Conformation of Amiodarone · HCl: A Solution and Solid State ^{13}C-NMR Study

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The comparison of ^{13}C-NMR spectra of Amiodarone · HCl in different solvents with the CPMAS spectrum and the crystal structure shows that the molecule adopts a single twist conformation both in the solid-state and in solution in nonpolar solvents.

KEY WORDS: Amiodarone · HCl; ^{13}C solid-state NMR; ^{13}C solution NMR; conformation.

INTRODUCTION

During the last ten years, the use of high-resolution solid-state NMR has found increasing application in the structural elucidation of drug powders, formulation, and solid dosage forms of drugs (1,2). ^{13}C CPMAS NMR is able to detect small differences in the chemical environment since carbon-13 chemical shift values in the solid-state are influenced by various factors such as crystal packing, hydrogen bonding, ionization effects, and conformational changes. To date, the comparison of ^{13}C CPMAS spectra with ^{13}C spectra obtained from solutions has not been systematically exploited, though the possibility of detecting molecular conformational differences between the solid and the solution states using the same technique, e.g. NMR spectroscopy, is clearly very important when studying drug active principles. The information thus gained is especially valuable when the crystal structure of the molecule under study is already known. Indeed conformational characterization on the base of conformation-dependent ^{13}C chemical shifts has been shown to be feasible as a complementary method to X-ray diffraction (3). Solid-state ^{13}C chemical shifts are characteristic of the solid state conformation, which is the conformation determined by single crystal X-ray diffraction. Carbon-13 CPMAS therefore fills the gap between ^{13}C NMR solution data and crystal structure information. This report describes the results we have obtained for Amiodarone · HCl.

Amiodarone · HCl (Fig. 1a) exhibits antianginal and antiarrhythmic properties (4,5), and is widely used in the treatment of ischemic heart diseases. Examination of the structure of the molecule (Fig. 1) shows that the orientation of the carbonyl oxygen and furan ring double bond may be either s-*cis* or s-*trans* (6,7). The structure of a series of compounds, including Amiodarone, presenting such a keto bridge has been studied by various methods (8–11). The data obtained for many of these compounds (8,11) suggest that the preferred conformation tends to be s-*trans* (Fig. 1b). The results of recent NMR studies on Amiodarone derivatives seem to support an equilibrium between the s-*cis* and s-*trans* conformers. However, in the case of Amiodarone itself, these studies failed to reveal two sets of lines, even at a temperature as low as 156 K. This result may be due either to the existence of a single conformation in solution (9) or to a conformational interconversion that is fast on the NMR time-scale (10). The present study, which compares solid-state and solution ^{13}C NMR data of Amiodarone · HCl with its single crystal structure, was then undertaken to help answer this question.

MATERIALS AND METHODS

Solution ^{13}C-NMR spectra were recorded at the Laboratoire de Chimie de Coordination (CNRS, Toulouse, France) on a BRUKER WM 250 spectrometer, operating at 62.89 MHz for carbon; the probe temperature was 310 K. Operating conditions were: spectral width 15 kHz; interpulse delay 1.5 sec; memory size 16 K; line broadening 1 Hz, except in the case of CD_3OD for which it was 2 Hz; number of scans 15000 for $CDCl_3$, 10000 for CD_2Cl_2, 116700 for acetone-d_6, 16500 for CD_3OD, and 7000 for DMSO-d_6. The chemical shifts were referred to TMS, taking the central line of the solvent signal at 77.0 ppm for $CDCl_3$, 53.6 ppm for CD_2Cl_2, 29.2 ppm for acetone-d_6, 39.6 ppm for DMSO-d_6, and 49.3 ppm for CD_3OD.

The concentrations of the solutions were: 3.8×10^{-2} M(14) and 6.3×10^{-2} M for $CDCl_3$, 6.5×10^{-2} M for CD_2Cl_2, 6.2×10^{-2} M for DMSO-d_6, and 6.2×10^{-2} M for CD_3OD. Amiodarone · HCl is only slightly soluble in Acetone-d_6, the solution used was therefore as concentrated as possible.

The ^{13}C CPMAS spectra were obtained at the Institut de Recherches de la Catalyse (CNRS, Villeurbanne, France) on a BRUKER MSL 300 spectrometer, operating at 75.47 MHz for carbon and at ambient probe temperature. Operating conditions were: spectral width 50 kHz; recycle time 9 sec; contact time 5 msec; number of scans 500; the dipolar dephasing pulse sequence was used (12) to suppress the resonances of the protonated carbon atoms, with the coupled delay lasting 70 usec. All CPMAS spectra were obtained with the TOSS sequence in order to suppress spinning-sidebands (13). The chemical shifts are referenced with respect to external adamantane for which δ_{CH2} was found to be 37.7 ppm.

