Molecular Determinants for Drug–Receptor Interactions. Part 7. 500 MHz ^{1}H Nuclear Magnetic Resonance Spectra of the Narcotic Agonists Morphine and Oxymorphone and of the Morphine-related Antagonist Nalorphine by Two-dimensional ^{1}H–^{1}H Chemical Shift Correlation Spectroscopy

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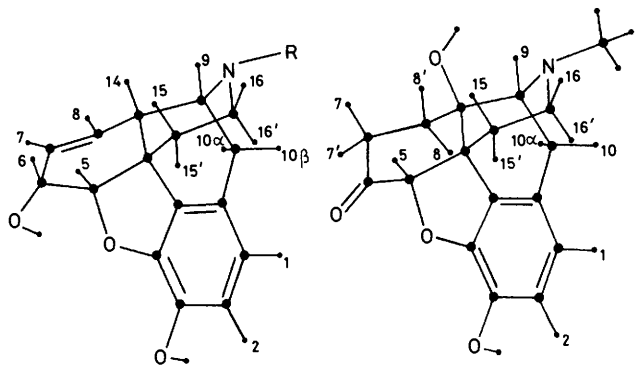
The high frequency (500 MHz) ^{1}H n.m.r. spectra of the narcotic agonists morphine and oxymorphone and of the mixed agonist–antagonist nalorphine (hydrochloride salts) were run in ^2H_2O and fully analysed in terms of chemical shifts and coupling constants. Detection of correlated resonances, relative signs of coupling constants and assignments of multiplets were made possible by two-dimensional (2D) homonuclear shift spectroscopy. The coupling constant determined provided evidence that the piperidine ring adopts a slightly distorted-chair conformation in all these compounds. Selective broadening of the signals of the protons in the 10, 15, 16, and 17 positions was observed in the morphine and nalorphine spectra at 296 K. This was consistent with an inversion process occurring between the two configurational isomers with axial and equatorial *N*-alkyl group. Resolved patterns were attained at 345 and 330 K for morphine and nalorphine, respectively. This demonstrated that the increase of temperature was sufficient to make the inversion process rates fast on the n.m.r. time-scale. All portions of the spectrum of oxymorphone were sharp at 296 K, showing that a single diastereoisomer (equatorial *N*-alkyl group) is present for this molecule. A possible correlation was proposed between restricted conformational freedom along the chain of the *N*-alkyl group and the relative narcotic antagonist potency of nalorphine, naloxone, and naltrexone.

As part of continuing extensive n.m.r. spectroscopic studies on the conformational and motional characteristics of narcotic agonists and antagonists,[1–3] we report here the complete analysis of the high-frequency proton spectra of the related pair morphine (**1**)–nalorphine (**2**) and of oxymorphone (**3**). By substitution of the *N*-methyl group in the pure agonist (**1**) with the *N*-methylallyl group, nalorphine (**2**) is obtained which has mixed agonist–antagonist activity. Compound (**3**), a more potent agonist than morphine, differs from the related very active pure antagonists naloxone and naltrexone only by having an *N*-methyl group in place of an *N*-methylallyl and *N*-methylcyclopropyl group, respectively.

A high frequency (600 MHz) ^{1}H n.m.r. study of (**1**) (in aqueous solution at variable pH) has been published,[4] providing new information on the conformational equilibria for this molecule.

Compounds (**1**)—(**3**) (hydrochlorides, in aqueous solution) were therefore examined in order to obtain a complete picture of their conformational features which, in the light of the ^{1}H n.m.r. results obtained for the antagonists naloxone and naltrexone,[2] could be useful in supporting a model of the mode of operation at the receptor site.

High frequency (500 MHz) and two-dimensional (2D) homonuclear shift spectroscopy were used to perform the full analysis of the highly complex spin systems of compounds (**1**)—(**3**).



