[c,d]
3	$Ph_2CCH(OAc)Et$	NMe_2	3*R*,5*S*[c,d]
4	$Ph_2CCH(OAc)Et$	NMe_2	3*S*,5*S*[c,d]
5	$PhCH_2C(Ph)OCOEt$	NMe_2	2*S*,3*R*[e]
6	PhNCOEt	NC_5H_{10}	2*R*[f]

[a] Configuration of more potent enantiomer. [b] A. H. Beckett, G. Kirk, and R. Thomas, *J. Chem. Soc.*, 1386 (1962). [c] Reference 34. [d] E. L. May and N. B. Eddy, *J. Org. Chem.*, 17, 1210 (1952). [e] H. R. Sullivan, J. R. Beck, and A. Pohland, *ibid.*, 28, 2381 (1963); A. F. Casy and J. L. Myers, *J. Pharm. Pharmacol.*, 16, 455 (1964). [f] P. S. Portoghese, *J. Med. Chem.*, 8, 147 (1965).

the more potent enantiomers possess very similar C(4)–C(5)–C(6)–N torsion angles, ~−150°. On the other hand, the equivalent group of atoms in salts of α-methadol[37] (**5**) and anilide[38] **10** (Table II), both of which display inverted receptor stereoselectivity, assume different conformations (116 and 54°, respectively). While such crystal-structure analyses do not provide definitive information on the conformations of these ligands in solution, these data are nevertheless consistent with the concept that different modes of ligand–receptor interaction arise as a consequence of constitutional and conformational differences among the opioid ligands.

In general, it might be expected that conformationally mobile ligands would be more likely to display dissimilar modes of interaction with opioid receptors than those that are less flexible or rigid. This would be due to the flexible congeners binding to opioid receptors in different conformations, as compared to conformationally restricted ligands. In this regard, a feature which distinguishes congeners related to methadone (Table II) from those of isomethadone (Table III) is that the more potent enantiomers in the former group do not all possess the same configuration at the equivalent chiral center, while in the latter group the receptor stereoselectivity is invariant. Indeed, the results of studies outlined below are consistent with the idea that the difference between these series is related in part to differences in conformational mobility.

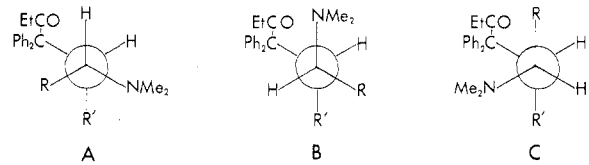


Figure 3. Projection formulas representing staggered conformations of the C(4)–C(5)–C(6)–N moiety of (6*R*)-methadone (R = Me; R′ = H) and (5*S*)-isomethadone (R = H; R′ = Me).

Circular dichroism studies[39] of methadone show a solvent-induced reversal in the sign of the Cotton effect associated with the n → π^* carbonyl transition when there is a change from aprotic to hydroxylic solvent. It is likely that this arises from a conformational change due to the disruption of the N:→C=O[40] interaction as a consequence of solvent hydrogen bonding with the carbonyl and amine functions. Unlike methadone, isomethadone shows no appreciable change in magnitude or sign of the Cotton effect. Another difference between these ligands consistent with the above is the rapid proton exchange at the C(2) position of methadone due to intramolecular catalysis by the basic nitrogen and the absence of facile proton exchange in isomethadone.[39] These data also are in harmony with the relative values of the dissociation constants for the salts of these compounds[33,34] in that methadone is considerably more basic than isomethadone (ΔpK_a = 0.86) due to intramolecular stabilization of the conjugate acid (C=O····HN^+). Finally, NMR studies[39] suggest that methadone base and its salt exist as a mixture of conformers (Figure 3) whose distribution is sensitive to change of solvent polarity. On the other hand, isomethadone appears to be less affected by solvation factors, with an antiperiplanar-type conformation, A, predominating for the Ph_2CCOEt and NMe_2 groups (Figure 3).

These studies suggest that methadone possesses greater conformational mobility than isomethadone (either as the base or salt) and that the greater propensity of the latter to reside in conformation A (Figure 3) is responsible for its weaker basicity. Analysis of the nonbonded interactions[41] in the projection formulas for

(38) P. Singh and F. R. Ahmed, *Acta Crystallogr.*, **25**, 1901 (1969).

(39) J. G. Henkel, K. H. Bell, and P. S. Portoghese, *J. Med. Chem.*, **17**, 124 (1974).

