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A COMPARISON OF SOLUTION, SOLID STATE AND THEORETICAL CONFORMATIONS OF MORPHINE

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SUMMARY: Using very high frequency ^{1}H nuclear magnetic resonance, nitrogen inversion in the N-substituted piperidine ring of a fused polycyclic molecule is demonstrated for the first time. The molecule discussed is the potent opiate agonist morphine where the piperidine ring is known to be of vital importance in receptor mediated pharmacological action. Previously, it has been accepted that the conformation of the piperidine ring was solely N-R, equatorial, on the basis of solid state and theoretical conformation studies. The present work demonstrates a relatively slow rate of interchange between the axial and equatorial forms and therefore requires a reappraisal of the previous literature as applied to the receptor active conformation of morphine.

There has been a great deal of recent interest in the topology of opiates with respect to acertaining the conformational features responsible for the receptor mediated actions of the molecules. This literature has taken the form of X-ray determinations of many of the important class representatives (1), quantum chemical calculations (2), and hypotheses based upon empirical structure activity relations (3,4). In particular, much of this work has centered on the role of the N-substituted piperidine ring in classical opiate agonists and antagonists since change in this section of the molecules is known to be very important in receptor-ligand recognition and subsequent biological effects. A stereoscopic view of the conformation of the archtypal agonist, morphine, as determined by X-ray crystallography is shown in Figure 1a (5,6). The formal structure and numbering system for morphine (I) is given below. A significant conformational feature of the structure is the wholly N-R, equatorial, chair, conformation of the E (piperidine) ring. This feature, apparently confirming theoretical calculations which make the N-R, equatorial, conformer overwhelmingly favored energetically (2), forms the basis of much current thinking on the nature of the opiate receptor (7).

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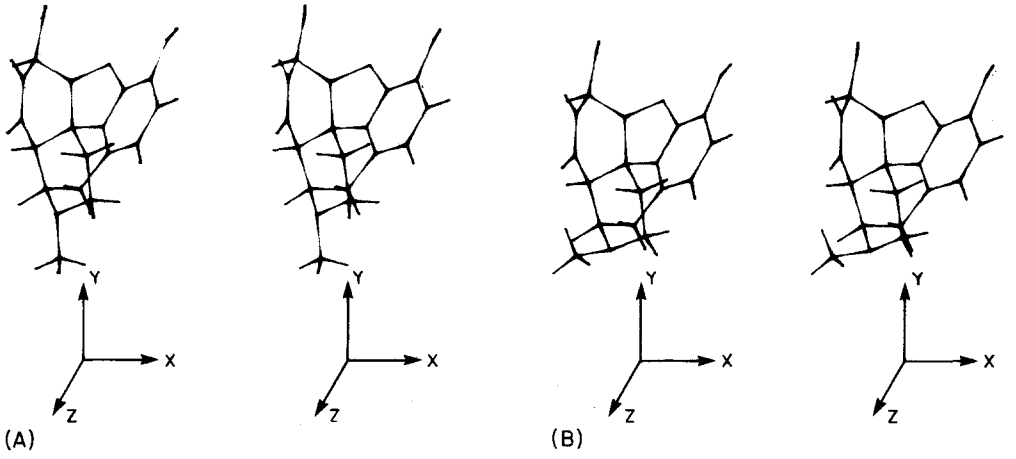

Figure 1. (A) Stereoscopic view of morphine conformation as determined by X-ray crystallography (the N-R, equatorial form). (B) Stereoscopic view of morphine in the N-R, axial, conformation.

In stark contrast to this voluminous solid state and theoretical literature little attention has been given to the solution conformations of alkaloid opiates and, in particular, none has been given to the interpretation of their relevant nuclear magnetic resonance (NMR) spectral features. NMR work on the opiates has been confined to some early 60 MHz ^{1}H work and later ^{13}C assignments in organic solvents (8,9).

