International Journal of Pharmaceutics, 46 (1988) 77–88
Elsevier

IJP 01531

Timolol prodrugs: synthesis, stability and lipophilicity of various alkyl, cycloalkyl and aromatic esters of timolol

Hans Bundgaard [1], Anders Buur [1], Shih-Chieh Chang [2] and Vincent H.L. Lee [2]

[1] *The Royal Danish School of Pharmacy, Department of Pharmaceutical Chemistry AD, Copenhagen (Denmark) and* [2] *University of Southern California, School of Pharmacy, Los Angles, CA 90033 (U.S.A.)*

(Received 21 January 1988)
(Accepted 14 February 1988)

Key words: Timolol; Prodrug; Timolol ester; Stability; Partition coefficient; Cyclodextrin

Summary

Some lower aliphatic esters of timolol have previously been developed as prodrugs to potentially diminish the systemic absorption and hence side-effects of topically applied timolol through increased corneal absorption. Although these esters are promising timolol prodrugs for ocular delivery, they suffer from instability in aqueous solution. In this study a large number of other alkyl, cycloalkyl and aromatic esters of timolol were prepared and their hydrolysis studied in aqueous solution and human plasma with the aim of developing chemically more stable yet enzymatically labile timolol prodrug esters. The stability of the esters varied widely and was increased greatly with increasing steric effects within the ester acyl groups. The most stable derivatives were found to be pivaloyl, 2-ethyl-butyryl, 3,3-dimethylbutyryl, cyclopropanoyl and substituted benzoate esters such as 4-methoxybenzoate and 2-amino-benzoate. Maximum stability of all esters occurred at pH 3–4. From a study of the influence of temperature on the stability it was shown that it is feasible to obtain shelf-lives greater than 5 years for solutions of the most stable esters at pH 3–4.5 and 5 °C.

Introduction

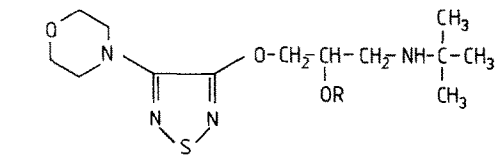
Timolol (**1**) is a non-selective β-adrenergic receptor blocker widely used in the treatment of glaucoma. A major problem in its use in glaucoma therapy is, however, its relatively high incidence of cardiovascular and respiratory side-effects (Nelson et al., 1986; Munroe et al., 1985). These effects arise as a result of absorption of the topically applied drug into the systemic circulation (Alvan et al., 1980; Schmitt et al., 1980; Chang and Lee, 1987).

In previous studies, four short chain esters (acetyl, propionyl, butyryl and pivaloyl) of timolol were developed as prodrugs to potentially diminish the systemic absorption and therefore side-effects of topically administered timolol through increased corneal absorption (Bundgaard et al., 1986; Chang et al., 1987a). Although the esters are promising timolol prodrugs for ocular delivery by virtue of enhanced permeability characteristics and unaltered or slightly reduced systemic absorption in comparison to timolol (Chang et al., 1987; 1988a and b), they suffer from instability in aqueous solution (Bundgaard et al., 1986).

The purpose of this study was to identify timolol ester prodrugs with improved chemical stability in aqueous solution, yet at the same time showing a sufficient enzymatic reactivity in order to ensure conversion to timolol in the eye. To this end a number of alkyl, cycloalkyl and aromatic esters of timolol (compounds **2–27**, see Table 1) were prepared and evaluated with respect to sta-

Correspondence: H. Bundgaard, The Royal Danish School of Pharmacy, Department of Pharmaceutical Chemistry AD, 2 Universitetsparken, DK-2100 Copenhagen, Denmark.

bility in aqueous solution as a function of pH and temperature. The hydrolysis kinetics of the esters in human plasma solutions as well as their lipophilicity were also determined. Along with studies on the corneal transport and systemic absorption characteristics of these esters which will be reported in a future communication, the information obtained may help to identify, in a rational manner, the optimal timolol prodrug ester in regard to both chemical stability and ocular delivery characteristics.



1 R = H

2 - 27 R = $-\overset{\overset{O}{\|}}{C}-R$

Materials and Methods

Apparatus

^{1}H-NMR spectra were run on a Varian 360 L Instrument using tetramethylsilane as internal reference. The pH measurements were made at the temperature of study using a Radiometer Type PHM 26 instrument. Melting points were taken on a capillary melting-point apparatus and are uncorrected. High-performance liquid chromatography (HPLC) was performed with a system consisting of a Waters pump model 6000 A, a variable wavelength UV-detector (Waters Type Lambda Max 480) and a 20-μl loop injection valve. The column used, 100 × 3.0 mm, was packed with CP SPHER C-8 (5-μm particles) (Chrompack). Microanalyses were performed at the Microanalytical Laboratory, Leo Pharmaceutical Products, Ballerup, Denmark.

Chemicals

Timolol maleate was kindly provided by Leo Pharmaceutical Products, Denmark or purchased from Sigma Chemical Co., U.S.A. All buffer substances and solvents used were of reagent grade. The acid chlorides used were either obtained commercially (Fluka AG, Switzerland or E. Merck, Darmstadt) or prepared from the corresponding acids by reaction with thionyl chloride.

Synthesis of timolol esters

The preparation of the timolol esters **2**, **3**, **4** and **7** (hydrochloride salts) was previously described (Bundgaard et al., 1986). The *O*-isobutyryl ester (**5**) was prepared and isolated as a hydrochloride salt in a similar manner. All other esters except compounds **25** and **26** were prepared by reacting timolol maleate with the appropriate acid chloride in acetonitrile and isolated as crystalline fumarate salts. Timolol maleate (1 mmol, 0.43 g) was slurried in 7 ml of acetonitrile. The appropriate acid chloride (10 mmol) was added and the mixture stirred at 80 °C for 4 h (aliphatic esters) or 20 h (aromatic esters). After these reaction times all timolol had reacted as evidenced by HPLC analysis of the reaction solution. The solution was evaporated under reduced pressure and the residue obtained washed with 20 ml of petroleum ether and taken up in water (40 ml) and ethyl acetate (50 ml). Sodium hydroxide (2 M) was added under stirring to give a pH of 9–9.5. The ethyl acetate layer was separated, washed with water (2 × 20 ml), dried over anhydrous sodium sulphate and evaporated in vacuo to give the timolol ester free base as an oil. This was dissolved in ether (40 ml) or, in some cases, in a mixture of ethyl acetate and ether and a solution of fumaric acid (1.1 mmol, 128 mg) in 2-propanol (2 ml) was added. After standing for about 3 h at 5 °C the crystalline fumarate salt of the timolol ester was isolated by filtration, washed with ether and recrystallized from ethanol–ether, acetone or acetonitrile–ether. Yields as well as physical and analytical data for the compounds are given in Table 1. For comparison some data for the previously described esters **2**, **3**, **4** and **7** are included. The NMR spectra of the new esters were consistent with their structures. The purity of the esters was greater than 98% as shown by HPLC analysis.

