The Structure of Morphine differs between the Crystalline State and Aqueous Solution

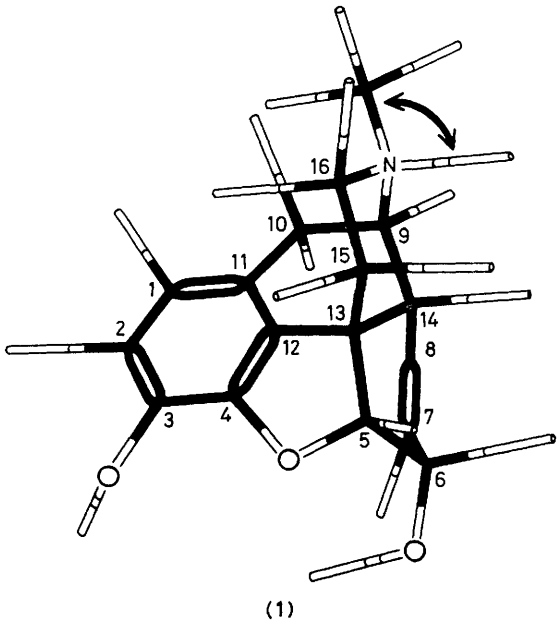
Charles E. Brown,*[a] Sandra C. Roerig,[b] James M. Fujimoto,[b] and Vern T. Burger[c]

[a] *Department of Biochemistry, Medical College of Wisconsin, Milwaukee, WI 53226, U.S.A.*
[b] *Research Service, Veterans Administration Center, Wood, WI 53193 and Department of Pharmacology and Toxicology, Medical College of Wisconsin, Milwaukee, WI 53226, U.S.A.*
[c] *Nicolet Analytical Instruments, 5225–1 Verona Road, Madison, WI 53711–0508, U.S.A.*

Cross-polarisation magic-angle-spinning and high resolution ^{13}C n.m.r. spectra indicate that hydrogen bonding and crystal packing forces alter the structure of morphine sulphate in the crystalline state from that available for receptor binding in aqueous solution.

The isomeric structure of (−)-morphine was first proven by *X*-ray crystallography of the hydroiodide dihydrate salt.[1] This and subsequent *X*-ray crystallography of the hydrochloride trihydrate salts[2] indicated that both morphine and codeine exist in the crystal as structure (**1**). The piperidine ring, which includes C-9, -14, -13, -15, and -16, exhibited a chair conformation in the solid state with the N–Me group oriented only in the equatorial direction as in (**1**).

It would appear that work on receptor recognition involving morphine derivatives is based, in part, on the assumption that this structure does not change when the crystal is put into solution,[3] even though there is only limited experi-



(1)

