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Journal of Pharmaceutical and Biomedical Analysis 43 (2007) 1335–1342
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
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NMR analysis of ion pair formation between timolol and sorbic acid in ophthalmic preparations

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Received 29 July 2006; received in revised form 27 October 2006; accepted 29 October 2006
Available online 4 December 2006

Abstract

Ion pair formation between timolol and sorbic acid was investigated using NMR spectroscopy in order to clarify their interactions within ophthalmic preparation. ^{13}C and ^{1}H NMR spectra of timolol, sorbic acid, and a mixture of the two were obtained, and the signal changes induced by pairing were observed. The carbon signals of the butylaminopropanol moiety of timolol were markedly shifted in the mixture, as were the carboxyl and conjugated carbons assigned to sorbic acid. The localizations of the changes in each molecule revealed the binding sites. The profiles of butylaminopropanol carbon chemical shifts plotted against a molar ratio of sorbate were synchronized, which suggested a single type of interaction with sorbic acid. The Job plot showed a typical pattern with a single–maximum at a mole function of 0.5, indicating the presence of a 1:1 complex of timolol and sorbic acid. The stability constants (K) of the timolol–sorbate and timolol–maleate pairs were 1.9×10^{1} and $2.2 \times 10^{2}\ M^{-1}$, respectively. The higher K value of the timolol–maleate interaction suggested that it was dominant to the timolol–sorbate interaction when maleate and sorbate coexisted within a timolol solution. Here, we demonstrated evidence of an interaction between timolol and sorbic acid using simple NMR measurements, which suggested the existence of ion pair formation derived from charge neutralization. Our analysis using NMR spectroscopy should advance the understanding and optimization of formulations that are based on ion pair.

Keywords: Timolol; Sorbic acid; ^{1}H NMR; ^{13}C NMR; Ion pair; Ophthalmic preparation

1. Introduction

Ophthalmic solutions have been widely used for simple medication; however, the low bioavailability of the instilled drugs has remained a hurdle to topical drug application [1]. The low bioavailability is mainly attributed to tear-flow drainage and the corneal barrier. Modifying the lipophilicity of a compound is a well-known method of addressing the issue of corneal permeation. Although the transcorneal route is a major means of drug distribution to the intraocular tissues, the cornea, especially the corneal epithelium is a severe barrier to hydrophilic and moderately lipophilic compounds due to the lipophilic property [2]. Ion pair formation has been used as a method of modifying the lipophilicity of ionizable drugs by shielding their charge with an oppositely charged ion without chemical modifications [3]. Experimental results of improvement of ocular bioavailability have been achieved using the ion pair approach [4–6]. However, it has not completely been contradicted that the counter ion might act singly without forming ion pair, and might modify the corneal integrity similar to enhancers [2].

Measuring the octanol/water partition coefficient is a basic method for evaluating the effects of ion pair formation on lipophilicity. However, this approach is based on the neutralization of the electric charge of each molecule when an ion pair is formed [3], so the binding site and stoichiometry of the complexes are not sufficiently described.

NMR spectroscopy has been used to analyze various complexations in term of changes in the chemical shifts, peak multiplicity, and other parameters associated with spin coupling [7]. Ogiso et al. investigated changes in the carbon chemical shifts of propranolol [8] and ozagrel [9] in the presence of fatty acids, which enhanced the skin penetration of drugs, and confirmed whether ion pairs were formed. The observed changes in the chemical shifts of the alkylaminoalcohol moiety of propranolol

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doi:10.1016/j.jpba.2006.10.043

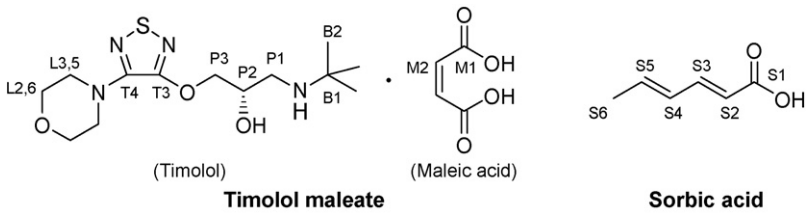

Fig. 1. Structures of timolol maleate and sorbic acid.

indicated an interaction with the fatty acid, whereas no changes were observed in ozagrel. Kikuchi et al. [10] and Wojciechowski and Buffle [11] determined the stoichiometries of host–guest complexes using Job plot [7] of the results obtained by ^{1}H and ^{13}C NMR, and calculated the stability constant. The Job plot for the complexation between resorcinol–dodecanal cyclotetramer and cyclohexanediol showed a typical single–maximum pattern, indicating 1:1 stoichiometry [10]. The Job plot for the complexation between azacrown ether and fatty acid exhibited a double–maximum pattern, suggesting a mixture of 1:1 and 1:2 stoichiometries [11]. Various other studies on native and modified cyclodextrin–inclusion complexes have been carried out. In addition, numerous methods for the analysis of complexes (including NMR spectroscopy, FT-IR spectroscopy, DSC, XRPD, CE, and isothermal calorimetry) have been investigated and used in combination to practically demonstrate the occurrence of complexation [12–16]. NMR spectroscopy in particular has provided unequivocal evidence of the detailed geometry of complexes formed between guests – some of which were close to the rim or outside of the cavity – and cyclodextrin, as well as details of their stoichiometries and affinities [17,18].