Experimental

Compounds (**1**)—(**3**) (hydrochlorides) were dissolved in ^2H_2O (CEA, France; 99.95% ^{2}H), at a final concentration of 10^{-2}M. A trace of sodium trimethylsilyl[2,2,3,3-$^2H_4$]propionate was added as internal reference. Spectra were obtained (32 768 data points) at 296, 330, and 345 K for (**3**), (**2**), and (**1**), respectively, using a Bruker WM500 spectrometer operating at 500.13 MHz. Further resolution enhancement was achieved by multiplication of the time domain by an optimized Lorentzian–Gaussian function.

Assignments were made by using two-dimensional shift correlation. Three types of experiments[5,6] were performed: (i)

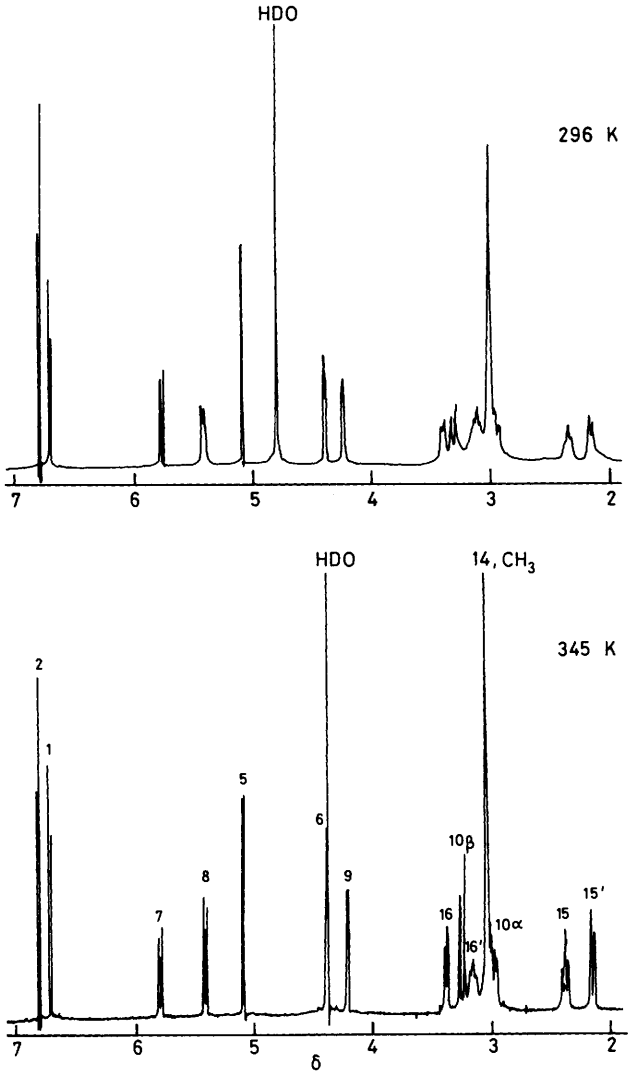


Figure 1. The 500 MHz ^1H n.m.r. spectrum of morphine (**1**) hydrochloride in ^2H_2O solution, at 296 (upper) and 345 K (below). Assignment of resonances is outlined in the resolved spectrum at higher temperature