O-(2-Aminobenzoyl) timolol fumarate (**25**) was prepared by reacting timolol maleate with isatoic anhydride according to a modification of the

TABLE 1

Physical and analytical data of various esters (hydrochloride or fumarate salts [a]) of timolol

Ester	R	Yield (%)	Melting point (°C)	Formula [c]
2 *O*-Acetyl [b]	$-COCH_3$	92	203–204	$C_{15}H_{27}ClN_4O_4S$
3 *O*-Propionyl [b]	$-COCH_2CH_3$	90	187–188	$C_{16}H_{29}ClN_4O_4S$
4 *O*-Butyryl [b]	$-CO(CH_2)_2CH_3$	88	158–159	$C_{17}H_{31}ClN_4O_4S$
5 *O*-Isobutyryl	$-COCH(CH_3)_2$	86	174–175	$C_{17}H_{31}ClN_4O_4S$
6 *O*-Valeryl	$-CO(CH_2)_3CH_3$	77	157–158	$C_{22}H_{36}N_4O_8S$
7 *O*-Pivaloyl [b]	$-COC(CH_3)_3$	65	146–147	$C_{18}H_{33}ClN_4O_4S$
8 *O*-2-Ethylbutyryl	$-COCH(C_2H_5)_2$	75	137–138	$C_{23}H_{38}N_4O_8S$
9 *O*-3,3-Dimethylbutyryl	$-COCH_2C(CH_3)_3$	81	173–174	$C_{23}H_{38}N_4O_8S$
10 *O*-Hexanoyl	$-CO(CH_2)_4CH_3$	80	176–177	$C_{23}H_{38}N_4O_8S$
11 *O*-Octanoyl	$-CO(CH_2)_6CH_3$	82	169–170	$C_{25}H_{42}N_4O_8S$
12 *O*-Cyclopropanoyl	$-COcC_3H_5$	79	172–173	$C_{21}H_{32}N_4O_8S$
13 *O*-1′-Methylcyclopropanoyl	$-COcC_3H_4$-1′-CH_3	74	174–175	$C_{22}H_{34}N_4O_8S$
14 *O*-2′-Methylcyclopropanoyl	$-COcC_3H_4$-2′-CH_3	77	161–162	$C_{22}H_{34}N_4O_8S$
15 *O*-Cyclobutanoyl	$-COcC_4H_7$	69	158–160	$C_{22}H_{34}N_4O_8S$
16 *O*-Cyclopentanoyl	$-COcC_5H_9$	78	172–173	$C_{23}H_{36}N_4O_8S$
17 *O*-Cyclohexanoyl	$-COcC_6H_{11}$	79	180–181	$C_{24}H_{38}N_4O_8S$
18 *O*-Benzoyl	$-COC_6H_{11}$	80	180–181	$C_{24}H_{32}N_4O_8S$
19 *O*-2-Methylbenzoyl	$-COC_6H_4$-*o*-CH_3	80	162–164	$C_{25}H_{34}N_4O_8S$
20 *O*-4-Methylbenzoyl	$-COC_6H_4$-*p*-CH_3	76	200–202	$C_{25}H_{34}N_4O_8S$
21 *O*-2-Methoxybenzoyl	$-COC_6H_4$-*o*-OCH_3	75	126–127	$C_{25}H_{34}N_4O_9S$
22 *O*-4-Methoxybenzoyl	$-COC_6H_4$-*p*-OCH_3	82	201–203	$C_{25}H_{34}N_4O_9S$
23 *O*-2-Acetoxybenzoyl	$-COC_6H_4$-*o*-$OCOCH_3$	60	135–136	$C_{26}H_{34}N_4O_{10}S$
24 *O*-2-Benzoyloxymethylbenzoyl	$-COC_6H_4$-*o*-$CH_2OCOC_6H_5$	55	150–152	$C_{31}H_{36}N_4O_{10}S$ [d]
25 *O*-2-Aminobenzoyl	$-COC_6H_4$-*o*-NH_2	67	169–170	$C_{24}H_{33}N_5O_8S$
26 *O*-2-Methylaminobenzoyl	$-COC_6H_4$-*o*-$NHCH_3$	70	165–167	$C_{25}H_{35}N_5O_8S$
27 *O*-3-Thienyl	$-COC_4H_3S$	79	149–150	$C_{22}H_{30}N_4O_8S$

[a] The esters **2**, **3**, **4**, **5** and **7** are hydrochloride salts whereas all other esters are salts with 1 equiv. fumaric acid.

[b] Data from a previous study (Bundgaard et al., 1986).

[c] Elemental analysis (C, H, N and S) was within ±0.4% of the theoretical values except when specifically noted.

[d] Calc.: C, 56.70; H, 5.53; N, 8.53; S, 4.88. Found: C, 55.89; H, 5.60; N, 8.37; S, 4.60.

method for preparing 2-aminobenzoate esters described by Venuti (1982). A mixture of timolol maleate (0.7 mmol, 302 mg), isatoic anhydride (0.9 mmol, 147 mg) and *N*,*N*-dimethylaminopyridine (0.07 mmol, 8.5 mg) in 2 ml of *N*,*N*-dimethylformamide was stirred at 60 °C for 5 h. After cooling, the solution was poured into a mixture of water (30 ml) and ethyl acetate (40 ml). Sodium hydroxide (2 M) was added under stirring to give a pH of 9–9.5 and the compound isolated as a fumarate salt following the procedure described above. It was recrystallized from acetone.

Ester 26 was prepared in a similar way using *N*-methyl-isatoic anhydride instead of isatoic anhydride.

Kinetic measurements

The hydrolysis of the timolol ester was studied in aqueous solutions at various temperatures. Hydrochloric acid, acetate, phosphate, borate, carbonate and sodium hydroxide solutions were used as buffers. The total buffer concentration was generally 0.02 M and a constant ionic strength (μ) of 0.5 was maintained for each buffer by adding a calculated amount of potassium chloride.

