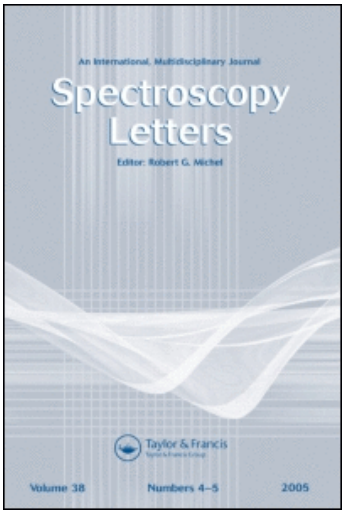

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^{1}H and ^{13}C NMR Conformational Analysis of Adrenergic Drugs. III. Dichloroisoproterenol, A Nonselective β-Blocking Agent

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^{1}H and ^{13}C NMR CONFORMATIONAL ANALYSIS OF ADRENERGIC DRUGS. Part 3. DICHLOROISOPROTERENOL, A NONSELECTIVE ß-BLOCKING AGENT.

Keywords: ^{1}H NMR, ^{13}C NMR, relaxation rates, adrenergic drugs

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ABSTRACT

^{13}C and ^{1}H NMR parameters were measured for dichloroisoproterenol in solution. Spin-lattice relaxation rates, nuclear Overhauser effects and J couplings were determined and compared to those obtained from isoproterenol. The t rotamer was shown to occur with a much higher probability than the two g rotamers. Dynamics in solution were interpreted in terms of a nearly isotropic motion of an extended molecular backbone. Some interesting differences were given evidence between the 'preferred' conformations in solution of dichloroisoproterenol and isoproterenol.

INTRODUCTION

Dichloroisoproterenol (4-{1-hydroxy-2[(1-methylethyl)-amino]ethyl}-benzene-1,2-dichloro (DCI) (Figure 1) is the first drug shown to exert ß-blocking adrenergic activity.[1] It was obtained by replacement of the cathecol hydroxyls of isoproterenol (ISP), a pure ß-agonist, by chlorine atoms. However DCI is also a partial ß-agonist; for this reason it has never been used as hypotensive drug. In spite of the poor therapeutic properties, DCI, when considered toghether with ISP, represents a suitable test for comparing substances having very similar chemical structures but rather different pharmacologic activities.

From this point of view, NMR parameters, especially J couplings spin-lattice relaxation rates and NOEs, provide the desired structural and dynamic information that can be used to acquire relevant conformational features in solution. Even in the case of small flexible molecules with multiple degrees of internal motional freedom and, hence, with many conformations separated by low energy barriers, it is possible to delineate some structural and/or motional characteristics that may be relevant for biological activity.[2,3]

The aim of this paper is to define the relevant NMR parameters for delineation of motional and structural features of DCI in solution and to compare the conformational properties of DCI and ISP.

EXPERIMENTAL

Dichloroisoproterenol was supplied by Sigma Chemical Co. and used without further purification. Solutions were prepared in $[^2H]_6$DMSO (99.96% from Merck) and were carefully deoxygenated.

The NMR spectra were recorded on a Varian VXR-200 and a Bruker AM 300 NMR spectrometers in the pulse-FT mode at a probe temperature of 298 ± 1 K; chemical shifts were referenced to internal TMS.