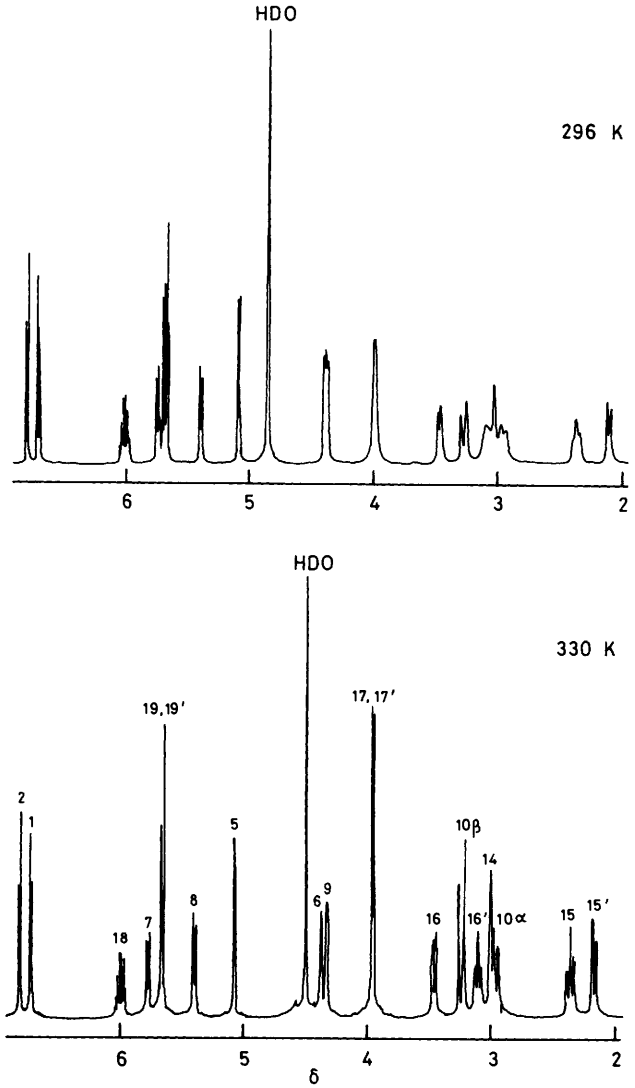


Figure 2. The 500 MHz ^1H n.m.r. spectrum of nalorphine (**2**) hydrochloride in ^2H_2O solution, at 296 (upper) and 330 K (below). Assignment of resonances is outlined in the resolved spectrum at higher temperature

COSY 45, sign sensitive shift correlation experiment (90, t_1, 45 acquisition), positive (^2J) and negative (^3J) couplings show reverse inclination of the corresponding cross-peaks on the correlation map; (ii) COSY with single relay correlation (90, t_1, 90, τ, 180, τ, 90 acquisition) with τ 30 ms; (iii) COSY LR with enhancement of small couplings (90, t_1, τ, 45 τ acquisition). The fixed delay τ was set to 150 ms to enhance small and long range couplings. In the pulse sequence t_1 represents the evolution period.

In all cases a total of 256 FIDs was collected. The final matrix sizes (magnitude mode) was 1K × 1K. All experiments were processed using square sine bell windows in both time domains. Homonuclear spin decoupling was used in some case to measure and confirm coupling constants.

The one-dimensional ^1H n.m.r. spectra of (**1**)—(**3**) are shown in Figures 1—3 along with a contour plot section of the shift correlation matrix of (**2**) (Figure 4). Some resonance patterns were simulated by using the LAOCOON III computer program,[7] in order to refine the spectral parameters. These are

Spectral Analysis

The analysis of the nalorphine (**2**) spectrum was initiated by assignment of the AB type spin-system at lowest field to H-1 and H-2. The relative assignment ($\delta_{H\text{-}1} < \delta_{H\text{-}2}$) was made on the basis of the small coupling of H-1 to both H-10α and H-10β detectable on the COSY LR correlation map (Figure 4).

The H-5, H-6, H-7, H-8, H-14 spin system was located on the same diagram (Figure 4). The H-5 resonance (doublet of doublets) was identified by decoupling on H-6. This latter nucleus appears as a complex multiplet, due to very small couplings (< 0.5 Hz) to H-7 and H-14, recognised by means of the COSY LR spectrum. Location of H-7 (doublet of sextuplets) and H-8 (doublet of triplets) was therefore straightforward as well as extraction of the value of the coupling constant J(7,8). The very small coupling constant J(8,14) which was detected in the COSY LR spectrum allowed the H-7 and H-8 resonances to be safely assigned. Inspection of the correlation diagram also showed that the H-14 (quintuplet) nucleus is coupled to H-9 (quadruplet).