Analyses of intermolecular interactions performed using NMR spectroscopy can provide valuable information about ophthalmic preparations based on the ion pair approach. We previously applied the ion pair concept to an ophthalmic solution and demonstrated that sorbic acid improved the ocular bioavailability of timolol [19]. However, as timolol is available in the form of a maleic salt [20,21], sorbic acid is present together with maleic acid as counter ions of timolol in the ophthalmic solution (Fig. 1). In vivo, we found that using greater than equimolar amount of sorbate relative to timolol maleate could effectively improve the ocular bioavailability of timolol. In the present study, we used ^{13}C and ^{1}H NMR spectra to evaluate the ion pair formation between timolol and its counter ions, and to determine the associated binding sites, stoichiometries and stability constants. The effect of the coexistent maleic acid in the ophthalmic solution is also discussed in relation to the octanol/water partition coefficient.

2. Materials and methods

2.1. Materials

Timolol maleate was purchased from LEIRAS (Turku, Finland). Sorbic acid was purchased from Daicel Chemical Industries, Ltd. (Osaka, Japan) or Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Potassium sorbate and maleic acid were purchased from Nacalai Tesque Inc. (Kyoto, Japan). Dimethyl sulfoxide (DMSO)-d_6 with tetramethylsilane (TMS) and chloroform-d ($CDCl_3$) were purchased from Cambridge Isotope Laboratories (Andover, MA, USA) and Wako Pure Chemical Industries, Ltd., respectively. All other chemicals used were reagent grade.

2.2. Preparation of timolol

Timolol maleate (200 mg) was added to 10 mL sodium hydroxide (0.05 mol/L). The solution was extracted with two 10-mL portions of chloroform. The chloroform layer was washed with saturated sodium chloride, dried over magnesium sulfate, and then concentrated in vacuo.

2.3. ^{13}C and ^{1}H NMR spectroscopy

NMR spectra were recorded on a Varian Gemini 2000 spectrometer (^{13}C at 75 MHz, ^{1}H at 300 MHz). TMS was used as an internal standard in $CDCl_3$. The chemical shifts were relative to the DMSO signal at 39.7 ppm for ^{13}C NMR and the TMS signal at 0 ppm for ^{1}H NMR in DMSO-d_6. The C–H correlation spectroscopy (COSY) spectra were recorded, in order to assign the carbon signals.

2.4. Continuous variation method

The continuous variation method (Job plot) was performed based on the differences in the chemical shift of timolol in the presence of sorbic acid, $\Delta\delta_{obs} = \delta - \delta_0$, where δ represents the shift in the presence of sorbic acid, and δ_0 represents the shift attributed to the free timolol in the absence of acid. Timolol solution (0.05 mol/L) and sorbic acid solution (0.05 mol/L) in DMSO-d_6 were prepared and mixed, keeping the total molar concentrations of timolol and sorbic acid at 0.05 mol/L in each. Subsequently, the $\Delta\delta/\Delta\delta_{max}[\text{Timolol}]_0$ was plotted as a function of r (=[Sorbic acid]/[Sorbic acid + Timolol]).

2.5. Titration method

A fixed concentration (0.05 mol/L) of timolol solution in DMSO-d_6 or $CDCl_3$ with varying concentrations of sorbic acid (0.012–0.81 mol/L) or maleic acid (0.008–0.23 mol/L) was prepared for the analysis. Each sorbic acid and maleic acid solution was also prepared at an equimolar concentration of free timolol. The ^{13}C and ^{1}H NMR spectra of these samples were recorded. The assignment of the signals was made based on C–H COSY

of a representative ion pair solution and of each component separately. The H–H COSY spectra of representative ion pair solutions were also recorded, in order to confirm the coupling protons.