The rates of hydrolysis were determined by means of a reversed-phase HPLC procedure capable of separating the esters from timolol. For all compounds except for the lipophilic *O*-octanoyl timolol ester (**11**), a mobile phase system consisting of acetonitrile–methanol–0.02 M acetate

buffer of pH 4.5 (10 : 45 : 45 v/v) was used. The flow rate was 1.0 ml/min and the column effluent was monitored at 294 nm. The retention times for the compounds were in the range of 1.5 min (for timolol) to 6.8 min (for *O*-hexanoyl timolol). For the determination of the octanoyl ester (**11**) a mobile phase system consisting of acetonitrile–methanol–0.02 M acetate buffer of pH 4.5 (15 : 60 : 25 v/v) was used, the retention time for the ester being 2.0 min. Quantitation of the esters as well as of timolol formed upon hydrolysis was done by measuring the peak heights in relation to those of standards chromatographed under the same conditions.

The reactions were initiated by adding 50 μl of a stock solution of the compounds in water or an acetonitrile–water mixture to 10.0 ml of buffer solution, pre-equilibrated at the temperature of study (i.e. 5, 37, 50, 60 or 70 °C), in screw-capped test tubes. The final ester concentration in the reaction mixture was in the range of 0.08–0.1 mM. The solutions were kept in a water-bath of constant temperature (±0.5 °C) and at appropriate times samples were taken and immediately chromatographed. Pseudo-first-order rate constants for the hydrolysis were determined from the slopes of linear plots of the logarithm of residual timolol ester against time.

Hydrolysis in human plasma solutions

The hydrolysis of the timolol esters was studied in 0.01 M phosphate buffer (pH 7.4) containing 80% human plasma at 37 °C, the initial ester concentration being 0.2–0.3 mM. At appropriate times, samples of 250 μl were withdrawn and deproteinized by mixing with 1000 μl of ethanol. After centrifugation for 2 min, 20 μl of the clear supernatant was analyzed by HPLC as described above.

Determination of partition coefficients

The apparent partition (distribution) coefficients (P) of the timolol esters were determined in the system octanol–0.05 M phosphate buffer (pH 7.40) at 22 °C as described previously (Bundgaard et al., 1986).

Results and Discussion

Kinetics and mechanism of hydrolysis of timolol esters

In agreement with the previous findings on the hydrolysis of the lower alkyl esters **2**, **3**, **4** and **7** (Bundgaard et al., 1986), all the new esters of timolol were hydrolyzed quantitatively (100 ± 3%) to timolol. As revealed by HPLC analysis, the disappearance of the esters in aqueous solutions at pH 1–13 was accompanied by the progressive appearance of free timolol. The quantitative conversion of the esters to timolol was also observed in 80% human plasma solutions. Apparently, the bulky tertiary butylamino group in the timolol molecule effectively prevents intramolecular O → N acyl transfer reaction as has been found to occur in esters of propranolol, which contains a less sterically hindered isopropylamino group (Buur et al., 1988).

At constant pH and temperature strict first-order kinetics was observed for the hydrolysis of all timolol esters for several half-lives. The influence of pH on the rates of hydrolysis of some esters at 37 °C is shown in Figs. 1 and 2 in which the logarithm of the observed pseudo-first-order rate constant (k_{obs}) has been plotted against pH. At the buffer concentration used (0.02 M) no significant general acid–base catalysis was observed. As previously discussed (Bundgaard et al., 1986) the shape of the pH–rate profiles can be accounted for by the following rate expression:

$$k_{obs} = k_H a_H \frac{a_H}{a_H + K_a} + k_0 \frac{a_H}{a_H + K_a} + k_{OH} a_{OH} \frac{a_H}{a_H + K_a} + k'_{OH} a_{OH} \frac{K_a}{a_H + K_a} \quad (1)$$

where a_H and a_{OH} refer to the hydrogen ion and hydroxide ion activities, respectively, $a_H/(a_H + K_a)$ and $K_a/(a_H + K_a)$ are the fractions of total ester in the protonated and free base forms, respectively, and K_a is the apparent ionization constant of the protonated NH-group in the esters. The rate constant k_0 refers to the spontaneous or

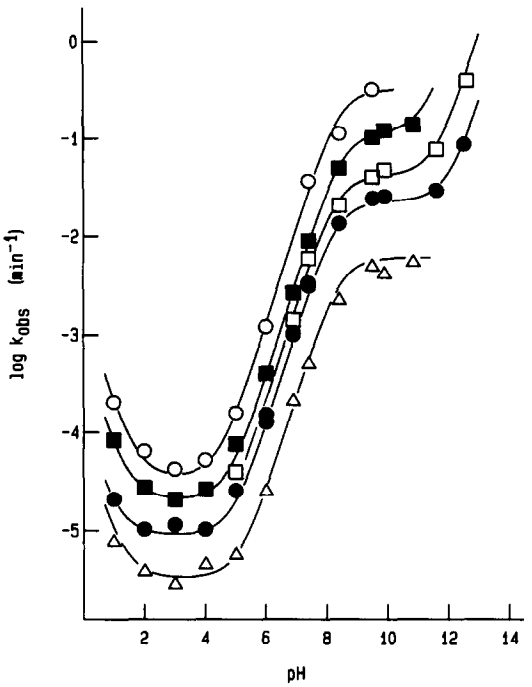


Fig. 1. The pH–rate profiles for the degradation of various timolol esters in aqueous solution at 37 °C. Key: ○, *O*-acetyltimolol (**2**); ■, *O*-hexanoyltimolol (**10**); ●, *O*-pivaloyltimolol (**7**); □, *O*-benzoyltimolol (**18**); △, *O*-2-ethylbutyryltimolol (**8**).

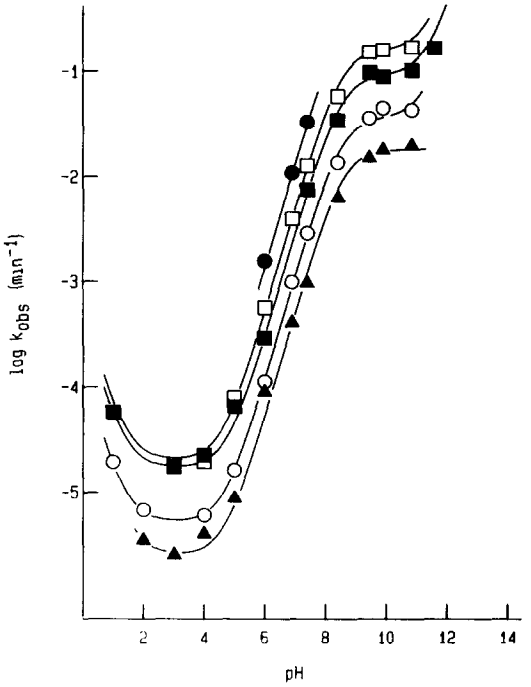


Fig. 2. The pH–rate profiles for the degradation of various timolol esters in aqueous solution at 37 °C. Key: ○, *O*-cyclopropanoyltimolol (**12**); ●, *O*-cyclobutanoyltimolol (**15**); □, *O*-cyclopentanoyltimolol (**16**); ■, *O*-cyclohexanoyltimolol (**17**); ▲, *O*-2′-methylcyclopropanoyltimolol (**14**).

