Molecular Determinants for Drug-Receptor Interactions. 6. Proton 500 MHz NMR Spectra of the Narcotic Antagonists Naloxone and Naltrexone by Two-Dimensional $^1H-^1H$ Chemical Shift Correlation Spectroscopy

Bruno Perly

C. E. N. Saclay, IRDI/DPC, 91191 Gif-sur-Yvette Cedex, 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 8, 95125 Catania, Italy

Z. Naturforsch. **41b,** 231–238 (1986); received August 22/November 4, 1985

NMR Spectra, Two-Dimensional NMR, Narcotic Antagonists, Naloxone, Naltrexone

The full analysis of the ^1H NMR spectra of naloxone and naltrexone (hydrochloride salts, in ^2H_2O solution) was performed by using an high-frequency (500 MHz) spectrometer and the recent technique of two-dimensional (2D) homonuclear shift spectroscopy. The $^1H-^1H$ connectivities allowed detection of correlated resonances and assignments of multiplets. The shapes of the contour levels of the COSY 45 spectra were also used to check the relative signs of coupling constants. The refinement of spectral parameters of some component spin-systems of the complex spectra was performed by computerized iterative simulation of patterns.

The spectral analysis provided proton coupling constants that allowed to establish a slightly distorted-chair conformation of the piperidine ring in both compounds.

The magnetic non-equivalence found for the protons bonded to C-17 atom (part of the N-alkyl fragment) was found to be larger in naltrexone than in the analogous naloxone. This fact, while no significant differences were observable in the chemical shifts of corresponding protons of the rigid molecular backbone of the two narcotic antagonists under study, was assigned to smaller degree of internal conformational flexibility of the N-methylcyclopropyl group in naltrexone with respect to that of the N-methylallyl group in naloxone.

The above findings appeared in good agreement with our previously proposed views based on results from ^{13}C relaxation times studies, which suggested the possible correlation of the motional rates of the N-methyl-R group to the pharmacological activity of antagonist compounds. This would consist in a direct correlation between decreasing flexibility of the N-bonded fragment and increasing antagonistic potency.

Introduction

A motional investigation [1] on two related pairs of agonist-antagonist narcotic analgesics (morphine – nalorphine; oxymorphone – naloxone)* was previously carried out by using ^{13}C NMR spin-lattice relaxation times measurements (T_1) in DMSO–$CDCl_3$ solution. The dynamics of internal motions appeared consistent with a possible operation mode of the "two-state" model of opiate receptor proposed by Snyder [3]. In the light of such a suggestive working hypothesis, it seemed preliminary to draw precise conclusions, through conventional ^1H NMR spectroscopy, on the conformations in aqueous medium of compounds having narcotic agonist and/or antagonist activities. The high complexity of the spin-systems and severe overlap of resonances have made impossible until now detection of accurate spectral parameters suited for conformational analysis of these molecules. The recent availability of spectrometers operating at very high field and more advanced techniques allowed us to initiate this work.

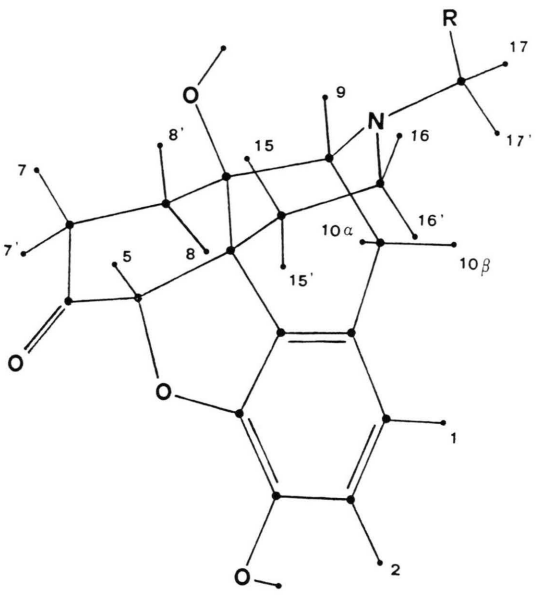
In this paper we present a complete analysis of the proton spectra at 500 MHz of two "pure" antagonists, namely naloxone (**1**) and naltrexone (**2**)**. The spectral analysis was performed by the aid of two-dimensional (2D) homonuclear shift spectroscopy. The determined conformational characteristics of **1** and **2** were discussed in terms of a possible correlation with their relative pharmacological potencies.

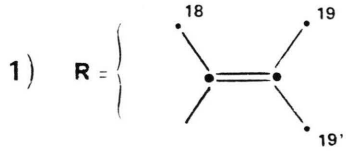
* While reference **1** was in print and this work in progress, a ^{13}C NMR spectroscopic study of the same pairs of compounds appeared [2], which investigated the conformation of the piperidine moiety using ^{13}C chemical shift data.

