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Synthesis, hydrolysis kinetics and lipophilicity of *O*-acyl esters of oxprenolol

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Summary

A number of β-adrenergic blockers, including timolol and propranolol, are administered in eye-drops for the treatment of glaucoma. Their therapeutic value is limited by a relatively high incidence of cardiovascular and respiratory side-effects. Because of poor ocular bioavailability, many ocular drugs are applied in high concentrations, which give rise to both ocular and systemic side-effects. Among the methods employed to increase ocular bioavailability are (a) the development of drug delivery devices designed to release drugs at controlled rates, (b) the use of various vehicles that retard precorneal drug loss and (c) the conversion of drugs to biologically reversible derivatives (prodrugs) with increased corneal penetration properties, from which the active drugs are released by enzymatic hydrolysis. A series of esters of oxprenolol were synthesised and investigated as potential prodrugs for ocular use. The stability of each *O*-acyl derivative was investigated in aqueous solutions over the pH range 2.2–9.0 at 37°C. The shelf-lives of the series of esters were determined at both 10 and 25°C.

Introduction

Several β-adrenergic blocking agents, notably propranolol (**I**) and timolol (**II**) are used in glaucoma therapy. The corneal penetration behaviour of a number of β-blockers has been investigated (Huang et al., 1983a,b; Schoenwald and Huang, 1983). Several of these compounds, including propranolol (Vale et al., 1972), have been shown to lower intraocular pressure. The hydrophobic properties of these drugs are apparently the primary determinants of their pharmacokinetic and corneal penetration behaviour. The development of prodrugs with improved corneal absorption characteristics has been used successfully to enhance the ocular bioavailability of a number of drugs (Bodor and Visor, 1984; Bundgaard et al., 1985), including adrenaline (**III**) and pilocarpine (**IV**).

Considerable attention has been focussed on the use of bioreversible derivatives (prodrugs) in order to improve the delivery characteristics of various drugs (Bundgaard, 1985). A fundamental requisite for the usefulness of the prodrug approach is the ready availability of chemical derivative types satisfying the prodrug require-

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ments, principally reconversion of the prodrug to the parent drug in vivo. Esters are the best known prodrugs due to the predominance of carboxylic and hydroxyl substituents in drug molecules along with the availability of enzymes in living systems capable of hydrolyzing them.

In previous studies, esters of timolol have been developed to potentially diminish the systemic absorption of topically added timolol through increased corneal absorption. The cardiovascular and respiratory side-effects (Velde and Kaiser, 1983; Munroe et al., 1985; Nelson et al., 1986) are thereby reduced. However, these esters are unstable in aqueous solutions. In respect of this a series of esters of both propranolol (Buur et al., 1988; Irwin and Belaid, 1988) and timolol (Bundgaard et al., 1986, 1988; Chang et al., 1987) were synthesised and the kinetics of degradation of the prodrugs in aqueous solution studied.

To further examine the basis of this instability, hydrolytic studies of a series of esters (compounds **VI–XIV**) of the structurally related β-adrenergic blocker oxprenolol (**V**), {1-[(1-methylethyl)amino-3-[2-(2-propenyloxy)phenoxy]-2-propanol}, are presented here. Oxprenolol is an important β-adrenergic blocking agent of the 3-aryloxy-1-(alkylamino)-2-propanol type. The overall conformation of the side chain in oxprenolol and its orientation relative to the aromatic ring is virtually identical to that observed in propranolol hydrochloride. Due to thermal agitation the bonds of the allyl group are unusually long (Leger et al., 1977). Oxprenolol is effective in reducing blood pressure and more particularly systolic levels in cats (Day and Roach, 1974). The feasibility of substituting oxprenolol for adrenergic blockers in the treatment of essential hypertension with relatively minor side-effects has been shown by Forrest (1976) in a trial comprising almost 900 patients. The configuration of the biologically more active (−)-isomer is *S* (Nelson and Burke, 1978).

Materials and Methods

Apparatus

The oxprenolol esters (**VI–XIV**) were characterised by a variety of analytical techniques. ^{1}H-NMR spectra were recorded in $CDCl_3$ solution with TMS as internal standard at 80 MHz by means of a Bruker Spectrospin spectrometer. Mass spectra were obtained using a Kratos M5902 instrument. Spectra were run in an electron impact mode using an ionization energy of 70 eV. The scan was taken over the range 720–30 amu using perfluorokerosene as the reference compound. Data were processed by a computer system based on an Arcom Stebus computer (80188 processor). UV spectral data were obtained using a Pye Unicam SP-8-100 double-beam spectrometer equipped with a thermostatically controlled cell compartment using 1 cm quartz cells. IR spectral data were recorded using a Pye Unicam SP-3-300 spectrometer with polystyrene as reference. A DSC analysis of each compound was carried out using a Perkin Elmer DSC-20 instrument with the Thermal Analysis Data Station (TADS) being employed for data collection, handling and presentation. Melting points determined from DSC analysis compared favourably with those obtained using a Gallenkamp melting point apparatus.