The pattern obtained after decoupling of H-9 was in acceptable agreement with the simulated one by the iterative

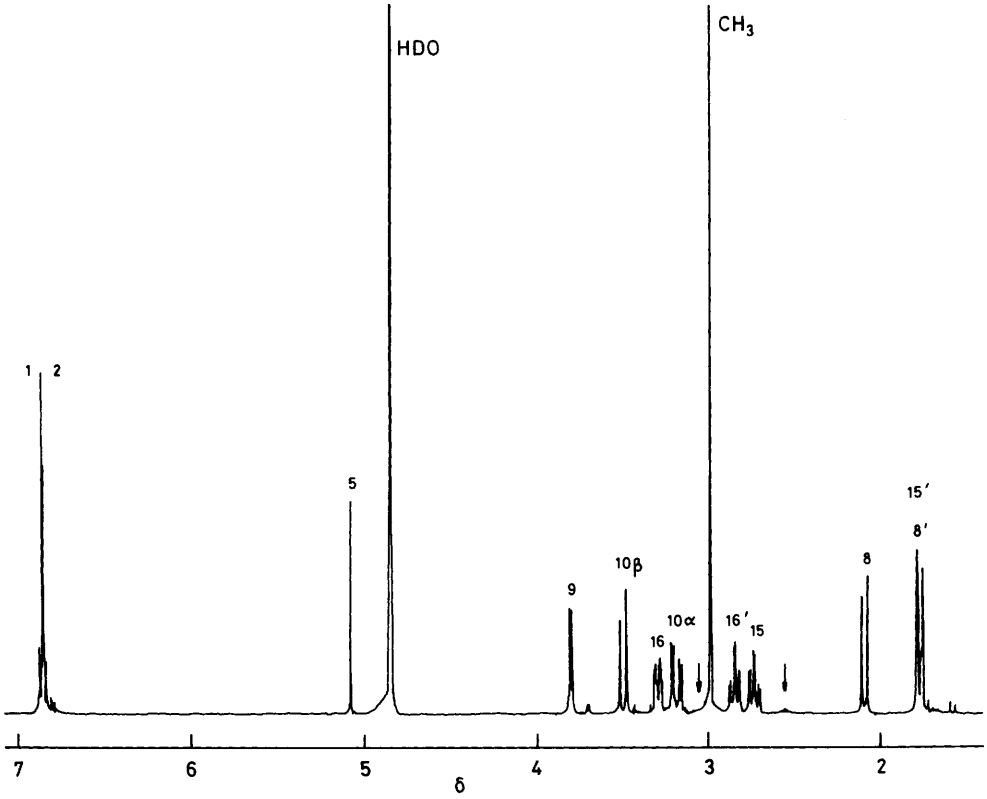


Figure 3. The 500 MHz ^1H n.m.r. spectrum of oxymorphone (**3**) hydrochloride in ^2H_2O solution, after complete deuterium exchange (at 320 K) of H-7 and H-7′. Assignment of resonances is outlined. The positions of the H-7 and H-7′ multiplets before deuteriation are denoted by the arrows