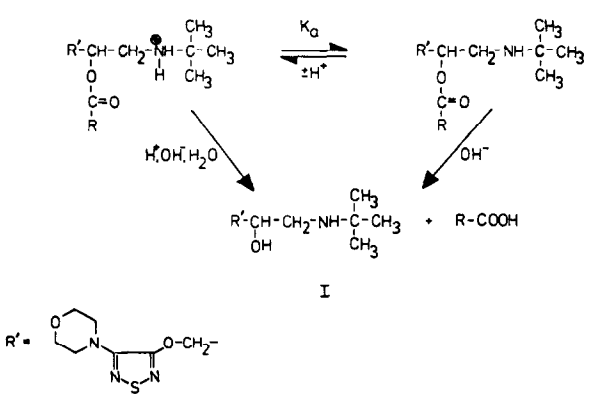
water-catalyzed hydrolysis of the protonated form of the ester, k_H is the specific acid-catalyzed rate constant for protonated ester, and k_{OH} and k'_{OH} are the second-order rate constants for the apparent hydroxide ion-catalyzed hydrolysis of the protonated and unprotonated species, respectively (Scheme 1).

The various rate constants derived from the pH–rate profiles are listed in Table 2. The kinetically derived pK_a values were in all cases 8.4 ± 0.1 which is in agreement with the pK_a value (8.4) determined by titrimetry at 37 °C ($\mu = 0.5$) for the ester **7** (Bundgaard et al., 1986). Due to the electron-withdrawing effect of the ester moiety, the timolol esters are less basic than the parent timolol, its pK_a value being 9.21 at 35 °C (Schoenwald and Huang, 1983).

As can be seen from the values of k_{OH} and k'_{OH} and from the shape of the pH–rate profiles, the ester with a protonated amino group is much more reactive than the free base form. This can most likely be ascribed to intramolecular general acid-catalysis by the protonated amino group of hydroxide ion attack on the ester moiety as discussed earlier (Bundgaard et al., 1986).

Structure–reactivity relationships

The stability of esters is a function of both steric and polar substituent effects. For the



Scheme 1.

TABLE 2

Rate data for the hydrolysis of various timolol esters in aqueous solution ($\mu = 0.5$) at 37 °C

Compound	k_H ($M^{-1}.min^{-1}$)	k_0 (min^{-1})	k_{OH} ($M^{-1}.min^{-1}$)	k'_{OH} ($M^{-1}.min^{-1}$)
2	1.8×10^{-3}	3.5×10^{-5}	5.2×10^{4}	
3			3.6×10^{4}	
4			3.0×10^{4}	
5			2.1×10^{4}	17
6			2.2×10^{4}	
7	1.2×10^{-4}	9.0×10^{-6}	4.7×10^{3}	0.9
8	7.6×10^{-5}	3.3×10^{-6}	1.1×10^{3}	
9			1.1×10^{3}	
10	6.1×10^{-4}	2.1×10^{-5}	1.5×10^{4}	27
11			1.7×10^{4}	
12	1.3×10^{-4}	5.3×10^{-6}	4.9×10^{3}	7.6
13			2.0×10^{3}	
14			1.9×10^{3}	
15			5.5×10^{4}	
16	5.5×10^{-4}	2.0×10^{-5}	2.1×10^{4}	26
17	4.1×10^{-4}	1.7×10^{-5}	1.1×10^{4}	15
18			8.6×10^{3}	4.6
19			2.6×10^{3}	
20			3.9×10^{3}	
21			4.2×10^{3}	
22			1.9×10^{3}	
23			3.7×10^{4}	
24			4.2×10^{3}	
25			5.6×10^{2}	
26			4.9×10^{2}	
27			5.6×10^{3}	

aliphatic esters the acyl groups possess almost the same polar effects as expressed in terms of σ^* values (Perrin et al., 1981). The steric properties, on the other hand, of the various acyl groups in these esters are quite different. As shown in Fig. 3, the observed differences in the stability of these esters in weakly acidic and neutral aqueous solutions (where k_{OH} is the main reaction taking place) can be ascribed to differences in the steric properties of the acyl groups, expressed in terms of the steric substituent parameter ν (Charton, 1977). The regression equation between log k_{OH} (at 37 °C) and ν for the esters is given by:

$$\log k_{OH} = -1.70(\pm0.11)\nu + 5.54(\pm0.10) \quad (2)$$
$$(n = 14;\ r = 0.977)$$

Regression analysis of the rate data showed that the inclusion of a σ^* term in Eqn. 2 did not improve the correlation.

At pH 3–4 the water-catalyzed hydrolysis of the esters is the predominant degradation reaction. Also, in this case the steric substituent effect is the most important factor determining the relative stability as seen from the plot in Fig. 4. The *O*-cyclopropanoyl ester (**12**) showed a significant negative deviation from the correlation line made for the other 6 esters for which k_o was determined. The regression equation between log k_o (at 37 °C) and ν for these esters is given by:

$$\log k_0 = -0.94(\pm0.09)\nu - 3.99(\pm0.09) \quad (3)$$
$$(n = 6;\ r = 0.982)$$

By comparing the coefficients to ν in Eqns. 2 and 3 it can be seen that the hydroxide ion-catalyzed hydrolysis is markedly more sensitive to steric effects than the neutral hydrolysis.

The most stable aliphatic ester is the *O*-2-ethylbutyryl derivative (**8**) for which the k_0 value is

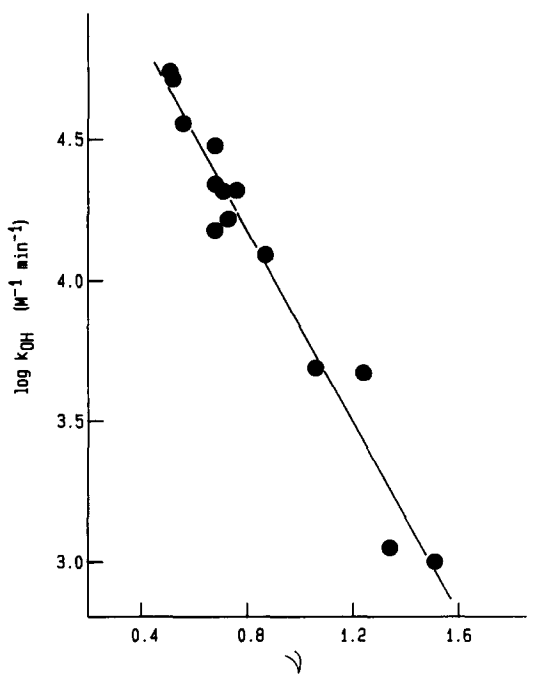


Fig. 3. Plot of log k_{OH} vs the steric parameter (ν) for various timolol esters. The ν values refer to the alkyl or cycloalkyl moiety in the acyl groups.

