Molecular Determinants for Drug Receptor Interactions, 9

¹H and ¹³C NMR Spectra of the Narcotic Antagonist N-Allyl-N-normetazocine by Two-Dimensional Chemical Shift Correlation Spectroscopy

Bruno Perly and Patricia Le Bail-Richer

CEN Saclay, DPC/SCM, F-91191 Gif-sur-Yvette, France

Giuseppe C. Pappalardo* and Antonio Grassi

Dipartimento di Scienze Chimiche, II Cattedra di Chimica Generale, Facoltà di Farmacia,
Università di Catania, Viale A. Doria 6, I-95125 Catania, Italy

Z. Naturforsch. **42b**, 1021–1026 (1987); received December 18, 1986

Drug Receptor, Interaction, ¹H NMR Spectra, ¹³C NMR Spectra, Narcotic Antagonist

The high frequency ¹H and ¹³C NMR spectra (at 500 and 75.47 MHz, respectively) of N-allyl-N-normetazocine (NAM) hydrochloride in ²H₂O solution were run and fully analyzed with the aid of both homonuclear ¹H–¹H and heteronuclear ¹³C–¹H chemical shift correlation experiments. Selective broadening of the signals of the protons in 3-, 4-, 8-, 9-, 12-positions was observed at temperatures < 350 K. This was found to be consistent with an inversion process occurring between the *N-equatorial* and *N-axial* configurational isomers of NAM whose rate of exchange is of the same order of magnitude of the chemical shifts differences at 500 MHz. The increase of temperature at 350 K is thus adequate to make “fast” the process in the NMR time scale and then to resolve the spectral portions.

The experimental proton-proton coupling constants were found to be consistent with a slightly distorted chair conformation of the piperidinic ring in NAM hydrochloride.

The ¹³C NMR spectrum showed the distinct spectra of the two conformers, which are present in the ratio of *ca.* 80 (*N-equatorial*) : 20 (*N-axial*). The field frequency employed for the experiment was therefore sufficient to make “slow” the exchange process between the two isomeric forms, whose resonance peaks could be isolated in the non-acidified solution.

Introduction

Our previous investigations [1–4] which were performed by using ¹³C NMR spin-lattice relaxation times and two-dimensional ¹H–¹H chemical shift correlation spectroscopy, suggested that the internal conformational dynamics of the N-alkyl group in morphine, oxymorphone, nalorphine, naloxone and naltrexone, could be important in determining the agonist-antagonist receptor mediated actions of these molecules. In particular, the observed trend of decreasing conformational flexibility about N–C and C–C bonds of the N-substituent, with corresponding increase of the relative antagonistic potency, appeared interesting and worthy of further investigations on additional compounds.

With this in mind, we initiated the study of a term of the benzomorphan series, namely N-allyl-N-nor-

metazocine* (NAM), whose synthesis and antagonistic activity were described some time ago [5–10]. In the present paper we therefore report the results of the ¹H and ¹³C NMR full spectral analysis of NAM in aqueous solution. Assignments and deduction of the spectral parameters for NAM were aided from both homonuclear [11] ¹H–¹H and heteronuclear [12] ¹H–¹³C chemical shift correlations.

A recent very high field ¹H NMR study [13] of morphine-nalorphine and oxymorphone-naloxone pairs in aqueous and non aqueous solutions gave a significant contribution, in agreement with some our observations, for the elucidation of the conformational properties of these molecules.

The ¹³C NMR spectra of a series of 6,7-benzomorphan derivatives have been reported and discussed by Casy in a recent study [14].

* Reprint requests to Prof. Dr. G. C. Pappalardo.

* Generic name for 2-allyl-2'-hydroxy-5,9-dimethyl-6,7-benzomorphan.

Experimental

The sample of NAM (m.p. 202 °C) was obtained from NIDA, USA.

The ^1H NMR spectra and shift correlation experiments (COSY 45 and COSY LR) [11] were run on a Bruker WM 500 spectrometer operating at 500 MHz, upgraded with an Aspect 3000 computer and with standard parameters [2, 3]. The solutions were 10 mM in $^2\text{H}_2\text{O}$ (CEA France) with a trace of sodium trimethylsilyl(2,2,3,3- $^2\text{H}_4$)propionate (TSP) as internal reference.

The ^{13}C NMR spectra were obtained on a Bruker MSL 300 spectrometer operating at 75.47 MHz, under broad band proton decoupling, on 0.08 M solutions in $^2\text{H}_2\text{O}$ at 300 K. Dioxane was used as chemical shift reference ($\delta = +67.8$ ppm from TMS).

