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THE CRYSTAL STRUCTURE AND MOLECULAR CONFORMATION OF *THREO*-5-METHYLMETHADONE IN RELATION TO OPIOID RECEPTOR BINDING

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Abstract—Crystals of the hydrochloride salt of the biologically inactive *threo* isomer of 5-methylmethadone, $C_{22}H_{30}ONCl$, are monoclinic space group $P2_1$ with unit cell dimensions $a = 11.019$ Å, $b = 8.6153$ Å, $c = 10.680$ Å and $\beta = 93.026°$. The observed conformation is one in which the nitrogen bearing chain is extended with the substituents on C(5) and C(6) nearly eclipsed, a feature compatible with NMR studies and molecular mechanics calculations. The very potent agonist (5*S*, 6*S*)-*erythro*-5-methylmethadone has a solid state conformation in which the N atom is rotated back toward the phenyl rings [C(4)–C(5)–C(6)–N = 97°] in agreement with molecular mechanics calculations. The fact that the more potent enantiomers, (6*R*)-methadone and (5*S*)-isomethadone, and the inactive *threo* isomer are observed in the extended solid state conformation in contrast to (5*S*, 6*S*)-*erythro*-5-methylmethadone is consistent with three different models for their interaction with opioid receptors. It is proposed that the more likely of these involves a receptor bound conformation of (6*R*)-methadone and (5*S*)-isomethadone that resembles the conformation of (5*S*, 6*S*)-*erythro*-5-methylmethadone or that opioid receptors recognize both *gauche*-like and extended conformations.

The activity of diphenylpropylamine analgetics is highly dependent upon the chirality at atomic positions C(5) and C(6) (Fig. 1). Although reports in the literature vary in their estimates of relative potency,[1-4] (6*R*)-methadone, (5*S*)-isomethadone, and (5*R*, 6*R*)-*erythro*-5-methylmethadone are found to have potencies comparable to morphine while (5*S*, 6*S*)-*erythro*-5-methylmethadone is from 10 to 70 times greater. In contrast, (6*S*)-methadone and (5*R*)-isomethadone[5] have less than one-tenth the activity of their respective isomers. Surprisingly the racemate of *threo*-5-methylmethadone is totally devoid of activity despite the presence of the combined chiralities of the most active methadone and isomethadone molecules in its (5*S*, 6*R*)-isomer. Because these molecules are potentially flexible, considerable effort has been directed toward determining the conformation or conformations responsible for opioid receptor binding. Thus far, solution spectral studies,[6,7] quantum mechanical[8] and force field empirical energy calculations,[9] and X-ray crystallographic investigations[10-13] have failed to provide a consistent picture of the conformational requirements for activity. It has been proposed that biological activity may depend upon either an electrostatic interaction between the amine and the carbonyl group in the free base structure[14] (Fig. 2a), or an H bond between these groups in protonated methadone[15] (Fig. 2b). Although this electrostatic interaction was demonstrated in the X-ray crystal structure determination of methadone free base,[12] the original postulate[14] that the protonated form of methadone is responsible for activity is now generally accepted.

Quantum chemical calculations for protonated methadone predicted six conformers within an energy range of 13 kcal/mole.[8] These conformations differ significantly in rotations about most single bonds in the structure, particularly the bonds of the quaternary C.

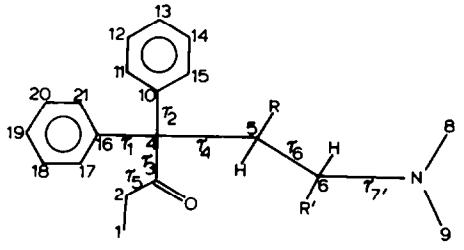

Fig. 1. Atomic numbering and torsion angles defining the molecular conformations of normethadone (R=R'=H), methadone (R=H, R'=CH_3), isomethadone (R=CH_3, R'=H) and 5-methylmethadone (R=R'=CH_3).

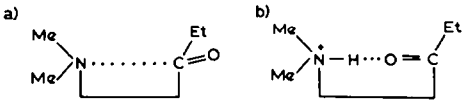

Fig. 2. Intramolecular interaction proposed to influence the conformation of (a) uncharged and (b) protonated methadone.

Molecular mechanics calculations also suggested that the diphenylpropylamines have a great deal of conformational heterogeneity.[9] For each of the common methadone analogues five or more grossly

different conformations were calculated to be within 5 kcal/mole energy of one another. No clear picture emerged of a single conformation that might be responsible for analgetic action. Three families of conformers were suggested as possible candidates for the active form and entirely different conformations were proposed to be responsible for the action of different isomers.