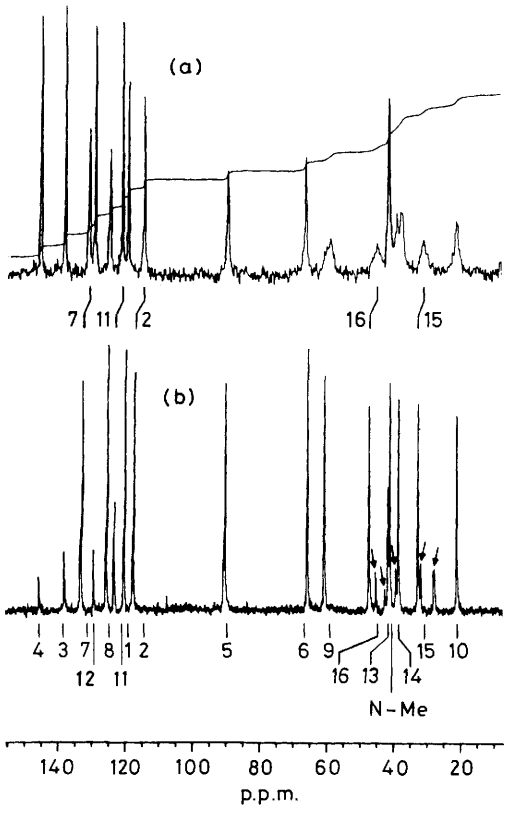


Figure 1. ^{13}C N.m.r. spectra of morphine sulphate (a) as a microcrystalline powder and (b) in saturated D_2O solution (pD *ca.* 7). The peak assignments below spectrum (b) are according to the numbering scheme in (**1**) and indicate the chemical shifts of peaks in the solid powder [spectrum (a)]. In both cases the samples were maintained at 20 °C. Chemical shifts are relative to Me_4Si. The observe frequency is 25.2 MHz in (a) and 37.7 MHz in (b).

mental support for this case.[4,5] We now present evidence to contend the assumption.

The major resonances in the natural abundance, high resolution ^{13}C n.m.r. spectrum of morphine sulphate dissolved in D_2O [Figure 1(b)] at ambient temperature correspond to

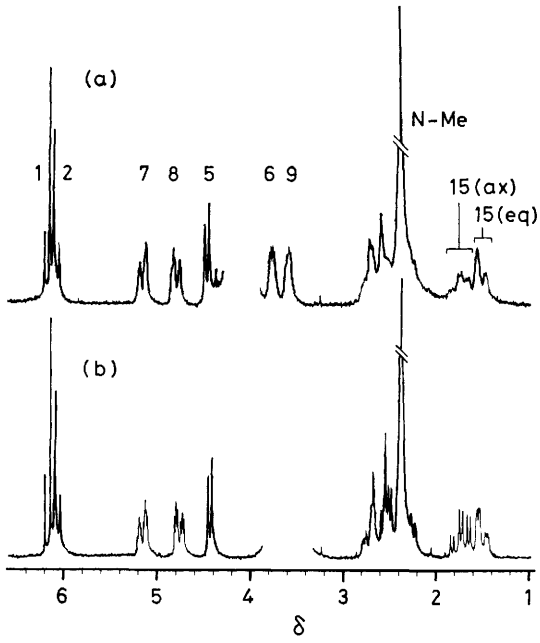


Figure 2. ^1H N.m.r. spectra (ν_{obs} = 150 MHz) of 10 mM morphine sulphate in D_2O at temperatures of (a) 22 °C and (b) 70 °C. Peak assignments are given by the numbers, which correspond to the numbering system in (**1**). The partially resolved peaks in the chemical shift range of δ 2—3 arise from the protons on C-10, -14, and -16. The open region in each spectrum is obscured by the large HOD peak. The pD values are (a) 7.0 and (b) 6.3.

the ^{13}C spectral assignments of Carroll *et al.*[6] for morphine dissolved in [$^2H_6$]dimethyl sulphoxide. However the minor peaks indicated by arrows do not. Furthermore, they can not be attributed readily to impurities [^1H n.m.r., Figure 2(a), *cf.* refs. 4 and 5; >99% pure by h.p.l.c.].

The anomalous peaks in Figure 1(b) appear to arise from minor components in the nitrogen invertoisomer equilibrium.[5] These peaks are not detectable when the temperature of the sample is raised to 70 °C, presumably because inversion at the nitrogen is more rapid at the higher temperature (data not shown). At this elevated temperature, the ^1H n.m.r. spectrum, Figure 2(b), exhibits marked narrowing of the resonances from the methylene protons at C-15 and -16, which suggests that inversion at the nitrogen is associated with a greater freedom of motion in the piperidine ring than previously believed.