^{13}C – ^1H chemical shift correlation was accomplished by using the pulse sequence described by Bax [12] in order to remove the ^1H – ^1H couplings from the proton dimension. In this experiment the ^{13}C and ^1H (via decoupler channel) 90 degree pulse lengths were 7 and 17 μs , respectively.

Simulations of some component patterns of the ^1H NMR spectrum were made by using the LAOCOON II computer program [15].

Results and Discussion

^1H NMR spectrum. – The one-dimensional spectra of NAM at 305 and 350 K are shown in Fig. 1. The analysis was performed on the spectrum run at higher temperature, because a severe broadening of the multiplets pertinent to the protons in 3-, 4-, 8-, 9- and 12-position occurs at temperatures lower than 350 K. The interchange, at neutral pH, between the *N*-axial and the *N*-equatorial configurations of NAM, is responsible of such a temperature effect, which is essentially analogous to that found [3, 13] in morphine and nalorphine, whose spectra sharpen at 345 and 330 K, respectively. At lower temperatures, the lines broaden due to the rate of the exchange process between the two diastereoisomers which, in the 500 MHz NMR time scale, is "slow", *i.e.*, of the same order of magnitude of the chemical shifts difference between the corresponding protons of the two configurational isomers.

The spectral analysis was started with identification and assignment of Me-10 and Me-11 resonances at highest field, that appear as a singlet and a doublet, respectively, owing to the coupling between H-11 and H-9 (6 Hz). Detection of H-9 (quartet of doublets) and H-1 (quadruplet, spaced by $J(1,9) \approx 3$ Hz,

$J(1,8\alpha) \approx 6.2$ Hz) multiplets was therefore easy on correlative basis. The H-1 coupled resonance of H-8 α (doublet of doublets) was then recognised together with the correlated one due to H-8 β (doublet, spaced by $J(8\alpha,8\beta) = 19$ Hz). The 2D method allowed also identification of the set of correlated resonances due to the nuclei H-3 α (triplet of doublets; $J(3\alpha,3\beta) = 13.5$ Hz; $J(3\alpha,4\alpha) = 14$ Hz; $J(3\alpha,4\beta) = 1.8$ Hz), H-3 β (quartet of doublets; $J(3\beta,4\alpha) = 5$ Hz; $J(3\beta,4\beta) = 3.5$ Hz), H-4 α (triplet of doublets; $J(4\alpha,4\beta) = 15$ Hz; $J(3\alpha,4\alpha) = 14$ Hz; $J(3\beta,4\alpha) = 4.8$ Hz), H-4 β (quartet of doublets). The relative assignment ($\delta_{\text{H-3}\alpha, \text{H-4}\beta} < \delta_{\text{H-4}\alpha, \text{H-4}\beta}$) was made on the basis of shielding effects considerations.

The analysis of the ABC-type spin system at lowest field, due to H-1', H-3', H-4', was straightforward. The long range coupling $J(1',4')$ of *ca.* 0.5 Hz

Table I. ^1H NMR spectral parameters (500 MHz) for N-allyl-normetazocine hydrochloride in $^2\text{H}_2\text{O}$ solution (350 K).

Chemical shifts (δ , ppm from TSP)		Coupling constants (Hz)	
H-1	3.78	$J(1,9)$	3.02
H-1'	6.89	$J(1,8\alpha)$	6.36
H-3'	6.83	$J(1,8\beta)$	< 0.5
H-4'	7.15	$J(1',3')$	2.44
H-3 α	2.72	$J(1',4')$	< 0.5
H-3 β	3.25	$J(3',4')$	8.16
H-4 α	1.99	$J(3\alpha,3\beta)$	17.2
H-4 β	1.64	$J(3\alpha,4\alpha)$	14.3
H-8 α	3.22	$J(3\alpha,4\beta)$	1.60
H-8 β	3.11	$J(3\beta,4\alpha)$	4.90
H-9	2.12	$J(3\beta,4\beta)$	3.65
Me-10	1.43	$J(4\alpha,4\beta)$	14.3
Me-11	0.91	$J(8\alpha,8\beta)$	19.7
H-12	3.85	$J(9, \text{Me-11})$	6.90
H-12'	3.89	$J(12,12')$	13.5
H-13	5.97	$J(12,13)$	7.13
H-14	5.61	$J(12',13)$	7.29
H-14'	5.62	$J(13,14)$	9.79
		$J(13,14')$	17.3
		$J(14,14')$	2.5

was detected by means of COSY LR experiment, being some fine structures not well resolved in the spectrum.