High-performance liquid chromatography (HPLC) was carried out using a system consisting of a Waters 501 HPLC pump, a variable-wavelength UV detector attached to a Houston omniscribe recorder and a 20 μl Rheodyne loop injection valve. The column used (100 × 4.6 mm) was packed with Spherisorb C-8 (5 μm particles). A pre-column (50 × 4.6 mm) was similarly packed. A sample of 10 μl was introduced by means of a Hamilton syringe.

The pH value of each solution was determined using a Radiometer M-26 pH meter fitted with a glass electrode (Radiometer G-202B) and a calomel reference electrode (Radiometer K-401). Reference buffers were Radiometer standard solutions (pH 4.00/22°C, pH 6.97/22°C and pH 8.86/22°C). A Heto thermostat water-bath with a Heto contact thermometer attached was used in all experiments.

Potentiometric titrations were carried out using a Mettler DL 25 automatic titrator fitted with an interchangeable burette and rod stirrer with a variable speed adjuster. Results were obtained in tabular form using a GA 44 printer and a dot

matrix graphics printer was used to plot the curve of the titration profile on a GA 14 recorder.

Chemicals

Samples of oxprenolol hydrochloride were obtained from both Ciba-Geigy Ltd and Sigma Chemical Co. (U.K.). The acid chlorides were obtained from Aldrich Chemical Co. (U.K.). All solvents used were either HPLC grade or distilled before use. Solid reagents were analytical or reagent grade and were used as supplied or were recrystallised before use.

All solvents used in HPLC (i.e., acetonitrile, methanol, acetone and tetrahydrofuran) were HPLC grade. All buffer substances used were of reagent or analytical grade. The ionic strength of each buffer solution was adjusted to 0.5 by adding a specific quantity of analytical grade potassium chloride. Commercial grade *n*-octanol was used in the partitioning experiments.

Synthesis of oxprenolol esters

The esters (**VI–XIV**) were prepared by heating oxprenolol hydrochloride (1 g) under reflux for a specified length of time with the appropriate acid chloride. Excess acid chloride was removed under vacuum. A solid product was obtained with the *O*-pivaloyl derivative (**X**). An oily residue was

TABLE 1

Physical and analytical data of various esters (hydrochloride salts) of oxprenolol

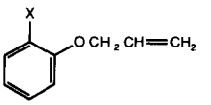
Ester	Yield (%)	Melting point (°C)	Formula	Analysis (%)		
				Calculated		Found
VI	82	118–119	$C_{17}H_{26}ClNO_4$	C	59.38	59.87
				H	7.56	7.64
				N	4.07	3.99
VII	71	64	$C_{18}H_{28}ClNO_4$	C	60.41	58.91
				H	7.83	7.86
				N	3.91	3.96
VIII	73	73	$C_{19}H_{30}ClNO_4$	C	61.37	60.58
				H	8.07	8.22
				N	3.76	3.52
IX	77	68	$C_{20}H_{32}ClNO_4$	C	62.25	61.59
				H	8.30	8.28
				N	3.63	3.36
X	78	105–106	$C_{20}H_{32}ClNO_4$	C	62.25	62.21
				H	8.30	8.43
				N	3.63	3.60
XI	65	94–95	$C_{19}H_{30}ClNO_4$	C	61.37	61.07
				H	8.08	8.27
				N	3.76	3.68
XII	64	87–88	$C_{19}H_{28}ClNO_4$	C	61.70	61.55
				H	7.57	7.69
				N	3.78	3.65
XIII	68	98	$C_{19}H_{28}ClNO_4$	C	61.70	61.00
				H	7.57	7.70
				N	3.78	3.67
XIV	66	154–155	$C_{22}H_{27}ClN_2O_6$	C	58.60	59.10
				H	5.99	4.56
				N	6.21	5.98

obtained in all other cases. The oil was crystallised by adding 5 ml acetone followed by 30 ml petroleum ether. Continuous removal of the acetone and petroleum ether under vacuum and repetition of the process resulted in the formation of a milky precipitate which was recrystallised from isopropanol. Physical and analytical data for the compounds are given in Table 1. The NMR, mass spectral and IR data were consistent with their structures. Elemental analyses were consistent with the molecular formulae.