(40) H. B. Bürghi, J. D. Dunitz, and E. Shefter, *Nature (London), New Biol.*, **244**, 186 (1973).

staggered conformations of the C(4)–C(5)–C(6)–N moiety suggests that, on the basis of group size, the mole fraction of conformation A in isomethadone should be greater than that of methadone. This approximation indicates that there should be a greater population of conformers B + C in methadone relative to isomethadone and it explains why methadone more readily undergoes intramolecular association (N:→C=O and $^{+}$NH····O=C)[33,40] in solution. If group size is an important factor which contributes to the difference in conformational flexibility between methadone and isomethadone as suggested above, then it would be expected that related ligands with common chiral centers should in general show qualitatively similar differences in conformational behavior. It is therefore conceivable that the inversions of receptor stereoselectivity which occur in the methadone series (Table II), but not with structures related to isomethadone (Table III), are due in part to the greater frequency of alternate modes of ligand–receptor interactions in the former series as a consequence of greater conformational flexibility.[39]

Another factor which could be responsible for the absence of inversion of receptor stereoselectivity in the isomethadone series (Table III) might be that the opioid receptor environment proximal to the C(5) (or its equivalent) chiral center is sterically more demanding than that in the vicinity of C(6). As conformational and configurational effects are not mutually exclusive, it is difficult to evaluate them independently. However, recent studies[42] with hybrids (**1a, 1b**) of methadone and isomethadone have shed some light on this subject. If the chiral centers in each of the diastereomers of 5-methylmethadone act as independent units, it would be expected that the threo racemate **1b** should be more active than the erythro racemate **1a** because one of its enantiomers contains the 6*R* and 5*S* chiral centers found in the more potent enantiomers of methadone and isomethadone, respectively. The erythro racemate **1a**, on the other hand, is a mixture of 5*R*,6*R*, and 5*S*,6*S* isomers and therefore one of the chiral centers in each of the enantiomers does not possess the "proper" configuration. That this is not the case is indicated by the fact that **1a** has approximately five times greater potency than methadone, whereas **1b** is inactive as an analgetic and has no antagonist action.[42] This clearly indicates that the vicinal chiral centers do not behave independently, but actually influence one another. An explanation for this stereostructure–activity relationship is that the methyl groups at C(5) and C(6) sterically prevent the threo racemate **1b** from assuming a pharmacophoric conformation, while no such constraints are present in the erythro diastereomer **1a**. Evidence indicating that the erythro and threo salts assume different preferred conformations has been obtained from their dissociation constants and from NMR studies.[42] In contrast to **1a**·HCl, which consists

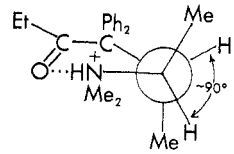


Figure 4. Projection formula representing protonated *threo*-5-methylmethadone in an internally hydrogen-bonded conformation.

of a mixture of rotamers, **1b**·HCl was found to be essentially conformationally homogeneous (Figure 4) as a consequence of intramolecular $^{+}$NH····O=C stabilization enhancement through nonbonded interaction between vicinal methyl groups.

If it is assumed that the protonated form of the ligands bind to opioid receptors,[16,18,43] then the large potency difference between **1a** and **1b** is explicable on the basis of the known stereostructure–activity relationship of methadone and isomethadone. Since the salts of methadone and **1a** each consist of a mixture of conformers whose interconversion barriers are low,[39,42] they can readily assume an antiperiplanar-type conformation which is similar to that favored for isomethadone·HCl (A, Figure 3). In contrast, the threo diastereomer **1b**·HCl is inactive because it is constrained in a conformation (Figure 4) which does not allow association with opioid receptors. Significantly, the conformations of methadone and isomethadone salts determined by x-ray crystallography[36,37] are very similar in that the C(4)–C(5)–C(6)–N moiety of the ligands approximate conformation A (Figure 3). This suggests that the intramolecular hydrogen bonding in methadone is not of sufficient strength to preclude the antiperiplanar-like conformation A. Quantum chemical calculations[44] also are consistent with this view. In light of these results it seems likely that a gauche-like relationship between the Ph_2CCOEt and $^{+}NHMe_2$ groups (Figure 4) is unfavorable for activity, in contrast to alternate conformations (e.g., antiperiplanar) which are more readily recognized by opioid receptors.