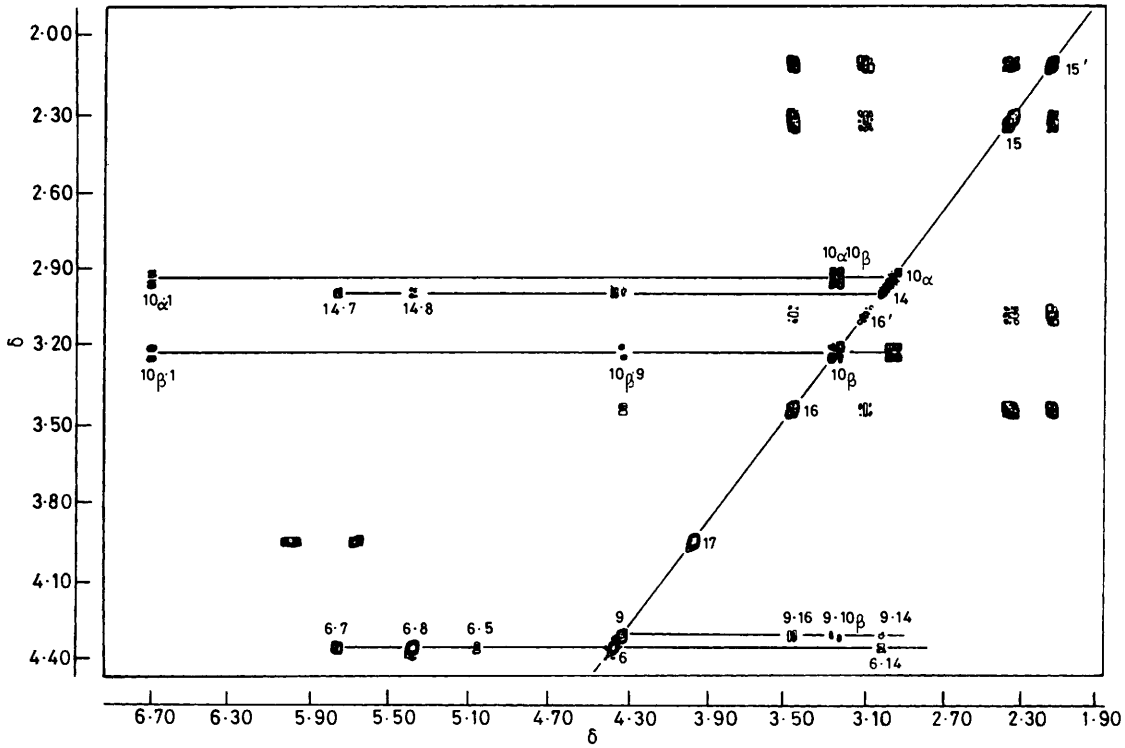


Figure 4. Contour plot of the ^1H COSY LR spectrum of nalorphine (**2**) hydrochloride, in the ranges δ 2.00—4.40 and 1.90—6.70. Assignments and correlation numbers are given. The matrix was symmetrized

fitting procedure for refinement of the whole spin-system. The irradiation of H-9 caused simplification of the H-10α signals (quadruplet) which were thus recognised unambiguously. The H-10β resonance (doublet) was then located on the map in Figure 4.

The resonances of the protons bonded to C-15 and C-16 were

Table. ^{1}H N.m.r. spectral parameters (500 MHz) for morphine (**1**), nalorphine (**2**), and oxymorphone (**3**) in ^2H_2O solution (10^{-2}M) at 345 (**1**), 330 (**2**), and 296 K (**3**)

