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# An NMR Spectrometric and Potentiometric Study on the Protonation of Timolol

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A study was undertaken to evaluate the protonation mechanism of (*S*)-(+)-1-[(1,1-dimethyl)-ethylamino]-3-[[4-(4-morpholino)-1,2,5-thiadiazol-3-yl] oxy]-2-propanol (Timolol) by means of $^{13}C$ and $^{15}N$ NMR spectrometry, and protonation constants by means of potentiometric pH titrations. The potentiometric data clearly showed that the title compound can add only one proton in water–ethanol solvent mixtures. The $^{13}C$ NMR measurements confirmed this result and in addition, revealed that the protonation takes place at NH nitrogen.

(*S*)-(+)-1-[(1,1-dimethyl)ethylamino]-3-[[4-(4-morpholino)-1,2,5-thiadiazol-3-yl] oxy]-2-propanol, more widely known as Timolol, is a β-adrenergic blocking agent used in the treatment of *angina pectoris, hypertension* and *glaucoma*.[1,2]

Prompted by some synthetic interests we have studied the protonation of Timolol using the potentiometric method for the determination of the protonation constant and both $^{13}C$ and $^{15}N$ NMR spectroscopy for evaluating the mechanism of the protonation.

## EXPERIMENTAL

*Preparation of Timolol.* Timolol was prepared according to a procedure described in the literature.[3]

*NMR measurements.* The $^{13}C$ and $^{15}N$ NMR spectra were recorded on a JEOL FX 100 spectrometer operating at 25.05 and 10.04 MHz, respectively.

The measurement conditions for $^{13}C$ NMR spectra were: ambient temperature; spectral width 6 kHz; data points 32 K; pulse width 5 μs (30°), and pulse repetition time 3 s for proton noise decoupled or 5 s for gated decoupled (with Nuclear Overhauser Enhancement, NOE) spectra. In addition, a $^{13}C$ NMR spectrum was recorded to suppress NOE and the gated decoupling was repeated at 60 s intervals. The unprotonated Timolol was measured as a 1 M solution in $CDCl_3$ and its protonation ability was studied by adding 1, 2 or 4 equivalents of trichloroacetic acid. Tetramethylsilane, TMS, was used as an internal reference.

The $^{15}N$ NMR chemical shifts were determined using a gated decoupling irradiation mode (without NOE) and the measurement conditions were: ambient temperature; spectral width 6 kHz; data points 16 K; pulse width 10 μs (30°); and pulse repetition time 6 s. Nitromethane in a coaxial inner tube was used for referencing $^{15}N$ chemical shifts. The 1.3 M Timolol in $CDCl_3$ and the external reference were doped with triacetylacetonato chromium(III), $Cr(acac)_3$, to a concentration of 0.05 M. A proton noise decoupled $^{15}N$ NMR spectrum of Timolol was recorded without $Cr(acac)_3$, and the pulse repetition time was 10 s in that experiment. As for the $^{13}C$ NMR part, the protonation was achieved by adding trichloroacetic acid.

*Potentiometric measurements.* A Radiometer PHM 64 digital pH meter, equipped with a glass electrode (Beckman N 40495) and Ag,AgCl reference electrode, was used in the emf titrations. The protonation of Timolol was studied in water–ethanol mixtures because of its limited solubility in pure aqueous solutions. Two differ-

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ent media were used in the measurements: the ethanol content was either 9.3 or 20 wt % and in both cases the solutions were 0.1 M with respect to $NaClO_4$. The electrode system was calibrated with the acetate buffer solutions (0.05 M acetic acid, 0.05 M sodium acetate) as reported by Bates *et al.*[4] Three separate titrations were carried out in both media with varying total Timolol concentrations. The titrant was an aqueous 0.1 M NaOH (Titrisol, Merck) solution which was 0.1 M with respect to $NaClO_4$. A threefold amount of $HClO_4$ with respect to Timolol was added to the initial solution to be titrated. Ethanol (94 wt %) was added to the solution to be titrated in the course of the titration to keep the ethanol content constant.

*Treatment of the potentiometric data.* The mean number of protons bound to each Timolol molecule, $\bar{n}_H$, were calculated from the eqns. (1) and (2).

$$\bar{n}_H(\text{exp})=[C_H-([H^+]-K_{HS}[H^+]^{-1})]/C_L \quad (1)$$

$$\bar{n}_H(\text{calc})=K_1[H^+]/(1+K_1[H^+]) \quad (2)$$

$C_H$ = the total concentration of free and dissociable protons
$[H^+]$= the free hydrogen ion concentration
$K_{HS}$ = the apparent autoprotolysis constant of the solvent
$C_L$ = the total Timolol concentration
$K_1$ = the first protonation constant, $K_1=K(L+H^+=HL^+)$

