Conformational Analysis of the Opioid Phenylmorphan and Its 9α-Methyl Analogue in Solution Using High-Resolution Nuclear Magnetic Resonance Spectroscopy

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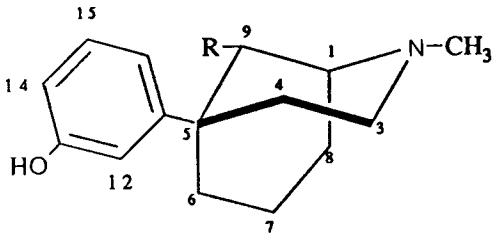
Received October 19, 1992; accepted February 1, 1993

The solution conformations of the opioid phenylmorphan (5-*m*-hydroxyphenyl-2-methylmorphan) and its 9α-methyl analogue were studied using one- and two-dimensional high resolution NMR techniques. The NMR spectra were analyzed by interpreting the phase-sensitive 2-D COSY and double quantum filtered COSY spectra, ^1H–^1H vicinal coupling constants, and nuclear Overhauser effects in the phase-sensitive 2-D NOESY spectra. The results show that, for both compounds, a chair–chair conformation of the cyclohexane and piperidine rings is exclusively preferred with some distortion of the rings from perfectly staggered chairs. For phenylmorphans, the phenyl ring is oriented to fit into the cleft formed by the cyclohexane and piperidine rings. Thus, for the (+)-enantiomer, the phenyl group assumes the same orientation with regard to the piperidine ring as morphine consistent with the morphine-like properties of the compound. For the 9α-methyl analogue, the plane of the phenyl ring essentially bisects the piperidine ring to which it is attached and is outside of the required range of opioid agonists. This is consistent with the atypical properties of the two enantiomers. The NMR results are compared to the conformations of (−)-phenylmorphan and the (+)-9α-methyl analogue in the crystal state and to the results of molecular mechanics (MM2) studies.

KEY WORDS: opioids; nuclear magnetic resonance; conformation; phenyl-equatorial; ligand model.

INTRODUCTION

The phenylmorphans (5-*m*-hydroxyphenyl-2-methylmorphans; Scheme I) are opioids in which the phenyl ring is constrained to be equatorial on the piperidine ring, whereas the phenyl ring of morphine is constrained to be axial. Despite this structural variation, (+)-1 exhibits antinociceptive activity three times that of morphine and has been shown to be morphine-like in its pharmacological properties (1,2). For example, the compound substitutes for morphine in morphine-dependent animals and displays a high capacity for inducing physical dependency. In contrast, (−)-1, with about the same antinociceptive potency as morphine, has atypical properties in that it does not substitute for morphine, precipitates withdrawal in addicted animals, and does not appear to induce physical dependency. Recently, it was shown that both enantiomers of 1 have considerable affinity for μ-receptors and that the antinociceptive activities of both are mediated through μ-receptors (3). The addition of a methyl group to the equatorial 9-position of the piperidine ring (2) produces compounds with different pharmacological profiles (4). The compound (+)-2, whose absolute configuration corresponds to (−)-1 (5,6), is a pure antagonist and the only phenylmorphan analog whose affinity for opioid κ-receptors is somewhat greater than its affinity for μ-receptors (3). The compound (−)-2, whose absolute configuration corresponds to (+)-1, is a weak, atypical agonist with codeine-like potency (4).



1: R = H

2 : R = CH_3

Scheme I

The above compounds are of interest with respect to a model which has been suggested for ligands which bind to opioid receptors (7–9). The ligand model, in which the phenyl ring of both phenyl-axial (morphine) and phenyl-equatorial (phenylmorphans) opioids binds to the same portion of the receptor, is successful in rationalizing the general structure–activity relationships of opioids. Thus, the phenyl ring can be considered to be the molecular anchor to the receptor. Superposition of the phenyl rings in phenyl-axial and phenyl-equatorial opioids shows that the ammonium nitrogens are situated in different portions of the receptor space. The ammonium hydrogens, however, are directed toward the same point in space and could, therefore, interact with the same negatively charged receptor site. Applying the ligand model (3,6,10), it appears that the orientation of the phenyl ring relative to the piperidine ring may be a more critical conformational factor in opioids.