Hydrolysis kinetics in aqueous solution

The decomposition of the oxprenolol esters (**VI–XIV**) was studied in aqueous buffer solutions over the pH range 2.2–9.0 at 37.0 ± 0.2°C. Phosphate and citrate buffers were used in the pH range 2.5–5.0, while borate buffers were used in the pH range 8.0–12.0. The total buffer concentration was 0.05 M and a constant ionic strength (μ) of 0.5 was maintained for each buffer.

The rates of hydrolysis were measured by means of a reversed phase HPLC procedure capable of separating the esters from the parent compounds. A mobile phase system consisting of acetonitrile/methanol/0.02 M phosphate buffer of pH 4.5 (65:5.0:30 v/v) was used. All solvents were previously degassed in an ultrasonic bath for 15 min. The phosphate buffer was filtered through a Millipore filter and was degassed. A flow rate of 1.0 ml min^{-1} achieved satisfactory results. The column effluent was monitored at 275 nm.

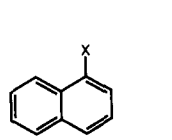


—X = —O CH_2 CH(OR)—CH_2 NH CH $(CH_3)_2$

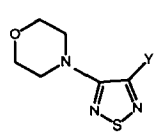
Compound	R
Oxprenolol (**V**)	H
O-Acetyl (**VI**)	- CO CH_3
O-Propionyl (**VII**)	- CO CH_2 CH_3
O-Butyryl (**VIII**)	- CO $(CH_2)_2CH_3$
O-Valeryl (**IX**)	- CO $(CH_2)_3CH_3$
O-Pivaloyl (**X**)	- CO C $(CH_3)_3$
O-Isobutyryl (**XI**)	- CO CH $(CH_3)_2$
O-Cyclopropanoyl (**XII**)	- CO - CH CH_2 CH_2
O-Crotonyl (**XIII**)	- CO CH = CH CH_3
O-*p*-Nitrobenzoyl (**XIV**)	- CO - C_6H_4 - NO_2

The retention times (t_r) for the compounds were in the range 2.65 min (compound **V**) to 9.91 min (compound **X**). In the case of the *O*-acetyl derivative (compound **VI**), a mobile phase consisting of acetonitrile/methanol/0.02 M phosphate buffer of pH 4.5 (55:5.0:40 v/v) was used to ensure separation. Hydrolysis was initiated by adding 7 ml of the stock solution (20 mg% of each compound in methanol) to 3 ml of buffer solution (pre-equilibrated at the appropriate temperature). At 20 min intervals, 10 μl samples were chromatographed. Quantitation of the compounds was carried out by measuring the peak heights in relation to those of standard solutions chromatographed under the same conditions.

Pseudo first-order rate constants for the hydrolysis were determined from the slopes of linear plots of the logarithm of residual oxprenolol ester vs time.



I



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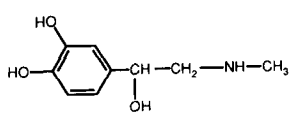
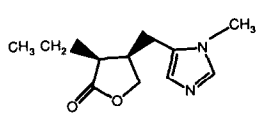


III



IV

—X = —O CH_2 CH(OH) CH_2 NH CH $(CH_3)_2$

—Y = —O CH_2 CH(OH) CH_2 NH C $(CH_3)_3$

Determination of partition coefficients

The apparent partition (distribution) coefficients, P_{app}, of the oxprenolol esters were determined in the *n*-octanol/buffer system (pH 7.40) at 22°C by potentiometric titration using a multiparametric curve-fitting technique (Clarke, 1984; Clarke and Cahoon, 1987). The method involves the potentiometric titration of the compound both in water and in a rapidly stirred mixture of water and *n*-octanol. The method is rapid and accurate for compounds with pK_a values between 4 and 10. Distribution coefficients calculated over a range of pH values may be presented graphically as distribution profiles. Subtraction of the titration curve of solvent alone from that of the compound in the solvent allows the calculation of pK_a values.

In the method of Kaufman et al. (1975), the ionization constant (pK_a) and the apparent ionization constant, $(pK_a)_{app}$, are first obtained from the results of two potentiometric titrations, one without and one with *n*-octanol present, respectively. The log *P* value is calculated from the difference between the pK_a and $(pK_a)_{app}$ values and the volume of water and *n*-octanol.

