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Study of carvedilol by combined Raman spectroscopy and *ab initio* MO calculations

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The novel cardioprotective drug carvedilol was studied by both Raman spectroscopy and *ab initio* molecular orbital methods (using the density functional theory approach). The spectra, acquired both for the solid samples and DMSO solutions as a function of pH, were assigned in view of the calculated wavenumbers and intensities, and also based on the experimental data obtained for individual compounds which comprise the molecule, namely carbazole and 1,2-dimethoxybenzene. The pH dependence of the Raman pattern of carvedilol was studied, and the pK_a value of its secondary amine group was determined (pK_a = 8.25) through pH titration experiments. This kind of information is of great significance for the understanding of the biochemical role of carvedilol, which is strongly determined by the acid–base behaviour of the molecule. Copyright © 2002 John Wiley & Sons, Ltd.

INTRODUCTION

Carvedilol ({1-[carbazolyl-(4)-oxy]-3-[2-methoxyphenoxyethylamino] propanol-(2)}) is a compound displaying antioxidant properties, used in clinical practice for the treatment of cardiovascular diseases (hypertension, congestive heart failure or myocardial infarction).[1,2] This drug was recently proposed to act through protection of the mitochondrial function,[3–6] as cardiac dysfunctions are often correlated with changes in mitochondrial bioenergetics. Carvedilol behaves as a weak protonophore, carrying protons through the mitochondrial membrane, thus causing a lowering of the electric membrane potential ($\Delta\Psi$) created by ejection of H^+ by the redox chain pumps.[7] In this way, carvedilol may be protonated in the cytoplasmic site, cross the membrane driven by the inner negative electric field and then release its amine proton into the matrix. This proposed cardioprotective effect is closely related to the acid–base characteristics of the molecule, which emphasizes the importance of a correct determination of its pK_a value.

In a previous study,[7] a conformational analysis of carvedilol was carried out by *ab initio* self-consistent field molecular orbital (SCF-MO) calculations for the different protonation states of the molecule, and the corresponding proton affinities were obtained. In the present work, a spectroscopic vibrational study of the molecule was carried out and its pK_a value was determined through analysis of the changes in the band intensities upon protonation.

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EXPERIMENTAL

Ab initio MO calculations

The *ab initio* SCF-MO calculations (total geometry optimization as well as harmonic vibrational wavenumbers and intensities) were performed using the Gaussian 98W program,[8] with the split valence basis set 3–21G.[9] Molecular geometries were fully optimized by the Berny algorithm, using redundant internal coordinates:[10] the bond lengths to within ca 0.1 pm and the bond angles to within ca 0.1°. The final root-mean-square (r.m.s.) gradients were always less than 3×10^{-4} hartree bohr^{-1} or hartree rad^{-1}.

Raman spectroscopy

The Raman spectra were recorded on a Jobin-Yvon T64000 triple monochromator Raman system (0.640 m, *f*/7.5) with holographic gratings of 1800 grooves mm^{-1}. The premonochromator stage was used in the subtractive mode. The detection system was a non-intensified CCD (charge-coupled device). Radiation of 514.5 nm line from an argon ion laser (Coherent Innova 300-05) was used for excitation, providing ca 80 mW at the sample position, and a 90° geometry was employed. The entrance slit was set to 200 μm and the slit between the premonochromator and the spectrograph was opened to 13.2 mm. An integration time of 5 s and 10–15 scans were used in all the experiments. Samples were sealed

in Kimax glass capillary tubes of i.d. 0.8 mm. Under these conditions, the error in wavenumbers was estimated to be within 1 cm^{-1}.

FT-Raman spectra were obtained for the carbazole sample with a Bruker RFS 100/S spectrophotometer. Radiation of 1064 nm line from an Nd:YAG laser was used for excitation, providing ca 100 mW at the sample position. The resolution was set at 2 cm^{-1} and a 180° geometry was employed.

Spectra were obtained for both solid samples (at 15 °C) and 0.5 mol dm^{-3} solutions (at 20 °C). Band intensity ratios were evaluated by fitting Lorentzian bands to the experimental spectra.

Sample preparation

Dimethyl-sulfoxide (DMSO) was used as a solvent for carvedilol (0.5 mol dm^{-3}), owing to the low water solubility of this compound. The pH of the solutions was adjusted between 2.5 and 13.5 with HCl and CO_2- free NaOH, using a Sargent-Welch pH-meter coupled to a Radiometer PHC3006-9 combined electrode.