Amiodarone · HCl used was an analytically pure sample. NMR solvents were purchased from SDS (Toulouse, France). Other solvents were of analytical grade and used without further purification.

RESULTS AND DISCUSSION

The reported crystallographic data (14) of Amiodarone · HCl were obtained by studying crystals grown from ethanol-

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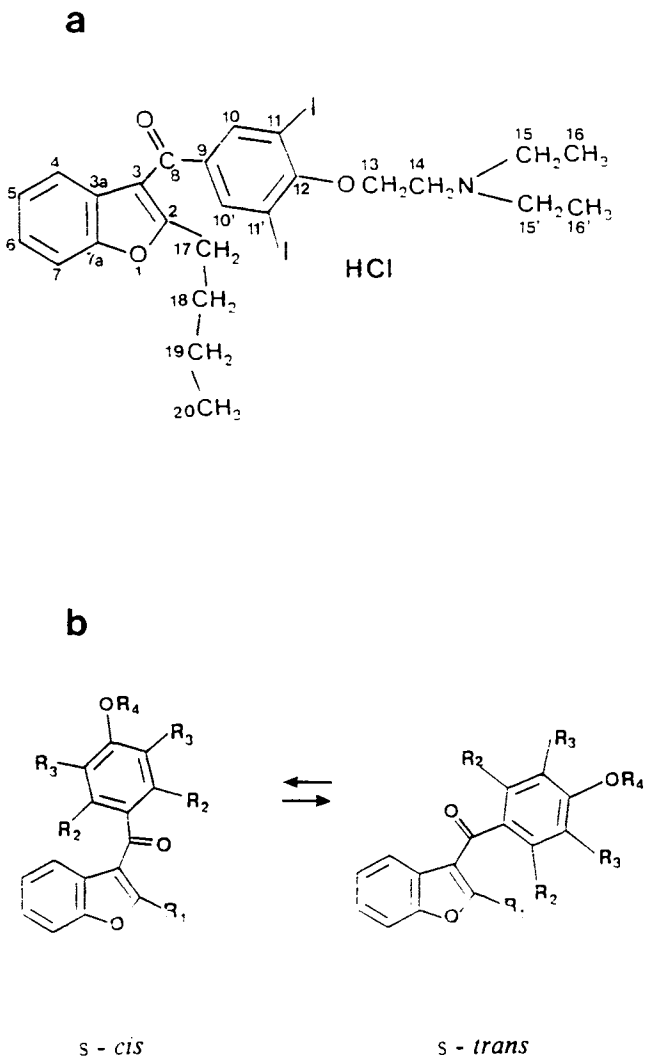

Fig. 1. (a) Structure of Amiodarone · HCl; (b) possible s-*cis* and s-*trans* equilibrium.

ic solutions. To ensure meaningful comparisons between the NMR data obtained for the solution and the solid state with the crystal structure, Amiodarone · HCl was recrystallized in various solvents: methanol, chloroform, methylene dichloride, and methyl ethyl ketone. The solids obtained were analyzed by Differential Scanning Calorimetry, Thermogravimetry, and powder X-ray diffraction and were all found to be identical to each other. Careful and prolonged grinding (circa 1 min.) of the solids in an agate mortar did not alter the results (i.e. no amorphous or other polymorphic structures was detectable).

The chemical shifts of Amiodarone · HCl in solution in a range of solvents ($CDCl_3$, CD_2Cl_2, Acetone-d_6, CD_3OD, and DMSO-d_6) are collected in Table I. It may be noticed that the data we obtained for the $CDCl_3$ solution compare well with those of Ribeiro and Jendrasiak (15), who reported recently the unambiguous assignment of the ^{13}C spectrum of Amiodarone · HCl in chloroform using a combination of various 1D and 2D NMR experiments. The assignments of the spectra in the other solvents were inferred from the results obtained for the solution in $CDCl_3$.

Assignment of the CPMAS spectrum was based upon comparison to the solution state results and the spectrum obtained under dipolar dephasing condition (Fig. 2b and c).