The above studies provide some insight concerning the possible role of a methyl group (attached to a chiral center) in conferring receptor stereoselectivity. The fact that 6-demethylmethadone (**2**) possesses a potency[45] comparable to that of the more active enantiomers of methadone and isomethadone indicates that a methyl group is not essential for the activity of these ligands. Therefore, we suggest that the methyl group promotes the stabilization of a chiral conformation in one of the enantiomers which in turn allows more facile association with the receptor. Accordingly, the less potent enantiomer assumes conformations which are the mirror images of those derived from the more potent enantiomer, but a significant energy barrier must be overcome to allow its pharmacophoric groups to attain orientations identical with those in the more potent enantiomer. Conformations identical with those in the more potent enantiomers of methadone and isomethadone are easily attained in the demethyl analogue **2** due to the molecules' plane of symmetry.

(41) The groups are designated as follows: large, Ph_2CCOEt (L) and NMe_2 (L′), with L > L′; medium, Me (M); small, H (S). Methadone gauche interactions (Figure 3): A = LM + LS + 2L′S + MS + SS; B = LL′ + LS + 2MS + SS; C = LL′ + LM + L′S + MS + 2SS. Isomethadone (Figure 3): A = L′M + 2LS + L′S + MS + SS; B = LL′ + LS + L′S + 2MS + SS; C = LL′ + L′M + LS + MS + 2SS. Accordingly, the sums of the nonbonded interactions are C > B > A with A (methadone) > A (isomethadone); see E. L. Eliel, "Stereochemistry of Carbon Compounds", McGraw-Hill, New York, N.Y., 1962, p 139.

(42) J. G. Henkel, E. P. Berg, and P. S. Portoghese, *J. Med. Chem.*, **19**, 1308 (1976).

(43) K. E. Opheim and B. M. Cox, *J. Med. Chem.*, **19**, 857 (1976).

(44) G. H. Loew, D. S. Berkowitz, and R. C. Newth, *J. Med. Chem.*, **19**, 863 (1976).

(45) The ED_{50} for 6-demethylmethadone is 2.5 mg/kg as compared to values of 0.8 mg/kg for (−)-methadone and 1.2 mg/kg for (−)-isomethadone: N. B. Eddy, H. Halbach, and O. J. Braenden, *Bull. World Health Org.*, **14**, 353 (1956).

It should be emphasized that this simple model does not necessarily imply that the more potent enantiomer is bound to the receptor in its preferred conformation. The important point is that the chiral center, which is created by addition of a methyl group to the ligand, introduces a bias against the less potent enantiomer's achieving a conformation which is identical with that of the more potent enantiomer when bound to the receptor.

Superimposed upon this effect is the possibility that the methyl group could play an obstructive role in the interaction of the less potent enantiomer with the opioid receptor. This would involve steric hindrance between the methyl group and the receptor when the less potent enantiomer is in a pharmacophoric conformation. It is also possible that the methyl group in the more potent enantiomer confers some affinity through hydrophobic bonding. This would lead to enhancement of the potency of the methyl-substituted ligand relative to the demethyl ligand.

Derivatives of 4-Phenyl-4-propionoxy-1-methylpiperidine. This class of opioid ligands is of interest because the unsubstituted molecule **3** possesses a plane of symmetry. The edges of the piperidine ring therefore are rendered enantiotopic. The terminology[46] used to identify each of the enantiotopic edges of **3** is *pro*-4*R* and *pro*-4*S*, and the C(4) position is referred to as a prochiral center. These features make derivatives of **3** intriguing opioid receptor probes, as it would be expected that the enantiotopic edges should be distinguishable in a chiral environment.

Although the nonequivalence of enantiotopic groups in substrates involved in enzyme-catalyzed reactions has received considerable attention since Ogston[47] first presented a conceptual model to illustrate how one of the two paired groups of citric acid is selected for enzymatic conversion, there has been no discussion of this phenomenon in connection with the interaction of drug molecules with noncatalytic recognition sites. One reason for this void is that the radiolabeling techniques employed to distinguish the enzymatic transformation of enantiotopic groups in a substrate cannot be applied to ligands whose pharmacologic effect is not dependent on a biochemical conversion at the receptor site. An alternate approach using the methyl group to label the *pro*-4*R* and *pro*-4*S* edges of the piperidine ring therefore was employed, as it was known that the methyl group attached to the chiral center in methadone or isomethadone apparently interferes in the association of the less potent enantiomer with the opioid receptor. Thus if a similar situation prevails with the C(3) methyl-substituted homologues of **3**, then it would be expected that the more potent enantiomeric diastereomers should be substituted on the same enantiotopic edge of the piperidine ring if the Ogston effect were in operation.