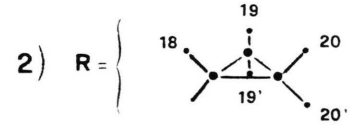
** The terms naloxone and naltrexone will refer throughout the whole text to the hydrochloride salts of both compounds.

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

Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
0340–5087/86/0200–0231/$ 01.00/0







Experimental

Naloxone hydrochloride and naltrexone hydrochloride (Salars S.p.A., Como-Camerlata, Italy) were dissolved in ^2H_2O (CEA-France, 99.95% D) and freeze dried. The deuterochloride was dissolved in ^2H_2O to obtain 3×10^{-2} M solution. A trace of trimethylsilyl 2.2.3.3 $^2H_4$ sodium propionate (TSP) was added as internal reference. The spectra were determined (32×2^{10} data point) at 295 K on a Bruker WM 500 spectrometer operating at 500.13 MHz. Further resolution enhancement was achieved by multiplication of the time domain by an optimized Lorentzian-Gaussian function.

Shift correlations were obtained using COSY 45 [4] or single relay COSY [5] with 32 ms relay time. The 1K×1K final matrices were obtained in both cases and are shown in the absolute value mode. Both time domains were processed using squared sine-bell windows.

The one dimensional proton spectra of **1** and **2** are shown in Figs. 1 and 2 along with assignments. Sections of the $^1H-^1H$ chemical shift correlation matrices are represented as contour plot in Figs. 3 and 4.

Simulation of resonance patterns and refinement of spectral parameters was performed using the LAOCOON III computer program [6]. Trial parameters for coupling constants were deduced from splitting patterns and, in some instances, after decoupling experiments. Centers of assigned multiplets were used as trial parameters for chemical shifts. Iterations were performed based on assignments of all the transitions observable. The calculated probable errors for the parameter sets were usually less than 0.1 Hz; the RMS deviations for the calculated and experimental lines were routinely 0.1 Hz or less. The spectral parameters derived from the complete analysis of each spectrum are presented in Table I.

Table I. ^1H NMR spectral parameters (500 MHz) for naloxone (**1**) and naltrexone (**2**) in ^2H_2O solution.

Chemical shifts (δ, ppm from TSP)			Coupling constants (Hz)		
	1	**2**		**1**	**2**
H-1	6.69	6.68	J(1,2)	8.28	8.33
H-2	6.72	6.71	J(7,7′)	14.86	14.91
H-5	5.08	5.08	J(7,8)	5.01	5.03
H-7	2.89	2.90	J(7,8′)	14.99	14.84
H-7′	2.20	2.21	J(8,8′)	14.66	14.45
H-8	1.91	1.95	J(8,7′)	3.08	3.03
H-8′	1.57	1.65	J(7′,8′)	3.12	3.15
H-9	3.75	4.04	J(9,10α)	6.03	6.10
H-10α	2.98	3.06	J(9,10β)	< 0.1	< 0.1
H-10β	3.31	3.28	J(10α,10β)	19.98	20.00
H-15	2.58	2.58	J(15,15′)	13.44	13.25
H-15′	1.64	1.54	J(15,16)	4.61	4.63
H-16	3.16	3.09	J(15,16′)	13.22	13.21
H-16′	2.71	2.66	J(16,16′)	13.17	13.25
H-17	3.80	3.25	J(16,15′)	< 0.1	< 0.1
H-17′	3.76	2.88	J(15′,16′)	3.60	3.49
H-18	5.79	1.13	J(17,17′)	13.72	13.66
H-19	5.81	0.85	J(17,18)	8.49	7.50
H-19′	5.55	0.51	J(17′,18)	6.44	7.33
H-20	–	0.77	J(18,19)	10.06	7.72
H-20′	–	0.48	J(18,19′)	16.72	4.75
			J(18,20)		7.73
			J(18,20′)		4.78
			J(19,19′)	1.86	3.26
			J(19,20)		7.96
			J(19,20′)		4.80
			J(19′,20)		4.88
			J(19′,20′)		8.54
			J(20,20′)		2.91