NMR, CD, and pKa investigations[6,7] have been interpreted to indicate that protonated methadone and *erythro*-5-methylmethadone are in equilibrium between the three staggered conformations shown in Fig. 3, whereas isomethadone and *threo*-5-methylmethadone are less flexible in solution with the antiplanar conformation of the Ph_2COOEt and $+NHMe_2$ groups (Fig. 3c) predominating in the case of methadone and the *gauche* form (Fig. 4) incorporating an intramolecular H–bond predominating in the case of the totally inactive *threo* isomer. This together with the observation of the extended conformer in the X-ray crystal structure determination of the hydrobromide salt of methadone[11] led to the conclusion that the extended form was one of the pharmacophoric conformations.

Model studies suggest that the presence of Me groups on both C(5) and C(6) should reduce the flexibility of the molecule. For this reason it was felt that X-ray analysis of the active and inactive isomers of 5-methylmethadone might help to identify the conformation(s) responsible for receptor binding. The observation that in the solid state (−)-(5*S*, 6*S*)-*erythro*-5-methylmethadone, the active isomer, has a conformation in which the substituted amine is in the (+)clinal orientation relative to the quarternary substituted carbon, led to the previously unconsidered possibility that this is an active conformation for diphenylpropylamine analgetics. The X-ray structure of the *threo* isomer therefore was undertaken in order to determine the conformation of a totally inactive compound.

RESULTS AND DISCUSSION

The X-ray crystallographically determined conformations of salts of normethadone, (6*R*)-methadone, (5*S*)-isomethadone, (5*S*, 6*R*)-*threo*-5-methylmethadone, and (5*S*, 6*S*)-*erythro*-5-methylmethadone defined by the eight torsion angles of Fig. 1 are compared in Table 1. The conformations of the molecules are remarkably consistent despite

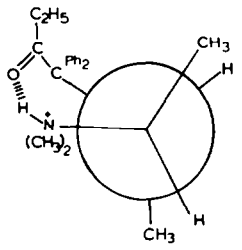

Fig. 4. The off staggered conformer of *threo*-5-methylmethadone presumed to account for the small coupling constant observed for the hydrogen on C(5) and C(6) in a wide variety of solvents.

variability in their anions, crystal habit, and intermolecular association. There is a minor difference observed in the position of the C(1) Me in the *threo* compound and variability is observed in the nitrogen methyl positions. The only significant difference is the (+)clinal conformation of τ_6 observed in the most active analogue, (5*S*, 6*S*)-*erythro*-5-methylmethadone.

The consistency of the conformations of these structures suggests that there may be less flexibility in the torsion angles involving the quaternary C(4) than is suggested by either the quantum chemical[8] or molecular mechanics calculation.[9]

When the *threo* and *erythro* structures are viewed in Newman projections along the C(5)–C(6) bond (Fig. 5), a remarkable similarity is seen in the relative positions of the vicinal hydrogens. This similarity, in spite of the interchange of the Me and amine substituents on C(6), suggests that the near eclipsing of the C(6) hydrogen and the phenyl substituted C(4) may be by far the most energetically acceptable arrangement for 5-methylmethadone isomers. The conformation is also compatible with the observed proton coupling constant[7] and the lowest energy molecular mechanics calculation for (5*S*, 6*R*)-methylmethadone.[9] In this connection, the previously assigned conformation (Fig. 4), which differs from the solid state conformation, was in part based on the higher pKa of the *threo* salt relative to its diastereomer, which suggested intramolecular stabilization of the conjugate acid of the *threo* isomer. A knowledge of the solid state conformation of the *threo* isomer (Fig. 6a) now provides an alternate

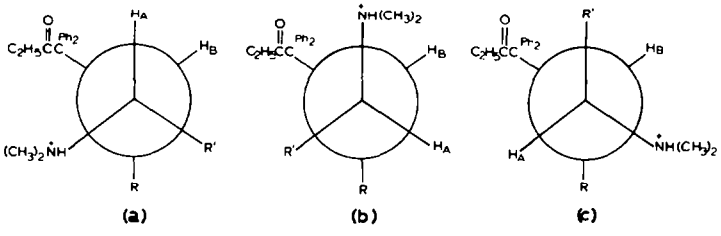

Fig. 3. Newman projection [C(6)→C(5)] of the three fully staggered conformers of protonated (6*R*)-methadone (R=H, R′=CH_3), (5*S*)-isomethadone (R=CH_3, R′=H) and (5*S*, 6*S*)-*erythro*-5-methylmethadone (R=R′=CH_3). On the basis of the NMR spectra the three conformers appear to be in equilibrium in methadone and *erythro*-5-methylmethadone but conformer 3c predominates in isomethadone.