Extensive classical NMR studies have, of course, been performed on nitrogen inversion and boat-chair interconversion in simple piperidine rings (10,11) but such interconversions have never been demonstrated in large

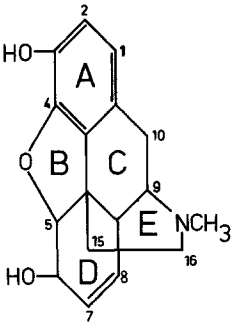

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TABLE I

Proton spin-spin coupling constants for the piperidine ring of morphine in aqueous (D_2O) solution, 30°C, pD_a=1.5 compared with those predicted from X-ray crystal structure of morphine hydrochloride.

(a)

derived dihedral angles from crystalline morphine hydrochloride structure, and observed coupling constants

			J(obs)	
dihedral angle	crystal	perfect chair	-NR,eq	-NR,ax
H16,eq-C16-C15-H15,ax	-54.19°	-60.00°	4.9 Hz	4.8 Hz
H16,eq-C16-C15-H15,eq	68.02	60.00	<1	<1
H16,ax-C16-C16-H15,ax	-166.19	-180.00	13.1	14.2
H16,ax-C16-C15-H15,eq	-43.98	-60.00	4.	4.

(b)

theoretical prediction vs. observation J(trans)/J(cis) for J(trans)=1/2(J_{aa}+J_{ee}), J(cis)=1/2(J_{ae}+J_{ea})

J(trans)/J(cis):	perfect chair	2.00 (ref. 13)
	crystal(predicted),	1.51
	-NCH_3,eq (obs)	1.51
	-NCH_3,ax (obs)	1.67

polycyclic fused ring systems such as the alkaloid opiates. Aided by the increase in chemical shift dispersion afforded by NMR spectroscopy at 600 MHz this report discusses and initial investigation of conformational equilbria in morphine in aqueous solution.

METHODS: ^1H assignments derive from homonuclear decoupling work performed initially at 100 MHz and later at 600 MHz. ^{13}C measurements, based on these assignments were performed at 25.2 MHz. 100 MHz measurements were taken in the Fourier transform mode while those at 600 MHz were done in the correlation mode. Samples were made up from recrystallized morphine (furnished as a gift from the State of Connecticut Department of Consumer Affairs, Drug Control Division) at a concentration of 50 mg/ml in 100% ^2H_2O with added ^2HCl to bring them to the desired acidity. An exception was the correlation mode experiment carried out in H_2O.

Figure 1, the dihedral angles reported in Table I, and the internuclear distances and moments of intertia quoted in the text were obtained using the program PEPTID developed in this laboratory and described previously (12) implemented on a Texas Instruments 980A minicomputer attached to a Hewlett-Packard 7220A graphics plotter.

RESULTS AND DISCUSSION: In this brief report only the resonances resulting from the N-CH_3, NH, H15,16 and ^{13}C15,16 nuclei will be discussed. At 100 MHz the lack of spectral definition, even at low pD, due to the complex ABCD spin system of H15,16 precludes further analysis. This complication is totally absent at 600 MHz at pD $\sim$ 1.5. Figure 2 shows the resulting spectra along with assignments, and is contrasted to the pD $\sim$ 7 spectrum at the same