For the titration of a salt of a weak base with a strong base in the presence of *n*-octanol, the following equations have been derived for the calculation of *P* and P_{app}.

$$P = \frac{V_w}{V_o}[10^{pK_a-(pK_a)_{app}} - 1] \quad (1)$$

$$P_{app} = P(1-\alpha) = P[10^{pK_a-pH} + 1]^{-1} \quad (2)$$

since

$$\alpha = [10^{pH-pK_a} + 1]^{-1} \quad (3)$$

where *P* is the partition coefficient, P_{app} denotes the distribution coefficient (apparent partition coefficient) at a specific pH, V_w is the aqueous volume and V_o represents the volume of *n*-octanol.

The value of log P_{app}, in terms of log *P*, is therefore given by the equation

$$\log P_{app} = \log P - \log(10^{pK_a-pH} + 1) \quad (4)$$

The apparent ionization constant $(K_a)_{app}$ is defined in terms of K_a by the equation

$$K_a = f^{-1}(K_a)_{app} \quad (5)$$

where *f* is a partition factor given by

$$f = \left(\frac{V_o P}{V_w} + 1\right). \quad (6)$$

Corresponding expressions may be derived for the titration of the salt of a weak acid with a strong acid. The value of K_a is given by

$$K_a = f(K_a)_{app} \quad (7)$$

where *f* is defined by Eqn 5. The partition coefficient is given by

$$P = \frac{V_w}{V_o}[10^{(pK_a)_{app}-pK_a} - 1] \quad (8)$$

and the apparent partition coefficient, P_{app} is given by

$$P_{app} = P[10^{pH-pK_a} + 1]^{-1}. \quad (9)$$

The value of log P_{app}, in terms of log *P*, is given by the equation

$$\log P_{app} = \log P - \log(10^{pH-pK_a} + 1) \quad (10)$$

Results and Discussion

Kinetics of degradation of oxprenolol esters

The degradation of all oxprenolol esters (**VI–XIV**) was studied in aqueous solution at 37°C over the pH range 2.2–9.0. The decomposition of the esters displayed strict first-order kinetics for several half-lives at constant pH and temperature. Typical first-order plots for the degradation of *O*-acetyloxprenolol (**VI**) are shown in Fig. 1. Pseudo-first-order rate constants (k_{obs}) were determined from plots of $\log(100[A]/[A]_0)$ vs time, where $[A]_0$ and [A] are the concentrations at time

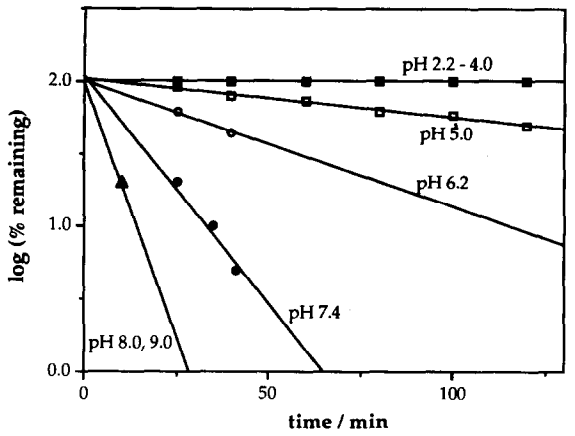


Fig. 1. First-order plots for the degradation of *O*-acetyloxprenolol (**VI**) over the pH range 2.2–9.0 at 37°C.

$t = 0$ and $t = t$, respectively. The value of the rate constant is equal to -2.3026 (slope). The values of the observed pseudo first-order rate constant (k_{obs}) at each pH value are given in Table 2.

The most stable compounds are the *O*-pivaloyl (**X**), *O*-cyclopropanoyl (**XII**) and *O*-*p*-nitrobenzoyl (**XIV**) derivatives and the least stable compound is the *O*-acetyl (**VI**) derivative. For the straight-chain aliphatic derivatives, their rate of degradation decreases with increasing chain length. Their shelf-lives (t_{90}) of degradation (times for 10% degradation) decrease as the temperature increases. These values are determined using Eqn 11 and are listed in Table 3.

$$t_{90} = \frac{\ln(1.11)}{k_{obs}} \qquad (11)$$

The results show that the shelf-lives at 37°C are greatly dependent on the ester structure. At pH 5.0 (37°C) most esters studied do not degrade, indicating a very high stability. The *O*-crotonyl derivative (**XIII**) exhibits remarkable stability. This is possibly due to the electron-releasing character of the double bond which slows down the rate of decomposition. The shelf-lives at this temperature are of the order of minutes at pH values higher than 5.0. The influence of pH on the rate of hydrolysis of the esters at 37°C is shown in Fig. 2.