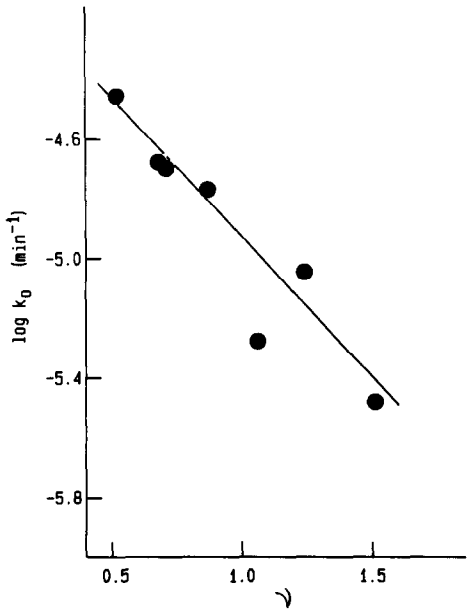
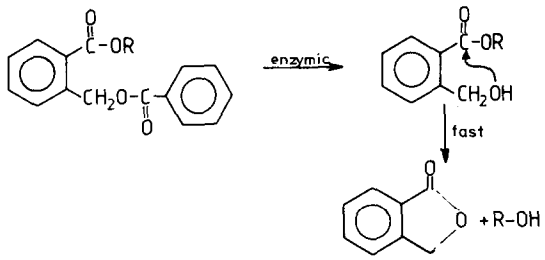


Fig. 4. Plot of log k_0 vs the steric parameter (ν) for various timolol esters. Compound **12** was excluded from the correlation plot.

10-fold lower than that of the *O*-acetyl ester. Furthermore, the 2-ethyl substituent in ester **8** is seen to bring about a 30-fold increase in stability as assessed by comparing the k_{OH} values for ester **8** and the linear *O*-butyryl ester **4**.

In the case of the aromatic esters, polar as well as steric effects are important. For the benzoate esters **18**, **20** and **22** the stability increases with decreased polar effects of the substituents as expressed by the Hammett σ-values (0.0 for H, -0.06 for 4-CH_3 and -0.28 for 4-OCH_3). The most stable ester in terms of k_{OH} values is the 2-aminobenzoate ester (**25**) and its *N*-methyl analog (**26**), which is in accordance with the electropositive character of the unprotonated amino group.

The *O*-2-benzoyloxymethylbenzoate ester (**24**) of timolol was prepared with the hope of obtaining a chemically stable, yet enzymatically very labile prodrug. Due to the presence of the rather bulky benzoyloxymethyl group in the ortho position it was thought that the ester would be considerably more stable than the unsubstituted benzoate ester (**18**). By the action of esterases in vivo the terminal ester group in compound **24** should be rather easily hydrolyzed to yield a 2-hydroxymethylbenzoate ester (Nielsen and Bundgaard, 1986). Such a compound is known (Fife and Benjamin, 1973, 1975) to undergo a rapid non-enzymatic intramolecular cyclization in aqueous solution at pH 7.4 with the formation of phthalide and concurrent release of the parent alcohol, i.e. timolol (Scheme 2). The stability experiments showed, however, that the compound (**24**) only possessed a 2-fold higher stability than the unsubstituted benzoate ester, i.e. a stability which is the same as that of the 2-methoxybenzoate ester (**21**) as seen from the k_{OH} values in Table 2.



ROH: Timolol

Scheme 2.

Effect of Tris and β-cyclodextrin on the stability

In a previous study (Bundgaard et al., 1986) Tris (tromethamine) was found to depress the hydrolysis of the esters **2**, **3**, **4** and **7** at pH 6–7.5 by a factor of 1.5–2 in a concentration of 0.005 M. This decelerating rate effect of Tris was shown to be due to complex formation. Since β-cyclodextrin is known to form inclusion complexes with various compounds in aqueous solution and such complexes of esters may protect against ester hydrolysis (Saenger, 1980; Uekama, 1981; Uekama and Otagiri, 1987) the influence of β-cyclodextrin on the stability of various timolol esters was examined along with studies on the effect of Tris. The results obtained at pH 4.0 and 60 °C are given in Table 3. As can be seen only a slight improvement in the stability of the esters studied was obtained in the presence of either 10^{-3} M Tris or 0.5% β-cyclodextrin.

Hydrolysis in plasma

As has been found before for the lower alkyl esters of timolol (Bundgaard et al., 1986) the rate

TABLE 3

Effect of 10^{-3} M Tris and 0.5% β-cyclodextrin on the rate of hydrolysis of various timolol esters in 0.02 M acetate buffer solutions (pH 4.0) at 60°C

Compound	Half-life (h)		
	Buffer	(+)-Tris	(+)-β-Cyclodextrin
4	92	103	97
5	88	101	108
6	100	114	132
7	274	307	354
10	92	127	120
18	180	206	256

of ester hydrolysis was reduced in 80% human plasma as compared to the rate of hydrolysis in the phosphate buffer without plasma (Table 4). This rate-retarding effect may be due to binding

TABLE 4

Rates of hydrolysis of various timolol esters in 0.02 M phosphate buffer (pH 7.4) and 80% human plasma (pH 7.4) at 37°C

Compound	Half-life (h)	
	Buffer	Plasma
2	0.47	0.58
3	0.67	0.75
4	0.83	1.80
5	0.94	1.30
6	1.0	2.40
7	3.6	8.8
8	23	–
9	23	105
10	1.3	3.1
11	1.2	7.4
12	4.1	4.9
13	9.9	11.5
14	12.1	13.2
15	0.35	0.65
16	0.92	2.2
17	1.6	4.5
18	2.0	4.4
19	10	52
20	4.9	20
21	4.1	7.3
22	4.9	20
23	0.51	–
24	4.5	–
25	36.6	70
26	41.8	–
27	3.8	7.2