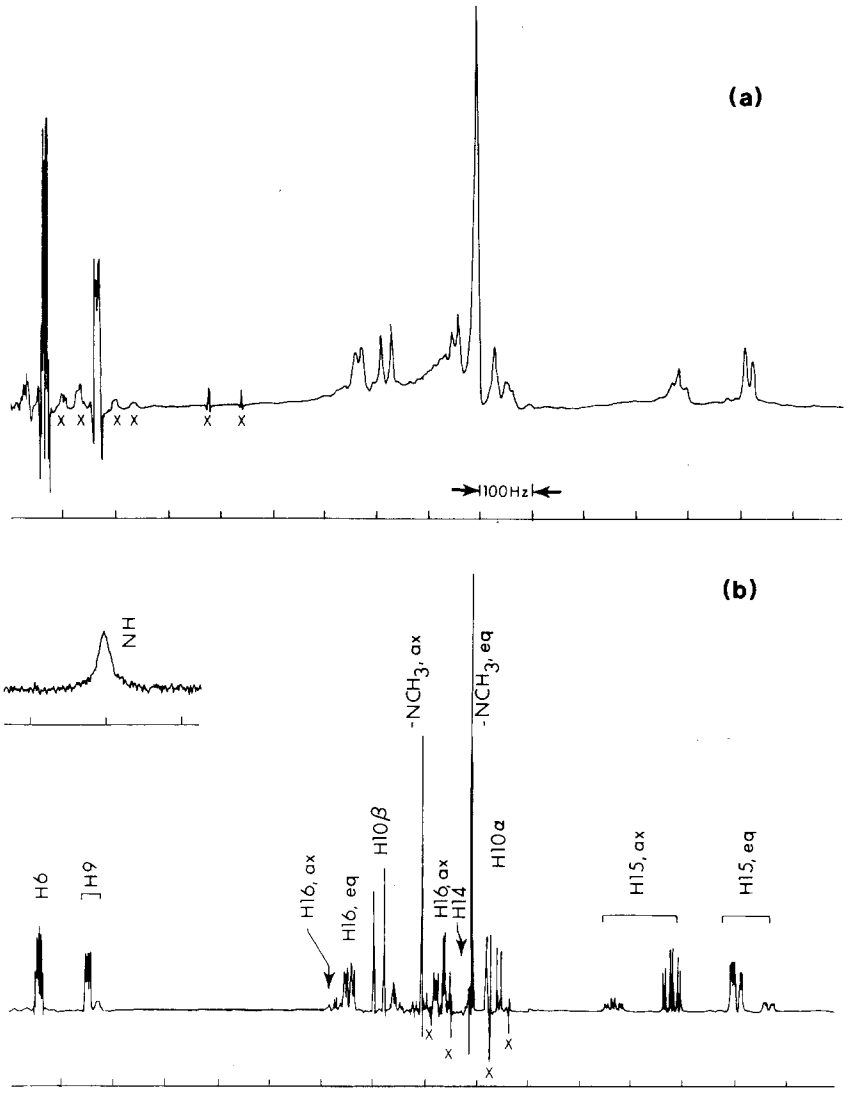

Figure 2. (A) 600 MHz proton spectrum of morphine in $^{2}H_2O$, pD ∿ 7, 25°C, in the region upfield from the water signal. This spectrum sharpens dramatically at 65°C. (B) Same region as (A), pD ∿ 1.5, inset shows NH peak, 9.5 ppm downfield from water signal taken in H_2O, pH=0.5. The major and minor components for H15 axial and H15 equatorial are bracketed and the major and minor components of the two methyl invertomers are labeled. On expanded scale (not shown) the major and minor components for H16 axial and H16 equatorial are visible. A minor component peak accompanying H9 is also seen in spectrum (B).

frequency. In H_2O at pH=0.5 the major and minor components of the N-methyl proton resonances are split into closely spaced doublets. Table I summarizes the coupling constant and chemical shift data resulting from

some of these experiments. The ^{13}C spectra at pD $\sim$ 1.5 show major and minor components for the methyl and 15,16 carbons.