2.6. Determination of stability constant

When the stoichiometry of the complex was 1:1, the concentration of pairing of sorbic acid (or maleic acid) (B_s) was equal to the concentration of pairing of timolol (B_t). The concentration of pairing of the compound ($B_s = B_t$) was calculated based on the changes in the chemical shifts of the tertiary butyl carbon induced by pairing with the acid, as follows:

$$B_t = \frac{\Delta\delta}{\Delta\delta_{max}}[\text{Timolol}]_0$$

The free sorbic acid concentration (F_s) was calculated as follows:

$$F_s = (\text{total concentration of sorbic acid}) - B_s.$$

The Scatchard function was plotted as B_s/F_s versus B_s using GraphPad Prism® (GraphPad Software Inc., San Diego, CA, USA). The slope of the line on the Scatchard plot was equal to dissociation constant $(K_d)^{-1}$ or stability constant (K).

2.7. Octanol/water partition coefficient

Potassium sorbate was added to timolol (15.7 mmol/L) in 0.1 mol/L hydrochloric acid solution at final concentrations of 0–157 mmol/L, and the solutions were adjusted to pH 7.0 using sodium hydroxide. The solutions were prepared without buffering agents, because some of the acids used for buffers can influence the octanol/water partition coefficient ($P_{O/W}$). Octanol (3 mL) was added to 3 mL solution, and the mixture was shaken for 2 h at room temperature. The timolol concentrations of the aqueous phase were determined by HPLC. The $P_{O/W}$ was calculated from the concentration in the aqueous phase before and after the addition of octanol. The same procedure was also used to determine the aqueous phase in timolol and maleic acid.

2.8. HPLC analysis of timolol

An HPLC system (CCP&8020 series) comprising an autosampler (AS-8020), pump (DP-8020), column oven (CO-8020), UV detector (UV-8020), data processing system (LC-8020), and octadecylsilica column (TSK-gel ODS-80Ts; 150 mm, 4.6 mm i.d.) was used (all from Tosoh, Tokyo, Japan). The analysis of timolol was carried out with a mixture of methanol and an aqueous solution of 50 mmol/L phosphoric acid containing 20 mmol/L sodium 1-pentansulfonate and 0.2% triethylamine (pH 3.0; 50:50, v/v, %) as the mobile phase, at a flow rate of 0.7 mL/min and a temperature of 40 °C. Detection was performed at 294 nm. Ethyl *p*-hydroxybenzoate was used as an internal standard. The injection volume was 20 μL.

3. Results

3.1. ^{13}C NMR spectra

The ^{13}C NMR spectra of timolol (held at a fixed concentration) were observed in the presence of sorbic acid or maleic acid at varying concentrations. Typical examples are shown in Fig. 2. The chemical shifts of individual carbon atoms in the free form of timolol and in mixtures with sorbic acid are summarized in Table 1. Large shifts were observed for the signals of the carbon atoms (P1, P2, P3, B1, and B2; Fig. 1) adjacent to the amino group in the tertiary butylaminopropanol moiety of timolol. A downfield shift was observed on the tertiary carbon (B1) in the tertiary butyl group; this signal was most obvious change seen among the timolol carbons. Upfield shifts were observed on the carbons adjacent to the amino group (P1 and P3) and hydroxyl group (P2), and on the methyl carbon (B2) in the tertiary butyl group. However, negligible shifts were seen in the carbons assigned to the morpholino (L3,5 and L2,6) and thiadiazolyl (T3 and T4) groups of timolol. The changes in the chemical shifts observed between timolol and maleic acid were similar to those observed between timolol and sorbate; however, the changes in chemical shifts induced by maleic acid were larger than those induced by sorbic acid.