For the apparent autoprotolysis of the solvent ($K_{HS}=[H^+]\ [OH^-]f_\pm^2$), the values 14.20 and 14.40 were used in the 9.3 and 20 wt % ethanol solutions, respectively.[5] The $f_\pm$ value was calculated from the Debye-Hückel equation:[6]

$$\log f_\pm = \frac{-354.5[d(s)/\varepsilon(s)^3]^{1/2}I^{1/2}}{1+13.31[d(s)/\varepsilon(s)]^{1/2}I^{1/2}} \quad (3)$$

The first protonation constant, $K_1$, was calculated from the eqn. (4),

$$K_1=(C_L+C_{HClO_4}-C_B-[H^+]+K_{HS}[H^+]^{-1})/$$
$$(C_B-C_{HClO_4}+[H^+]-K_{HS}[H^+]^{-1})\,[H^+] \quad (4)$$

$C_{HClO_4}$ = the total perchloric acid concentration
$C_B$ = the NaOH concentration added in the solution to be titrated

## RESULTS AND DISCUSSION

*$^{13}C$ NMR.* The $^{13}C$ chemical shifts for Timolol and protonated Timolol with structurally indicative carbon-proton coupling constants are presented in Table 1. While the coupled spectrum immediately permitted the assignment of C-1, C-2 and C-4 by their multiplicity, the NOE suppressed decoupled $^{13}C$ NMR spectrum was needed to interpret the methylene carbon signals at 44.4, 47.8, 66.3 and 72.7 ppm. This experiment unambiguously showed that the lines with twofold intensities at 47.8 and 66.3 ppm belonged to C-8/C-8′ and C-9/C-9′ respectively. Hence the remaining signals at 44.4 and 72.7 ppm were safely assigned to C-3 and C-5, respectively, by the chemical shift criteria. The two olefinic carbons at 149.5 and 153.4 ppm could be assigned to C-7 and C-6, respectively, by their three-bond couplings. While the resonance signal of C-7 was only a broadened multiplet, that of C-6 was a

*Table 1.* $^{13}C$ chemical shifts and carbon–proton coupling constants for Timolol in $CDCl_3$.

| Carbon | No $Cl_3CCOOH$ $^{13}C$ chemical shift[a] | C–H coupling constants[b] | | | | 1 equivalent of $Cl_3CCOOH$ $^{13}C$ chemical shift[a] | $\Delta$[c] |
|---|---|---|---|---|---|---|---|
| 1 | 28.9 | $^1J_{CH}$ | 125.0 | $^3J_{CH}$ | 4.4 | 25.7 | −3.2 |
| 2 | 50.3 | | | $^2J_{CH}$ | 2.2 | 57.2 | +6.9 |
| 3 | 44.4 | $^1J_{CH}$ | 133.4 | $^2J_{CH}$ | 2.5 | 44.5 | +0.1 |
| 4 | 67.8 | $^1J_{CH}$ | 143.4 | | | 65.6 | −2.2 |
| 5 | 72.7 | $^1J_{CH}$ | 147.6 | $^2J_{CH}$ | 2.6 | 71.6 | −1.1 |
| 6 | 153.4 | | | $^3J_{CH}$ | 2.6 | 152.7 | −0.7 |
| 7 | 149.5 | | | | | 149.4 | −0.1 |
| 8 | 47.8 | $^1J_{CH}$ | 138.3 | | | 47.8 | 0.0 |
| 9 | 66.3 | $^1J_{CH}$ | 143.7 | $^3J_{CH}$ | 3.3 | 66.3 | 0.0 |

[a] In ppm, downfield from TMS. [b] In Hz. [c] Expressed as a chemical shift difference (in ppm) from the unprotonated Timolol.

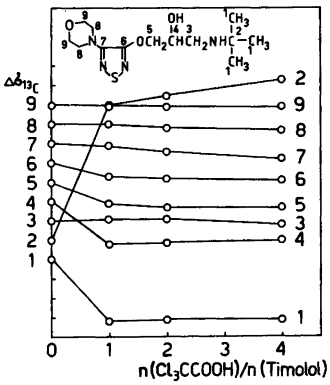


*Fig. 1.* The $\Delta\sigma_{13_C}$ values *versus* the added amount of $Cl_3CCOOH$.

nicely resolved triplet with a coupling constant of 2.6 Hz.

The site of protonation was studied by adding trichloroacetic acid to the $CDCl_3$ solution of Timolol. The addition of one equivalent of acid caused a deshielding effect of 6.9 and 0.1 ppm for C-2 and C-3, respectively. The acid had no observable effect on the chemical shifts of the carbon atoms in the morpholine ring, but it shielded the carbons $\beta$ from the NH-nitrogen and also slightly from C-5, C-6 and C-7 (Table 1).

When more acid was added, the resonance signal of C-2 moved further downfield and the chemical shift was 58.6 ppm with four equivalents of trichloroacetic acid. Interestingly, the acid excess only caused a minor upfield effect of 0.1–0.3 ppm for the other carbon atoms (Fig. 1).

According to these changes in the $^{13}C$ chemical shifts, the protonation takes place merely at the secondary nitrogen atom.