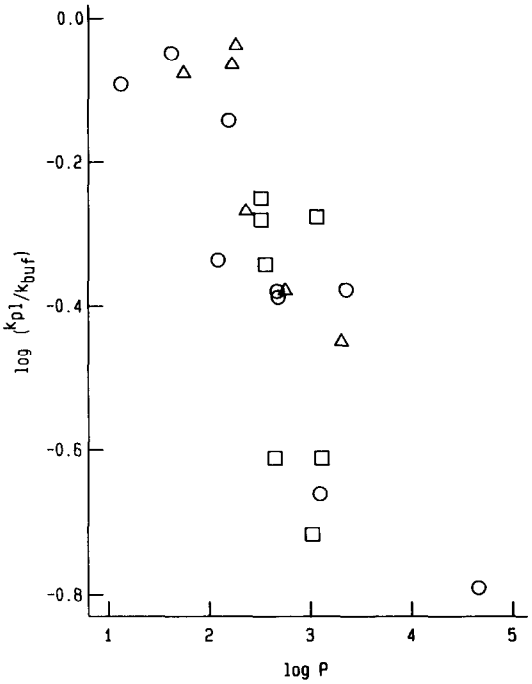


Fig. 5. Plot of the logarithm of the ratio of the observed pseudo-first-order rate constants for the hydrolysis of timolol esters in 80% human plasma and 0.02 M phosphate buffer (pH 7.4) (data from Table 4) vs log P where P is the partition coefficient for the esters between octanol and pH 7.4 phosphate buffer. Key: ○, aliphatic esters; △, cycloalkyl esters; □, aromatic esters.

of the esters to plasma proteins. In the presence of more dilute plasma, however, the rate of ester hydrolysis is accelerated as it is in the presence of homogenates of various rabbit eye tissues (Bundgaard et al., 1986; Chang et al., 1987). Steric effects generally alter non-enzymatic and enzymatic ester hydrolysis rates in the same direction (Charton, 1977). In accordance with this the sterically hindered esters **7** and **9** are only slowly hydrolyzed in 80% plasma solutions. Interestingly, however, is the behaviour of the *O*-cyclopropanoyl esters **12–14** since the hydrolysis of these sterically hindered esters is only very slightly retarded in the presence of 80% plasma.

A plot of the ratio of the rate constants for the hydrolysis in plasma and buffer against the octanol–pH 7.4 buffer partition coefficients for the esters is shown in Fig. 5. It appears from the plot that the rate-retarding effect of plasma increases

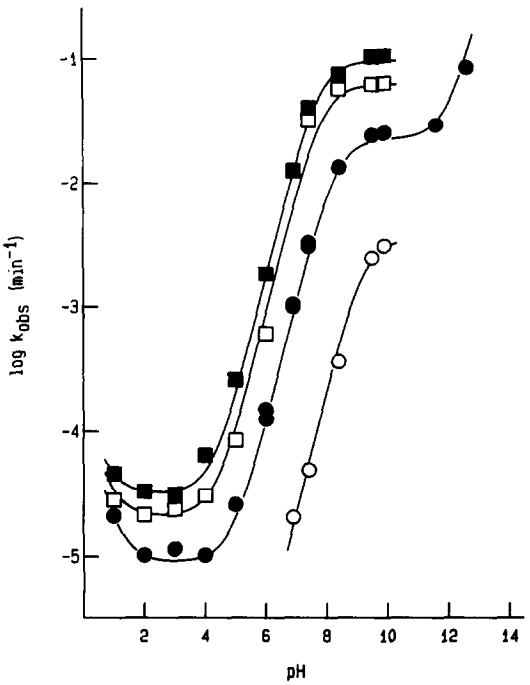


Fig. 6. The pH–rate profiles for the hydrolysis of *O*-pivaloyltimolol (**7**) at various temperatures. ○, 5 °C; ●, 37 °C; □, 50 °C; ■, 60 °C.

with increasing lipophilicity in the case of the aliphatic esters whereas for the aromatic and cycloalkyl esters the effect of plasma is not related to the lipophilicity.

Prediction of shelf-lives

Due to their weak basic character the timolol esters are readily soluble in aqueous solutions of pH 3–6 and may, like timolol, form salts with various acids, e.g. fumaric and hydrochloric acid. As seen from the pH–rate profiles, the stability of the esters is maximal at pH about 3. As shown with the *O*-pivaloyl ester (**7**) this broad pH-maximum does not change appreciably with the temperature (Fig. 6) which is in accordance with the predominance of the water-catalyzed hydrolysis at pH about 3.

The influence of temperature on the rate of hydrolysis of a number of the esters prepared was studied at pH 5.0 (0.02 M acetate buffer, $\mu = 0.5$) over the temperature range 37–70 °C. In some cases a temperature of 5 °C was also used. At this pH the predominant degradation reaction is the apparent hydroxide ion-catalyzed hydrolysis of the protonated form of the timolol esters (the k_{OH}-reaction in Scheme 1). In Fig. 7 the rate data obtained for the esters **7**, **10**, **12** and **18** are plotted according to the Arrhenius equation:

$$\log k_{obs} = \log A - \frac{E_a}{2.303R} \cdot \frac{1}{T} \qquad (3)$$

where A is the frequency factor, E_a is the apparent energy of activation, R is the gas constant and T is the absolute temperature in °K. From such plots the Arrhenius parameters A and E_a were obtained and are listed in Table 5. On the basis of these values it is possible to estimate the shelf-life of aqueous solutions of the esters at various temperatures. The calculated shelf-lives in terms of $t_{10\%}$, i.e. times for an extent of degradation of 10%, are listed in Table 6. From a comparison of the rate constants observed at pH 4 and 5 at 37 °C shelf-life predictions were also made at

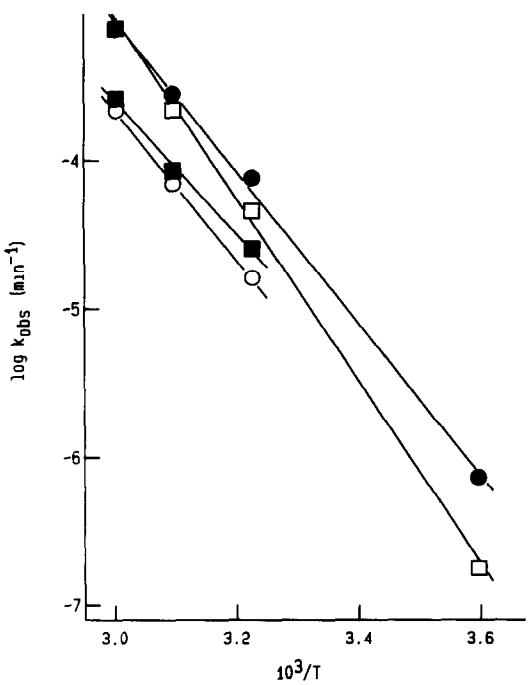


Fig. 7. Arrhenius plots of the rates of hydrolysis of *O*-hexanoyltimolol (●), *O*-pivaloyltimolol (■), *O*-cyclopropanoyltimolol (○) and *O*-benzoyltimolol (□) in aqueous buffer solution of pH 5.0.