In this report, high-resolution ^1H NMR spectroscopic techniques were used to determine the preferred conformations of 1 and 2 in solution and the results were considered with respect to their pharmacological properties and the ligand model. The analytical process first required the complete assignment of chemical shifts by the interpretation of the 500-MHz 1-D and phase-sensitive 2-D COSY and double quantum filtered (DQF) COSY spectra. Vicinal ^1H–^1H coupling constants (^3J) were then estimated from the 1-D and

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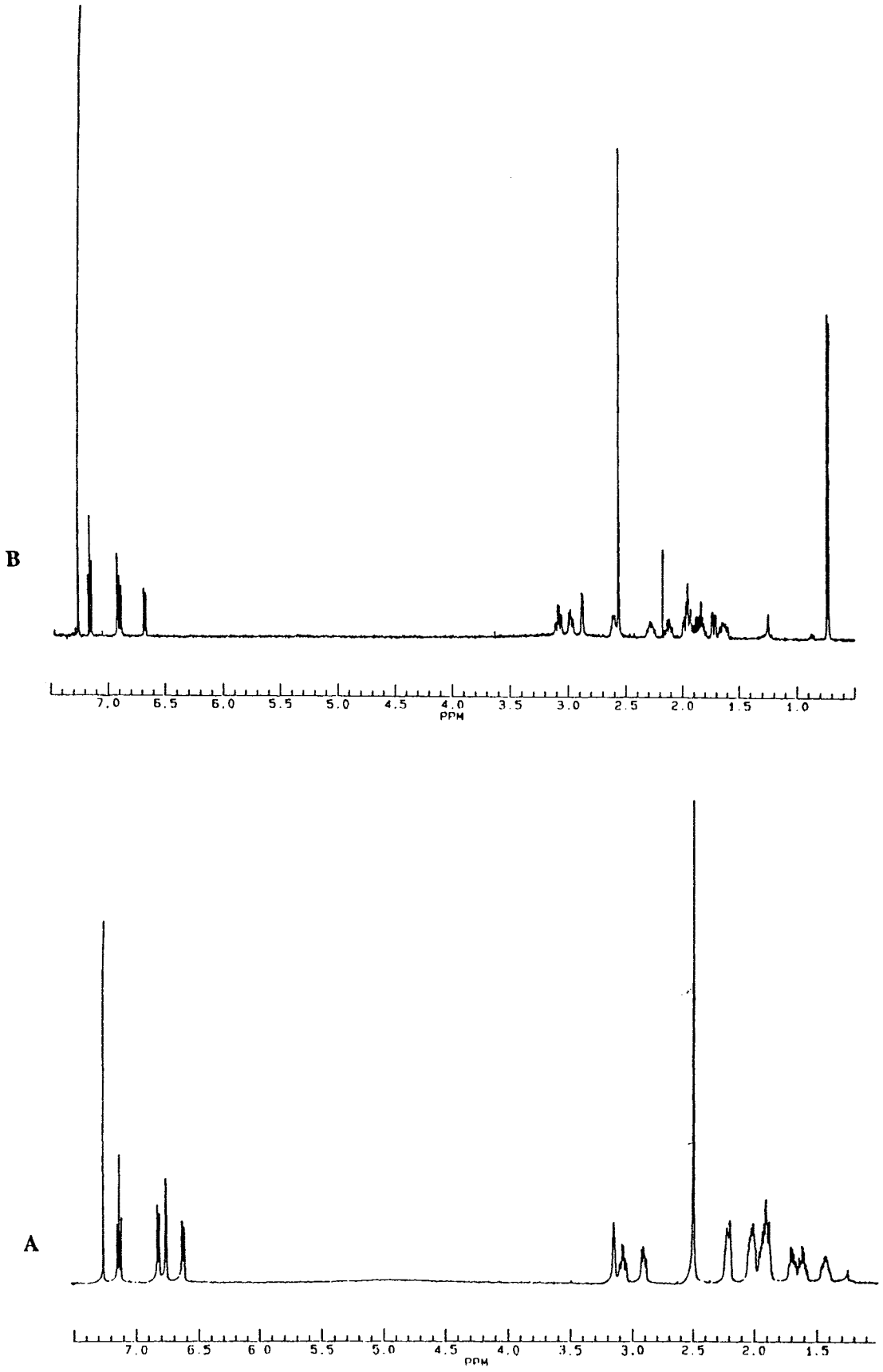


Fig. 1. Full-scale 500-MHz ^{1}H NMR spectra of phenylmorphan (1;A) and 2,9α-dimethyl-5-*m*-hydroxyphenylmorphan (2;B), both prepared as 0.08 *M* in $CDCl_3$.