The shape of the pH-rate profiles indicates that (a) the free base and the protonated forms of the esters undergo hydrolysis at different rates and (b) the hydrolysis can be described in terms of specific base-catalysed reactions involving both species as well as a specific acid-catalysed reaction involving the protonated ester (Scheme 1). All esters studied are quite stable at pH values less than 5. Consequently, rate constants were not determined in this pH range. Oxprenolol esters are generally more stable than the corresponding propranolol esters, due to the overrid-

TABLE 2

Observed pseudo first-order rate constants (k_{obs}) for the degradation of various oxprenolol esters in aqueous solution at different pH values and at 37°C ($\mu = 0.5$)

Ester	k_{obs} ($\times 10^3$) (min^{-1}) at pH							
	2.2	3.0	4.0	5.0	6.2	7.4	8.0	9.0
VI	–	–	–	6.7	21.4	75.4	161.1	160.9
VII	–	–	–	3.8	18.1	66.2	86.3	91.5
VIII	–	–	–	–	15.6	36.1	43.8	48.0
IX	–	–	–	–	12.7	32.8	40.6	40.9
X	–	–	–	–	–	0.3	0.4	0.4
XI	–	–	–	–	6.8	10.6	13.3	25.6
XII	–	–	–	–	3.8	10.6	12.5	17.5
XIII	–	–	–	0.1	4.1	5.8	9.8	12.3
XIV	–	–	–	–	–	–	1.7	2.0

ing steric effects (shielding) of the *ortho* substituent on the benzene ring.

The reactivity of the esters is a function of steric and polar factors. Since the polar effects of the acyl groups in the aliphatic esters are similar, the observed differences in reactivity in neutral and alkaline solutions may be ascribed to differences in the steric properties. Charton (1975) showed that the rates of acid-catalysed esterification are solely a function of steric effects. The relationship between the steric substituent parameter, υ (Charton, 1975, 1976, 1977), and the logarithm of the shelf-life, log t_{90}, was investigated. The literature values of this parameter are listed in Table 4. The relationship (Fig. 3) was shown to be reasonably linear ($r = 0.92$). The linearity is greatly improved with the omission of the *O*-cycloalkyl group (*O*-cyclopropanoyl ester, **XII**) whereby the correlation coefficient is increased to 0.994 ($n = 6$).

Polar effects also influence the rate of hydrolysis of the *O*-crotonyl (**XIII**), *O*-cyclopropanoyl (**XII**) and *O*-*p*-nitrobenzoyl (**XIV**) derivatives. The relative stability of the *O*-crotonyl ester (**XIII**) is due to the greater electron-releasing character of the unsaturated carbon-carbon bond in the side chain, resulting in a decreased interaction with a nucleophilic reagent (OH^-). The relative stability of the *O*-cyclopropanoyl ester (**XII**) is due to steric effects of the cyclic side chain. The aromatic ester *O*-*p*-nitrobenzoyl (**XIV**) should exhibit a higher rate of hydrolysis at elevated pH values, as the *p*-NO_2 group deactivates the aromatic ring by electron withdrawal. Such enhanced reactivity is not, however, observed. This is a consequence of the presence of the $OCH_2CH = CH_2$ group in the *ortho* position on the phenyl ring. Steric effects are predominant when considering reasonably bulky *o*-substituents and therefore, this group prevents interaction between the *O*-acyl carbon atom and the NO_2-group.

As demonstrated by HPLC, the disappearance of almost all the esters studied was accompanied by the progressive appearance of free oxprenolol and a very small trace of a third product was also observed in all esters except the *O*-pivaloyl derivative. This unknown product was observed at higher pH values (pH > 6). It was very stable and had a short retention time.