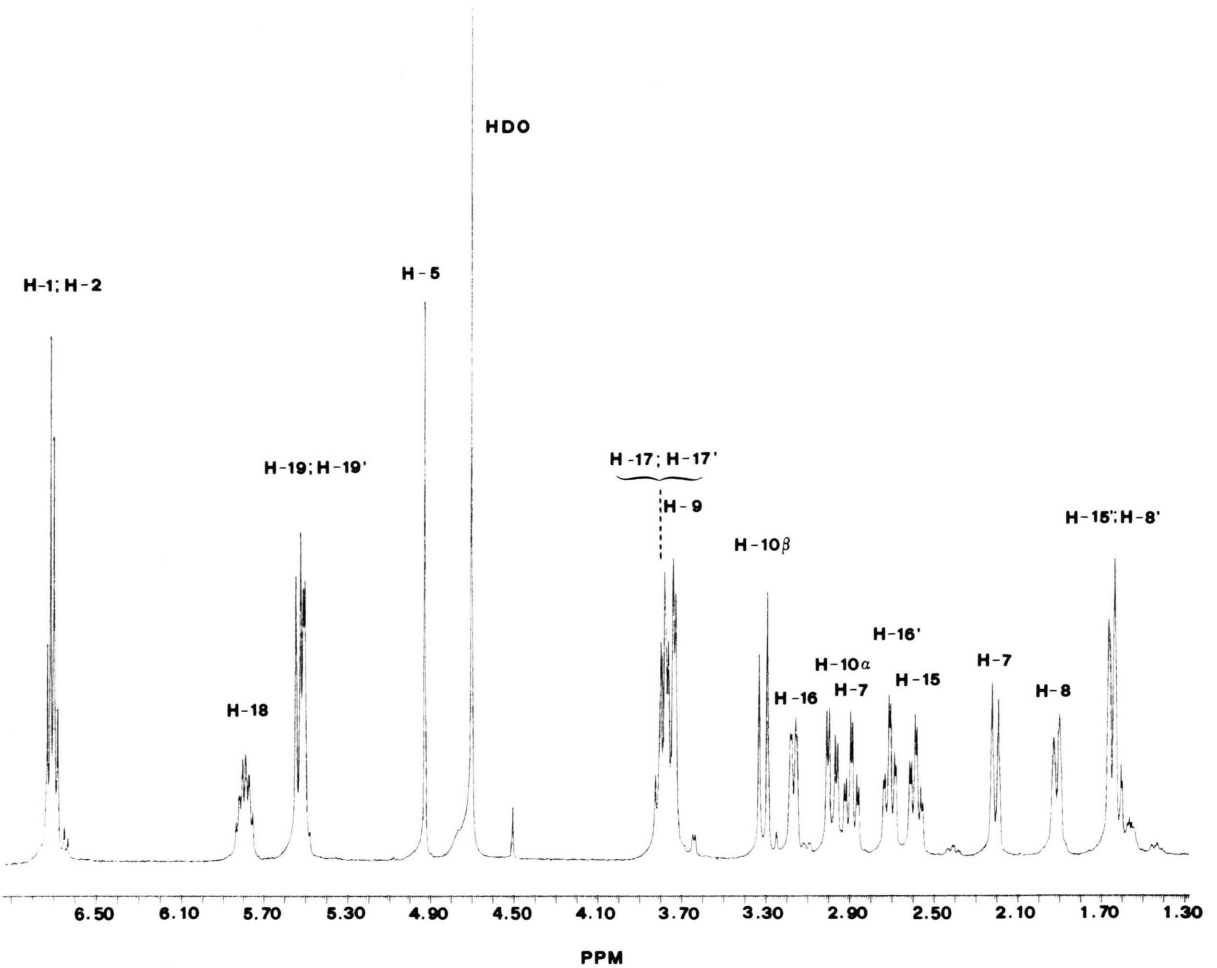


Fig. 1. The ^1H NMR spectrum at 500 MHz of naloxone in ^2H_2O solution. Assigned resonances are outlined.

Spectral analyis

Naloxone: Assignment of the H-5 signal (singlet, partially deuterated after heating at 60° for 24 h) is straightforward. The H-9 signal appears as a doublet centered at 3.73 ppm. Its identification is safely confirmed by evaluation of the contour plot level of COSY spectrum (Fig. 3) that shows coupling of H-9 to H-10α [quadruplet; $J(9, 10\alpha) \simeq 5.9$ Hz] and, in turn, of H-10α to H-10β [doublet; $J(10\alpha, 10\beta) \simeq 20$ Hz, $J(9, 10\beta) \simeq 0$ Hz]. The well resolved AB pattern [$J(1, 2) \simeq 8.3$ Hz] at lowest field can be easily assigned to the remaining methine protons ($\delta_{H\text{-}1} > \delta_{H\text{-}2}$).

The identification of the resonances of protons that are part of methylen groups in 7 and 8 positions was first based on the total and partial deuteration occurring for H-7 and H-7′, respectively, after heating at 60° (<8 h)*. The doublet of triplets due to H-7′ appears as a triplet after H-7 exchange, while the multiplet lines are shifted due to isotopic effects. The observed splittings allow extraction of $J(7, 7') \simeq 14.8$ Hz, $J(7', 8) \simeq J(7', 8') \simeq 3$ Hz values. Correlated H-8, H-8′ resonances, that can be also detected through $^1H-^1H$ connectivities contained in the COSY spectrum (Fig. 3), are located in the spectrum after deuteration of H-7 (triplet of doublets) that in-

* The exchange of H-7 and H-7′ nuclei was expectable because of the mobilizing effect exerted by the carbonyl group bonded to C-7.