Table I. ^{1}H NMR Chemical Shifts for Compounds 1 and 2 in $CDCl_3$, Relative to Internal TMS

Proton	1 (ppm)	2 (ppm)
H12	6.76	6.92
H14	6.62	6.68
H15	7.13	7.14
H16	6.82	6.89
2-CH_3	2.49	2.57
9-CH_3	—	0.73
H1eq	3.15	2.88
H3ax	3.07	3.08
H3eq	2.90	2.98
H4ax	2.00	2.29
H4eq	1.92	1.73
H6ax	1.60	1.96
H6eq	2.05	2.11
H7ax	1.90	1.84
H7eq	1.67	1.88
H8ax	1.42	1.64
H8eq	2.18	1.96
H9ax	2.21	2.62
H9eq	1.89	—

phase-sensitive 2-D COSY spectra (11) and refined by iterative simulation of subspectra. The ^3J values so obtained were subsequently correlated to the corresponding dihedral angles using the empirical Karplus equation (12). Finally, the ^{1}H–^{1}H NOE cross-peaks in the phase-sensitive NOESY spectra, which reflect close through-space interactions of protons within a molecule, were interpreted in order to provide information about the three dimensional geometries of 1 and 2. The results of this study are compared to the conformation of (−)-1 and (+)-2 in their crystal states (5,6) and to the molecular mechanics (MM2) calculations (3,6,10) in order to provide a more complete picture of the conformational preferences of compounds 1 and 2.

MATERIALS AND METHODS

Materials. The compounds (+)-5-*m*-hydroxyphenyl-2-methylmorphan (1) and (±)-2,9-α-dimethyl-5-*m*-hydroxyphenylmorphan (2) were obtained from Prof. E. L. May of the Medical College of Virginia. The NMR solvent $CDCl_3$ (99.8 atom%) was purchased from Aldrich Chemical Company (Milwaukee, WI). The NMR samples were prepared in $CDCl_3$ as 0.08 *M* drug, thoroughly degassed, and sealed in precision 5-mm NMR tubes (728-pp; Wilmad Glass Company, Buena, NJ).

^{1}H NMR Spectra. The high-resolution 500 MHz 1-D and 2-D spectra were recorded with a Bruker MSL-500 FT spectrometer equipped with an Aspect 3000 computer. The spectra were recorded at 21.7°C and all ^{1}H chemical shifts were referenced to internal tetramethylsilane (TMS). Typical acquisition parameters for recording 1-D spectra were as follows: spectral width, 4464 Hz (8.9 ppm); data size, 16 K; recycle delay, 5 sec; pulse width, 6.0 μsec (90° tip angle); and 128 transients. Typical acquisition parameters for recording the phase-sensitive double quantum filtered COSY (DQF-COSY) and phase-sensitive 2-D NOESY spectra were as follows: 90° pulse width, 6.0 μsec; initial t_1 value, 3 μsec; 64 transients/t_1 value; sweep width in f_1 and f_2, 4464 Hz; recycle delay, 5 sec; and data size, $f_1 \times f_2$, 256*w* × 4 K. The NOESY spectra were recorded with a mixing time of 0.4 sec, which was randomly varied for suppression of zero-order *J* cross-peaks. Data processing of the 2-D spectra included application of a sine-bell window function in f_1 and f_2 and 2-D Fourier transformation.

NMR Spectral Simulations. Spectral regions of the 1-D 500-MHz spectra of compounds 1 and 2 corresponding to the cyclohexyl and piperidinyl rings were simulated with an Aspect 3000 computer and the LAOCOON-based PANIC software from the Bruker library of computer programs. The program accepts a maximum of nine different nuclear spins and requires the input of chemical shifts and coupling constants. Linewidth, spectral width, and spectral density are also adjustable parameters. Chemical shifts and estimated ^2J and ^3J coupling constants estimated using established procedures (13) were used as the starting point for an iterative simulation of subspectra. Dihedral angles were calculated from the Karplus equation ($^3J = K\cos^2\theta$) (12) using *K* values (K_{aa} = 13.31 Hz, $K_{ae} = K_{ea}$ = 14.36 Hz, K_{ee} = 12.24 Hz) calculated from ^3J values measured in 1,1′,4,4′-tetradeuterocyclohexane (14) and dihedral angles determined independently for cyclohexane (15).