Table 1. Torsion angles of crystallographically observed conformations of methadone and its congeners, τ_1 = C(17)–C(16)–C(4)–C(5), τ_2 = C(15)–C(10)–C(4)–C(5), τ_3 = C(2)–C(3)–C(4)–C(5), τ_4 = C(3)–C(4)–C(5)–C(6), τ_5 = C(1)–C(2)–C(3)–C(4), τ_6 = C(4)–C(5)–C(6)–N, τ_7 = C(5)–C(6)–N-C(8), $\tau_{7'}$ = C(5)–C(6)–N-C(9)

Compound	τ_1	τ_2	τ_3	τ_4	τ_5	τ_6	τ_7	$\tau_{7'}$
normethadone[13]	-20°	98°	175°	74°	168°	-165°	71°	-53°
	-22	100	172	75	177	-167	73	-53
(6*R*)-methadone[11]	-34	96	-174	76	157	-146	75	-53
(5*S*)-isomethadone[10]	-25	86	-167	66	176	-152	81	-154
(5*S*,6*R*)-*threo*-5-methylmethadone	-38	93	-166	71	-123	-138	78	-52
(5*S*,6*S*)-*erythro*-5-methylmethadone[3]	-40	100	175	64	175	97	92	-145

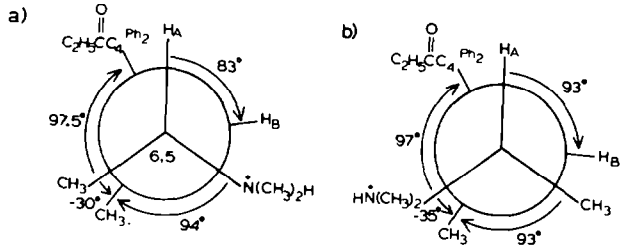

Fig. 5. Newman projection [C(6)→C(5)] of (a) (5*S*, 6*R*)-*threo* and (b) (5*S*, 6*S*)-*erythro*-5-methylmethadone illustrating orthogonality of the hydrogen atoms in the nearly eclipsed observed conformations.

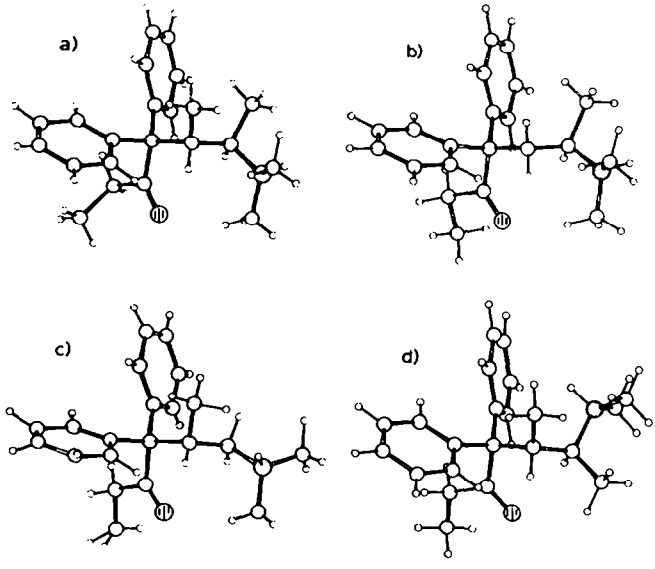

Fig. 6. Observed conformation of (a) (5*S*, 6*R*)-*threo*-methylmethadone, (b) (6*R*)-methadone, (c) (5*S*)-isomethadone, and (d) (5*S*, 6*S*)-*erythro*-5-methylmethadone.

explanation, since its NH^+ appears to be more exposed than that in the *erythro* isomer (Fig. 6d). This suggests that the greater basicity of the *threo* isomer is related to greater stabilization of the conjugate acid as a consequence of its greater accessibility to water.

Comparison of the solid state and solution data with molecular mechanics calculations suggests that while a conformation observed in the solid state may be at or very near the global minimum energy conformation, it may not be the active conformation, particularly in the case of relatively flexible molecules of moderate activity. The data suggest that for protonated (6*R*)-methadone and (5*S*)-isomethadone the lowest energy conformation is one in which the N is in the extended conformation but that in solution some fraction of the population of molecules is in the (+)clinal conformation.