Reagents

Carvedilol and carbazole (vetranal) were obtained from Boehringer and Fluka/Riedel-de Haën, respectively. DMSO (HPLC grade), 1,2-dimethoxybenzene (veratrole, 99%), HCl and NaOH (analytical grade) were purchased from Aldrich.

RESULTS AND DISCUSSION

Carvedilol was found to have two distinct conformers in simultaneous equilibria in its unprotonated state, with relative populations of 71% [(N)H···O distance equal to 229 pm] and 29% [((N)H···O distance equal to 239 pm], and only one stable conformation in the protonated form (displaying a significatively shorter (N)H···O distance of 184 pm).[7] Protonation of the molecule was found to induce a severe change in the $C_{carbazol}OCC$ dihedral angle, from −79° (unprotonated compound) to 177° (protonated species) (Fig. 1). In all cases, the molecule is stabilized through intramolecular (O)H···O and/or (N)H···O hydrogen-type interactions, which are determinant of its acid–base characteristics and consequently of its biochemical effect in living cells. Tables comprising the optimized geometric parameters of carvedilol are available from the authors upon request.

Figure 2(A) displays the Raman spectrum of solid carvedilol, that was assigned based on its calculated harmonic Raman wavenumbers and intensities (Table 1), as well as on the comparison with the experimental spectra obtained separately for two compounds which comprise the molecule, 1,2-dimethoxybenzene and carbazole [Fig. 2(B) and (C)]. Interestingly, these aromatic systems, situated at two opposite ends of the carvedilol molecule, hardly interact with each other, yielding rather localized vibrational modes, which may actually be considered as group wavenumbers.

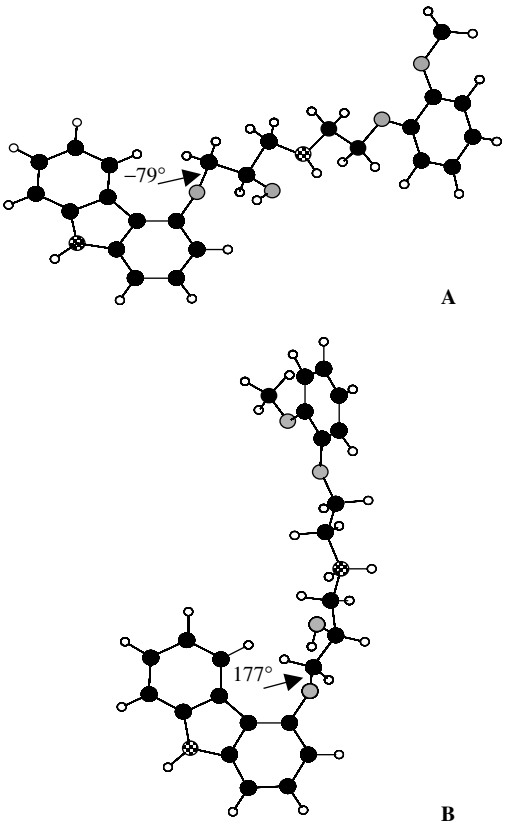

Figure 1. Schematic representation of the lowest energy conformations of carvedilol, in its unprotonated (A) and *N*-protonated (B) forms.

In fact, the spectrum obtained for carvedilol can be viewed as the result of a superposition of the individual spectra of carbazole and 1,2-dimethoxybenzene (Fig. 2). A tentative assignment of the Raman patterns corresponding to these molecules is given in Table 1. Good agreement is found between the present values and those recently reported for carbazole by Lao *et al*.[11]

Figure 3 displays several Raman spectra of carvedilol solutions in DMSO. Apart from the features due to the solvent, the pattern observed is in close accord with the results obtained for the solid sample (Fig. 2) and thus with the assignments presented in Table 1.

The spectroscopic results obtained for these solutions, at distinct pH values, undergo clear changes in the region around 750 cm^{-1} (Fig. 3), which reflect the presence of the protonated and unprotonated forms of a secondary amine group in the molecule (Fig. 1). The corresponding protonation constant (pK_a) was therefore determined through Raman pH titration experiments. Through analysis of the spectra, it was verified that the protonation equilibrium can be monitored through the band at ca 747 cm^{-1}, assigned to the RNH_2^+ group, as its intensity increases steadily on lowering the pH of the solution (Fig. 3). This feature may then be used unequivocally as a measure of the concentration of the