*$^{15}N$ NMR.* Due to a possibility of observing the site of protonation by direct NMR measurement of the nitrogen nucleus,[7] we measured the $^{15}N$ chemical shifts for Timolol and for Timolol with an equimolar amount of trichloroacetic acid in $CDCl_3$ (Table 2).

In the proton noise decoupled $^{15}N$ NMR spectrum of Timolol, only the NH and the morpholine nitrogen resonance signals are clearly visible. The NH nitrogen signal at −321.7 ppm shows the highest intensity due to its dominating $^{15}N-^{1}H$ dipole–dipole relaxation and, consequently, an almost maximum nuclear Overhauser enhancement factor, NOEF, of $-4.5\pm0.5$.[8] The morpholine nitrogen resonates at −307.2 ppm shielded by 40.9 ppm with respect to the unsubstituted morpholine.[9] This kind of a strong shielding effect is typical for numerous cyclic amines conjugated by a double bond.[10]

The addition of some parametric relaxation reagent, for example $Cr(acac)_3$, decreases $^{15}N$ spin-lattice relaxation times and suppresses the NOE. In the gated decoupled (without NOE) $^{15}N$ NMR spectrum of Timolol all the nitrogen resonances are visible. The signals at −102.5 and −98.2 ppm belong to N-5 and N-2, respectively, in the 1,2,5-thiadiazole ring. This assignment, which is based on inductive effects, can be, however, converse, but the unambiguous assignment with the aid of four-band $^{15}N-^{1}H$ coupling constants is undoubtedly impossible without an enriched sample. When 1 equivalent of trichloroacetic acid was added to the $CDCl_3$ solution of Timolol, the NH nitrogen was deshielded by 3.7 ppm. However, downfield shifts of 0.8 and 2.1 ppm for N-2 and N-5, respectively, were also found and only the morpholine nitrogen showed an upfield shift of 0.1 ppm. Although the NH nitrogen was mostly deshielded, thus corroborating the results obtained by $^{13}C$ NMR measurements, we made an additional study by poten-

*Table 2.* $^{15}N$ Chemical shifts for Timolol and protonated Timolol[a] in $CDCl_3$.

| Nitrogen | No $Cl_3CCOOH$ $^{15}N$ chemical shift[b] | 1 equivalent of $Cl_3CCOOH$ $^{15}N$ chemical shift[b] | $\Delta$[c] |
|---|---|---|---|
| −NH− | −321.7 | −318.0 | +3.7 |
| morpholine nitrogen | −307.2 | −307.3 | −0.1 |
| N-2 | − 98.2 | − 97.4 | +0.8 |
| N-5 | −102.5 | −100.4 | +2.1 |

[a] Protonation was achieved by adding 1 equivalent of trichloroacetic acid. [b] In ppm, upfield from external nitromethane. [c] Expressed as a chemical shift difference (in ppm) from the unprotonated Timolol (a positive sign corresponds to a downfield shift).

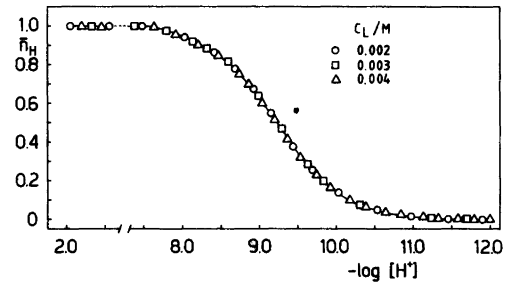


*Fig. 2.* Part of the potentiometric data presented as an $\bar{n}_H(-\log [H^+])$ plot at 25 °C and $I = 0.1$ ($NaClO_4$; 20 wt % $CH_3CH_2OH$).

tiometric methods to verify if there were more possible sites for the protonation.

*Potentiometry.* The experimental data obtained potentiometrically were visualized with the $\bar{n}_H(-\log [H^+])$ plot shown in Fig. 2. It is clear from the figure that in the studied media and concentration range, $\bar{n}_H$ is a function of $-\log [H^+]$, indicating that polymerization is negligible. Further, Fig. 2 suggests that Timolol can add only one proton in the ethanol–water mixtures studied and the pH range over which the measurements were made, because all the experimental $\bar{n}_H$ values lie between 1 and 0.

For the logarithms of the first protonation constant of Timolol, the values 9.29±0.03 and 9.23±0.04 were obtained in solutions containing 9.3 and 20 % ethanol, respectively. The quoted errors are three times the standard deviations. The protonation constant in 20 wt % ethanol is a little lower than that in 9.3 wt % ethanol. The protonated form has a positive charge ($HL^+$) whereas the unprotonated form is uncharged (L). When the ethanol content in the solvent increases, the reciprocal value of the permitivity of the solvent decreases, which favours the formation of neutral species.

## CONCLUSIONS

Potentiometric titration shows that for ethanol–water mixtures, Timolol can add only one proton. The $^{13}C$ NMR measurements corroborate this result and also reveal that protonation takes place at NH nitrogen. If in an NMR study the protonation is achieved by adding trichloroacetic acid, the $^{15}N$ chemical shifts are not so indicative as the $^{13}C$ chemical shifts for Timolol.

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