RESULTS

Chemical Shift Assignments. The ^{1}H NMR chemical shift assignments of 1 and 2 were made on the basis of a

Table II. Selected ^{1}H NMR ^2J (Hz) and ^3J (Hz) Coupling Constants and the Corresponding Dihedral Angles (°) for Compounds 1 and 2

Ha–Hb	1 (Hz)[a]	Dihedral angle[b]	2 (Hz)[a]	Dihedral angle[b]
^2J				
3ax–3eq	−10.1	—[c]	−9.8	—
4ax–4eq	−10.2	—	−10.5	—
6ax–6eq	−11.4	—	−11.5	—
7ax–7eq	−11.6	—	−10.3	—
8ax–8eq	−10.6	—	−10.3	—
^3J				
3ax–4ax	9.4	147	9.8	149
3ax–4eq	2.9	63	3.0	63
3eq–4ax	6.0	50	6.6	47
1eq–8ax	4.0	58	4.4	56
1eq–8eq	2.0	66	1.7	68
1eq–9ax	1.9	69	2.2	67
6ax–7ax	10.5	153	10.0	150
6ax–7eq	6.1	49	6.2	50
6eq–7ax	6.1	49	6.3	49
6eq–7eq	2.2	65	1.9	67
8ax–7ax	9.4	147	9.7	148
8ax–7eq	8.8	38	8.5	39
8eq–7ax	6.3	49	6.5	47
8eq–7eq	1.9	66	1.7	68

[a] ^2J and ^3J values determined from spectral simulations of the 500-MHz 1-D NMR spectra (see text).

[b] Dihedral angles calculated from the Karplus equation using constants as discussed under Materials and Methods.

[c] These dehedral angles cannot be quantitatively determined from ^2J values (15).

first-order analysis of the 1-D spectra and interpretation of the 2-D COSY and 2-D NOESY cross-peak patterns. The full-scale 500-MHz spectra are shown in Fig. 1. The phenyl protons H12, H13, H15, and H16, the piperidinyl protons H1 and H3, and the methyl protons 2-CH_3 and 9-CH_3 (for compound 2) were assigned on the basis of integrated chemical shifts and analysis of 2-D DQF-COSY cross-peak patterns. The remaining protons were assigned on the basis of coupling networks identified by the phase-sensitive COSY experiments. For example, H1 of compound 2 couples through a vicinal mechanism to H9 and the two H8 protons. In a similar analysis, the two H3 protons couple to the two vicinal H4 protons. Assignment of protons oriented either axially or equatorially was accomplished by measurement of their respective coupling constants and by comparison of their individual NOESY cross-peak patterns. As an example, H3ax experiences NOE interactions with H8eq and H7ax, while H3eq does not. H4ax shows an NOE interaction with H9ax and H3eq, while H4eq shows an NOE cross-peak with H7ax. Chemical shift assignments are summarized in Table 1.

Coupling Constants. Examination of the 1-D spectra for 1 and 2 (Fig. 1) shows that many resonances overlap, preventing the direct determination of $^2J_{HH}$ and $^3J_{HH}$ coupling constants. Thus, ^2J and ^3J values were first approximated by first-order analysis of the individual multiplets in the f_1 cross sections of the 2-D COSY spectra. The chemical shift values (Table I) and approximated J values were then

