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CONFORMATIONAL AND DYNAMIC FEATURES OF COCAINE IN DMSO-d_6 SOLUTION

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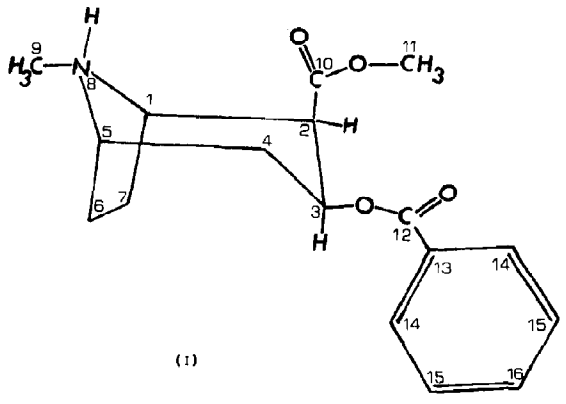
^{13}C spin-lattice relaxation rates, ^{13}C {^1H} NOEs, ^1H spin-spin relaxation rates and ^1H two-dimensional magnetization transfer spectroscopy were used for delineating conformational features of cocaine in DMSO-d_6 solution. Two main conformations differing in the orientation of the plane made by the benzoxy substituent with respect to the piperidine ring principal axis were observed. Relatively slow interconversions of the piperidine ring were delineated together with the main motional features of the whole molecule.

1. Introduction

Cocaine (I) is one of the most popular drugs of abuse, exhibiting a stimulatory effect on the central nervous system [1], beyond a local and regional anesthetic activity [2]. In spite of the biological relevance, very few reports have appeared on the conformational and dynamic features responsible for the receptor-mediated actions of the molecule. X-ray determinations of the free base [3] and of its salts [4,5] have shown that, in the solid state, the molecule exists in a piperidine chair conformation, the methoxycarbonyl substituent at C^2 is axial, the benzoxy substituent at C^3 is equatorial, and the methyl substituent at the nitrogen is also equatorial, in agreement with the (−)-2*R*-methoxycarbonyl-3*S*-benzoxytropane absolute configuration of (−)-cocaine [6].



(I)

In heavy contrast to the solid state and physiologic literature, very little attention has been paid to the solution conformation of the alkaloid and, in particular, none has been directed toward exploiting the NMR parameters. NMR study has been confined to some early ^1H work [7]. In this report we present extensive ^1H- and ^{13}C-NMR relaxation rate and NOE measurements, as well as ^1H-NMR two-dimensional magnetization transfer experiments which allow relevant conformational and dynamic features of cocaine in solution to be delineated.

2. Methods

NMR spectra were recorded on a Varian XL-200 spectrometer. The spin-lattice relaxation rates were measured with the inversion recovery pulse sequence. The spin-spin relaxation rates were measured with the Carr-Purcell-Meiboom-Gill pulse sequence. The values of $1/T_1$ (R_1) and $1/T_2$ (R_2) were calculated from exponential regression analysis. ^{1}H-^{1}H NOEs were determined by applying a 10 s low-power saturating pulse at the appropriate peak position, followed immediately by a high-power observing pulse. Difference spectra were obtained by subtracting 40 on-resonance FIDs from 40 off-resonance FIDs followed by Fourier transformation. ^{13}C-^{1}H NOEs were determined by presaturating the chosen proton resonance with a 10 s decoupler pulse, followed by a ^{13}C observing pulse under conditions of high-power broad-band decoupling. Magnetization transfer ^{1}H-NMR two-dimensional experiments were performed using pulse sequences as in refs. 8 and 9. The spectral width was 2000 Hz. The data set consisted of 512 points in the t_1 dimension and 1024 points in the t_2 dimension. Eight FIDs were accumulated for each value of t_1, and the total accumulation time was approx. 2 h.