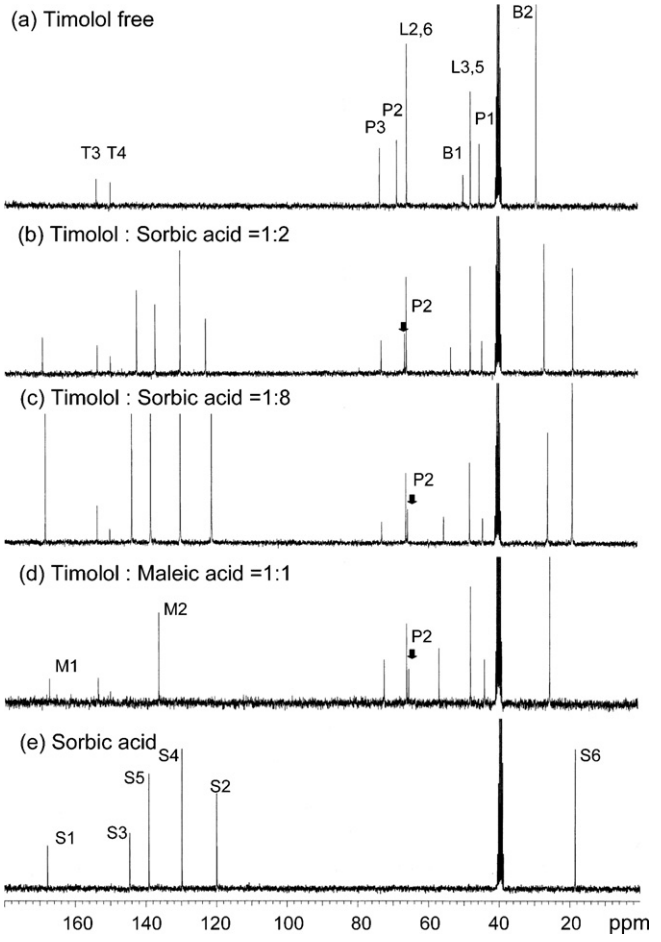

Fig. 2. The ^{13}C NMR spectra of timolol free, mixture with sorbic acid, mixture with maleic acid and sorbic acid (free) measured in DMSO-d_6.

Table 1
Chemical shifts (δ, ppm) of timolol, sorbic acid and maleic acid obtained from ^{13}C NMR spectra in DMSO

Compound	Timolol carbon					Sorbate carbon						Maleate carbon	
	B1	B2	P1	P2	P3	S1	S2	S3	S4	S5	S6	M1	M2
Timolol	49.73	28.96	45.16	68.64	73.52	–	–	–	–	–	–	–	–
Timolol/sorbate (1/1)	52.42	27.18	44.58	66.94	73.15	169.58	123.43	142.22	130.28	136.99	18.47	–	–
Timolol/sorbate (1/8)	55.11	25.52	44.04	65.40	72.77	168.45	121.16	143.88	130.05	138.50	18.53	–	–
Sorbic acid	–	–	–	–	–	167.88	112.00	144.66	129.88	139.24	18.54	–	–
Timolol/maleate (1/1)	56.69	25.12	43.75	65.23	72.26	–	–	–	–	–	–	167.36	136.32
Maleic acid	–	–	–	–	–	–	–	–	–	–	–	166.90	130.35

The ^{13}C NMR spectra of sorbic acid are also shown in Fig. 2. The carbons assigned to the carboxyl group (S1) and adjacent to the carboxyl group (S2) were shifted downfield; similar changes were observed upon addition of maleic acid (M1 and M2; Table 1). The methyl carbon (S6) at the other end of the carboxyl group showed a minimal change of shift. These findings provided evidence of the alteration of electron densities on the carbon atoms. The localization of the changes on each molecule revealed the binding site. Our results suggest that the carbon atoms are involved in the interaction between the amino group of timolol and the carboxyl group of sorbic acid.

The significant changes in the chemical shifts ($\Delta\delta$) of the timolol carbons (P1, P2, P3, B1 and B2) assigned to tertiary butylaminopropanol were plotted against the molar ratio of sorbic acid (Fig. 3(a)). The $\Delta\delta$ of the five carbons changed as the concentration of sorbic acid increased. All of the profiles showed synchronous curves, which reached individual saturation ($\Delta\delta_{max}$) at the same concentration of sorbic acid. This finding suggests that timolol interacts with sorbic acid at only one binding site. The $\Delta\delta$ profiles observed with maleic acid were also plotted (Fig. 3(b)). Again, the curves were synchronous and reached $\Delta\delta_{max}$ at the same concentration of maleic acid. Thus, timolol appears to interact with both maleic acid and sorbic acid at only one binding site. The molar ratio required to achieve $\Delta\delta_{max}$ with sorbic acid was ~8, whereas, $\Delta\delta_{max}$ with maleic acid was reached at a roughly equal molar ratio. This finding suggests that sorbic acid and maleic acid have different affinities for interacting with timolol.

3.2. ^{1}H NMR spectra

The ^{1}H NMR spectra of timolol (held at a fixed concentration) were observed in the presence of sorbic acid at varying concentrations. Representative spectra are shown in Fig. 4. The signals of the protons assigned to the aminopropanol (P1, P2, and P3) and tertiary butyl group (B2) were shifted downfield in the mixture, whereas an upfield shift was observed for the sorbic acid protons. Negligible changes were observed in the chemical shifts of the morpholino group in timolol. Unique ^{1}H NMR spectroscopy results were observed for the spin–spin coupling of methylene protons. The timolol molecule contains four types of methylene group: two in the aminopropanol moiety (P1 and P3) and two in the morpholino group (L3,5 and L2,6). The chemical shifts, spin–spin coupling, and coupling constants of the methylene protons in the mixtures are summarized in Table 2. The chemical shifts of the morpholino methylene protons (L3,5 and L2,6), and their multiplet patterns and coupling constants remained unchanged. By contrast, the P1 and P3 protons, which were attached to the chiral carbon, showed notable changes in coupling as the concentration of sorbic acid in the mixture increased. The geminal protons of P1 in free timolol were observed as a doublet at 2.56 ppm, and looked as if they were equivalent. Two protons on the P1, which were designated as P1a and P1b, were distinguished in the mixture as a double-doublet signal produced from both vicinal and geminal coupling. The coupling constants varied as the concentration of sorbic acid in the mixture was increased: one P1a coupling constant varied from 6.0 to 9.6 Hz while the other varied around 12 Hz; and one