The analysis of the remaining set of correlated multiplets due to the N-methyl-allyl group protons showed that H-12 and H-12' nuclei are almost, but

Fig. 1. The 500 MHz ^1H NMR spectrum of NAM hydrochloride in $^2\text{H}_2\text{O}$ solution, at 305 (upper) and 350 K (below). Assignment of resonances is outlined in the resolved spectrum at higher temperature.

not entirely, isochronous: their resonances are very close (differential shift 0.04 ppm), so that, in the place of the two expected doublets of doublets, seven resonance lines symmetrically centered with respect to the highest intensity one are produced. The H-10 nucleus gives rise to ten lines, of separate intensities, in the place of the expected 16 lines, while each proton 14 and 14' gives rise to a quadruplet. Selective decouplings produced simplification of the multiplets thus allowing detection of J values useful for simulation and iterative refinement of spectral parameters for the whole spin system. Very small long-range couplings between the protons in 12- and 14-positions were detected on the COSY LR spectrum.

The experimental J values for the protons in 1-, 3-, 4- and 9-positions did not allow to establish, in unambiguous way, by the use of the Karplus relationship [16], the conformation about C(1)–C(9) and C(3)–C(4) bonds.

Based on the experimental $J(1,8\alpha)$ and $J(1,8\beta)$ values, the angles of *ca.* 28° and *ca.* 90° could be estimated between the axes C–H(1), C–H(8 α) and C–H(1), C–H(8 β), respectively, which fit a slightly distorted chair conformation of the C(1)–N–C(3)–C(4)–C(5)–C(9) ring much better than a boat form. This finding is consistent with retention of the chair conformation found in the crystal

[17]; a chair conformation of the piperidinic ring, moreover, was suggested by ^1H and ^{13}C NMR investigations on the related morphine, oxymorphone, naloxone and metazocine molecules [2, 3, 13, 14] to be retained in the solution state.

The small differential shift of H-12 and H-12' appears of interest and worthy of further discussion, when considering that the $\Delta\nu$ value of 0.04 ppm is smaller than that found [2] for the N-methylenic protons in the more rigid analogous fragment of naloxone (0.08 ppm) and naltrexone (0.37 ppm). Even if a free rotation or, alternatively, an almost equivalent shielding in a rigid conformation about N–C(12) bond, cannot be excluded *a priori*, the H-12 and H-12' nuclei could be not so far from to be isochronous due to conformational flexibility of the N-methyl-allyl group in NAM. Such a flexibility should be more marked than in naloxone and naltrexone, and less marked than in nalorphine. A check by means of ^{13}C NMR spin lattice relaxation times measurements and analysis for NAM (in the course) is needed, in order to verify, as made for previously investigated [3, 4] compounds, the above suggested relationship between conformational flexibility and differential shift of N-methylenic protons.

^{13}C NMR spectrum. — The high frequency (75.47 MHz) spectrum of NAM (Fig. 2) in aqueous

Fig. 2. The overlapped ^{13}C NMR spectra of the N-equatorial (high intensity peaks) and N-axial (low intensity peaks) diastereoisomers of NAM hydrochloride in $^2\text{H}_2\text{O}$ solution at 75.47 MHz. Assignment of resonances is outlined.

medium shows the separate resonances of corresponding carbons of the N-*equatorial* (major) and N-*axial* (minor) configurations of the molecule. Consequently there is, at such high field, no need for strong acidification in order to make "slow" the rate of the *axial-equatorial* interconversion process in the NMR time scale. The addition of acid was used before for morphine and nalorphine to identify and isolate [4, 18] the spectral components. Now by means of the high field spectrometer the configurational isomers could be located at the physiologically more significant neutral pH value: the chemical shift differences between the carbons involved in the configurational change (Table II) are, at 75.47 MHz, larger than the rate of exchange between the two isomeric forms. The ratio of the populations was evaluated as *ca.* 80:20, based on peak intensities.

The peak assignments, which were firstly made on the basis of chemical shifts data for 6,7-benzomorphans [14], were confirmed by using ^{13}C - ^1H correlation experiments (Fig. 3).

Table II. ^{13}C NMR chemical shifts (δ , Me_4Si) for major (*equatorial*) and minor (*axial*) isomers of N-allyl-normetazocine determined at 75.47 MHz in $^2\text{H}_2\text{O}$ solution at 300 K.