TABLE 5

Arrhenius parameters for the hydrolysis of various timolol esters in aqueous solution at pH 5.0 ($\mu = 0.5$)

Compound	log A	E_a (kJ·M^{-1})	r [a]	n [b]
4	12.38	98.0	1.000	3
7	9.90	86.1	0.997	3
8	10.06	91.2	0.999	4
10	12.30	98.0	0.999	4
12	11.48	96.7	1.000	3
13	12.06	97.7	0.998	4
14	11.62	99.2	1.000	4
16	11.29	91.7	1.000	3
17	10.67	89.4	1.000	3
18	15.38	117.4	0.999	4
22	13.26	109.0	0.999	3

[a] Correlation coefficient for the Arrhenius-type plot.
[b] Number of different temperatures used in the Arrhenius-type plot.

pH 4 and are included in Table 6. The stability at pH 4 is very similar to that at pH 3 and therefore, the $t_{10\%}$ values given at pH 4 represent the maximal stabilities that can be achieved by adjusting pH of aqueous eye-drop formulations.

The results show that the shelf-lives at 25 °C are greatly dependent on the ester structure. The most stable esters are the 2-aminobenzoate (**25**) and 2-methylaminobenzoate (**26**) esters and these compounds can be estimated to have shelf-lives of about 1 year at 25 °C and pH 4. At similar conditions the 2-ethylbutyryl (**8**), 3,3-dimethylbutyryl (**9**) and the methyl-substituted cyclopropanoyl (**13**, **14**) esters are predicted to have shelf-lives of 4–6 months. In contrast, the *O*-butyryl timolol ester (**4**) possesses a shelf-life of only 12 days.

At 5–10 °C the stability is much improved and it is readily possible to select esters with shelf-lives exceeding 2 years at pH 4. As seen from Table 6 the esters **8**, **13**, **14** and **22** all have predicted shelf-lives greater than 2 years at pH 4 and 10 °C. When stored at 5 °C solutions of these esters of pH 3–4.5 can be predicted to have shelf-lives greater than 5 years.

A long-term stability study of some of the esters at 5 °C and 21 °C has been initiated. Measurements of the amount of timolol formed in aqueous solutions (pH 3.5) of the esters indicate that the shelf-lives are actually somewhat higher than predicted. Thus, the 3 month study indicates that the esters **12** and **13** possess shelf-lives of 10 and 18 months, respectively, at 21 °C.

Lipophilicity of the timolol esters

Partition coefficients (P) for the timolol esters and timolol between octanol and aqueous phosphate buffer of pH 7.40 are listed in Table 7. The lipophilicity of the derivatives was also evaluated by means of reversed-phase HPLC using a solvent system of methanol–acetonitrile–0.02 M acetate

TABLE 6

Predicted values of $t_{10\%}$ for various timolol esters in aqueous solutions ($\mu = 0.5$) of pH 4 and 5

Ester	$t_{10\%}$ (days)					
	pH 4			pH 5		
	5 °C	10 °C	25 °C	5 °C	10 °C	25 °C
4	213	101	12	76	36	4.4
7	1.1 years	213	33	137	71	11
8	7.2 years	3.6 years	180	2.4 years	1.2 years	60
10	273	129	16	91	43	5.3
12	2.7 years	1.3 years	60	334	160	20
13	6.9 years	3.3 years	130	2.3 years	1.1 years	44
14	6.0 years	2.8 years	120	2.0 years	340	41
16	171	84	12	61	30	4.3
17	273	136	20	94	47	7.0
18	3.0 years	1.2 years	36	1.0 years	150	12
22	9.6 years	4.2 years	150	3.2 years	1.4 years	50

buffer pH 4.00 (10:45:45 v/v). In this method the capacity factor (k') of a solute is taken as a measure for the relative lipophilicity where k' is defined as:

$$k' = (t_r - t_0)/t_0 \quad (4)$$

where t_r is the retention time of the solute and t_0 is the elution time of the solvent. The k' values obtained are also given in Table 7. As appears from the data all the esters studied are more lipophilic than the parent timolol. This greater lipophilicity is partly due to a decreased pK_a value of the esters relative to timolol, thus affording a greater proportion of the lipophilic free base form at pH 7.4, and partly due to the conversion of the hydroxyl group to an ester group. The log P values of the esters are mutually in good agreement with values calculated on basis of π substituent values (Hansch and Leo, 1979).

TABLE 7

Partition coefficients (P) and capacity factors (k') of timolol and various timolol esters at 22°C

Compounds	log P [a]	log k'
Timolol	−0.04	−0.03
2	1.12	0.13
3	1.62	0.30
4	2.08	0.47
5	2.19	0.45
6	2.67	0.66
7	2.68	0.62
8	3.26	0.83
9	3.09	0.78
10	3.35	0.89
11	4.66 [b]	1.31
12	1.74	0.33
13	2.22	0.48
14	2.26	0.49
15	2.36 [b]	0.52
16	2.75	0.68
17	3.30	0.89
18	2.55	0.55
19	3.02	0.73
20	3.11	0.74
21	2.51	0.59
22	2.65	0.61
23	1.21 [b]	0.13
24	4.37 [b]	1.21
25	2.51	0.49
26	3.04	0.76
27	2.27	0.46

[a] P is the partition coefficient between octanol and 0.05 M phosphate buffer (pH 7.4).

[b] Log P value estimated from Eqn. 5.

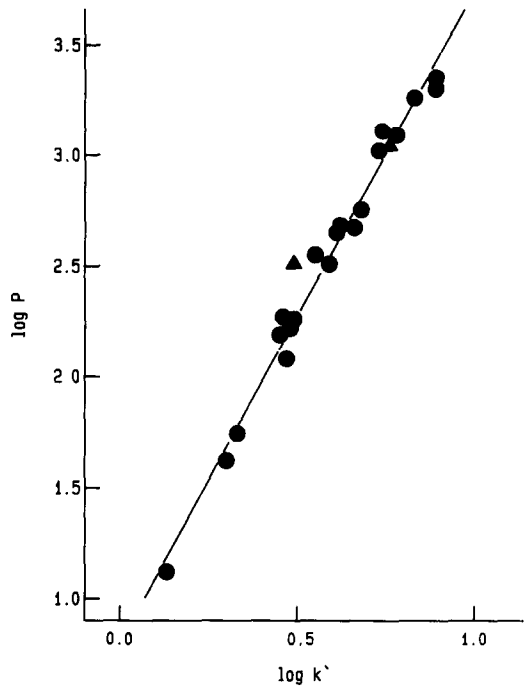


Fig. 8. Plot of log P against log k' for timolol and 21 timolol esters. The values are taken from Table 7. The triangles refer to the aminobenzoate esters **25** and **26** and these compounds are excluded from the correlation plot. The deviation of the data for these compounds from the correlation line is due to the fact that the aminobenzoyl group is partly protonated in the solvent used for determination of k' whereas it is unprotonated at the pH value (7.4) used in the determination of P.