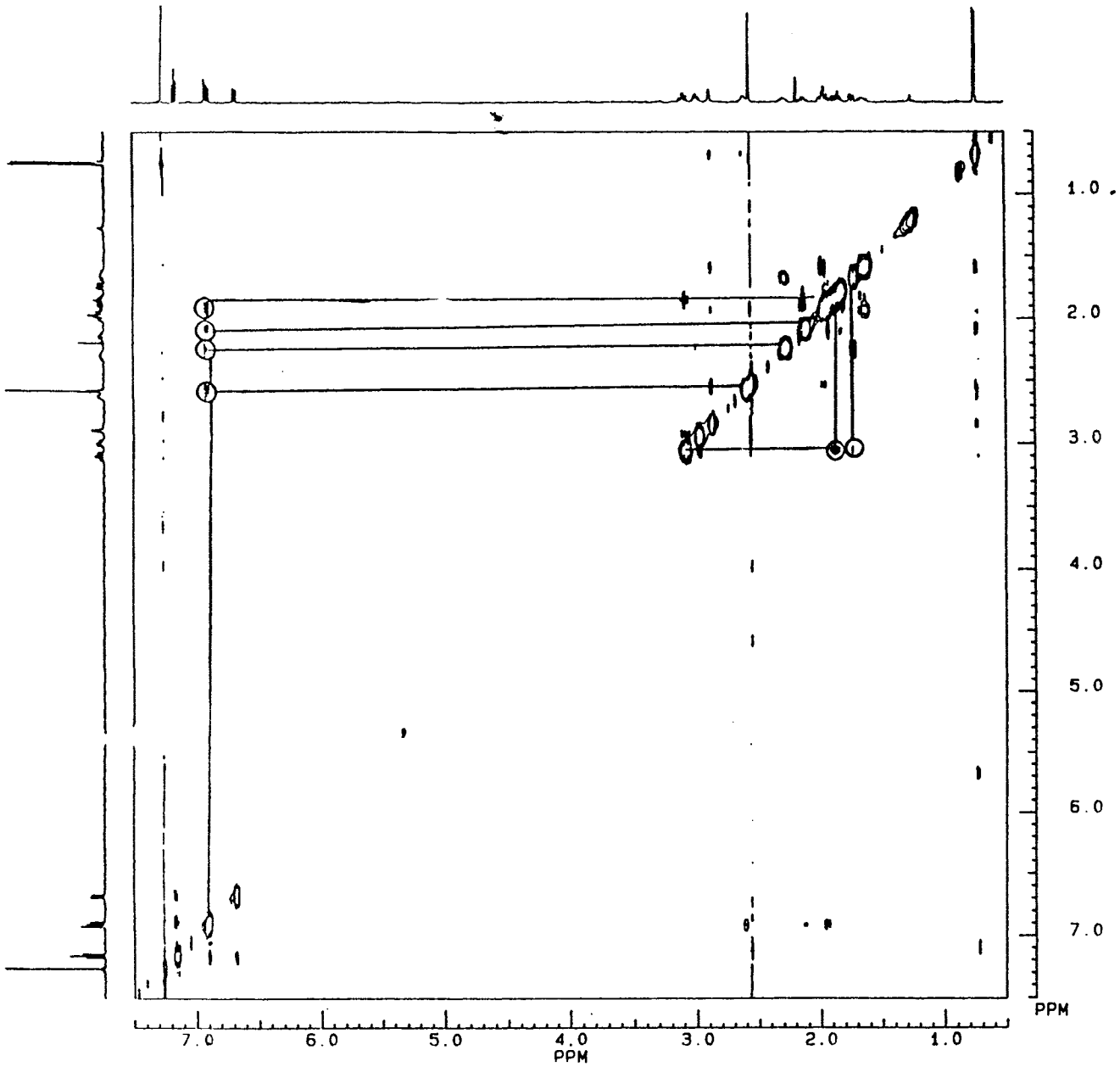


Fig. 2. The full-scale 500-MHz ^{1}H 2-D NOESY contour map for the 9α-methyl analogue (2). NOE cross-peaks between H12/H16 and H9ax, H6eq, H4ax and between H3ax, H7ax are highlighted.

used as the starting point for iterative simulation of subspectra. This simulation procedure allowed determination of selected geminal and vicinal coupling constants between protons of the piperidine and cyclohexane rings. The ^3J values so obtained are then incorporated into the Karplus equation (12). Coupling constant values and the calculated dihedral angles for 1 and 2 are summarized in Table II.

NOE Interactions. Nuclear Overhauser effects (NOE) can be used to determine the spatial relationships of protons within a molecule and thus the NOE is one of the most important NMR parameters used in conformational analysis. Two-dimensional ^{1}H NOESY experiments were carried out for both 1 and 2 in order to determine the spatial relationship of all hydrogens in the molecule. The resulting full-scale NOESY 2-D contour map for 2 is shown in Fig. 2. NOE interactions of particular significance to the conformational analysis of 1 and 2 are listed in Table III.