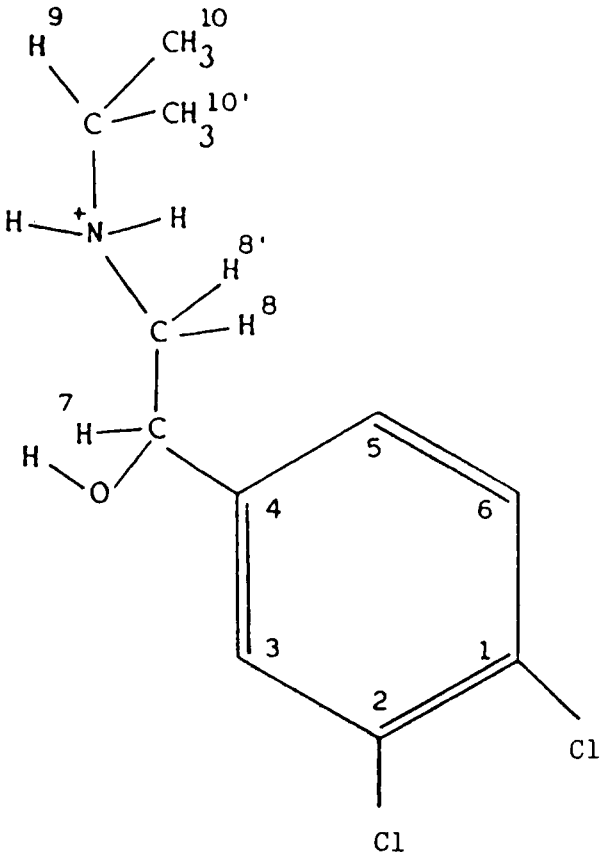

Figure 1 - Molecular formula of dichloroisoproterenol.

Non-selective spin-lattice relaxation rates were measured with the inversion recovery pulse sequence $(t\text{-}180°\text{-}\tau\text{-}90°)_n$; selective and double-selective spin-lattice relaxation rates were measured with inversion recovery pulse sequences where the 180° pulse was given by the proton decoupler at the chosen frequency(ies) at low power for relatively long times. All the spin-lattice relaxation rates were calculated in the initial rate approximation[4] from exponential regression analysis of the

recovery curves of longitudinal magnetization components by using the computer of the spectrometer.

2D J resolved ^1H NMR spectra were obtained by using the pulse sequence described in Ref. 5. the time domain data size was 128 points in the t_1 dimension and 512 points in the t_2 dimension. 16 FIDs were accumulated for each value of t_1 for a total accumulation time of ca. 1 h. 2D NOE spectra were measured by using the pulse sequence described in Ref.6. The spectral width was 2000 Hz, and the data set consisted of 512 points in the t_1 dimension and 1024 points in the t_2 dimension for a total accumulation time (16 FIDs collected) of ca. 4 h.

RESULTS AND DISCUSSION

It has been previously shown, for isoproterenol,[2] that the molecule is highly flexible in solution and, hence, that what is dected by NMR is not the "minimum energy" conformation but just a highly probable molecular arrangement that arises from motional averaging of several equally or almost equally populated structures. In the case of isoproterenol it was found that:

(i) when looking on the C_7-C_8 bond, the t rotamer is more populated ($\sim$ 60%) than hte g^+ ($\sim$ 26%) or the g^- ($\sim$ 14%) rotamers;

(ii) the α-hydroxyphenethylamine moiety almost isotropically reorients around a molecular axis that connects C_4 and C_1;

(iii) the isopropyl moiety is somehow folded towards the phenyl ring.

The ^1H NMR parameters of DCI in DMSO are reported in Table 1. The coupling constants within the ABX pattern of H_7 and H_8 protons and within the ABC pattern of the H_3, H_5, H_6 protons were determined from the 2D J spectrum (figure 2) and ratified by spectral simulation.

By considering electronegativity differences[7,8] to correct the rotamer couplings obtained by Feeney for aminoacid side chains,[9] the same equations as in the case of isoproterenol were used[2] to calculate the fractional populations of the three minimum energy staggered conformati-

TABLE 1

^{1}H NMR parameters for DCl 0.1 mol.dm^{-3} in [^{2}H]$_6$DMSO at T=298 K

Peak	δ (ppm)	J (Hz)	ν = 200 MHz R^{nsel} (s^{-1})	ν = 200 MHz R^{sel} (s^{-1})	ν = 300 MHz R^{nsel} (s^{-1})	ν = 300 MHz R^{sel} (s^{-1})
NH	8.8		3.02		8.70	
H_3	7.7	J_{35}=2.6	0.59	0.53	0.54	
H_6	7.6_5	J_{65}=8.6	0.68	0.56	0.57	
H_5	7.4		1.16	0.90	0.95	
OH	6.4		2.80	2.89	3.88	
H_7	5.0	J_{78}=9.7	2.37	1.75	2.08	1.72
H_9	3.3		2.35		2.15	
H_8	3.1	$J_{88'}$=12.7	4.72		4.61	3.74
$H_{8'}$	2.9_5	$J_{8'7}$=4.1			4.44	3.67
H_{10}	1.25	J_{910}=6.6	2.75		2.78	
$H_{10'}$	1.2	$J_{910'}$=6.6			2.67	

ons around the C_7-C_8 bond, as shown in figure 3. The t rotamer was found to have a much higher probability of occurence (~70%) than the g^+ (~17%) and g^- (~13%) rotamers.