	Chemical shifts (δ)				Coupling constants (*J*/Hz)		
	(1)	(2)	(3)		(1)	(2)	(3)
H-1	6.71	6.71	6.87	*J*(1,2)	8.17	8.19	8.46
H-2	6.80	6.80	6.85	*J*(5,6)	6.35	6.40	
H-5	5.07	5.08	5.08	*J*(5,7)	1.14	1.28	
H-6	4.38	4.38		*J*(6,7)	*	*	
H-7	5.79	5.78	3.04	*J*(6,8)	*	*	
H-7′			2.36	*J*(6,14)	†	†	
H-8	6.40	5.40	2.09	*J*(7,7′)			14.82
H-8′			1.77	*J*(7,8)	9.90	9.89	4.99
H-9	4.21	4.33	3.80	*J*(7,8′)			14.78
H-10α	2.98	2.96	3.18	*J*(7′,8)			2.93
H-10β	3.25	3.24	3.49	*J*(7′,8′)			3.23
H-14	3.04	3.01		*J*(8,8′)			14.54
H-15	2.38	2.36	2.74	*J*(8,14)	†	†	
H-15′	2.15	2.17	1.79	*J*(9,10α)	6.08	6.22	6.10
H-16	3.38	3.46	3.30	*J*(9,10β)	†	†	†
H-16′	3.15	3.11	2.85	*J*(9,14)	3.10	3.20	
N-Me	3.04		3.00	*J*(9,16)	*	*	
H-17		3.96[a]		*J*(10α,10β)	20.10	19.98	20.02
H-17′		3.96[a]		*J*(15,15′)	14.16	14.02	13.53
H-18		6.00		*J*(15,16)	4.72	4.90	4.69
H-19		5.67[b]		*J*(15,16′)	13.76	13.65	13.33
H-19′		5.67[b]		*J*(15′,16)	†	†	†
				J(15′,16′)	3.16	3.62	3.66
				J(16,16′)	13.40	13.38	13.04
				J(17,18)		~7.2[c]	
				J(17′,18)		~7.2[c]	
				J(18,19)		~10.1[d]	
				J(18,19′)		~17.2[d]	
				J(19,19′)		*e*	

* Value *ca.* 1 not refined, determined in the COSY LR spectrum. † Very small value ≤0.5.
[a] Overlapped with HDD signal at 330 K; determined at 296 K. [b] Centre of overlapped H-19, H-19′ multiplets. [c] Deduced from splitting pattern of H-17. [d] Deduced from splitting pattern of H-18 (quadruplet) after decoupling of H-17. [e] Not detectable due to overlapped resonances.

identified on the basis of ^{1}H–^{1}H connectivities. H-15 (triplet of doublets) and H-15′ (not well resolved doublet of quadruplets) were distinguished from H-16 (doublet of doublets) and H-16′ (triplet of doublets) on the bases of the small coupling of H-16 to H-9 which was detected in the COSY LR correlation diagram.

The remaining groups of signals in the spectrum of (**2**), centred at δ 3.96, 6.00, and 5.67, belong to the *N*-methylallyl group protons in positions 17, 18, and 19, respectively. The doublet* due to H-17, H-17′ becomes a singlet by irradiating H-18, thus providing evidence that these nuclei are isochronous.

The lack of the multiplets due to the protons of the *N*-methylallyl group made easy the analysis of the analogous spectrum of morphine (**1**). This showed differences consisting only in (i) the overlap between H-14 and H-17 (protons of the *N*-methyl group) resonances and (ii) the partial overlap of the H-6 multiplet by the HDO line at a given temperature.

Also in the case of (**3**), the lack of resonances due to the *N*-methylallyl and *N*-methylcyclopropyl groups, made its spectrum less complex than the spectra of the analogoues naloxone and naltrexone, respectively, which were previously reported by us.[2] The spectral analysis for (**3**) was therefore straightforward. It should be noted however that, as for naloxone and naltrexone, in (**3**) the H-7 and H-7′ (and to a lesser extent H-5) nuclei exchange readily with ^2H_2O. The exchange rates observed for oxymorphone were even faster than that for previously studied analogues.

* Broadening of the component lines of the doublet indicated unresolved very small couplings.

Discussion

The Karplus relationship[8] was used to deduce the conformations about the C(9)–C(10), C-7)–C(8) [only for (**3**)], and C(15)–C(16) bonds, on the basis of the coupling constants. Exactly the same conformational conclusions drawn before for naloxone and naltrexone[2] were found to be valid for molecules (**1**)—(**3**). The results are summarized in Figure 5. In particular, when considering the conformation about the C(15)–C(16) bond, the *J* values do not discriminate between conformations A and B. A choice was made[2] in favour of the staggered conformation A which corresponds, as may be checked by use of molecular models, to a distorted-chair conformation of the C(13), C(14), C(15), C(16), N, C(9) ring for all compounds studied. It should be noted that this finding agrees well with previous results from *X*-ray diffraction analysis of the crystal for morphine and oxymorphone.[9,10] It suggests, interestingly, that the relative pharmacological potency of (**2**) and (**3**) and its related analogues cannot be correlated with the occurrence of one of the possible chair or boat conformations of the piperidine ring in these molecules.