This extremely simple experiment, made possible by the dispersion of high frequency NMR, has a straightforward, unambiguous, interpretation. The major and minor components in the acidified ^{1}H spectrum of morphine have nearly identical coupling constants which are consistent with the slightly distorted chair conformation (13) exhibited by the piperidine ring of (I) in the solid state. Processes such as ring inversion and chair-boat interconversion are precluded by the rigidity imposed by the fused ring molecular skeleton of (I). The results are, however, all completely consistent with a nitrogen invertomer equilibrium "frozen out" at low pH as previously found by the same method in small piperidine ring systems (11). The equilibrium ratio found corresponds to an axial-equatorial energy difference of approximately 1 Kcal/mole and the rate of interchange is on the NMR time scale at neutral pH (as evidenced by temperature dependent, differential, line broadening). Both the energy difference and barrier between the invertomers are inconsistent with theoretical estimates (14).

A stereoscopic view of the N-axial conformer of morphine is shown in Figure 1b. As a further aid in establishing molecular dimensions the following figures are pertinent. The distances between the methyl carbon and the tyrammine oxygen are: 8.177 A (N-R, equatorial) and 8.215 A (N-R, axial). The distances between the methyl carbon and the D ring oxygen are: 7.895 A (N-R, equatorial), 6.768 A (N-R, axial). The traces of the moment of intertia tensor in the principal axes systems show the N-R axial conformation to be slightly more compact with an inertial ratio of axial to equatorial of 0.93 for these quantities.

CONCLUSIONS: Previous hypotheses and theoretical studies on the topology of this molecule at the receptor which have accepted the N-R, equatorial-

only, conformer must be re-examined. This is because the on-rate to the receptor for opiate agonists such as morphine are many orders of magnitude more rapid than the invertomer interchange rate implied by the spectra shown. Hence there are effectively two different molecules of morphine being presented to the receptor at any given instant. Certain important interatomic distances vary considerably between the two invertomers.

Finally, we have observed similar effects in other opiate agonists and antagonists and will report on these along with quantitative kinetics (15), investigated using rotating frame NMR techniques (16) in more extensive reports to follow.

Acknowledgements

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References

1. Review: J.P. Tollenaere, H. Moereels and L.A. Raymaekers, "Atlas of The Three-Dimensional Structure of Drugs", Elsevier/North-Holland Biomedical Press, New York, 1979.

2. Review: S.K. Burt, G.H. Loew and G.M. Hashimoto, Ann. N.Y. Acad. Sci., 367, 219-239 (1981).

3. A.P. Feinberg, I. Creese and S.H. Snyder, Proc. Natl. Acad. Sci. (USA), 73, 4215-4219 (1976).

4. B. Belleau, T. Conway, F.R. Ahmed and A.D. Hardy, J. Med. Chem., 17, 907-908 (1974).

5. E. Bye, Acta Chem. Scand., B30, 549-554 (1976).

6. L. Gylbert, Acta Cryst., B29, 1630-1635 (1973).

7. W.G. Richards, "Quantum Pharmacology", Butterworths, Boston, 1977, pgs. 170-175.

8. S. Okuda, S. Yamaguchi, Y. Kawazoe and K. Tsuda, Chem. Pharm. Bull. (Japan), 12, 104-112 (1964).

9. F.I. Carroll, C.G. Moreland, G.A. Brine and J.A. Kepler, J. Org. Chem., 41, 996-1001 (1976).

10. J.B. Lambert, R.G. Keske, R.E. Carhart and A.P. Jovanovich, J. Am. Chem. Soc., 89, 3761-3767 (1967).

11. J.L. Sudmeir and G. Occupati, J. Am. Chem. Soc., 90, 154-159 (1968).

12. E.L. Becker, H.E. Bleich, A.R. Day, R.J. Freer, J.A. Glasel and J. Visintainer, Biochemistry, 18, 4656-4668 (1979).

13. J.B. Lambert, J. Am. Chem. Soc., 89, 1836-1840 (1967).

14. G.H. Loew and D.S. Berkowitz, J. Med. Chem., 18, 656-662 (1975).

15. E. Grunwald and E.K. Ralph III, J. Am. Chem. Soc., 89, 4405-4411 (1967).

16. H.E. Bleich and J.A. Glasel, Biopolymers, 17, 2445-2457 (1978).