As has been observed for many different types of compounds (e.g. Hafkenscheid and Tomlinson, 1983) a linear relationship was found to exist between log P and log k' for the esters (Fig. 8):

$$\log P = 2.95(\pm 0.09) \log k' + 0.80(\pm 0.06) \quad (5)$$
$$(n = 20,\ r = 0.982)$$

Conclusions

This study shows that it is possible to design esters of timolol with stability characteristics that makes it feasible to formulate ready-to-use aque-

ous eye-drop preparations with acceptable shelf-lives at 5–10 °C. The stability of the esters varies widely with the straight chain alkyl esters being the most unstable compounds. Sterically hindered esters such as the 2-ethylbutyryl, 3,3-dimethylbutyryl and cyclopropanoyl derivatives show greatly improved stability. Along with the substituted benzoate esters these compounds are the prime candidates as timolol prodrugs from the point of view of chemical stability. Studies are going on to examine the ocular hydrolysis and delivery characteristics of these esters.

Acknowledgement

This work was supported in part by Grant EY-03816 from the National Institutes of Health, Besthesda, MD, U.S.A.

References

Alvan, G., Calissendorff, B., Seideman, P., Widmark, K. and Widmark, G., Absorption of ocular timolol. *Clin. Pharmacokin.*, 5 (1980) 95–100.

Bundgaard, H., Buur, A., Chang, S.-C. and Lee, V.H.L., Prodrugs of timolol for improved ocular delivery: synthesis, hydrolysis kinetics and lipophilicity of various timolol esters. *Int. J. Pharm.*, 33 (1986) 15–26.

Buur, A., Bundgaard, H. and Lee, V.H.L., Prodrugs of propranolol: hydrolysis and intramolecular aminolysis of various propranolol esters and an oxazolidin-2-one derivative. *Int. J. Pharm.*, 42 (1988) 51–60.

Chang, S.-C., Bundgaard, H., Buur, A. and Lee, V.H.L., Improved corneal penetration of timolol by prodrugs as a means to reduce systemic drug load. *Invest. Ophthalmol.*, 28 (1987) 487–491.

Chang, S.C. and Lee, V.H.L., Nasal and conjunctival contributions to the systemic absorption of topical timolol in the pigmented rabbit: implications in the design of strategies to maximize the ratio of ocular to systemic absorption. *J. Ocular Pharmacol.*, 3 (1987) 159–169.

Chang, S.C., Chien, D.S., Bundgaard, H. and Lee, V.H.L., Relative effectiveness of prodrug and viscous solution approaches in maximizing the ratio of ocular to systemic absorption of topically applied timolol. *Exp. Eye Res.*, 46 (1988a) 59–69.

Chang, S.C., Bundgaard, H., Buur, A. and Lee, V.H.L., Low dose *O*-butyryl timolol improves the therapeutic index of timolol in the pigmented rabbit. *Invest. Ophthalmol.*, 29 (1988b) 626–629.

Charton, M., The prediction of chemical lability through substituent effects. In Roche, E.B. (Ed.), *Design of Biopharmaceutical Properties through Prodrugs and Analogs*, American Pharmaceutical Association, Washington, DC, 1977, pp. 228–280.

Fife, T.H. and Benjamin, B.M., General base catalyzed intramolecular transesterification. *J. Am. Chem. Soc.*, 95 (1973) 2059–2061.

Fife, T.H. and Benjamin, B.M., Intramolecular general base catalyzed transesterification. The cyclization of ethyl 2-hydroxymethylbenzoate and ethyl-2-hydroxymethyl-4-nitrobenzoate to phthalide and 5-nitrophthalide. *Bioorg. Chem.*, 5 (1976) 37–50.

Hafkenscheid, T.L. and Tomlinson, E., Correlations between alkane–water and octan-1-ol/water distribution coefficients and isocratic reversed-phase liquid chromatography factors of acids, bases and neutrals. *Int. J. Pharm.*, 16 (1983) 225–239.

Hansch, C. and Leo, A., *Substituent Constants for Correlation Analysis in Chemistry and Biology*, Wiley, New York, 1979.

Munroe, W.P., Rindone, J.P. and Kerschner, R.M., Systemic side effects associated with the ophthalmic administration of timolol. *Drug Intell. Clin. Pharm.*, 19 (1985) 85–89.

Nelson, W.L., Fraufelder, F.T., Sills, J.M., Arrowsmith, J.B. and Kuritsky, J.N., Adverse respiratory and cardiovascular events attributed to timolol ophthalmic solution, 1978–1985. *Am. J. Ophthalmol.*, 102 (1986) 606–611.

Nielsen, N.M. and Bundgaard, H., Prodrugs as drug delivery systems. Part 42. 2-Hydroxymethylbenzamides and 2-acyloxymethylbenzamides as potential prodrug forms for amines. *Int. J. Pharm.*, 29 (1986) 9–18.

Perrin, D.D., Dempsey, B. and Serjeant, E.P., *pK_a Prediction for Organic Acids and Bases*, Chapman and Hall, London/New York, 1981, pp. 109–126.

Saenger, W., Cyclodextrin inclusion compounds in research and industry. *Angew. Chem. Int. Edn.*, 19 (1980) 344–362.

Schmitt, C.J., Lotti, V.J. and LeDouarec, J.C., Penetration of timolol into the rabbit eye. Measurements after ocular instillation and intravenous injection. *Arch. Ophthalmol.*, 98 (1980) 547–551.

Schoenwald, R.D. and Huang, H.-S., Corneal penetration behaviour of β-blocking agents I: Physicochemical factors. *J. Pharm. Sci.*, 72 (1983) 1266–1272.

Uekama, K., Pharmaceutical applications of cyclodextrin complexations. *J. Pharm. Soc. Jap.*, 101 (1981) 857–873.

Uekama, K. and Otagiri, M., Cyclodextrins in drug carrier systems. *CRC Crit. Rev. Ther. Drug Carrier Syst.*, 3 (1987) 1–40.

Venuti, M.C., Isatoic anhydride/4-dimethylaminopyridine as a reagent for ortho-aminobenzoylation. *Synthesis*, 1982, 266–268.