Initial studies utilizing this approach involved preparation of the prodine enantiomers (**4**, **5**) and the determination of their absolute stereochemistry.[48] It was found that the analgetically potent enantiomers (**4a**, **4b**) of each racemate have the C(3) methyl group attached to the *pro*-4*S* enantiotopic edge of the piperidine ring (Table IV). The fact that the demethyl compound **3** is considerably more potent than the less active enantiomers (**5a**, **5b**) and nearly as potent as **4a** indicates that the methyl group at C(3) interferes with ligand–receptor association when it is located on the *pro*-4*R* enantiotopic edge. Since distribution and metabolism studies[31] have suggested that the potencies of **3**–**5** are good indicators of receptor-related events, the data are consistent with the ability of opioid receptors to distinguish between the C(3) and C(5) positions in **3**.

Table IV
Relative Analgetic Potencies of
4-Phenyl-4-propionoxy-1-methylpiperidine Derivatives

Compd[a]	Configuration	Relative molar potency[b]	Enantiomeric potency ratio[c]	
3[d]		0.7	....	
4a[d]	3*R*,4*S*	1.0	**4a/5a**	25
4b[d]	3*S*,4*S*	3.8	**4b/5b**	13
6[e]	3*S*,5*S*	1.0	**6/7**	5
8[f]	2*S*,4*S*,5*R*	1.0	**8/9**	12
10a[g]	3*R*,4*S*	1.0	**10a/11a**	25
12a[h]	3*R*,4*S*	1.0	**12a/13a**	25
14a[h]	3*R*,4*S*	34	**14a/15a**	260
16[i]	2*R*,4*S*,5*S*	17	**16/17**	>800

[a] Tested as HCl salts. [b] Relative to morphine HCl = 1; tested subcutaneously in mice by the hot-plate procedure. [c] Ratio of more potent over less potent enantiomer. [d] Reference 48. [e] Reference 49. [f] Reference 54. [g] Reference 56. [h] Reference 57. [i] Reference 62.

Additional information concerning the role of methyl substitution in relation to the Ogston effect was obtained from studies with enantiomers **6** and **7**.[49] These ligands were chosen for study because each contains a combination of equatorial and axial methyl groups which flank the aromatic ring. Note that, in addition to the inverted axial–equatorial relationship between these enantiomers, the C(4) center is achiral.

The analgetic potency of **6** is five times that of its enantiomer **7** and about one-quarter the potency of **4b** (Table IV). The lower potency of **6** relative to **4b** and its lower enantiomeric potency ratio are consistent with the Ogston effect, as in this particular case both enantiotopic edges of the piperidine ring are substituted. However, the fact that the potency of **6** is considerably greater than that of **5a** suggested still another factor had to be considered in order to explain why **6** is more potent than its enantiomer **7**. A coherent stereostructure–activity relationship was obtained when the conformational features from single-crystal x-ray studies were considered together with chirality.[49-51] The principal feature distinguishing the more potent enantiomers from the corresponding less potent enan-

(46) R. Bentley, "Molecular Asymmetry in Biology", Vol. 1, Academic Press, New York, N.Y., 1969, Chapter 4; W. L. Alworth, "Stereochemistry and Its Application in Biochemistry", Wiley-Interscience, New York, N.Y., 1972, Chapters 5 and 6.

(47) A. G. Ogston, *Nature (London)*, **162**, 963 (1948).

(48) D. L. Larson and P. S. Portoghese, *J. Med. Chem.*, **16**, 195 (1973).

(49) P. S. Portoghese, Z. S. D. Gomma, and D. L. Larson, *J. Med. Chem.*, **16**, 199 (1973).

(50) G. Kartha, F. R. Ahmed, and W. H. Barnes, *Acta Crystallogr.*, **13**, 525 (1960).

(51) F. R. Ahmed and W. H. Barnes, *Acta Crystallogr.*, **16**, 1249 (1963); F. R. Ahmed, W. H. Barnes, and L. D. Masironi, *ibid.*, **16**, 237 (1963).

(52) In this discussion the quadrant system is defined by considering C(4) as the intersection point when the ligand is viewed as a Newman projection formula (Table V).