3. Results and discussion

Since the ^{13}C spin-lattice relaxation rates are essentially determined by ^{13}C-^{1}H dipole-dipole interactions, dynamic features were readily recognized by the R_1 analysis. The ^{13}C-NMR spectrum of cocaine is shown in fig. 1 where the R_1 value of each carbon is also reported. Since all the carbons exhibited complete ^{13}C {^{1}H} NOE independent of the number and distance of nearest hydrogens, only the dipolar mechanism could be considered. As a consequence the motional correlation time of ring carbons was calculated ($\tau_c = 8.02 \pm 0.40 \times 10^{-11}$ s at 298 K) by ignoring contributions from protons other than those directly attached [10]. The calculation assumed $r_{C,H} = 1.05$ Å and the R_1 value averaged on R_1 of methynes and $R_1/2$ of methylenes. The R_1 values of all ring carbons were absolutely comparable such that no preferential

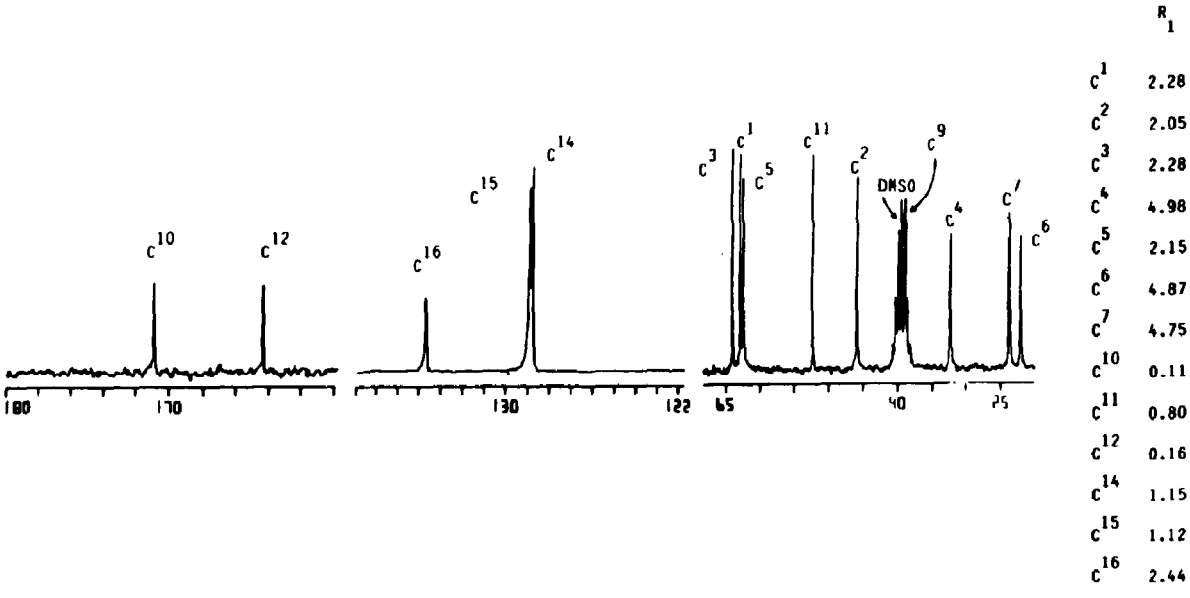


Fig. 1. ^{13}C-NMR spectrum and ^{13}C spin-lattice relaxation rates (R_1) for cocaine (0.1 mol dm^{-3}) in DMSO-d_6 (degassed). $T = 298$ K. The C^9 carbon is underneath the C^{10} carbon. The aromatic part of the spectrum is shown on the reduced vertical scale.