As has been noted with other biomolecules,[7] use of the cross-polarization magic-angle-spinning (CP/MAS) ^{13}C n.m.r. technique to obtain narrow line spectra of crystalline morphine sulphate, Figure 1(a), and comparison of these with the high resolution ^{13}C n.m.r. spectrum of the same sample dissolved in D_2O, Figure 1(b), permits a more direct assessment of possible changes that may occur upon crystallization. Virtually identical conditions were used for recording spectra of both the solid samples and aqueous solutions; Nicolet S-100 (MAS at 2.9 kHz and 1.1 × 10^{-3} T of proton decoupling field) and NT-150 n.m.r. spectrometers were used, respectively. Our assignment of Figure 1(a) (solid sample) is based on the assignment of Carroll *et al.*[6] for the high resolution ^{13}C n.m.r. spectrum of morphine, and is shown, for comparison, on the scale below Figure 1(b) (dissolved sample). In most cases, the peak positions line up remarkably well and permit direct assignment. These assignments were then corroborated: (i) using the pulse sequence of Opella and Frey[8] to suppress signals from protonated carbons and (ii) dipolar coupling to

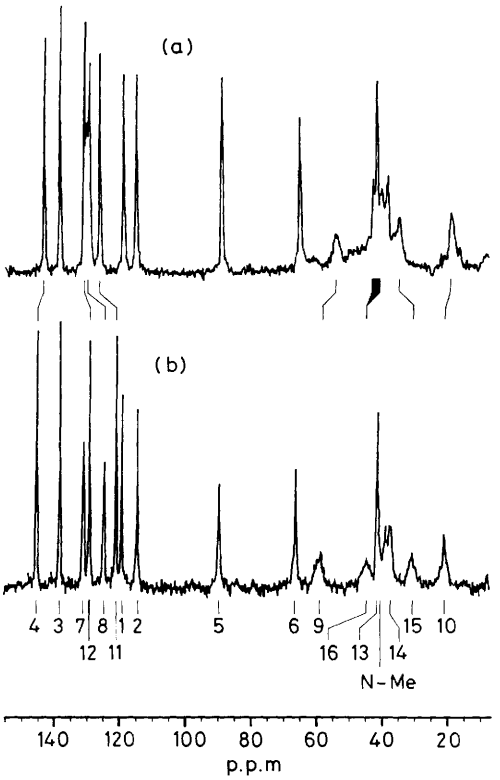


Figure 3. ^{13}C N.m.r. spectra (ν_{obs} = 25.2 MHz) of solid samples of (a) morphine free base and (b) morphine sulphate. Peak assignments are as in (**1**). In both cases the samples were maintained at 20 °C.

the ^{14}N quadrupole moment causes broadening of ^{13}C resonances in the solid that can not be removed by magic-angle sample spinning,[9] and thus the broadened resonances in the upfield region of the spectrum, Figure 1(a), were required to arise from carbons α or β to the nitrogen. The only confusion that arises involves the methyl group. This resonance is expected to be broadened by the quadrupole moment of the ^{14}N, and indeed this resonance is not resolved from the other broadened resonances in this region of the spectrum. The chemical shift indicated in Figure 1 for the methyl group is based on the position of this resonance with morphine in solution and may be off by a few p.p.m.

The significance of the fact that only five particular carbons change chemical shift† upon crystallization should not be overlooked. Not all of the carbons α and β to the nitrogen and hydroxy groups change chemical shift upon crystallization (*e.g.* in agreement with the ^1H n.m.r. evidence that C-5 maintains the same conformation in the crystal and solution,[4] C-4 and -5, which also are β to the hydroxy groups, exhibit little change in chemical shift). Thus it is not possible to attribute the observed chemical shift changes solely to inductive and long-range electric field effects that might arise from changes in charge at the nitrogen and hydroxy groups. Comparison of the CP/MAS ^{13}C n.m.r. spectrum of morphine free base with that of morphine sulphate (Figure 3) indicates that the charge on the nitrogen affects very different carbons. The fact that the chemical shifts of C-9, -10, -11, -12, and -4 all vary with the charge on the nitrogen agrees with the finding of Kolb[3] that the acidity of the phenolic hydroxy group at C-3 depends on the identity of the substituent on the nitrogen. These carbons form the most direct link between C-3 and the nitrogen, and the electron densities at their nuclei appear to depend on the charge at the nitrogen.