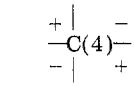
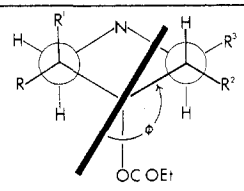
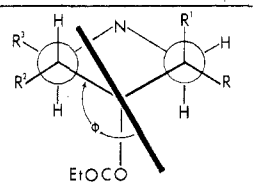


Table V
Torsion Angle ϕ in Enantiomers of Alkyl-Substituted 4-Phenyl-4-propionoxypiperidines



A



B

More potent enantiomer A	Substitution	Torsion angle ϕ,[a] deg	Less potent enantiomer B
4a	R = Me; $R^1 = R^2 = R^3 = H$	152[b]	5a
4b	R^1 = Me; $R = R^2 = R^3 = H$	167[c]	5b
6	$R^1 = R^2$ = Me; $R = R^3 = H$	164[d]	7
8	$R = R^3$ = Me; $R^1 = R^2 = H$	139[e]	9
14a	R = $CH_2CH{=}CH_2$; $R^1 = R^2 = R^3 = H$	128[f]	15a

[a] With the exception of trimeperidine (6, 7), ϕ values were obtained from x-ray data of the racemate HCl. [b] Reference 50. [c] Reference 51. [d] Reference 49. [e] Value obtained from free base of the alcohol.[55] [f] Reference 58.

tiomers is the sign of the quadrant[52] in which the phenyl group resides (Table V). The data suggest that the quadrant sign is determined by substitution of groups vicinal to C(4) on one of the enantiotopic edges of the piperidine ring. Thus the C(3) methyl in **4a** and **4b** induces the phenyl group to adopt conformation A due to intramolecular steric hindrance. Similarly, the phenyl group in **6** falls into a quadrant of the same sign (negative) due to the greater hindrance of an axial vs. an equatorial methyl group. Quantum chemical studies[53] are consistent with the x-ray data.

Since all of the derivatives of **3** which have been discussed thus far have methyl groups adjacent to the C(4) center, trimeperidine enantiomers **8** and **9** were investigated[54] in order to evaluate the relative importance of the C(2) position with respect to C(5). The more potent enantiomer **8** possesses analgetic activity equal to that of **4a** whose configuration is identical at the common chiral centers (Table IV). X-ray crystallography[55] also presents a consistent picture with regard to the aromatic ring of the more potent enantiomer being situated in a negative quadrant (Table V). As expected, the C(2) methyl group plays a minor role because it is remote from the C(4) center and overshadowed by the effect of the C(5) methyl group. Consequently, the C(2) chiral center only modifies the stereoselectivity of trimeperidine, probably through a direct interaction with the opioid receptor.

The stereostructure–activity relationship also holds for higher homologues of prodine provided that replacement of the C(3) methyl group with a longer alkyl chain does not interfere with ligand–receptor association. Thus, the more potent enantiomers of the α diastereomers (trans phenyl:R) which contain ethyl, propyl, and allyl groups (**10a**, **12a**, **14a**) all possess the 3*R*,4*S* configuration.[56,57] Interestingly, all of the ligands with saturated alkyl groups (**4a**, **10a**, **12a**) have enantiomeric potency ratios, (3*R*,4*S*)/(3*S*,4*R*), of about 25, with the 3*R*,4*S* enantiomers having nearly identical potencies (Table IV). The trimeperidine enantiomer **8** also possesses a similar potency, and its enantiomeric potency ratio, **8**/**9**, though somewhat lower, is in the same range. These data suggest that saturated alkyl substituents which are equatorial and situated on the *pro*-4*S* enantiotopic edge of the piperidine ring are functioning similarly in the binding of these ligands (**4a**, **8**, **10a**, **12a**) to opioid receptors.

Although the receptor stereoselectivity for the allyl compound **14a** is qualitatively in harmony with that of the saturated analogues, its unusually high potency (34 times that of morphine) and enantiomeric potency ratio (260) indicate that the allyl group confers substantial affinity to the binding of **14a** by the receptor (Table IV). The enhanced affinity is not related to chain length, but is due specifically to the double bond. An x-ray crystallographic study[58] suggests that the allyl substituent induces a negative torsional relationship between the phenyl group and the piperidine ring in conformity with other more potent enantiomers (Table V), but the only feature which differentiates this enantiomer from the others is the smaller magnitude of the torsion angle. The possibility that the allylic double bond causes stabilization of the phenyl group through π overlap was discounted because none of the distances are shorter than normal van der Waals contacts. These data suggest that the high potency of **14a** is due to a highly specific interaction between the allylic double bond and an accessory site on the receptor. The main distinction between the role of the C(3) substituent in **14a** and that in the other 3*R*,4*S* isomers is therefore one of direct interaction with the receptor in the former, vs. indirect interaction in the latter. In other words it appears that the equatorial C(3) alkyl groups in **4a**, **8**, and **12a** contribute to receptor binding only to a minor extent.