Table 1

^{13}C $\{^1H\}$ NOE upon selective saturation of cocaine protons

Carbon observed	Proton irradiated	
	H^3	H^{14}
C^3	0.623	0.016
C^{12}	0.333	0.283
C^{10}	0.021	0.053
C^{14}	0.014	0.777
C^{15}	–	0.377
C^2	–	0.012

axis of rotation could be suggested. The reported correlation time was therefore identified with the molecular tumbling motional time. Information about the motion of the two substituents could also be gained. In fact, the R_1 value of C^{16} was only slightly different from those of ring methynes, while the R_1 values of C^{14} and C^{15} were much shorter. As already stated in other cases [10], one may interpret that a main molecular axis exists, passing through C^{16} and C^{13}, tumbling at almost the same rate as the pyrrolidine-piperidine ring. The dynamics of C^{14} and C^{15} was therefore elucidated in terms of librational motions around the axis of rotation [11] ($\tau_G = 4.21 \pm 0.28 \times 10^{-11}$ s at 298 K). In contrast the R_1 value of the C^{11} methyl which is much slower than $3R_1$ of every methine suggested that the methyl behaves as a completely unlocked free rotor tumbling with $\tau_c = 9.9 \pm 0.5 \times 10^{-12}$ s at 298 K.

Relevant details of the conformation were obtained by measuring the ^{13}C NOE upon selective irradiation of properly chosen protons as shown in table 1. In fact the NOE of a certain C^d nucleus upon saturation of a particular H^s resonance can be reasonably approximated by [12]:

$$f_{C^d}(H^s)R_{1C^d} = \frac{\hbar^2\gamma_H^3\gamma_C}{10r_{C^dH^s}^6} f(\tau_c) \qquad (1)$$

where r is the C^d-H^s distance and $f(\tau_c)$ is known provided that τ_c is known [12]. The following conformational features were therefore inferred by using the values of τ_c, or alternatively τ_G, calculated from ^{13}C R_1 analysis.

(i) The C^{12} carbonyl is 2.23 Å apart from the H^3 proton which brings the plane of the benzoxy substituent into a position almost parallel to the axial position at C^3.

(ii) Since $r_{C^{14}H^3} = 2.45$ Å and $r_{C^{14}H^2} = 2.20$ Å are found, two main conformations differing in the location of the benzoxy plane can be hypothesized in which the C^{13}-C^{16} axis makes different angles with the C^3-N axis. Librational jumpings of C^{14} and C^{15} around the C^{13}-C^{16} axis bring H^3 or H^2 quite close to C^{14}. Both conformations are characterized, as demonstrated in item (i), by $r_{C^{12}H^3} = 2.23$ Å.

Combining the ^{13}C-NMR analysis with ^1H-NMR data yielded further insight into the solution behavior of cocaine. The ^1H-NMR spectrum, shown at the top of fig. 2, by presenting nonequivalence among the aromatic protons, ratifies the coplanarity between the C^{12} carbonyl and the aromatic ring. The two geminal H^4 protons, near two asymmetric centers, are intrinsically nonequivalent and do not provide information about processes such as ring inversion and chair-boat interconversion. The relatively small values of the H^{5eq}-H^{4eq} and H^{4ax}-H^{3ax} couplings agree with the equatorial configuration of the methyl at the nitrogen [13].

Extensive investigation of the ^1H-^1H NOEs was performed but it was not possible to determine the conformational parameters since it came out that saturation transfer between the NH and the resid-

Table 2

Relaxation parameters of cocaine (0.1 mol dm^{-3}) in DMSO-d_6 at 298 K

± values denote approximate 95% confidence limits of the regression analysis.

Proton resonance	T_1 (s)	T_2 (s)
NH	0.72 ± 0.05	0.01 ± 0.005
H^{14}	1.27 ± 0.02	0.56 ± 0.06
H^{16}	1.25 ± 0.03	0.67 ± 0.04
H^{15}	1.01 ± 0.02	0.57 ± 0.05
H^3	0.33 ± 0.01	0.18 ± 0.02
H^1	0.42 ± 0.02	0.16 ± 0.01
H^5	0.34 ± 0.01	0.16 ± 0.01
H^{11}	0.61 ± 0.03	0.38 ± 0.03
H^2	0.53 ± 0.03	0.07 ± 0.01
H^9	0.31 ± 0.01	0.12 ± 0.01
H^{4ax}	0.17 ± 0.01	0.08 ± 0.01
H^{4eq}	0.14 ± 0.01	0.07 ± 0.01