DISCUSSION

Cyclohexane and Piperidine Rings. The 2-D NOESY spectra for 1 and 2 clearly show that the piperidine and cyclohexane rings are exclusively in a chair–chair conformation. This is confirmed by the NOE cross-peaks observed in the corresponding 2-D contour maps. In the case of compound 2, for example (Fig. 2, Table III), there are NOE interactions between NCH_3 and H8eq but not between NCH_3 and H8ax. However, there are NOE cross-peaks between 9-CH_3 and H8ax but not with H8eq. Very strong evidence is also provided by the cross-peaks between H4ax and H9ax and the large NOE cross-peak between H3ax and H7ax. Taken together, the NOE cross-peak pattern rules out the presence of chair–boat or boat–chair conformers and supports an exclusive preference for a chair–chair conformation. This results in the formation of a cleft at the junction of the cyclohexane and piperidine rings (Fig. 3). NOE data for 1 (Table III) lead to a completely analogous conclusion. The crystal structures of (−)-1 and (+)-2 also show a chair–chair conformation in the solid state (5,6). MM2 calculations for 1 (10) also suggest a strong preference for a chair–chair conformer.

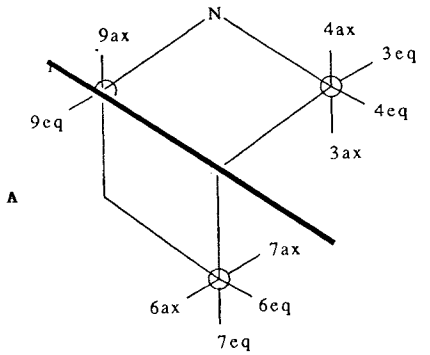
The conformations of the cyclohexane and the piperidine rings were also analyzed by interpretation of the vicinal coupling constants between the aliphatic ring hydrogens in the context of the Karplus equation (Table II). These results show that the cyclohexane and piperidine rings deviate significantly from perfectly staggered geometries, particularly in the region of the cleft formed at the juncture of the two rings. In the case of compound 2, for example, the dihedral angles between H7ax, H7eq, H8ax, and H8eq (148, 39, 47, 68°) differ considerably from the almost perfectly staggered conformation observed for cyclohexane (175, 55, 55, 65°) (15). This geometrical distortion occurs to relieve the unfavorable steric crowding in the chair–chair conformation between H7ax and H3ax. A similar result is found for compound 1 (Table II). The structures of (−)-1 and (+)-2 in the crystal state also show that the two chairs are distorted due to a close intermolecular contact of 3.08 and 3.06 Å between

Table III. Selected NOE Interactions Relevant to the Conformational Analysis of Compounds 1 and 2

Ha:Hb interaction	Cross-peak (ppm, ppm)	
	1	2
H16:H6eq	6.82, 2.05[a]	6.89, 1.96
H16:H6ax	None	6.89, 2.11
H16:H4eq	6.82, 1.92[b]	None
H16:H4ax	6.82, 2.06[a]	6.89, 2.29
H16:9-CH_3	—	None
H16:H9eq	6.82, 1.89[b]	—
H16:H9ax	6.82, 2.21	6.92, 2.62
H12:H6eq	6.72, 2.05[a]	6.92, 1.96
H12:H6ax	None	6.92, 2.11
H12:H4eq	6.76, 1.92[b]	None
H12:H4ax	6.76, 2.06[a]	6.92, 2.29
H12:9-CH_3	—	None
H12:H9eq	6.76, 1.89[b]	—
H12:H9ax	6.76, 2.21	6.92, 2.62
H8eq:NCH_3	2.18, 2.49	1.95, 2.57
H8ax:NCH_3	None	None
H8eq:9-CH_3	—	None
H8ax:9-CH_3	—	1.64, 0.73
H7ax:H3-ax	3.07, 1.90	3.08, 1.84

[a] Spectral overlap H6eq and H4ax precludes assignment of this cross-peak.

[b] Spectral overlap H9eq and H4eq precludes assignment of this cross-peak.