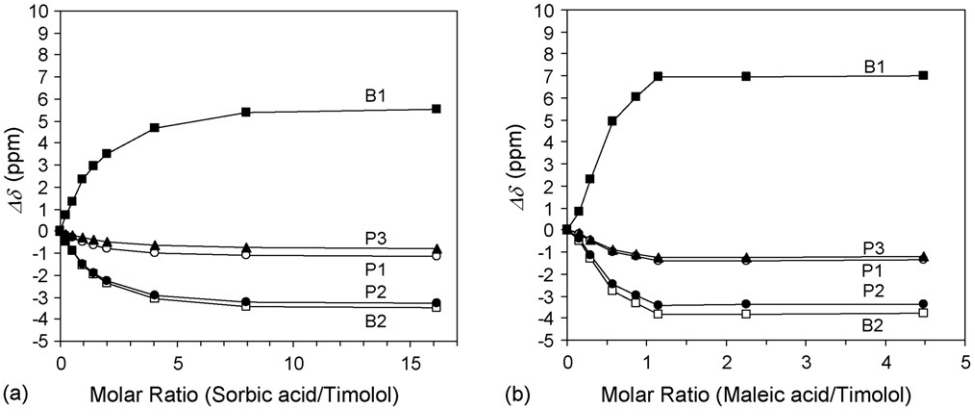

Fig. 3. Changes in chemical shifts of timolol carbons induced by (a) sorbic acid and (b) maleic acid as a function of acid/timolol ratio.

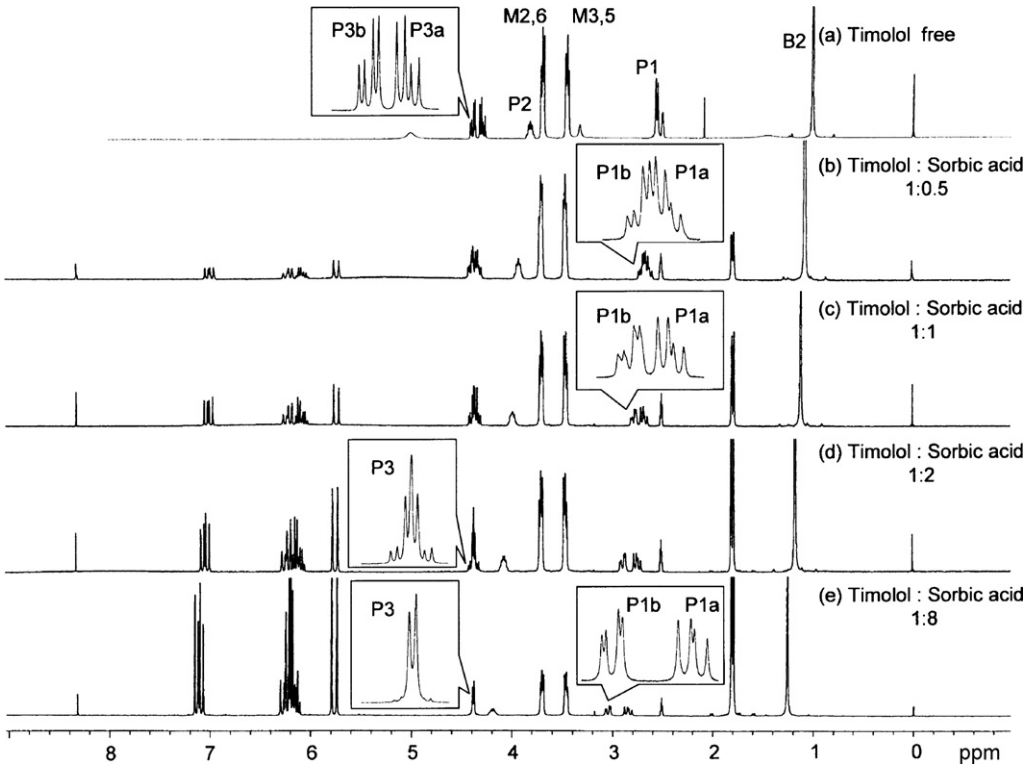

Fig. 4. The ^{1}H NMR spectra of timolol free, and the mixture with sorbic acid measured in DMSO-d_6.