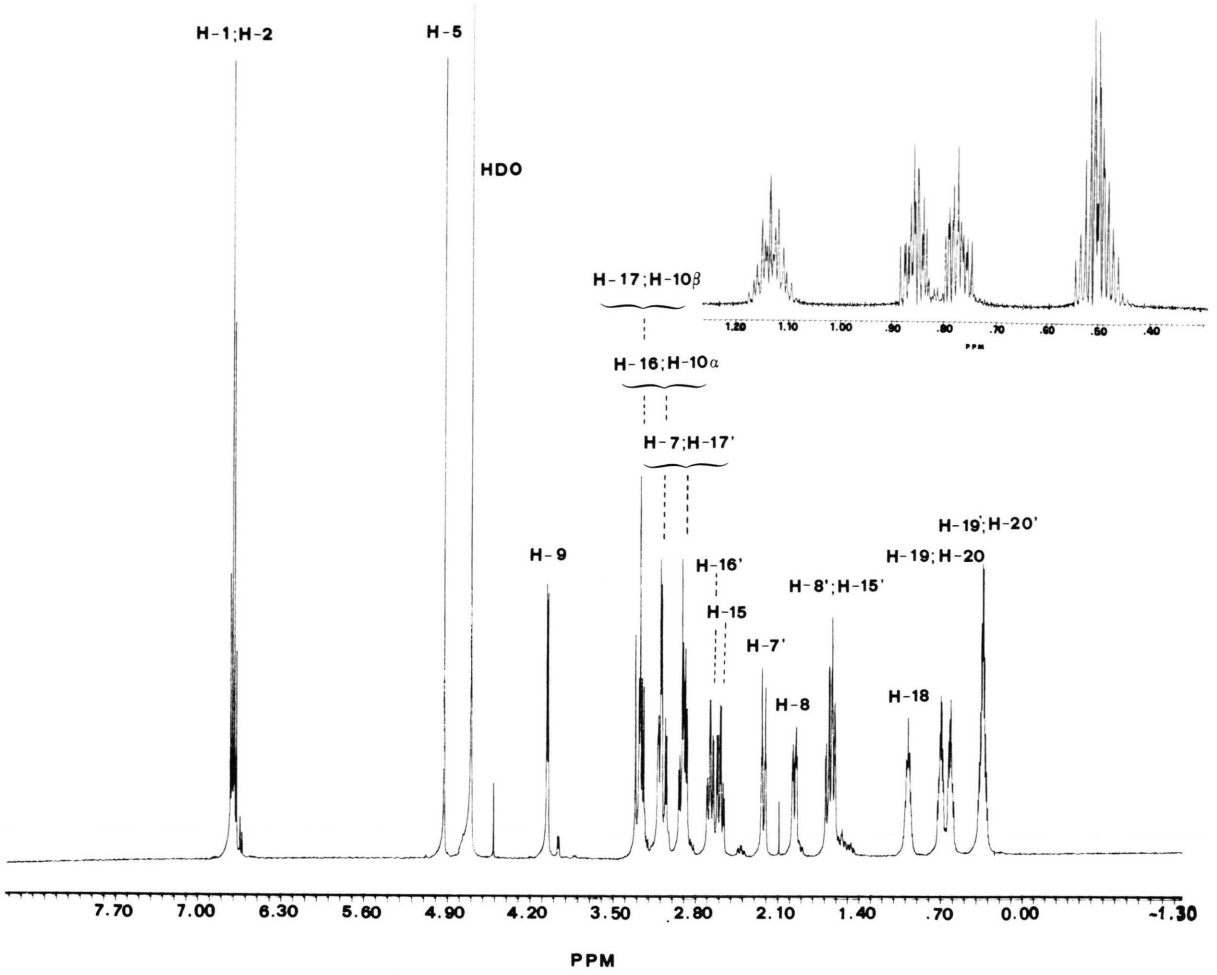


Fig. 2. The ^1H NMR spectrum at 500 MHz of naltrexone in ^2H_2O solution. Assigned resonances are outlined.

duces simplification of their splitting patterns. The H-8 and H-8′ resonances (H-8: doublet of quadruplets; H-8′: triplet of doublets)** appear, after complete deuterium exchange of H-7, as an asymmetric quadruplet and a doublet, respectively. Such multiplets structure, in addition to tests of irradiation effects on H-8′, allowed the extraction of coupling constants $J(7, 8') \simeq 14.8$ Hz, $J(7, 8) \simeq 5.1$ Hz and $J(8, 8') \simeq 14.4$ Hz.

** Although H-8′ and H-15′ patterns are partially overlapped, the simulation of the whole high field pattern was possible after refinement of the H-15, H-15′, H-16, H-16′ spin-system.