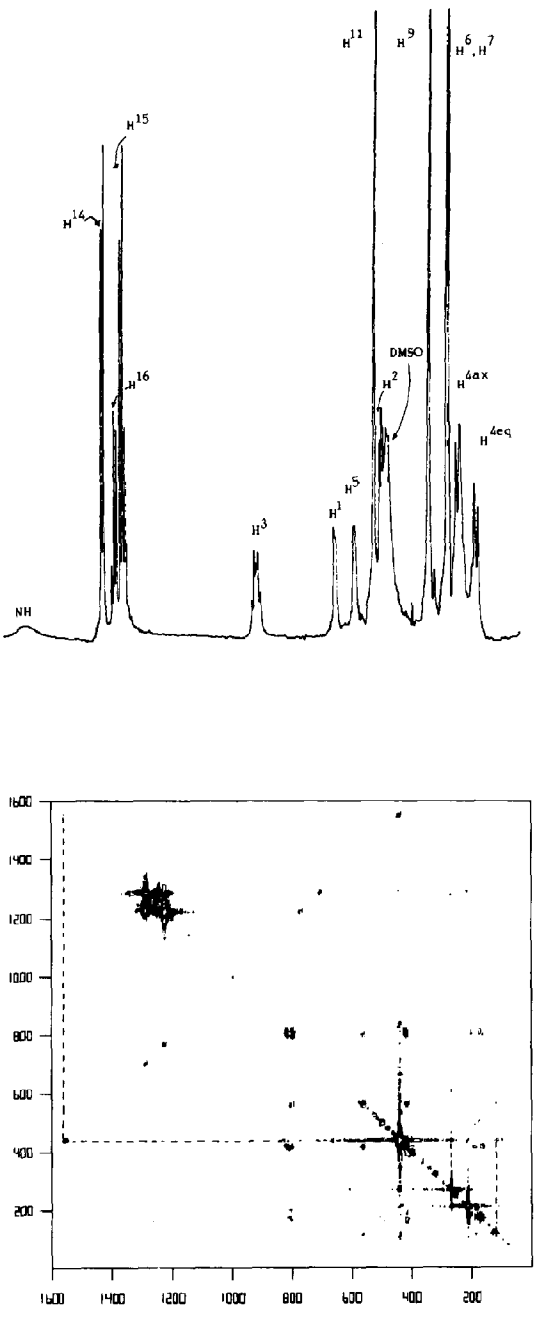


Fig. 2. Contour plot of a two-dimensional magnetization transfer spectrum of cocaine (0.1 mol dm^{-3}) in DMSO-d_6 (degassed) at $T = 298$ K. The mixing time was 100 ms. The dashed lines show ^1H-^1H connectivities to the H^{11} methyl.

ual H_2O in the solvent, as well as cross-saturation among ring protons, strongly affected the observed NOE. As a matter of fact, the spin-spin and spin-lattice proton relaxation rates, summarized in table 2, clearly showed that at least two motions, one of them relatively slow, must be taken into account for the interpretation of the relaxation data. The short T_2 values, especially for H^2, H^3 and H^4, are consistent with effective cross-saturation yielding negative NOEs. The inference can be advanced that relatively slow piperidine ring interconversions modulate the dipolar interactions in which H^2, H^3 and H^4 are involved, thus determining a certain slow motion contribution to T_2.

In spite of such a complicated situation, ^1H-NMR two-dimensional magnetization transfer experiments, shown in fig. 2, allowed other conformational details to be clarified. In fact, ^1H-^1H connectivities were observed between the C^{11} methyl and the NH, as well as with H^{4ax} and, also, the C^9 methyl, suggesting that the methoxycarbonyl substituent somehow folds toward the piperidine ring, such that the ring interconversions are slowed further by steric hindrance. All the other several ^1H-^1H connectivities observable in fig. 2 can be suitably classified among the short-range dipole-dipole interactions among nearby protons and do not deserve any particular attention.

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