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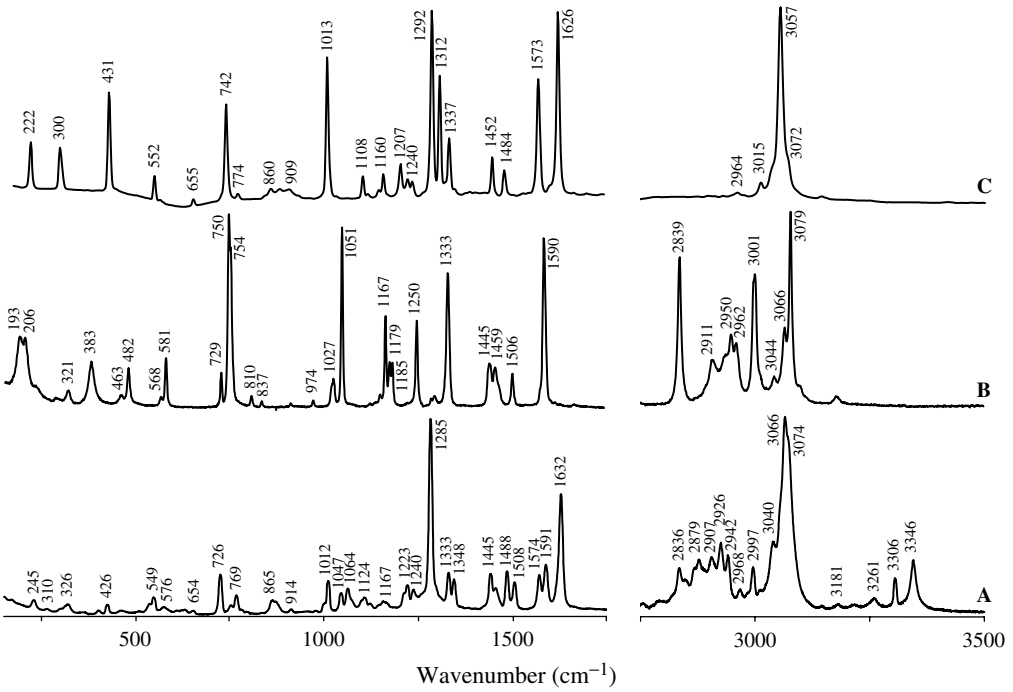

Figure 2. Raman spectra (175–1750, 2750–3500 cm^{-1}) of carvedilol (A), 1,2-dimethoxybenzene (B) and carbazole (C), in the solid phase.

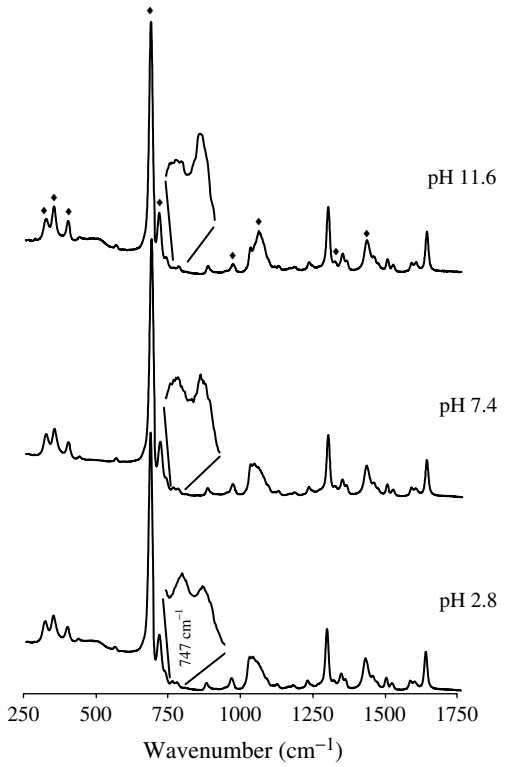

Figure 3. Raman spectra (250–1750 cm^{-1}) of 0.5 mol dm^{-3} solutions of carvedilol in DMSO, at 20 °C and distinct pH values. The bands due to the solvent are marked with ♦.