The ^{13}C NMR parameters are summarized in table 2. ^{13}C-{^{1}H} NOEs were found at their maximum values, thus implying that the ^{13}C-^{1}H dipolar interaction provides the main relaxation mechanism. Accordingly, the R_1/n_H values are determined by the one bond ^{13}C-^{1}H interaction and can be used to gain some knowledge of reorientational dynamics.[10]

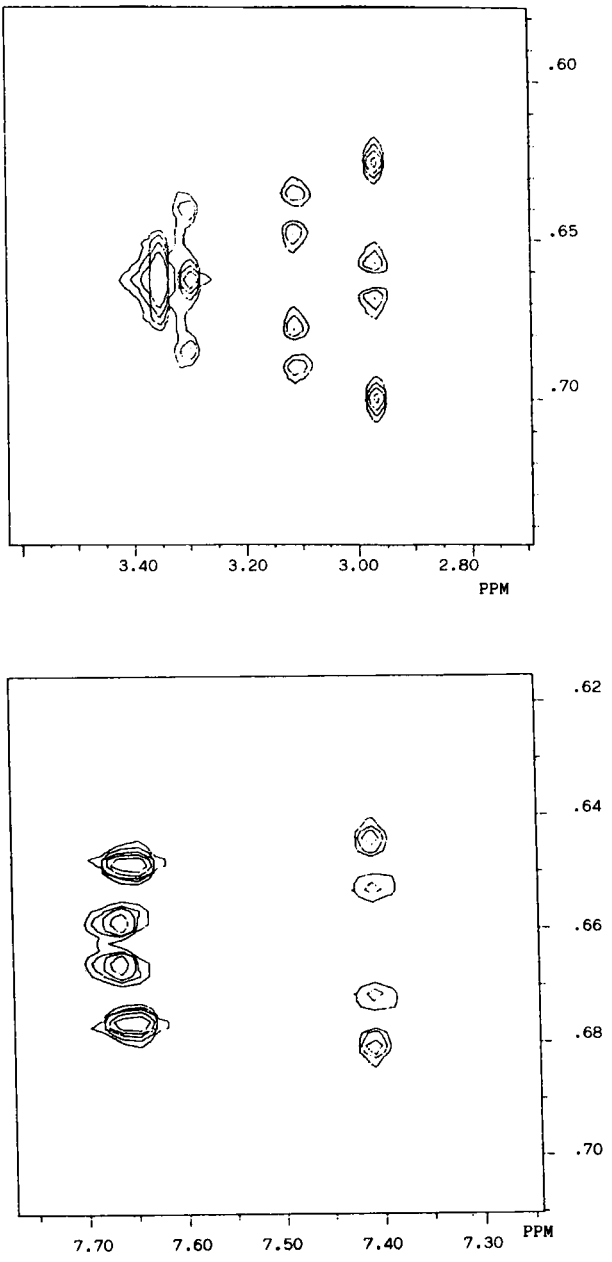

Figure 2 - Contour plots of selected regions of the J-resolved 2D NMR spectrum of DCI 0.1 mol dm^{-3} in $|^{2}H|_{6}$DMSO showing the ABX pattern of H_7-H_8 protons (upper spectrum) and the ABC pattern of aromatic protons (lower spectrum).