From the examination of Table II it can be seen that the overall conformation of the molecule is only slightly solvent-dependent, as the differences induced in the chemical shifts do not exceed 3 ppm. Indeed the most affected signals are those of carbons α to the nitrogen atom (2.9 ppm for C(14) and 2.2 ppm for (C(15) and C(15')), which give sharp lines in the solvent range used. The trends observed in their chemical shifts may thus be explained by an increasing interaction between the ammonium ion and the solvent while going from $CDCl_3$ to CD_3OD. The position of the signals of carbon atoms α to an oxygen atom presents some differences (2.2 ppm for C(9), 2.4 ppm for C(2) and C(7a)). The comparison of the chemical shift differences observed from $CDCl_3$ to CD_3OD suggests the existence of some intermolecular hydrogen-bond interactions between the solvent and the solute in the case of CD_3OD.

The ^{13}C NMR spectra of Amiodarone · HCl in $CDCl_3$ and in the solid phase are shown in Fig. 2a and b, respectively. Compared to the solution, the solid state spectrum shows some unique and interesting features. The resonance signals of carbons directly bonded to quadrupolar nuclei having nonzero electric field gradients are often split or broadened. This effect becomes more important as the quadrupolar coupling constant increases. Indeed, with the spin of the quadrupolar nucleus being not quantized along B_0 (the magnetic field direction), the magic angle spinning is then unable to fully average the dipolar interaction between the quadrupolar nucleus and the carbon attached to it. The Amiodarone molecule contains several such nuclei. Firstly, the spin-5/2 ^{17}O nucleus the natural abundance of which (3.7×10^{-2}) is too low to increase the linewidth of the resonances of the carbons (natural abundance 1.1%) C(2), C(7a), C(8), C(4') or C(13) attached to it. Secondly, the spin-5/2 ^{127}I (100% natural abundance) which has a very large quadrupolar coupling constant. As a result the resonances of the directly bonded C(11) and C(11') are so broadened that they are barely detectable in the solid state spectrum (Fig. 2b). This effect is indeed very important since the resonances of the carbons β to C(11) and C(11') (i.e. C(10), C(10') and C(12)) are also affected. Thirdly, the linewidth of the carbons β to the ^{14}N spin-nucleus (99.6 3% natural abundance) is also increased. This may be explained by a residual (^{14}N, ^{13}C) dipolar coupling. However, since the resonance of C(13) exhibits a similar broadening, probably due to restricted motion of the ethoxy side-chain in the solid phase, it cannot be ruled out that the linewidths of C(14), C(15) and C(15') may be the consequence of the reduced mobility of the chain. The results of the crystallographic study (11) show indeed that the amine group is fully protonated. In this case it is expected that the resonances of the carbons α to the nitrogen should be very sharp since the symmetry about the ^{14}N is then nearly tetrahedral (the field gradient being small, the spin-1 ^{14}N nuclei are quantized along B_0).

Another interesting feature of the solid state spectrum is the splitting of the resonance of C(16) and C(16'). This splitting can be easily explained by the hindered motion of the ethoxy side-chain due to the much more encumbered environment of the molecules in the solid state. However, unlike

Table I. Solution and Solid State ^{13}C NMR Data (δ/ppm) for Amiodarone · HCl

Solvent carbon	$CDCl_3$[a]	$CDCl_3$	CD_2Cl_2	DMSO-d_6	Acetone-d_6	CD_3OD	None
2	166.52	166.57	166.52	165.61	[b]	167.98	165.06
3	115.67	115.67	116.00	115.56	[b]	117.32	115.21
3[a]	126.20	126.22	126.70	126.06	[b]	127.90	125.85
4	120.91	120.91	121.17	120.82	121.42	122.35	124.19
5	123.92	123.93	124.00	123.91	124.12	125.29	122.66
6	124.78	124.79	124.91	124.87	125.11	126.38	122.66
7	111.21	111.22	111.28	111.17	111.31	112.46	108.55
7[a]	153.66	153.70	154.00	153.08	[b]	155.52	151.97
8	187.31	187.28	187.53	187.31	[b]	189.43	186.04
9	139.15	139.23	139.46	138.61	[b]	140.91	139.85
10	140.70	140.66	140.96	139.74	140.86	142.08	139.86
10′							
11	90.72	90.53	90.75	92.16	90.90	91.90	[c]
11′							
12	160.13	160.11	160.53	160.28	[b]	162.08	159.91
13	67.17	67.17	66.52	67.22	68.59	68.07	67.48
14	50.35	50.42	50.63	49.88	50.50	52.80	49.31
15	48.00	47.92	48.01	47.13	47.73	49.30	47.24
15′							
16	9.31	9.22	9.14	8.70	8.95	9.58	11.40[e]
16′							9.30
17	28.17	28.16	28.33	27.50	[d]	29.31	24.81
18	30.01	30.01	30.19	29.34	[d]	31.35	33.29
19	22.48	22.46	22.67	21.89	22.54	23.79	20.11
20	13.71	13.66	13.67	13.40	13.35	14.27	11.81[e]

[a] From ref. 14.
[b] Not detected.
[c] Too broad.
[d] Peaks obscured by the solvent signal.
[e] Assignment may be reversed.

the resonances of C(13), C(14), and C(15), the lines of C(16) and C(16′) are sharp indicating that the CH_3 end groups are rotating freely. The restricted motion of the n-butyl side chain may similarly explain the broadening of the C(17), C(18), and C(19) resonances, whereas free rotation of the end group is responsible of the sharpness of the C(20) line.