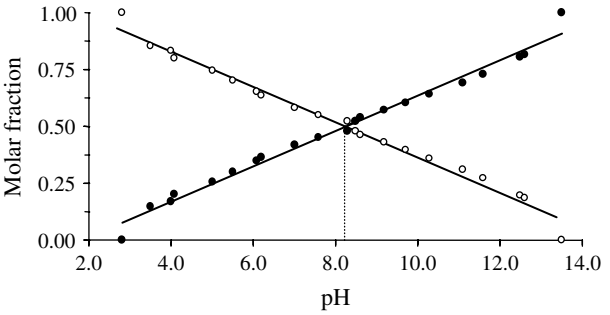

Figure 4. Molar fraction of the unprotonated and protonated forms of carvedilol (DMSO solution, 0.5 mol dm^{-3}) as a function of pH, and corresponding linear fit to the data. ●, Unprotonated species; ○, protonated species.

protonated form of the molecule. Upon obtaining spectra in a broad region of pH (from 2.5 to 13.5), the molar fractions of both the unprotonated and protonated species in solution were calculated from the Raman intensity of that band, and plotted as a function of pH (Fig. 4). Finally, the pK_a was determined as being 8.25, from the pH value at the crossing points of those curves. The band at ca 867 cm^{-1}, that remained unchanged, both in wavenumber and intensity, throughout the whole pH range studied, was used as an internal reference. Actually, the fact that this band, which was previously ascribed to a mixed mode due to the indole NH bending and ring deformation,[13,14] is not affected by the variation in pH is good evidence that protonation occurs only in one of the nitrogen atoms of the carvedilol molecule (within its

Table 1. Raman experimental wavenumbers (cm^{-1}) for carvedilol, 1,2-dimethoxibenzene (1,2-DMB) and carbazole, in the solid state, with *ab initio* MO-calculated harmonic wavenumbers and intensities (IR, Raman) for carvedilol

Carvedilol		1,2-DMB	Carbazole	Approximate description
Experimental	Calculated[a]			
3346	3515 (80;92)			OH stretching
3306	3501 (96;111)			NH stretching (carbazole)
3261	3365 (14;39)			NH stretching (chain)
3181				2×1590
3074	3093 (0;205)	3079		CH sym. stretching (1,2-DMB)
3066	3089 (4;145)	3066	3057	CH sym. stretching (carbazole; 1,2-DMB)
3040	3061 (13;109)	3044	3038 sh	CH antisym. stretching (carbazole; 1,2-DMB)
3013	3048 (1;53)		3015	CH antisym. stretching (carbazole)
2997	3010 (26;98)	3001		CH_3 antisym. stretching
2968	2971 (15;50)	2962		CH stretching (chain); CH_2 antisym. stretching (ethoxy)
2942	2949 (48;54)	2950		CH_3 antisym. stretching
2926	2933 (19;35)			CH_2 (central) antisym. stretching
2907	2911 (22;71)	2911		CH_2 sym. stretching
2879	2886 (47;85)			CH_2 sym. stretching
2849	2852 (46;56)			CH_2 (central) sym. stretching
2836	2902 (40;96)	2839		CH_3 sym. stretching
1632	1626 (10;174)		1626	CC stretching (carbazole)
1591	1608 (57;44)	1590		CC stretching (1,2-DMB)
1574	1583 (8;31)		1573	CC stretching (carbazole)
1508	1530 (94,19)	1506		CC stretch. (1,2-DMB) + CH_3 antisym. bend. + CH_2 scissor.
1488	1500 (24;19)		1484	CC stretch., CH in-plane bend. (carbazole) + CH_2 scissoring
1458	1483 (21;5)	1459		CC stretch., CH in-plane bend. (1,2-DMB) + CH_3 sym. bend.
1445	1471 (30;2)	1445		CC stretch., CH in-plane bend. (1,2-DMB) + CH_3 sym. bend.
1445	1454 (45;35)		1452	CC stretching, CH/NH in-plane bending (carbazole)
1348	1325 (30;13)			CH_2 wagging + CH_2 twisting
1333	1315 (198;25)	1333		CC–OC sym. stretch., CH in-plane bending (1,2-DMB)
1333	1311 (6;11)		1337	CC stretching; CH in-plane bending (carbazole)
	1299 (10;59)		1312	CN/CC sym. stretch.; CH in-plane bending (carbazole)
1285	1281 (3;17)		1292	CN sym. stretch., CC stretch., CH in-plane bending (carbazole)
1240	1248 (18;6)		1240	CC/CO stretch., CH in-plane bend. (carbazole) + CH_2 twisting
1240	1241 (400;1)	1250		CC–OC antisym. stretching, ring antisym. bend. (1,2-DMB)
1223	1223 (24;20)		1226	CN antisym. stretching, NH in-plane bending (carbazole)
1216	1203 (31;81)		1207	CH in-plane bending (carbazole) + CC sym. stretching (pyrrole)
	1182 (18;4)	1185/1179		CH_3 rocking
1167	1169 (35;28)	1167	1160	CC stretching, CH in-plane bending (carbazole)
1145	1143 (1;7)		1149	CC stretching, CH in-plane bending (carbazole)
1124	1118 (54;16)		1108	CN antisym. stretch. (chain) + CC stretch., CH bend. (carbazole)
1064	1085 (62;8)			CC/CN/C–OH stretching (chain) + CH_2 rocking
1047	1053 (22;24)	1051		C–C/O–CH_3 stretching, CH in-plane bending (1,2-DMB)
	1022 (18;16)	1027		O–CH_3 stretching (1,2-DMB)
1012	1005 (16;10)		1013	CC stretching (phenyl ring breathing, carbazole)
1000	981 (8;16)			CH out-of-plane bending (carbazole) + CC/CN stretching (chain)
914	925 (27;8)		909	CCC/CNC deformation (carbazole) + CH_2 rocking (linear chain)
865, 873	865 (8;13)		860/884	CCC/CNC deformation, NH in-plane bending (carbazole)
769	787 (111, 2)			CH out-of-plane ('butterfly' of carbazole)
754	760 (33;24)			NH perp. bend. (chain) + CCC in-plane bend. (1,2-DMB)
726	747 (96;9)	729/750	742	NH perp. bend. (chain) + CCC in-plane bend. (1,2-DMB, carbazole)
654	670 (1;4)		655	CCC/CNC in-plane deformation (carbazole)