Carbon	<i>equatorial</i> Isomer δ	<i>axial</i> Isomer δ
C-1	60.05	60.41
C-1'	113.50	—
C-2'	155.90	—
C-3'	115.35	—
C-4'	130.25	—
C-3	47.25	44.09
C-4	40.17	34.86*
C-5	36.06	36.45
C-6	142.20	—
C-7	125.90	—
C-8	23.19	29.57
C-9	40.56	33.78*
C-12	57.45	55.41
C-13	127.29	127.93**
C-14	127.20	125.95**
Me-10	24.73	24.91
Me-11	13.70	13.18

* Assignments uncertain; ** assignments uncertain.

Fig. 3. The ^{13}C - ^1H shift correlation of NAM hydrochloride in $^2\text{H}_2\text{O}$ solution.

The criteria of configurational assignment of substituted piperidines [19], which were already used for distinguishing N-methyl resonances of N-*axial* and N-*equatorial* isomers of quaternary salt of α -metazocine [14], applied also in the case of the related N-allyl derivative NAM. Therefore, of the two N-methylenic (C-12) resonances observed for NAM, that at higher field (minor form, 55.41 ppm) and that at lower field (major form, 57.45 ppm) could be safely assigned to the N-*axial* and N-*equatorial* configurations, respectively. Such an assignment of N-dia-

stereoisomers is corroborated by the X-ray structure analysis of NAM [17] showing that this molecule adopts in the crystal also the N-*equatorial* configuration. Previous [18] interpretation of ^{13}C NMR data led to the assignment of the major isomer (N-*equatorial*) for the structurally related to NAM morphine and nalorphine molecules.

In the spectrum of NAM the peaks pertinent to the carbons of the single N-*equatorial* diastereoisomer can be thus selected for spin lattice relaxation times (T_1) measurements. The analysis of these data in terms of conformational flexibility of NAM is expected to provide new additional information supporting our recently suggested [4] Snyder's-type modified model of dynamic regulation of the opioid receptor.

Thanks are due to National Institute of Drug Abuse, USA, and to Mr. Kenneth H. Davis (Jr.) (Research Triangle Park, North Carolina, USA) for the gift of the sample. This work was supported by M. P. I. (Italy) and, in part, by C. N. R. (Italy).

- [1] G. C. Pappalardo, L. Radics, M. Baldo, and A. Grassi, *J. Chem. Soc., Perkin II* **1985**, 955.
- [2] B. Perly, G. C. Pappalardo, and A. Grassi, *Z. Naturforsch.* **41b**, 231 (1986).
- [3] B. Perly, G. C. Pappalardo, and A. Grassi, *J. Chem. Soc., Perkin II* **1986**, 1413.
- [4] G. C. Pappalardo, B. Perly, and A. Grassi, *Eur. J. Med. Chem.*, in press.
- [5] M. Gordon, J. J. Lafferty, D. H. Tedeschi, N. B. Eddy, and E. L. May, *Nature (London)* **192**, 1089 (1961).
- [6] S. Archer, N. F. Albertson, L. S. Harris, A. K. Pierson, and J. G. Bird, *J. Med. Chem.* **7**, 123 (1964).
- [7] P. S. Portoghese, *J. Med. Chem.* **8**, 609 (1965).
- [8] P. S. Portoghese, *J. Pharm. Sci.* **55**, 865 (1966).
- [9] S. Archer, N. Albertson, and A. Pierson, in H. W. Kosterlitz, J. Villareal, and H. O. J. Collier (eds): *Agonist and Antagonist Actions of Narcotic Analgesic Drugs*, p. 27, Macmillan, London (1973).
- [10] C. B. Pert, S. H. Snyder, and E. L. May, *J. Pharmacol. Exp. Ther.* **196**, 316 (1976).
- [11] A. Bax and R. Freeman, *J. Magn. Reson.* **44**, 542 (1981).
- [12] R. Freeman and G. A. Morris, *J. Chem. Soc. Chem. Commun.* **1978**, 684.
- [13] J. A. Glasel and H. W. Reiher, *Magn. Reson. Chem.* **23**, 235 (1985).
- [14] A. F. Casy, A. O. Ogundaini, and R. T. Parfitt, *Org. Magn. Reson.* **20**, 254 (1982).
- [15] A. A. Bothner-By and S. Castellano, Program 111, QCPE, Indiana University.
- [16] M. Karplus, *J. Chem. Phys.* **30**, 11 (1959).
- [17] W. Fedeli, G. Giacomello, S. Cerrini, and A. Vacciago, *J. Chem. Soc. B* **1970**, 1190.
- [18] E. L. Eliel, S. Morris-Natschke, and V. M. Kolb, *Org. Magn. Reson.* **22**, 258 (1984).
- [19] A. F. Casy and F. O. Ogungbamila, *Org. Magn. Reson.* **18**, 171 (1982).