Conversely, a peculiar feature was found for the spectra of (**1**) and (**2**) initially run at 296 K, *i.e.* a severe broadening of the spin patterns assigned to H-15, H-15′, H-16, H-16′, H-10α, H-10β and, mainly, to H-17, H-17′ [in (**2**)], while the resonance lines due to the remaining protons were sharp† (see Figures 1 and 2).

† The H-14 signal (Figure 2) is slightly broadened at 296 K. However, at higher temperature, only its intensity increases, while it remains a well resolved singlet.

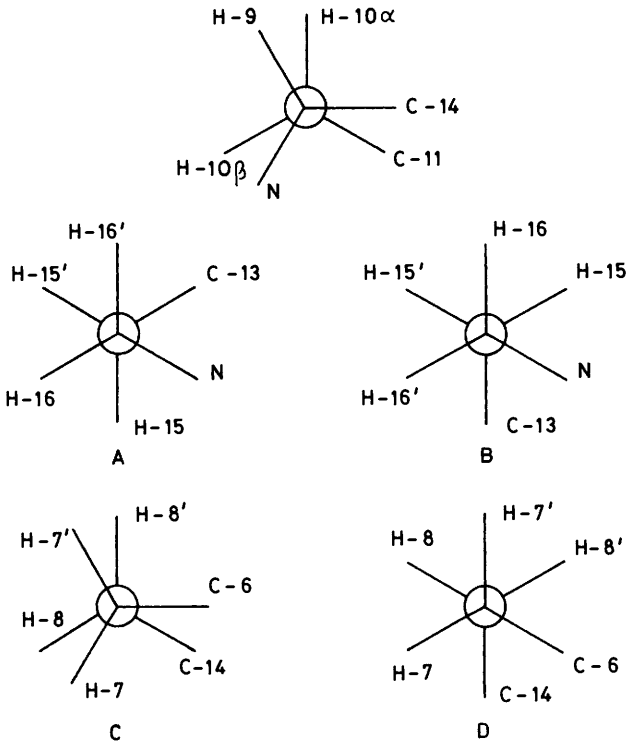


Figure 5. Newman projections for conformations about C–C bonds for compounds (**1**), (**2**) (A,B,E), and (**3**) (A—E). Both conformations A and B are compatible with J_{exp} values. Calculation of the rotation angle for which $J_{calc} = J_{exp}$ in A gives an angle for a distorted-chair conformation of the piperidine ring. Conformation D agrees selectively with J_{exp} data for (**3**): this molecule will adopt a chair conformation of the ring C(5), C(6), C(7), C(8), C(13), C(14), corresponding to the staggered conformation D about the C(7)–C(8) bond. The J_{exp} value in (**1**)—(**3**) fits the single possible conformation E allowed by this fixed molecular portion (an angle of *ca.* 30° was evaluated between H-9 and H-10α axes in arrangement E)

The increase of temperature caused an effective increase of resolution of the patterns which were back to normal at 345 and 330 K for (**1**) and (**2**), respectively. These therefore were the temperatures at which the spectra used for analysis were run.