Table 2
^{1}H NMR chemical shifts and coupling constants of spin coupling proton of timolol

Ratio (sorbate/timolol)	Chemical shift, δ [ppm] (multiplet patterns by spin-spin coupling, coupling constant, J [Hz])						
	P1a	P1b	P2	P3a	P3b	L3,5	L2,6
0 (timolol free)	2.56 (d, J = 6.0)	–	3.82 (m)	4.30 (dd, J = 6.2, 10.7)	4.40 (dd, J = 4.2, 10.5)	3.45 (t, J = 4.8)	3.70 (t, J = 4.8)
0.5	2.62 (dd, J = 7.2, 11.4)	2.70 (dd, J = 5.1, 11.7)	3.92 (m)	4.32 (dd, J = 5.9, 10.4)	4.39 (dd, J = 4.4, 10.4)	3.46 (t, J = 4.7)	3.70 (t, J = 4.5)
1	2.68 (dd, J = 7.8, 11.7)	2.78 (dd, J = 4.4, 11.9)	3.98 (m)	4.33 (dd, J = 5.6, 10.7)	4.39 (dd, J = 4.5, 10.5)	3.46 (t, J = 4.8)	3.70 (t, J = 4.8)
2	2.74 (dd, J = 8.4, 12.0)	2.89 (dd, J = 3.8, 11.9)	4.07 (m)	4.34 (dd, J = 5.1, 10.2)	4.39 (dd, J = 4.7, 10.7)	3.46 (t, J = 4.7)	3.70 (t, J = 4.7)
8	2.85 (dd, J = 9.6, 12.3)	3.05 (dd, J = 3.2, 12.2)	4.19 (m)	4.39 (d, J = 5.1)	–	3.46 (t, J = 4.8)	3.70 (t, J = 4.8)

P1b coupling constant varied from 6.0 to 3.2 Hz while the other varied around 12 Hz. The value of 12 Hz was a typical coupling constant observed for geminal coupling [22]. The other coupling constants varied from 6 Hz upwards or downwards resulted from vicinal coupling with the asymmetric proton assigned to the P2 position. The geminal protons assigned to P3 in free timolol were observed as distinct double-doublet signals at 4.30 ppm (P3a) and 4.40 ppm (P3b). These gradually merged as the sorbic acid ratio increased. Eventually, these protons were observed as a doublet without geminal coupling, as a set of equivalent protons.

3.3. Stoichiometry of ion pair formation

The stoichiometry of the ion pair formation between timolol and sorbate was examined using the continuous variation method (Job plot) based on the chemical shifts of tertiary butyl carbon in the ^{13}C NMR spectra; this was chosen because the maximum change in chemical shift was observed in the mixture (Fig. 3). The total initial concentrations of timolol and sorbate were kept constant, and the ratio (r) of the two varied between 0 and 1.

The Job plot showed a typical single–maximum pattern at a mole function of 0.5, which indicated the formation of a 1:1 pairing between timolol and sorbic acid.

3.4. Stability constant

As mentioned above, timolol and sorbic acid paired with a 1:1 stoichiometry. The equilibrium condition and the stability constant (K) of the timolol–sorbate pairing can be described as follows:

$$\text{Timolol} + \text{Sorbic acid} \overset{K}{\rightleftarrows} \text{Timolol–Sorbate} \quad (1)$$

$$K = \frac{[\text{Timolol–Sorbate}]}{[\text{Timolol}]\,[\text{Sorbic acid}]} \quad (2)$$

The K values are summarized in Table 3. The timolol–sorbate interaction had a lower K than the timolol–maleate interaction.

Table 3
Stability constant of ion pairs

Counter ions	Solvent	Stability constant [M^{-1}]
Sorbic acid	DMSO-d_6	1.9×10^1
Maleic acid	DMSO-d_6	2.2×10^2
Sorbic acid	$CDCl_3$	4.1×10^1

This finding suggested that the timolol–maleate ion pair was dominant to the timolol–sorbate ion pair when maleate coexisted with sorbate. The K value for the timolol–sorbate interaction in $CDCl_3$ was also determined. Representative spectra for free timolol, a timolol/sorbate (1:2) mixture, and free sorbic acid in $CDCl_3$ are shown in Fig. 5. The changes in the chemical shifts were similar to those observed in DMSO-d_6, suggesting a single type of interaction with one binding site near the amino atom. The K of the timolol–sorbate interaction in $CDCl_3$ was higher than that in DMSO-d_6. This was due to the higher hydrophobicity of chloroform compared with DMSO. These data indicate the predominant presence in lipophilic environment.