Unlike the 3*R*,4*S* isomers, lengthening the C(3) group in the β diastereomers (cis phenyl:R′) leads to a great decrease in potency and in the (3*S*,4*S*)/(3*R*,4*R*) potency ratios for **12b**/**13b** and **14b**/**15b**.[56,57] The fact that **4b** is three to four times more potent than **4a** suggests that the axial C(3) methyl group in the former enhances activity, perhaps by binding in a hydrophobic pocket of limited size on the receptor. An axial propyl (**12b**) or allyl (**14b**) cannot be accommodated by the pocket, and consequently the affinity of these ligands is greatly reduced. As axial propyl or allyl substitution interferes with ligand–receptor association when located on either enantiotopic edge of the piperidine ring, the net result is low potency and low stereoselectivity.

All of the aforementioned investigations suggest that (1) opioid receptors are capable of distinguishing between the enantiotopic edges of the piperidine ring in 4-phenylpiperidines and (2) the more potent enantiomers have their aromatic ring located in a negative quadrant[52] with respect to the piperidine ring. Moreover, these studies have drawn attention to the

(53) G. H. Loew and J. R. Jester, *J. Med. Chem.*, 18, 1051 (1975).
(54) D. Fries and P. S. Portoghese, *J. Med. Chem.*, 17, 990 (1974).
(55) W. H. DeCamp and F. R. Ahmed, *Acta Crystallogr., Sect. B*, 28, 1791 (1972).
(56) K. H. Bell and P. S. Portoghese, *J. Med. Chem.*, 16, 589 (1973).
(57) K. H. Bell and P. S. Portoghese, *J. Med. Chem.*, 17, 129 (1974).
(58) P. S. Portoghese and E. Shefter, *J. Med. Chem.*, 19, 55 (1976).

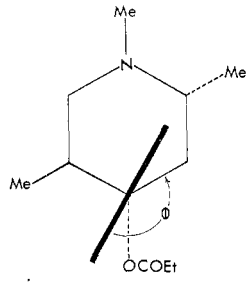


Figure 5. Projection formula of the axial-phenyl enantiomer **16**. Note that ϕ is of the same sign in **16** and in projection formula A (Table V).

fact that the roles of absolute configuration and conformation are not easily factored in the analysis of stereostructure–activity relationships. Inasmuch as these studies utilized 4-phenylpiperidines whose phenyl group is in a preferred equatorial conformation,[2,49-51,53,55,58-60] it was therefore of interest to employ a receptor probe containing an axially preferred aromatic ring as well. This would reveal whether there is an identical recognition locus on the receptor for a moiety common to both axial and equatorial 4-phenylpiperidines.

The ligands which were employed for this study are enantiomers (**16**, **17**) of (±)-α-promedol, a potent analgetic agent whose phenyl group is known to reside preferentially in the axial conformation.[2,60,61] The 2*R*,4*S*,5*S* isomer (**16**) is 17 times more potent than morphine, and its enantiomer (**17**) is inactive at 50 mg/kg (Table IV).[62] Comparison of the absolute stereochemistry of **16** with that of the equatorial-phenyl congeners reveals that they all possess the 4*S* configuration. In other words, the more potent enantiomers of both the axial- and equatorial-phenyl congeners contain a C(3) or C(5) alkyl group on the *pro*-4*S* edge of the piperidine ring. In addition, x-ray data[61] suggest that the torsional relationship between the aromatic group and piperidine ring in the active enantiomer **16** is of the same sign as the more potent enantiomers that reside preferentially in the equatorial-phenyl conformation (compare Figure 5 with projection formula, A, Table V). This is believed[61] to arise from a combination of conformational distortion of the piperidine ring and to the steric interaction between the C(5) methyl and phenyl group of **16**.