The chemical shifts observed in the CPMAS spectrum differ little from those obtained from the solutions, except in the case of CD_3OD, and the differences ($\Delta\delta < 2$ ppm) may be ascribed to the molecular packing in the crystal lattice. For the resonance position of some carbons, $\Delta\delta$, however, exceeds 2 ppm. The carbons concerned are C(17), C(18), and C(19) along with C(4), C(6), and C(7a). This observation may indicate that the orientation of the n-butyl side chain is somewhat different in the solution, where it is expected to reorient rapidly without interfering with the benzofuran or the iodophenyl rings, whereas in the crystal (11) it is folded back over the iodophenyl ring. These results suggest that the conformations of the skeleton of the Amiodarone · HCl molecule in the solid state and in solution in $CDCl_3$, CD_2Cl_2, Acetone-d_6, and DMSO-d_6 are very close.

In the case of CD_3OD, $\Delta\delta > 2$ ppm for most of the resonances. This may be due to the polarity of the solvent and, as already noted above, to the formation of hydrogen bonds between the solvent and the molecule under study (e.g. C(8) is more shielded in the absence of the solvent) therefore bringing some modifications to the conformation of the Amiodarone · HCl molecule.

From the comparison of the data obtained in a range of solvents and in the solid state it is thus possible to deduce some very important results that would be difficult to attain otherwise. The results described in this paper provide therefore an interesting example of the benefit that can be gained in using solid-state NMR for the study of the conformation of drug active principles. Actually the confrontation of solid and solution state ^{13}C NMR results with single crystal X-ray data offers a unique possibility to detect conformational changes induced by going from the solid to the solution state and thus to evidence the influence of the solvent.

For Amiodarone · HCl it is then possible to conclude that, except for the increased mobility of the two side-chains in solution and the more rigid orientation of the n-butyl chain in the solid, the conformation of the molecule is very similar in both phases, in the case of nonpolar solvents. The crystallographic structure being known, it may thus be deduced that in nonpolar solvents the molecule adopts a single conformation with the carbonyl oxygen s-*trans* to the benzofuran C(2)-C(3) double bond, the phenyl ring being in a twist conformation about the carbonyl bridge to the benzofuran ring system, while the amine is fully protonated.

In conclusion, the correlation of the solid state and solution ^{13}C NMR data shows that the solid and solution con-