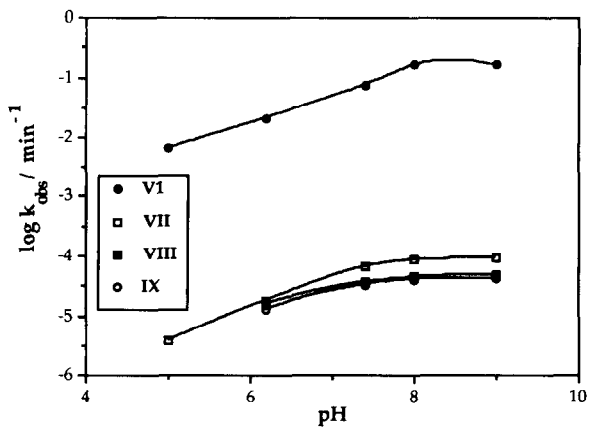
The shape of the pH-rate profiles indicates that the overall degradation process can be described in terms of specific-acid and base-catalysed reactions of the protonated species along with a specific base-catalysed reaction of the free base form of the esters according to the equation

$$k_{obs} = k_H a_H \left(\frac{a_H}{a_H + K_a} \right) + k_0 a_H \left(\frac{a_H}{a_H + K_a} \right) + k_{OH} a_{OH} \left(\frac{a_H}{a_H + K_a} \right) + k'_{OH} a_{OH} \left(\frac{K_a}{a_H + K_a} \right) \quad (12)$$

TABLE 3

Predicted values of the shelf life (t_{90}) for various oxprenolol esters in aqueous solution at different pH values and at 37°C ($\mu = 0.5$)

Ester	t_{90} (min) at pH							
	2.2	3.0	4.0	5.0	6.2	7.4	8.0	9.0
VI	–	–	–	15.7	4.9	1.4	0.7	0.7
VII	–	–	–	27.7	5.8	1.6	1.2	1.2
VIII	–	–	–	–	6.8	2.9	2.4	2.2
IX	–	–	–	–	8.3	3.2	2.6	2.6
X	–	–	–	–	–	309.9	257.0	266.7
XI	–	–	–	–	15.5	9.9	7.9	4.1
XII	–	–	–	–	27.7	9.9	8.4	6.0
XIII	–	–	–	810.5	25.3	18.0	10.7	8.5
XIV	–	–	–	–	–	–	62.0	52.7



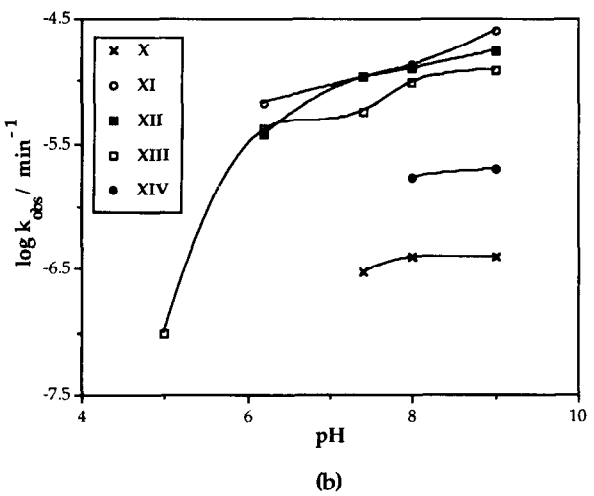
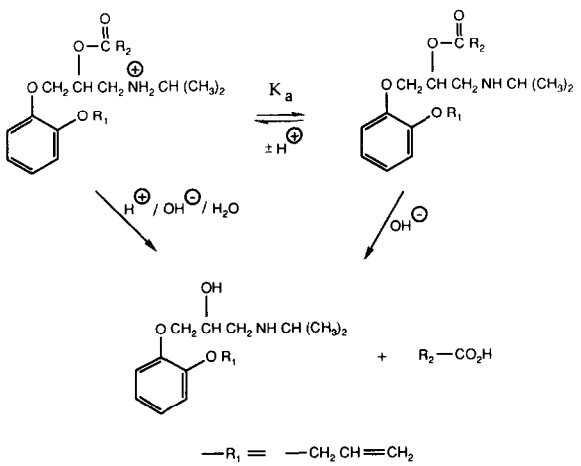


Fig. 2. pH-rate profiles for the degradation of *O*-acyl esters (a) **VI–IX** and (b) **X–XIV** of oxprenolol ($\mu = 0.5$) at 37°C.