The observed shifts of resonances from C-2, -7, -11, -15, and -16 upon crystallization of morphine sulphate (Figure 1) also can not be attributed in a direct fashion to restriction of the sulphate counter ion to fixed loci within the crystal. The chemical shifts of C-2 and -7 are the same for solid morphine free base as for solid morphine sulphate (Figure 3); if these were dictated primarily by proximity to a sulphate group, then they should be different in solid morphine free base than they are in solid morphine sulphate. Furthermore, direct covalent attachment of a sulphate group to the oxygen at C-6 of morphine was found to have little effect on the chemical shifts of carbons other than the one at which the substitution was made.[10]

In the crystal, the charged nitrogen and the two hydroxy groups are expected to participate in hydrogen bonding.[1,2] Thus it is interesting to note that four of the five carbons to change chemical shift upon crystallization are α or β to these functional groups. Conformational strain at these four carbons, which could account for their changes in chemical shifts from those in solution, might be produced by a combination of intermolecular hydrogen bonding and crystal packing forces. A change in conformation at C-15 and -16 from that in (**1**) would also account for the observed change in chemical shift of C-11, since in the crystal structure of protonated morphine (**1**) the through-space distance C-11–16-H_{ax} represents the closest interaction between the aromatic and piperidine rings. This agrees with the observation that the distance between the piperidine nitrogen and the aromatic ring may be important for stereochemically controlled binding of some opiate analgesics at the receptor site.[11]

We find it hard to avoid the conclusion that crystal packing forces when combined with hydrogen bonding at the nitrogen and hydroxy groups alter the conformation of morphine from that in solution. These results put into serious question the assumption that the opiate receptor(s) in the central nervous system recognizes morphine and its chemical analogues with the ring structure having the same conformation as in the crystal.

This work is supported by grants from N.I.D.A. and V.A.

Received, 5th July 1983; Com. 901

† C-2 2.6, C-7 2.0, C-16 2.2, C-15 1.6, and C-11 2.2 p.p.m.

References

1 M. Mackay and D. C. Hodgkin, *J. Chem. Soc.*, 1955, 3261.
2 L. Gylbert, *Acta Crystallogr., Sect. B*, 1973, 1630.
3 V. M. Kolb, *J. Pharm. Sci.*, 1979, **68**, 1250; S. D. Darling, V. M. Kolb, G. S. Mandel, and N. S. Mandel, *ibid.*, 1982, **71**, 763.
4 S. Okuda, S. Yamaguchi, Y. Kawazoe, and K. Tsuda, *Chem. Pharm. Bull.*, 1964, **12**, 104.
5 J. A. Glasel, *Biochem. Biophys. Res. Commun.*, 1981, **102**, 703.
6 F. I. Carroll, C. G. Moreland, G. A. Brine, and J. A. Kepler, *J. Org. Chem.*, 1976, **41**, 996.
7 C. J. Groombridge, R. K. Harris, K. J. Packer, B. J. Say, and S. F. Tanner, *J. Chem. Soc., Chem. Commun.*, 1980, 174; M. H. Frey and S. J. Opella, *ibid.*, 1980, 474; L. G. Pease, M. H. Frey, and S. J. Opella, *J. Am. Chem. Soc.*, 1981, **103**, 467.
8 S. J. Opella and M. H. Frey, *J. Am. Chem. Soc.*, 1979, **101**, 5854.
9 J. G. Hexem, M. H. Frey, and S. J. Opella, *J. Am. Chem. Soc.*, 1981, **103**, 224; G. E. Balimann, C. J. Groombridge, R. K. Harris, K. J. Packer, B. J. Say, and S. F. Tanner, *Philos. Trans. R. Soc. London, Ser. A*, 1981, **299**, 643.
10 C. E. Brown, S. C. Roerig, J. M. Fujimoto, and V. T. Burger, *Bull. Magn. Reson.*, 1983, **5**, 184.
11 S. Shiotani, T. Kometani, Y. Iitaka, and A. Itai, *J. Med. Chem.*, 1978, **21**, 153.