3.5. Octanol/water partition coefficient

The $P_{O/W}$ of timolol in the presence of maleate or sorbate were plotted against the molar ratio of sorbate or maleate (Fig. 6). An increase in the $P_{O/W}$ was observed with increasing concentrations of both types of acid, suggesting that they formed ion pairs by neutralizing their charges. The $P_{O/W}$ of timolol with sorbic acid gradually increased as of sorbate ratio rose, and reached a 90-fold higher value than that of free timolol when the molar ratio was 10. By contrast, the $P_{O/W}$ of timolol with maleate was three-fold higher than that of free timolol, and reached a maximum at an equimolar ratio of maleic acid. These results indicate that ion pair formation with sorbate increases the lipophilicity of timolol more effectively than ion pair formation with maleate.

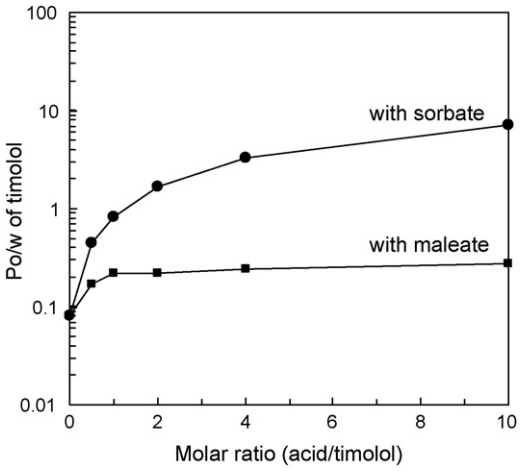

Fig. 6. Partition coefficient ($P_{O/W}$) of timolol.

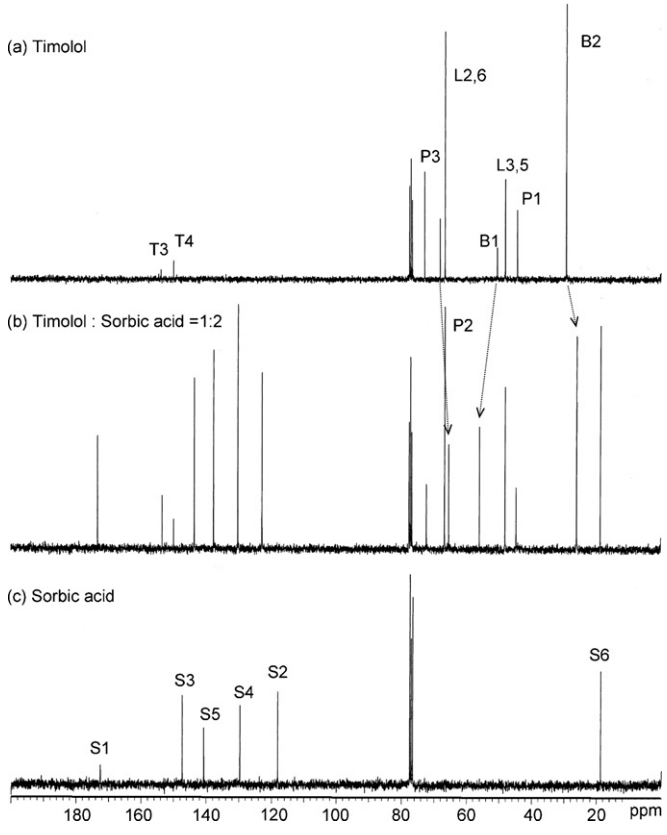

Fig. 5. The ^{13}C NMR spectra of timolol free, the mixture of timolol/sorbic acid (1:2), and sorbic acid measured in $CDCl_3$.

4. Discussion

The present study examined ion pair formation between timolol and sorbic acid using ^{13}C and ^{1}H NMR spectroscopy. Complex formation is generally driven by overall forces such as electrostatic attraction, hydrophobic interaction and hydrogen bonding. Electrostatic force dominates the interactions between polar molecules [7]. Although solvents with low dielectric constant are favored for ion pair formation, the contribution of hydrophobic interaction is relatively large as well as electrostatic force once the ion pair is formed in aqueous solution [3]. DMSO was chosen as the solvent in the current study not only to provide sufficient solubility for timolol, sorbic acid, maleic acid, and their complexes, but also to allow the simple evaluation of ion pair formation.