The remaining coupled methylenic protons H-15, H-15′, H-16, H-16′ can be identified by inspection of the correlation map in Fig. 3, their relative assignment being made on the basis of shielding effects considerations ($\delta_{H\text{-}16,H\text{-}16'} > \delta_{H\text{-}15,H\text{-}15'}$). Irradiation of H-16, that appears as a doublet of doublets, eliminated the vicinal coupling to H-15 whose multiplet [triplet of doublets, with doublet splittings due to $J(15, 16) \simeq 4.7$ Hz] is simplified to a triplet [splitted by $J(15, 16') \simeq 13$ Hz]. The value of $J(15, 15') \simeq 13$ Hz can be deduced from this splitting pattern.

The H-16′ resonance multiplet [triplet of doublets with doublet splittings due to $J(15', 16') \simeq 3.8$ Hz], after decoupling on H-16 is modified into a quadruplet. This multiplet must therefore arise from cou-

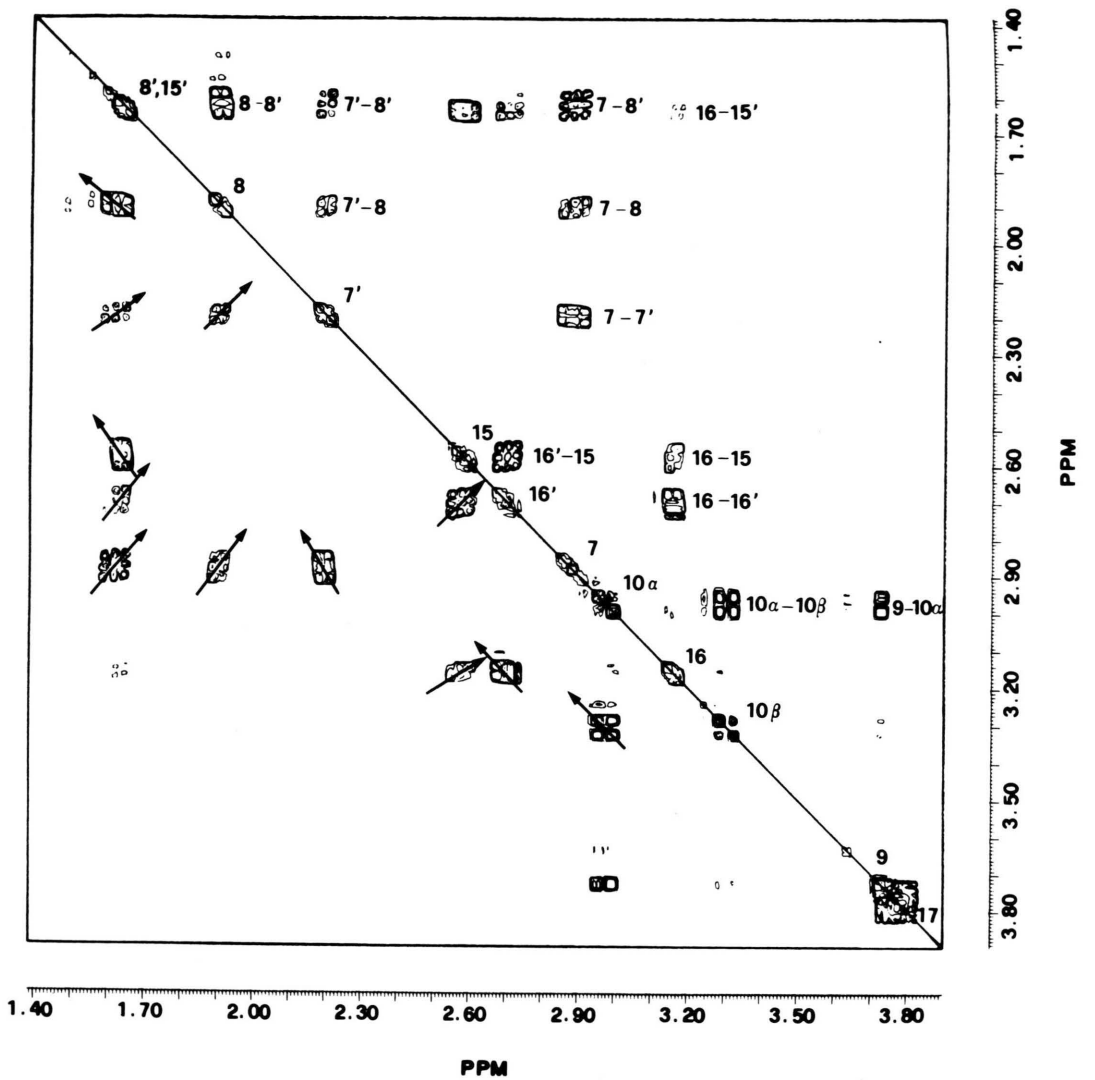


Fig. 3. Contour plot of the ^{1}H COSY 45 spectrum, in the range 1.40–3.80 ppm, of naloxone. Assignments and correlation numbers are shown. The direction of the arrows denote the vicinal and geminal couplings ($\nwarrow = {}^2J$; $\nearrow = {}^3J$).