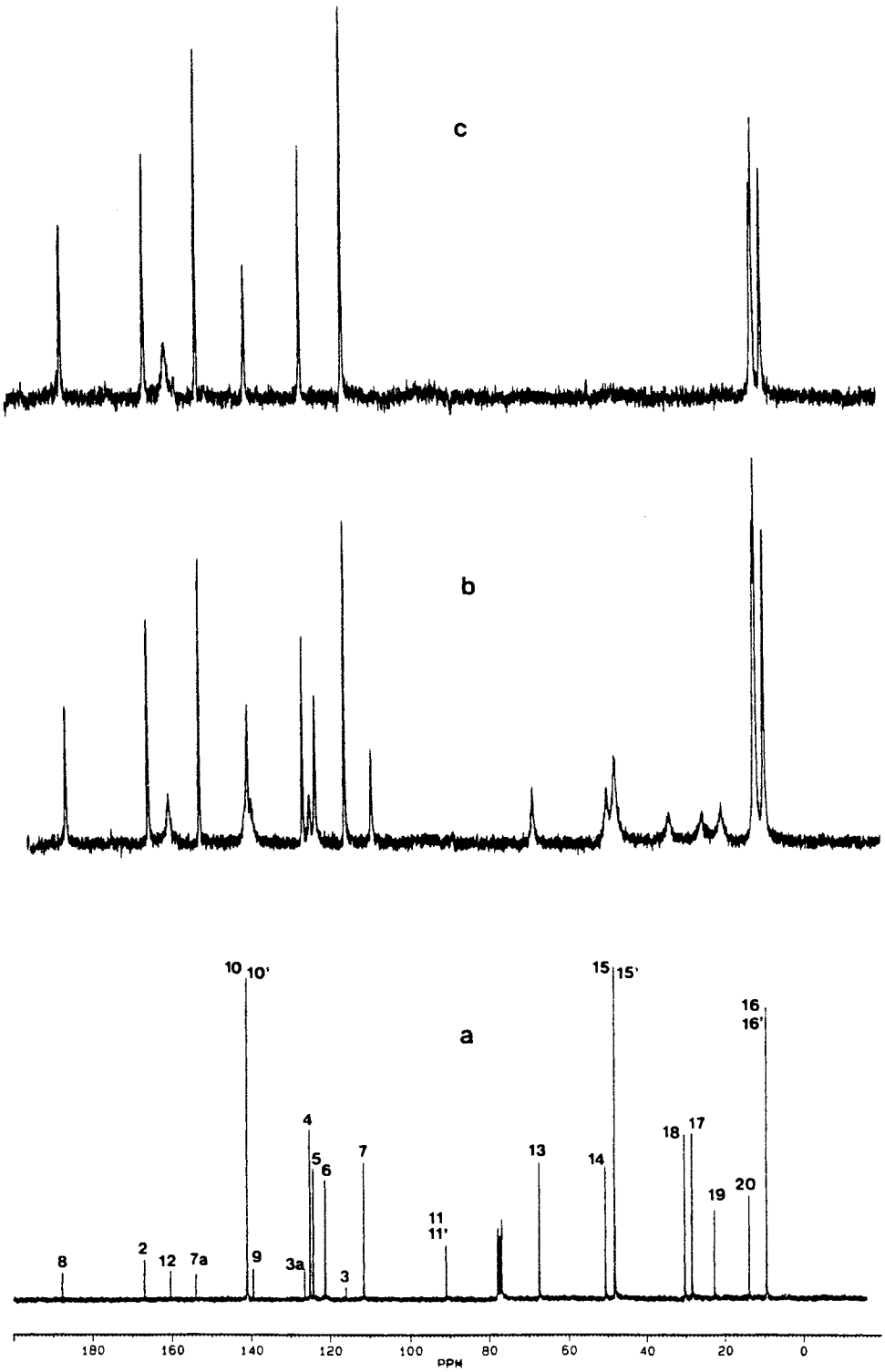

Fig. 2. ^{13}C NMR spectra of Amiodarone · HCl (a) was obtained from a $CDCl_3$ solution; (b) is the CPMAS spectrum obtained using the TOSS (13) sequence; (c) was obtained under dipolar diphasing conditions.

formational properties of Amiodarone · HCl are similar when nonpolar solvents are considered. These results provide further evidence of the usefulness of solid-state NMR spectroscopy to establish whether or not the conformation of the active principle of a drug is retained in solution. The answer to this question is clearly of utmost importance for conformational:activity investigations.

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Table II. Chemical Shift Difference $\Delta\delta = (\delta_{solid} - \delta_{solution})$/ppm

Solvent carbon	$CDCl_3$	CD_2Cl_2	DMSO-d_6	Acetone-d_6	CD_3OD
2	+1.5	+1.4	+0.5	—	+2.9
3	+0.5	+0.8	+0.4	—	+2.1
3[a]	+0.3	+0.8	+0.2	—	+2.0
4	−3.3	−3.0	−3.4	−2.8	−1.8
5	+1.2	+1.3	+1.2	+1.4	+2.6
6	+2.1	+2.2	+2.2	+2.4	+3.7
7	+2.6	+2.7	+2.6	+2.7	+3.9
7[a]	+1.7	+2.0	+1.1	—	+3.5
8	+1.3	+1.5	+1.3	—	+3.4
9	−0.7	−0.4	−1.3	—	+1.0
10	+0.9	+1.1	−0.2	+1.0	+2.2
10′	+1.0	+1.2	−0.1	+1.1	+2.3
11	—	—	—	—	—
11′	—	—	—	—	—
12	−0.2	+0.6	+0.4	—	+2.2
13	−0.8	−0.5	−0.8	+0.6	+0.1
14	+1.1	+1.3	+0.6	+1.2	+3.5
15	+0.7	+0.8	−0.1	+0.5	+2.1
15′					
16	0.0	+0.1	+1.6	+0.6	+0.4
16′					
17	+3.4	+3.5	+2.7	—	+4.5
18	−3.3	−3.1	−4.0	—	−1.9
19	+2.4	+2.6	+1.8	+2.4	+3.7
20	+1.9	+1.9	+1.6	+1.6	+2.5

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