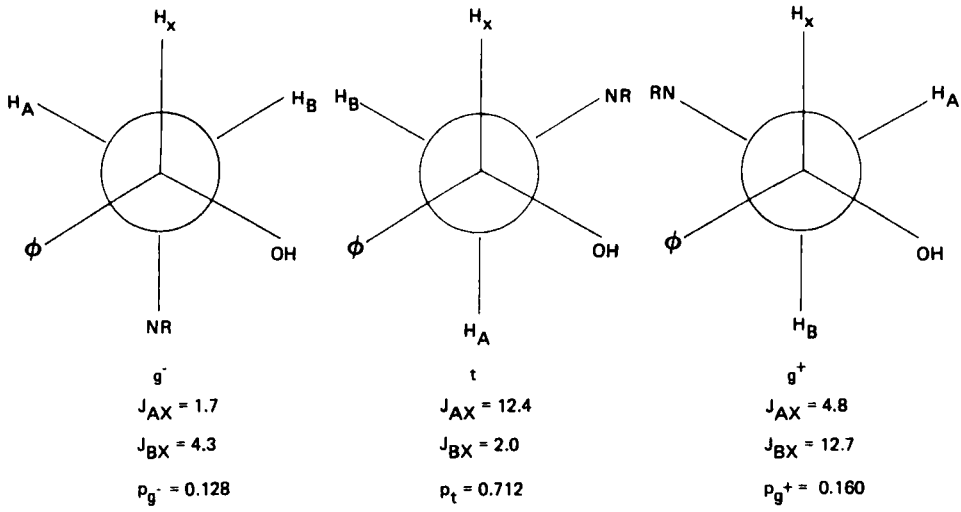

Figure 3 - Minimum energy staggered conformations around the C_7-C_8 bond in dichloroisoproterenol. Coupling constants and fractional populations were calculated as explained in the text.

TABLE 2

^{13}C NMR parameters of DCI 0.1 mol dm^{-3} in $[^2H]_6$DMSO at T=298 K

Peak	δ (ppm)	R_1 (s^{-1})	R_1/n_H (s^{-1})
C_4	143.0	0.33	----
C_2	131.0	0.50	----
C_6	130.5	1.34	1.34
C_1	130.1	0.42	----
C_3	128.1	1.20	1.20
C_5	126.4	1.27	1.27
C_7	67.4	1.91	1.91
C_8	50.2	3.73	1.86
C_9	49.8	1.84	1.84
C_{10}	18.7	1.52	0.51
$C_{10'}$	18.1	0.98	0.32

From this point of view the α-hydroxyphenethyl amine moiety appears to almost isotropically reorient in solution, only the two methyl groups retaining some degrees of internal mobility. By considering the average R_1/n_H of C_7, C_8 and C_9, $\tau_c = 92\pm2$ ps was calculated for the "isotropic" reorientational carrelation time of DCI in solution at 298 K. The following features of ^{13}C and ^{1}H relaxation rates were supporting this value of τ_c:

(i) if one considers non selective and selective ^{1}H relaxation rates, the difference between them yields the sum of cross-relaxation terms σ_{ij} contributing the overall rate of the observed proton.[11,12] In the case of H_6 only the dipolar interaction with H_5 is likely to be effective. This being the case, $\sigma_{5,6} = R^{nsel}-R^{sel} = 0.12\ s^{-1}$ can be calculated, wherefrom $\tau_c = 90\pm2$ ps is evaluated ($r = 2.43$ Å).[13]

(ii) Consideration of the one bond $^{13}C-^{1}H$ dipolar interaction for the C_3, C_5 and C_6 ring carbons leads to calculate $\tau_{ceff} = 62\pm3$ ps at 298 K for the effective reorientational motion of the ring. The effective correlation time can be suitably interpreted in terms of the model of Woessner[14] for relaxation of a C-H vector that reorients with an internal correlation time τ_i around an axis, at an angle $\theta = 60°$ in our case, that, in turn, isotropically reorients with an overall correlation time τ_o. By taking $\tau_o = 90$ ps and $\tau_i = 57$ ps, $\tau_{ceff} = 62$ ps is obtained.

(iii) the same model of Woessner[14] can be considered for interpreting ^{13}C relaxation rates of the two methyl groups. $\tau_{ceff} = 25$ ps at 298 K is obtained when $\tau_o = 90$ ps and $\tau_i = 7.5$ ps are taken into account.

In can be concluded that, in spite of the high intrinsic flexibility, the molecule presents an extended backbone that almost isotropically reorients with $\tau_o = 90$ ps. Librational motion of the aromatic ring and free rotation of the methyl groups apparently provide the only degrees of internal freedom.