Scheme 1.

where a_H and a_{OH} refer to the hydrogen ion and hydroxide ion activities, respectively, $a_H/(a_H + K_a)$ and $K_a/(a_H + K_a)$ represent the fractions of total ester in the protonated and free base forms, respectively, and K_a is the ionization constant of the protonated NH group in the esters. Thus α, the degree of ionization, may be identified with the term $a_H/(a_H + K_a)$ while $(1 - \alpha)$ is equal to $K_a/(a_H + K_a)$. The rate constant k_0 refers to the spontaneous or water-catalysed hydrolysis of the protonated form of the ester, k_H is the specific acid-catalysed rate constant for the protonated ester form and k_{OH} and k'_{OH} denote the second-order rate constants for the apparent hydroxide ion-catalysed hydrolysis of the protonated and unprotonated species, respectively. The processes described by Eqn 12 may be represented schematically (Scheme 1). In this scheme, the R_2CO group corresponds to the R group defined previously, e.g., $R_2 = CH_3$ for the *O*-acetyl ester.

The pK_a values of the esters (Table 5) were lower than that of the parent compound (**V**), indicating that they are less basic than oxprenolol. The difference may be ascribed to the greater polar effect of the ester moiety relative to a hydroxyl group. The electron-withdrawing effect of the ester moiety decreases the basicity of the oxprenolol esters relative to the parent ox-

TABLE 4

Literature values for the steric substituent constant (v) of various alkyl groups (the alkyl moiety R_2 refers to that shown in Schemes 1 and 2)

Compound	R_2	v
VI	$-CH_3$	0.52 [a]
VII	$-CH_2CH_3$	0.56 [a]
VIII	$-(CH_2)_2CH_3$	0.68 [a]
IX	$-(CH_2)_3CH_3$	0.68 [a]
X	$-C(CH_3)_3$	1.24 [a]
XI	$-CH(CH_3)_2$	0.76 [a]
XII	$c-C_3H_5$	1.06 [b]

[a] Charton (1975).
[b] Charton (1976).

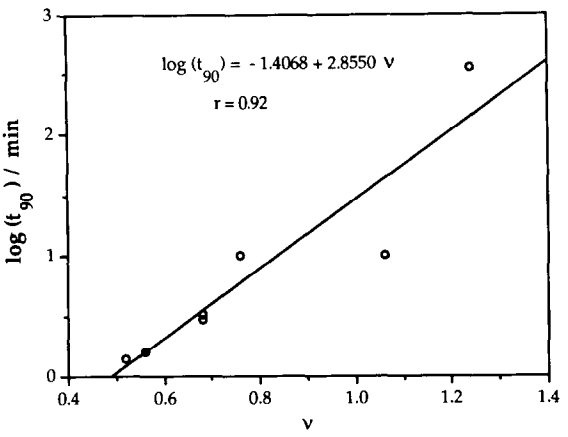


Fig. 3. Plot of log t_{90} (at pH 7.40 and 37°C) vs the steric parameter (v) for various oxprenolol esters. The v values refer to the alkyl or cycloalkyl moiety in the acyl groups (Table 4).

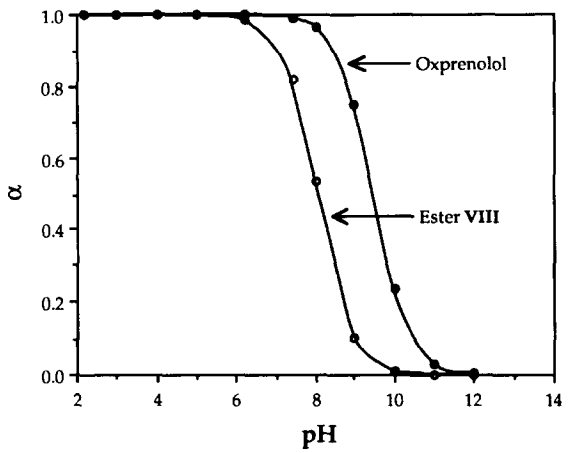


Fig. 4. Variation of the degree of ionization (α) with pH at 37°C. The curve for oxprenolol is compared with that for the *O*-butyryl ester (**VIII**).

prenolol. The pK_a value of the *O*-*p*-nitrobenzoyl ester is particularly low, presumably due to the strong electron-withdrawing effect of the *p*-NO_2 substituent.

The effect of changing the pK_a value upon esterification is shown in Fig. 4. The variation of α, the degree of ionization, with pH is shown and the curve for the *O*-butyryl ester (**VIII**) is compared with the parent oxprenolol (**V**).