While it is conceivable that a receptor-induced conformational change of the ligand might force all of the more potent enantiomers to assume a similar conformation, this appears unlikely in view of the comparable analgetic potency of related ligands whose aromatic ring is fixed in axial and equatorial conformations[63] (also see Table I). Rather, our results are consistent with a similar recognition locus on the receptor for the C(3)–C(4)–C(5) moiety and its C(4) substituents in both the equatorial and axial phenyl analogues.[62] Because this recognition locus plays a dominant role in the binding of **3** and its derivatives to

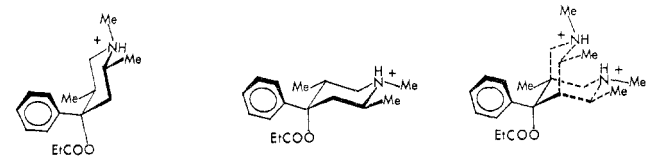


Figure 6. The more potent enantiomers of conformationally preferred equatorial-phenyl (A) and axial-phenyl (B) diastereomers **8** and **16**, respectively, superimposed upon one another (C). Portions which are not superimposed are designated by dashed lines and represent the C(2)–N–C(6) moieties of **8** and **16** which are located in different receptor environments.

opioid receptors, it is likely that the C(2)–N–C(6) moieties of axial-phenyl and equatorial-phenyl conformers are bound in different loci on the receptor. This is illustrated in Figure 6 which shows the different orientation of C(2)–N–C(6) relative to the superimposed portion, C(3)–C(4)–C(5), of two diastereomeric ligands (**8**, **16**). The dissimilar modes of interaction therefore arise by virtue of the different orientations of the C(2)–N–C(6) moiety.

Also consistent with this model is the absence of similar rank order potencies between axial- and equatorial-phenyl analgetics whose N substituent has been varied in an identical fashion (Table I). This is readily understandable if the N substituent is viewed as being projected into different receptor environments due to the dissimilar orientation of the C(2)–N–C(6) moiety in each conformer. The mechanism by which the C(2)–N–C(6) moiety is accommodated in both equatorial and axial 4-phenylpiperidines remains to be clarified. One possibility is a conformational change of the anionic site on the receptor. Another is the juxtaposition of the anionic site between the two binding loci of the C(2)–N–C(6) moiety.

Summary and Conclusions

The mode of interaction of opioids with its receptors is a function of the constitution and geometric disposition of key groups in the ligands. Two approaches have been employed to determine whether different ligands have similar or divergent modes of interaction with opioid receptors. The first involves the use of the N-substituent residue of the ligand as a "reporter" group, and the second utilizes enantiomers as probes to uncover changes in the absolute stereoselectivity of opioid receptors. The data suggest that conformationally mobile ligands generally exhibit a greater frequency of divergent modes of interaction than those that are conformationally restricted. Moreover, it appears that multiple modes of interaction arise by virtue of the basic nitrogen and its contiguous moieties having several alternate loci for interaction with the receptor.

The potency difference between enantiomers is suggested to arise primarily from a combination of two factors. The first involves the induction of dissymmetric conformations by a chiral center in the ligand. The difference between the enantiomers in achieving an identical, dissymmetric pharmacophoric conformation when bound to the receptor contributes in part to the potency difference. The second factor is related to steric hindrance between the substituent (attached to the chiral center and often a methyl group) and the receptor when the ligand is in a pharmacophoric

(59) A. J. Jones, A. F. Casy, and K. M. J. McErlane, *Can. J. Chem.*, **51**, 1782 (1973).
(60) A. J. Jones, C. P. Beeman, A. F. Casy, and K. M. J. McErlane, *Can. J. Chem.*, **51**, 1790 (1973).
(61) W. H. DeCamp and F. R. Ahmed, *Acta Crystallogr., Sect. B*, **28**, 1796 (1972).
(62) D. S. Fries and P. S. Portoghese, *J. Med. Chem.*, **19**, 1155 (1976).
(63) E. E. Smissman and M. Steinman, *J. Med. Chem.*, **9**, 455 (1966).

conformation. Finally, converging lines of evidence indicate that the influence of absolute configuration on conformational dissymmetry requires that the effects of chirality and conformation be dealt with together in the analysis of the stereostructure–activity relationship of opioid ligands.