TABLE 3

Cross-relaxation rates ($\sigma'_{ij} = R_i^{i,j} - R_i^{sel}$) between selected proton pairs of DCI 0.1 mol dm^{-3} in $[^2H]_6$DMSO at T=298 K

Proton pair	σ'_{ij} (s^{-1})
H_5-H_7	0.064
H_3-H_7	0.060
H_7-OH	0.058
H_7-H_8,	0.052
H_7-H_8	0.091

A comparison with isoproterenol does not disclose many differnces; it is however interesting to observe that the R_1/n_H values of C_9 are somehow diverse. In the case of DCI this carbon atom reorients in coherence with the other backbone carbons; whereas in the case of isoproterenol, the same carbon atom was found to possess some degrees of internal freedom.

The dynamic picture, as obtained by interpretation of ^{13}C relaxation rates, can be used to account for observed ^1H spin-lattice relaxation rates. In fact, whatever is the relevance of the ^1H-^1H dipole-dipole interaction in determining the relaxation mechanism for any observed proton, double irradiation techiques allow to selectively excite any pair of proton resonances, wherefrom the corresponding dipolar cross-relaxation term can be obtained:[11,12]

$$\sigma'_{ik} = R_i^{i,k} - R_i^{sel} \qquad (1)$$

where $R_i^{i,k}$ and R_i^{sel} are the initial rate constants for recovery of the ith longitudinal magnetization component after double selective or single selective excitation respectively (Table 3).

From this point of view the fact that $\sigma_{5,7} \simeq \sigma_{3,7}$ discloses the interesting feature that H_7 has to be at almost the same distance from H_3 and H_5. By assuming that both these dipolar interactions are modulated by the correlation time for internal motion of the aromatic ring, it is found that $r_{5,7} = r_{3,7} = 2.55$ Å. Since there is not an unique conformation that can account for such set of distances, one has to consider at least two different conformations that are almost equally populated and that are originated by rotation around the C_1-C_4 axis. These findings are verified by the ^1H-$\{^1H\}$ NOE's that are measured on H_5 (0.058) and H_3 (0.053) upon presaturation of H_7.

By the same way consideration of the cross relaxation term between H_7 and the hydroxyl proton allows to locate the two protons at a distance of 2.7 A from each other (τ_c = 92 ps was assumed in this case), that is to say in a mostly anti conformation).

Interpretation of the $\sigma_{7,8}$ and $\sigma_{7,8'}$ cross-relaxation terms requires to take the following averaging equations into account, as previously done far isoproterenol:[2]

$$\langle \sigma_{7,8} \rangle = p_{g-}\sigma_g + p_t\sigma_t + p_{g+}\sigma_g \quad (2)$$

$$\langle \sigma_{7,8'} \rangle = p_{g-}\sigma_g + p_t\sigma_g + p_{g+}\sigma_t \quad (3)$$

Where σ_g and σ_t are the cross-relaxation rates of two protons in gauche and in trans respectively. If the three fractional populations calculated by J coupling analysis (Figure 3) are considered together with τ_c = 92 ps at 298 K, a good fitting of experimental values is obtained ($\sigma_{7,8}$ = 0.054 and $\sigma_{7,8'}$ = 0.096).

These findings, as compared to those obtained with isoproterenol, suggest that the conformations around C_7-C_8 are somehow different in the two cases. The hydroxyl group, in respect of H_7, is anti for DCI and syn for isoproterenol and the high probability of occurrence of the t rotamer around the C_7-C_8 bond is emphasized in DCI where it gets at more than 70%. This feature is verified also by the absence of dipolar connectivities between ring and sidechain protons. Such connectivities were showing up for isoproterenol in solution[2] and were interpreted in terms of a certain folding of the isopropyl residue towards the ring. It is obvious that such folding is possible only in a gauche conformation (either g^- or g^+) that, as a matter of fact, does occur with a much higher probablility in the case of isoproterenol ($p_{g+} + p_{g-} = 0.41$) than in the case of DCI.

All the NMR findings can be summarized by stating that DCI possesses fewer degrees of conformational freedom than isoproterenol. Both these substances, in spite of their intrinsic high flexibility, are characterized by an extended backbone that reorients almost isotropically in solution; however, the backbone is more extended in the case of DCI, involving also the isopropyl moiety in contrast with isoproterenol where the same moiety was found to retain some degrees of internal motional freedom. These features reflect into diverse fractional rotamer populations and in a relatively high probability of occurrence of folded conformations in the case of isoproterenol.

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