plings $J(15', 16') \simeq 3.8$ Hz and $J(15, 16') \simeq 13$ Hz, as these can be extracted from the lines separations. The geminal coupling $J(16, 16') \simeq 12$ Hz can be thus easily obtained from splittings of both undecoupled H-16 and H-16′ resonances. However, irradiation of H-15 that eliminates the small coupling to H-16 does again $J(16, 16')$ measurable, while strong perturbation of the closely resonating H-16′ does not permit analysis of the effects on H-16′ multiplet. Decoupling on H-16 does not affect the H-15′ multiplet and therefore a $J(15', 16) < 2$ Hz is deduced. Such a small coupling can be also picked out in the H-16 multiplet, in which the resolved fine structure of the component doublets shows a separation <2 Hz.

The remaining groups of signals (centered at 3.78, 5.50 and 5.79 ppm) must belong to the protons of the N-alkyl fragment. The 2 D method provides unequivocal proof that all five spins belong to the same coupling network. In particular, the diagram allows detection of both the correlated H-17, H-17′, H-18 and H-19, H-19′, H-18 spin systems as well as safe assignment of H-18 to lower field. The order of occurrence in the field of methylenic (17, 17′) and

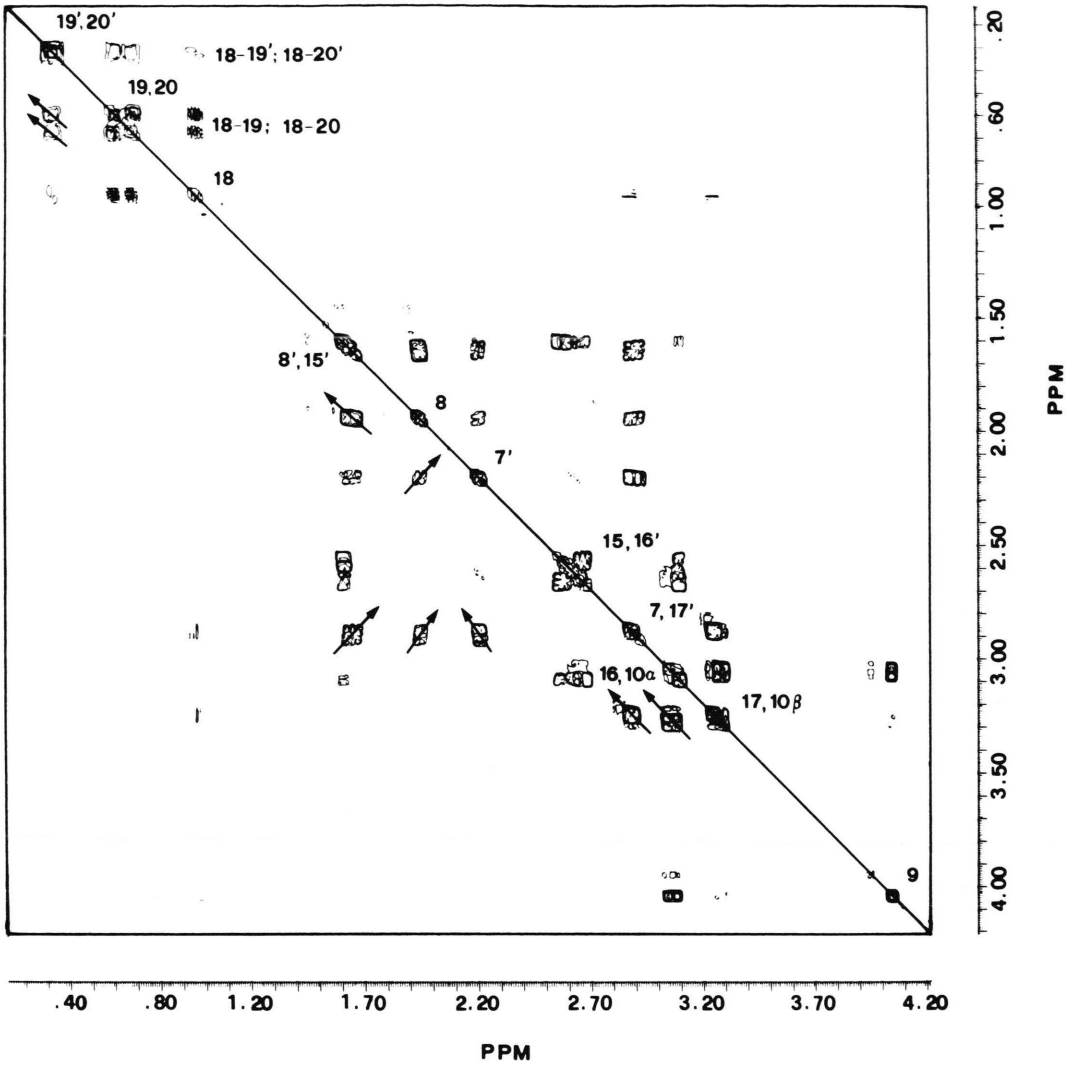


Fig. 4. Contour plot of the ^{1}H COSY 45 spectrum, in the range 0.40–4.20 ppm, of naltrexone. Assignments and correlation numbers (for protons of the cyclopropyl ring) are shown. The direction of the arrows denote the vicinal and geminal couplings ($\nwarrow = {}^2J$; $\nearrow = {}^3J$).