Such a temperature effect was expectable on the basis of the results of a recent ^{13}C n.m.r. study[11] showing that a mixture of the two diastereoisomers, corresponding to the axial and equatorial *N*-methyl (**1**) or *N*-methylallyl (**2**) substituent, exists in solution for both compounds. The temperature effect exerted on the shape of the proton signals clearly indicates that these nuclei are part of a molecular portion (*N*-alkyl group, the chair-boat interconversion of the piperidine ring being excluded) that undergoes a configurational change at a rate that, at 296 K, is of the same order of magnitude as the chemical shift differences between the corresponding protons of the two configurational isomers. This difference was found to be of *ca.* 0.15 p.p.m. at 600 MHz[4] in (**1**). The related exchange rate (*ca.* 10^2 Hz) leads in our case, at 500 MHz, to intermediate exchange causing the observed broadening of the resonance lines. At higher temperatures the inversion process rates become fast enough to give well resolved, averaged resonance lines for the nuclei. Freezing of the process and thus detection of the two separate isomers could be attained at low pH values.[4,11] However, to avoid pH changes far from that of the medium, we used higher temperatures for obtaining sharp spectra suitable for analysis.

The observed lack of a temperature effect on the spectra of (**3**) and its *N*-alkyl derivatives naloxone and naltrexone, in combination with the results of ^{13}C n.m.r. experiments carried out in very acidic solution,[11] indicates that these molecules are configurationally rigid and homogeneous in solution (as in the crystal[10,12]) with an equatorial *N*-alkyl group.

Of interest is the fact that in (**2**) the H-17, H-17′ nuclei (at 296 K) are isochronous. The chemical shift difference between H-17 and H-17′ decreases on going from naltrexone to (**2**) [*i.e.* the difference is 0.37 in naltrexone,[2] 0.16 p.p.m. in naloxone,[3] and 0 in nalorphine (**2**)]. An interpretation of this trend in terms of an increasing degree of rotational freedom, along the above series, about the N–C(17) and C(17)–C(18) bonds, is possible. This interpretation is consistent with a quantitative estimate of the relative diffusion rates for internal motions, which was made by us on the basis of ^{13}C n.m.r. spin–lattice relaxation time studies[1,3] for these molecules. The calculated diffusion coefficients, in fact, showed that the conformational flexibility of the *N*-alkyl group increases in the order: naltrexone < naloxone < nalorphine. Therefore the hypothesis according to which the relative antagonistic pharmacological potency of these compounds can be correlated (in inverse sense) to the degree of internal conformational flexibility of the *N*-alkyl group can be considered.

More data are still needed to add definitive reliability to such a correlation. For this reason, further conformational and motional studies by n.m.r. spectroscopic methods are in course on additional compounds having different narcotic–antagonist potency.

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References

1 G. C. Pappalardo, L. Radics, M. Baldo, and A. Grassi, *J. Chem. Soc., Perkin Trans. 2*, 1985, 955.
2 B. Perly, G. C. Pappalardo, and A. Grassi, *Z. Naturforsch.*,1988, **41b**, 231.
3 B. Perly, G. C. Pappalardo, and A. Grassi, to be submitted.
4 J. A. Glasel, *Biochem. Biophys. Res. Commun.*, 1981, **102**, 703.
5 (*a*) W. P. Aue, E. Bartholdi, and R. R. Ernst, *J. Chem. Phys.*, 1976, **64**, 2229; (*b*) A. Bax and R. Freeman, *J. Magn. Reson.*, 1981, **44**, 542.
6 (*a*) G. Eich, G. Bodenhausen, and R. R. Ernst, *J. Am. Chem. Soc.*, 1982, **104**, 3731; (*b*) G. Wagner, *J. Magn. Reson.*, 1983, **55**, 151.
7 A. A. Bothner-By and S. Castellano, Program 111, QCPE, Indiana University.
8 M. Karplus, *J. Chem. Phys.*, 1959, **30**, 11.
9 E. Bye, *Acta Chem. Scand.*, 1976, **B30**, 549.
10 R. J. Sime, M. Dobler, and R. L. Sime, *Acta Crystallogr.*, 1976, **B32**, 2937.
11 E. L. Eliel, S. Morris-Natschke, and V. M. Kolb, *Org. Magn. Reson.*, 1984, **22**, 258.
12 R. L. Sime, R. Forehand, and R. J. Sime, *Acta Crystallogr.*, 1975, **B31**, 2326.

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