The chemical shifts observed in our study represent an average of the complex and free compounds, which were not distinguished by ^{13}C and ^{1}H NMR due to the rapid exchange on the NMR time scale. The changes in the chemical shifts of carbon provided powerful evidence of ion pair formation by timolol. Several previous NMR studies on the interactions of compounds involving aromatic rings have reported plane–plane stacking based on π-electron systems [23]. The thiadiazolyl group of timolol, and both sorbic acid and maleic acid contain more than one double bond (Fig. 1). We found that the chemical shifts of sorbic acid were altered in the mixture, whereas those of the thiadiazolyl group remained the same. This finding suggests that the π-electron systems contribute little to the intermolecular interaction of present timolol ion pairs. By contrast, the chemical shifts of the tertiary butylaminopropanol moiety

of timolol varied markedly. Ogiso and Shintani [8] demonstrated an interaction between propranolol, which contains a dimethylaminopropanol moiety, and lauric acid in both $CDCl_3$ and ethanol-D. The chemical shifts of the aminopropanol moiety showed similar changes to those observed in the present study of timolol, indicating that the characteristic change limited at the amionopropanol moiety was induced by carboxylate. Overall, our ^{13}C NMR spectra indicate that the interaction between timolol and sorbic acid can be attributed to ion pair formation based on electrostatic force.

In addition, our ^{1}H NMR spectra showed notable changes. The coupling constants of two pairs of geminal protons in the methylene groups of timolol were altered induced by the addition of sorbic acid. The change appeared as a rotation isomer, that is "*cis*-gauche-*trans* form" represented by relationship between Karplus equation and dihedral angle [22]. This finding suggests that the regulation of rotation occurs around the single bonds between P3 and P2, and between P2 and P1 of the alkanol chain. Kraszni et al. [24] determined the rotamer population of clenbuterol – which contains the same tertiary butylaminoalcohol as the timolol P2-P1-N-B1 (B2) structure – based on the vicinal coupling constants. Their finding also suggested the possibility of intramolecular hydrogen bond formation between the tertiary butylamino and the hydroxyl group. Fang et al. [25] observed that the molecular conformation of mefenamic acid was altered due to ion pair formation with alkanolamines, based on an analysis of torsion angles using X-ray diffraction. Their results demonstrated that interion hydrogen bonds were established through the hydroxyl groups, as well as the interaction between anion and cation in the ion pair complex. Our present analysis indicates that not only electrostatic force between anion and cation, but also hydrogen bonding involving hydroxyl group play a role in interaction between timolol and sorbic acid.

We determined the stability constant of the interactions between timolol and sorbic acid or maleic acid. The K values observed in DMSO were $1.9 \times 10^{1}\ M^{-1}$ with sorbate and $2.2 \times 10^{2}\ M^{-1}$ with maleate. These were relatively low compared with the previous results of Al-Ghannam [26], who reported stability constants in the order of $\sim 10^{3}$ between timolol and acidic dyes (such as bromophenol blue, bromothymol blue, and bromocresol purple). The equilibrium between ions and ion pairs is dependent upon the dielectric constant (ε) of the solvent: a high dielectric constant such as water ($\varepsilon = 78.5$) is unfavorable for ion pair formation based on the Bjerrum theory, whereas the interaction is increasingly important for solvents in which $\varepsilon < 40$ [3]. In the present study, the K value for the interaction between timolol and sorbate was $4.8 \times 10^{1}\ M^{-1}$, even in chloroform ($\varepsilon = 4.80$).

Ion pairs are neutral species that are formed by electrostatic attraction between oppositely charged ions in solution. Competitive exchange between sorbate or maleate and timolol can therefore occur if sorbate is present in timolol maleate ophthalmic solutions. The K values in this study suggest that timolol–maleate ion pair formation is dominant to timolol–sorbate ion pair formation when sorbate and maleate are present at equimolar concentrations in solution. However, an increase of the sorbate ratio in the solution can shift the equilibrium (Eq. (1)) to forming timolol–sorbate ion pair. Sorbate greatly increases the lipophilicity of timolol compared with maleate. Thus, despite its lower affinity, an excess of sorbate can promote ion pair formation with sorbate and incrementally improve the transcorneal absorption of timolol.

Timolol is a beta-adrenergic receptor blocking agent, and its binding site for ion pairing is also a characteristic moiety in molecular structure of this type of drugs [27]. Complete shielding of the timolol binding site by counter ions could block to bind to beta-adrenergic receptor. Thus, the rapid exchange and low affinity characterizing this ion pair might be advantageous to timolol to bind to the target receptor. Indeed, sorbic acid did not influence the in vitro binding of timolol to the ocular beta-adrenergic receptor (unpublished data).

5. Conclusion

Based on simple ^{13}C and ^{1}H NMR observations of binding site, stoichiometries and affinities, we have elucidated the interaction between timolol and sorbic acid, and our findings support the notion of ion pair formation derived from charge neutralization. These results will advance our understanding and optimization of ophthalmic preparations involving ion pairs.

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