allylic (19, 19′) proton resonances ($\delta_{\text{H-19,H-19'}} > \delta_{\text{H-17,H-17'}}$) was established on the basis of simple chemical shift considerations. Conversely, the results of subsequent spectral analysis confirmed such assignment on the basis of separate characteristic couplings of these nuclei to H-18 (see Table I). To fully analyse the whole three-multiplets pattern, this was considered as arising from two combined ABX spin systems, in which the H-18 to lower field can be treated as the X nucleus and the AB nuclei are, in turn, H-17, H-17′, and H-19, H-19′. In the case of system H-17, H-17′, H-18, all eight lines in the AB region are resolved*, and therefore the whole set of couplings [$J(17, 17') \simeq 14$ Hz, $J(17, 18) \simeq 8.4$ Hz, $J(17', 18) \simeq 6.2$ Hz] could be checked on the splitting patterns, while the corresponding four transition lines expectable for the X nucleus were picked out from the symmetric multiplet centered at H-18. Iterative fitting of this system confirmed identification of these lines and therefore, at the same

* The partial overlap of H-9 doublet was eliminated by decoupling on H-10α.

time, the remaining H-18 quadruplet pertinent to the other H-19, H-19′, H-18 ABX-type system could be isolated. When considering this latter, the AB portion of the spectrum can be recognised as portion of a "deceptively simple" ABX spectrum [7], in which four of the eight theoretically allowed transitions were not observable. The detection of small intensity signals was achieved by expanding sensitivity and width scale. This made the spectrum over-determined and therefore the exact analysis could be performed by iterative fitting procedure that produced an unique solution for parameters set and a simulated pattern in acceptable agreement with experimental. The final refinement was made by iterating lines of the best trial spectrum closely reproducing the experimental, obtained by using as trial parameters approximate coupling constants of literature for allyl protons [8] and chemical shifts values deduced by tentative approximations to the optimum distance between H-19 and H-19′ resonance frequencies.

The relative signs of coupling constants could be obtained by inspection of contours of the COSY 45 correlation map. As expected [4], positive (^3J) and negative (^2J) couplings show reverse inclination of the corresponding cross peaks. This is shown in Fig. 3 and Fig. 4 for **1** and **2,** respectively.

Naltrexone: The spectrum is closely similar to that of naloxone, with the exception of the high field portion corresponding to resonances of protons of the cyclopropyl group. The assignment is therefore straightforward for protons that are part of the fixed backbone similar to that of **1.** However, with respect to the spectrum of **1,** the most striking feature shown by the naltrexone spectral pattern is the more marked difference between H-17 and H-17′ resonances causing partial overlap with H-10β and H-7 doublets, respectively. The quadruplet due to H-17 can be recognised owing to the characteristic splitting of the H-10β doublet [$J(10\alpha, 10\beta) \simeq 20$ Hz] that therefore is picked out. All transition lines due to H-17′ resonance can be isolated by total deuterium exchange of H-7.

The only task to accomplish is thus exact analysis of the spectral pattern arising from H-18, H-19, H-19′, H-20, H-20′, that can be dealt with as an AA′BB′C type system. The fine structure of component multiplets is shown in Fig. 2. Evaluation of contour plot levels (Fig. 4) made possible preliminary assignment of resonances due to protons *cis* (H-19, H-20) and *trans* (H-19′, H-20′) with respect to the correlated H-18. Refinement procedure gave coupling constant values that are in agreement with literature data [9] for cyclopropyl ring.

Discussion

The use of Karplus relationship [10] for ^3J(H,H) allows to interpret these spectral parameters in terms of conformation about C(9)–C(10), C(7)–C(8) and C(15)–C(16) bonds and, accordingly, to define the configuration of either C(5), C(6), C(7), C(8), C(13), C(14) and C(13), C(14), C(15), C(16), N, C(9) rings. The conformations about pertinent C–C bonds are presented as Newman projections in Fig. 5.