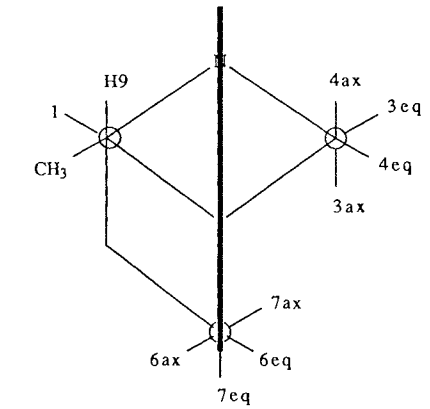


Fig. 3. The preferred orientation of the phenyl ring about the phenyl–piperidine bond for 1 (A) and 2 (B).

C3 and C7, respectively (5,6). The results from MM2 calculations are in agreement with those of the experimental methods (3,6,10).

Phenyl Ring. For the phenylmorphans, a major conformational degree of freedom is rotation about the phenyl–piperidine bond. Interpretation of NOE interactions between the phenyl and aliphatic ring protons was required in order to elucidate the orientation about the phenyl–piperidine bond. It is important to note that both ortho phenyl protons (H12/H16) have similar NOEs, suggesting that a 180° rotation of the phenyl ring has little effect on the conformational energy. The energy equivalence of flipping the phenyl ring is also demonstrated by MM2 calculations (3,6,10). The orientation of the phenyl ring in the crystal structure of (+)-2 is opposite of that observed in the crystal structure of (−)-1 and may be obtained from the biologically active form by 180° rotation (6).

For compound 2, the ortho phenyl protons H12/H16 show 2-D NOE cross-peaks with aliphatic ring protons H9ax, H4ax, H6ax, and H6eq (Fig. 2, Table III). No NOE cross-peaks are observed between H12/H16 and 9-CH_3 or H4eq. Taken together, this cross-peak pattern is consistent with a conformation in which the plane of the phenyl ring essentially bisects the piperidine ring to which it is attached (Fig. 3B).

In the case of compound 1, two NOE cross-peaks can be unambiguously assigned to interactions between the ortho phenyl protons H12/H16 and H9ax (Table III). Unfortunately, spectral overlap of several aliphatic protons (H4ax and H6eq; H4eq and H9eq) precludes the unambiguous assignment of NOE cross-peaks at (6.8, 2.0 ppm), (6.7, 2.0 ppm), (6.8, 1.9 ppm) and (6.7, 1.9 ppm). This complicates assignment of the phenyl ring conformation. However, a clue to the orientation of the phenyl ring for this compound can be gained from the observation that phenyl protons H12/H16 do not exhibit NOE interactions with H6ax. The only phenyl ring conformation consistent with an unambiguous cross-peak between H12/H16 and H9ax and the absence of NOE between H12/H16 and H6eq is one in which the phenyl ring fits into the cleft formed by the two aliphatic rings (Fig. 3A). Although the evidence is circumstantial, this conclusion is supported by the observation of a similar conformation in the solid-state crystal structure of (−)-1 (5) and by MM2 calculations (10).

CONCLUSION

In the present study, the preferred phenyl orientations for phenylmorphan (1) and its 9α-methyl analogue (2) in solution have been examined by high-resolution ^1H NMR spectroscopy. In previous reports, phenyl orientation has been examined in the solid state by X-ray crystallography (5,6) and by MM2 calculations (3,6,10). There is excellent agreement among the three methods, and considered together, they provide a more complete picture of the relationship between pharmacological activity and phenyl orientation. The opioid ligand model discussed above proposes that morphine-like properties require a narrow range of phenyl orientations. For (+)-1, all three methods show a similar preferred phenyl conformation (Fig. 3B) that is likely to be responsible for its morphine-like pharmacological profile. The weaker μ-affinity of (−)-1 is consistent with the extra 1.0 kcal/mol necessary to achieve the required orientation of the phenyl ring (6,10). For 2, the NMR, X-ray, and MM2 results show the preferred phenyl orientation (Fig. 3A) as being out of the pharmacologically active range. Achieving the required phenyl orientation in (−)-2 and (+)-2 requires an additional 1 and 3 kcal/mol, respectively (6). This can account for the weak atypical agonist properties of (−)-2 and the pure antagonist properties of (+)-2. Thus, the results of this study along with those of the previous studies (3,6,10) indicate that as the phenyl ring deviates from a particular orientation (Fig. 3B), μ-receptor affinity decreases and a non-morphine-like pharmacological profile emerges.

ACKNOWLEDGMENT

This work was supported by NIDA Grant DA 04762.

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