The crystallographically observed conformations of (6*R*)-methadone, (5*S*)-isomethadone, and (5*S*, 6*S*)-*erythro*-5-methylmethadone are illustrated in Fig. 6. The (5*S*, 6*R*) isomer of the *threo* structure has a conformation nearly identical to that of (6*R*)-*methadone* and differs from that of (5*S*)-isomethadone only in the orientation of the amine Me substituents. This demonstration of the stability of extended conformers of the active compounds, (6*R*)-methadone and (5*S*)-isomethadone, and the totally inactive (5*S*, 6*R*)-*threo*-5-methylmethadone suggests that opioid receptors are capable of adjusting to the presence of a 5*S* or 6*R* substituted Me, but not to both in the same molecule.

The fact that (5*S*, 6*S*)-*erythro*-5-methylmethadone is observed crystallographically as a unique conformation relative to the other methadones (Fig. 6) has special significance and might account for its greatly enhanced potency. One possibility is that this combination of chiral centers is responsible for facilitating a conformation which may have greater affinity for opioid receptors than other methadones. Several plausible possibilities for the dramatic difference in the potency of the *erythro* and *threo* isomers are presented below.

(1) (6*R*)-Methadone, (5*S*)-isomethadone and (5*S*, 6*S*)-*erythro*-5-methylmethadone bind to opioid receptors in the extended conformation whereas the combined presence of the C(5) and C(6) Me's in the *threo* isomer interfere with its binding while in that conformation. While this possibility implies that the (5*S*, 6*S*)-*erythro* isomer readily attains an extended conformation, it does not account for the greatly enhanced potency of this ligand unless it is assumed that the additional Me group somehow confers increased affinity over (5*S*)-isomethadone and (6*S*)-methadone, and interferes in the case of the *threo* isomer.

(2) The (+)clinal conformation observed for (5*S*, 6*S*)-*erythro*-5-methylmethadone is active, while the extended conformation is not one involved in receptor binding. This model attributes the greater potency of the (5*S*, 6*S*)-*erythro* isomer to its greater *gauche*-like conformational stability over the other active methadones or inactive *threo* isomer. The implication is that the *threo* isomer cannot bind in a *gauche*-like conformation due to its preferred extended conformation and to steric interference between one of its Me groups and the receptor.

(3) Both the extended and (+)clinal conformations are capable of being accommodated by opioid receptors. This suggests that the lack of correlation between chirality among the methadone congeners is related to the involvement of more than one type of receptor bound conformation. In this regard, it is conceivable that both the extended and *gauche*-like conformations are recognized by opioid receptors and that the facility with which a ligand binds is dependent on both conformational preference and the constitution of the ligand. For example, if (6*R*)-methadone were to bind in a *gauche*-like conformation and (5*S*)-isomethadone in an extended conformation, then this would explain why the more potent enantiomer of 5-methylmethadone possessed the *erythro*-(5*S*, 6*S*) stereochemistry rather than having chiralities identical to these ligands at both C(5) and C(6). Evidence for this possibility has been reported for receptor recognition of conformationally restricted 4-phenylpiperidine diastereomers of different geometry.[16-18]

It is noteworthy that all three models imply or suggest that the C(5) or C(6) chiral centers of 5-methylmethadone behave differently from those in methadone or isomethadone. While none of these models can be eliminated as possibilities, we presently favor models 2 or 3 as more plausible.

EXPERIMENTAL

A single crystal of the hydrochloride salt from a racemic mixture of *threo*-5-methylmethadone was found to belong to the monoclinic space group $P2_1$ with one molecule in the asymmetric unit, indicating spontaneous separation of enantiomers upon crystallization. The unit cell dimensions were measured to be $a = 11.019(1)$ Å, $b = 8.6153(6)$ Å, $c = 10.680(1)$ Å and $\beta = 93.026(7)°$. The intensities of 1494 reflections having $\sin\theta/\lambda < 0.55$ Å^{-1} were measured with CuKα radiation (1.5418 Å) on a Syntex P_3 automated four-circle diffractometer using θ-2θ scans. The structure was solved by direct methods[19] and refined by full-matrix least-squares. The H atoms were located from difference maps and assigned isotropic thermal parameters equivalent to those of carbon to which they are bonded and only their positional coordinates were refined. All non H atoms were refined anisotropically. The final reliability index was 3.8% for the 1479 reflections having intensities greater than twice the standard deviation of their measurement [2σ(I)]. The bond lengths in the benzene rings range from 1.364 to 1.397 Å and the carbon–hydrogen bond lengths range from 0.86 to 1.10 Å. For purposes of comparison with the most active isomers of methadone, isomethadone and *erythro*-5-methylmethadone, the (5*S*, 6*R*)-*threo*-isomer is illustrated in Fig. 1(d), although the absolute configuration was not determined.

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