Biosynthesis of Vitamin B_{12}. In Search of the Porphyrin–Corrin Connection

A. IAN SCOTT

Sterling Chemistry Laboratory, Yale University, New Haven, Connecticut 06520

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The history of corrin biosynthesis spans the relatively brief passage of 12 years, yet even in this period several distinct phases can be clearly recognized. By 1968, when our researches were inititated, the origin of the corrin nucleus had been defined, since the building blocks δ-aminolevulinic acid (ALA; **1**), porphobilinogen (**2**), and methionine were clearly involved[1,2] in the makeup of cobyrinic acid (**3a**), the "simplest" of the cobalt-containing corrins. To this key heptacarboxylic acid is added the nucleotide loop, whose components are derived from threonine, guanosine triphosphate, and the unusual base, dimethylbenzimidazole, and the primary amide functions.[3] The major technical problem in obtaining rigorous evidence of nonrandom incorporation of regiospecifically labeled carbon, however, was the lack of degradative chemistry of vitamin B_{12}, a void which had been created by solution of the structural problem by x-ray diffraction analysis.

In this Account we shall discuss both experimental and theoretical aspects developed recently in our laboratory and elsewhere which are attempting to solve several fascinating mechanistic problems in the unknown territory between the reduced type III porphyrin, uro'gen III (**4**), and the first fully corrinoid intermediate cobyrinic acid (**3a**). We begin our discussion with an account of the experiments designed to establish both the number and the mode of insertion of the methionine-derived methyl groups in the corrin nucleus as a background for the development of the mechanistic proposals for the uro'gen–corrin transformation.

Origin of the Methyl Groups in Vitamin B_{12}

Of the eight methyl groups attached to the periphery of **3** it was suggested[1] that those at C-1 and C-12 stem from C-5 and C-2 of ALA, respectively, the latter by a well-documented decarboxylation of acetate attached to the uro'gen system, while the derivation of the former (C-1) methyl group could be envisioned either as a reduction of a $-CH_2-$ bridge of uro'gen III or as a result of direct cyclization of a linear tetrapyrrole,[4] the six remaining methyl groups arising from methionine. Support for these ideas came from Kuhn–Roth oxidation of corrinoids labeled with [5-^{14}C]- and [2,3-^{14}C]ALA and [^{14}C-methyl]methionine.[1]

When the problem was reexamined using ^{13}C Fourier transform NMR, administration of [2-^{13}C]ALA to *P. shermanii* afforded a sample of vitamin B_{12} in which eight high-field signals in the $-CH_2-$ and $-CH_3$ region were enriched. Assignments of the eight ^{13}C resonances were made to the seven $-CH_2CONH_2$ methylenes and one of the *gem*-dimethyl groups of ring C, in full accord with earlier ^{14}C studies. It is evident, however, that the methyl signal appears at lower field than the methyl region assigned by Doddrell and Allerhand.[5] A sample of B_{12}, enriched by feeding [5-^{13}C]ALA, provided the surprising result that, of the eight anticipated enriched carbons, only seven signals appeared in the low-field region associated with sp^2 (C=C and C=N) functions. The splitting pattern predicted for the distribution of label illustrated in **3c** (Figure 2) was indeed obtained. Such an array is in harmony with current ideas on the mechanism of type III uro'gen formation, and this result was simultaneously discovered in Shemin's laboratory[6] in 1972. However, there was no ^{13}C-enhanced signal above 95 ppm downfield from HMDS, showing that no enrichment of the C-1 methyl occurred. This indicates that one of the $-^{13}CH_2NH_2$ termini of ALA (and hence of PBG or uro'gen III) has been extruded in the formation of the vitamin. The origin of the "missing" C-1 methyl group was demonstrated to be methionine. Inspection of the integrated spectrum after feeding [^{13}C-methyl]methionine left no doubt that seven methionine methyl groups have been incorporated (see Figure 2). This result, which is of considerable significance for the mechanism of corrin synthesis, was to receive confirmation from the work of the Cambridge group,[7] and extension of these studies led to the ab-

A. Ian Scott was born in Scotland and received B.Sc., Ph.D., and D.Sc. degrees from Glasgow University, where he taught from 1957 to 1962, before holding professorships at the University of British Columbia, University of Sussex, and Yale University. In 1977, he was appointed a Distinguished Professor at Texas A&M University, where he continues his research on natural product biosynthesis in cell-free systems from fungi, bacteria, and plant tissue cultures. Professor Scott was recipient of the 1976 Ernest Guenther Award in the Chemistry of Essential Oils and Related Products sponsored by Fritzsche Dodge & Olcott Inc., and this Account is based on his award address. The author wishes to dedicate this work to the celebration of the birthday of another, but much younger, producer of the vitamin, R. B. Woodward.

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