Table 1. (*Continued*)

Carvedilol		1,2-DMB	Carbazole	Approximate description
Experimental	Calculated[a]			
630	619 (7;3)			CCC in-plane deformation (1,2-DMB)
576	571 (4;7)	581		COC deformation
549	557 (11;4)		552	Chain deformation + CCC deformation (carbazole)
538	539 (20;5)			Chain deformation + CCC deformation (carbazole)
		482		CCC deformation
462	453 (38;3)			COH bending + chain deformation
426	420 (4;4)		431	Pyrrole ring breathing
402	394 (8;1)	383		COC/CCN/CCC chain deformation + COH bending
364	363 (3;4)			Chain deformations
326	331 (0;7)	321	300	Phenyl–O–C deformations + out-of-plane phenyls bend. (carbazole)
310	280 (6;2)			Chain deformations
276	272 (9;1)			Chain deformations
245	234 (5;1)			Chain torsions + chain deformations
			222	Out-of-plane phenyls bending
		206		O–CH_3 torsion
191	200 (1;2)	193		Chain torsions

[a] For the most populated conformer, at the 3–21G level of calculation; scaled by a factor of 0.9085,[12] for all wavenumbers above 400 cm^{-1}. IR intensities in km mol^{-1}; Raman scattering activities in Å u^{-1}.

linear chain), the carbazole group remaining unchanged. In fact, the wavenumber of this Raman pattern is considered by some workers[14] as a probe of H-bonding occurring at the nitrogen atom of the indole group.

The pK_a value now obtained in DMSO solution is in fairly good accord with that found in the literature for carvedilol in phosphate saline medium: pK_a = 7.9.[15] Also, the results from the present study agree completely with those that we previously reported for the proton affinity of carvedilol.[7] In fact, having a weak affinity for protons (amine pK_a = 8.25) and carrying a positive charge at physiological pH, this compound will be able easily to release H^+ within the mitochondrial matrix, acting as an efficient cationic protonophore, with relevance for a cardioprotection effect.[7]

CONCLUSIONS

The Raman spectra of carvedilol were obtained and assigned for both the solid state and DMSO solution. Interpretation of the spectroscopic data was based on the calculated vibrational wavenumbers and intensities, obtained through *ab initio* SCF-MO methods, and on the experimental spectra for individual compounds which comprise the carvedilol molecule, namely 1,2-dimethoxybenzene and carbazole.

The present work clearly evidences the utility of Raman spectroscopy in the determination of pK_a values in solution, provided that a clear pH dependence is detected in the spectroscopic pattern during the protonation process, and an unequivocal correlation between the bands undergoing variation and a particular degree of protonation of the molecule may be achieved. The pK_a value of the secondary amine group of carvedilol, in DMSO solution, was now determined to be 8.25, in fairly good accord with that previously reported using analytical methods.[15]

Moreover, the fact that the experimental and calculated Raman spectra found for carvedilol in the present work display very good agreement reflects a close similarity between the theoretical geometry and the real conformation of the molecule. This may be of significance in future studies aimed at the understanding of the structure–activity relationships associated with the biological role of this compound, already in clinical use.

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