The $J(9,10\alpha)$ in both **1** and **2** fits the single possible conformation allowed by this fixed molecular portion. The measured J's for protons bonded to C(15) and C(16) are compatible with both staggered forms **A** and **B,** corresponding to the chair and skew-boat conformation, respectively, of the ring C(15), C(16), N, C(9), C(14), C(13). A choice can be however made in the favour of **A,** *i.e.* of the chair conformation (with N–R *equatorial* [2]), because the relative orientation of hydrogens in such a staggered arrangement is the only one apt to account for the observed lack of

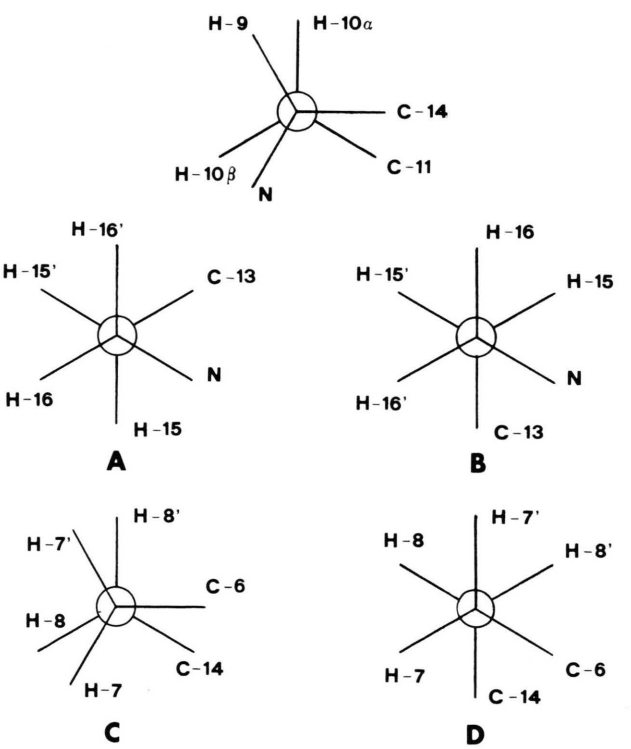


Fig. 5. Newman projections for conformations about C–C bonds. The drawings apply to both **1** and **2** compounds.

magnetic equivalence between H-16 and H-16′. Calculation of the angle for which is $J_{calcd} = J_{exp}$ gives for **1** and **2** an angle value that, more precisely, is related to a distorted-chair conformation of the ring. The remaining ring also adopts the chair conformation in both **1** and **2** molecules. This is demonstrated by the selected agreement of the inherent J's values with the staggered form **D,** while the **C** one, that is related to a shew-boat conformation, can be ruled out on the basis of the large disagreement between J_{calcd} and J_{exp} values.

The chemical shift difference between H-17 and H-17′ is larger in **2** than in **1.** This is of interest, in that it can be interpreted essentially in terms of relative lower degree of internal rotational freedom of the methylenic fragment in the former compound **2,** because the assumption of magnetic equivalence implies that the energy difference between isomers is small. The substitution of N-methyl-cyclopropyl for N-methyl-allyl in naloxone yields naltrexone (**2**) in which the antagonistic properties are still increased. This appears in line with the hypothesis based on results of previous ^{13}C NMR relaxation times analysis [1], suggesting that the relative capabilities of molecules active as antagonists to evoke such a pharmacological response increase with corresponding decrease of degree of internal motion of the N-alkyl group, namely in the order: nalorphine > naloxone > naltrexone.

Work supported by Ministero della Pubblica Istruzione (fondi 60% art. 65 DPR 382) and, in part, by Consiglio Nazionale delle Ricerche (contributo 82.03042.03).

[1] G. C. Pappalardo, L. Radics, M. Baldo, and A. Grassi, J. Chem. Soc. Perkin II **1985,** 955.
[2] E. L. Eliel, S. Morris-Natschke, and V. M. Kolb, Org. Magn. Reson. **22,** 258 (1984).
[3] C. B. Pert and S. H. Snyder, Mol. Pharm. **10,** 868 (1974).
[4] a) W. P. Aue, E. Bartholdi, and R. R. Ernst, J. Chem. Phys. **64,** 2229 (1976);
b) A. Bax and R. Freeman, J. Magn. Reson. **44,** 542 (1981).
[5] a) G. Eich, G. Bodenhausen, and R. R. Ernst, J. Am. Chem. Soc. **104,** 3731 (1982);
b) G. Wagner, J. Magn. Reson. **55,** 151 (1983).
[6] A. A. Bothner-By and S. Castellano, Program 111, QCPE, Indiana University.
[7] R. J. Abraham, The Analysis of High Resolution NMR Spectra, Elsevier, Amsterdam 1971.
[8] J. A. Pople, W. G. Schneider, and H. J. Bernstein, High Resolution Nucleare Magnetic Resonance, McGraw-Hill Inc., New York 1959, p. 238.
[9] J. W. Emsley, J. Feeney, and L. H. Sutcliffe, High Resolution NMR Spectroscopy, Vol. 2, Pergamon Press, London 1966, p. 694.
[10] M. Karplus, J. Chem. Phys. **30,** 11 (1959).