TABLE 5

Ionisation constants (pK_a) and apparent ionisation constants ($pK_a)_{app}$ of oxprenolol and its esters at 22°C

Compound	pK_a	(pK_a)$_{app}$
V	9.48; 9.50 [a]; 9.60 [b]; 9.32 [c]	7.18
VI	8.70	5.06
VII	8.58	4.99
VIII	8.06	4.29
IX	7.83	4.00
X	8.07	4.71
XI	8.52	4.93
XII	8.79	5.96
XIII	8.74	5.26
XIV	4.66	9.71

[a] Betageri and Rogers (1987).
[b] Mannhold et al. (1990) – potentiometric titration (20°C).
[c] Schoenwald and Huang (1983) – potentiometric titration (35°C).

In their studies of propranolol esters, Buur et al. (1988) concluded that the possible spontaneous (water-catalyzed) reaction of the protonated species (described by the second term on the right-hand side of Eqn 12) is insignificant to the overall reaction. This is in contrast to the findings for the timolol esters (Bundgaard et al., 1986).

Previous studies on both propranolol (Buur et al., 1988) and timolol (Bundgaard et al., 1986, 1988) esters have indicated that the values of k_{OH} are much greater than k_{II}. These values are of the order 10^7–10^8 greater than k_H. Since values of k_{obs} at pH values less than 5.00 have not been determined, the data as presented would not provide reliable values of k_H. Thus, the first term (on the right-hand side) in Eqn 12 cannot be isolated. For example, in the case of *O*-acetyloxprenolol the value of the degree of ionization $[a_H/(a_H + K_a)]$ at pH 5.0 is approximately equal to 0.9998. The difference between this value and unity is large by comparison with the ratio k_H/k_{OH}. This term would not be the predominant term under these conditions, since the value of $k_{OH}\ a_{OH}$ would not be negligible by comparison with $k_H\ a_H$. Only at very low pH values can the value of k_H be assigned unambiguously.

TABLE 6

Second-order rate constants (k_{OH}) for the hydroxide ion-catalyzed hydrolysis of the protonated species (values are quoted at two pH values: 6.2 and 7.4)

Compound	k_{OH} ($\times 10^{-5}$) (M^{-1} min^{-1})	
	pH 6.2	pH 7.4
VI	6.34	1.48
VII	7.95	1.33
VIII	4.68	0.81
IX	3.87	0.86
X	–	0.0008
XI	2.04	2.14
XII	1.13	0.21
XIII	1.23	1.92
XIV	–	–

The values of k_{OH}, listed in Table 6, were estimated at the pH values 6.2 and 7.4. These values are much more reliable since the third term in Eqn 12 under these conditions is the predominant term. Thus,

$$k_{obs} \cong k_{OH} a_{OH} \left(\frac{a_H}{a_H + K_a} \right). \qquad (13)$$

At pH 6.2, the value of k_{OH} determined using Eqn 13 is 6.39×10^5 for the *O*-acetyl ester. The corresponding value at pH 7.4 is 1.48×10^5.

As shown in Fig. 5, the observed differences in the stability of the esters in weakly acidic and slightly alkaline aqueous solution can be ascribed to differences in the steric properties of the acyl groups, expressed in terms of the steric substituent parameter (v). The correlation coefficients at pH 6.2 and 7.4 are 0.95 and 0.93, respectively. The relationship is quite linear. The $k_{OH}^{|}$ term may not be estimated accurately, since sufficiently high pH values were not studied. This term becomes increasingly important as the pK_a value is lowered. At a particular pH value, the proportion of the unprotonated form increases as the pK_a is lowered. For example, at pH 7.4, $\alpha = 0.992$ for oxprenolol while $\alpha = 0.834$ for ester **VIII** (Fig. 4). The term $[a_{OH}(K_a/(a_H + K_a))]$ increases by a factor of about 240 from pH 6.2 to 7.4 for esters **VI**, **VII** and **XI–XIII**. The increase is less significant for esters **VIII–X** (approx. 200).

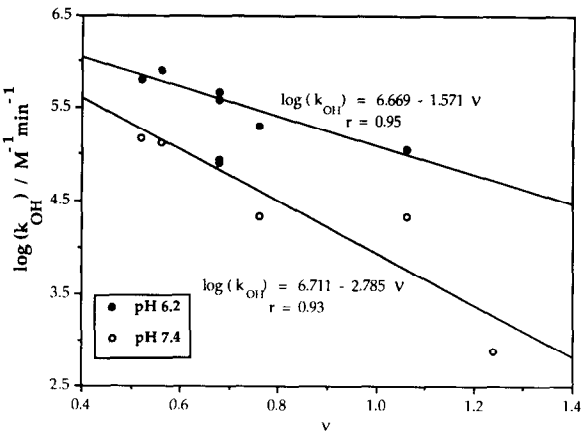
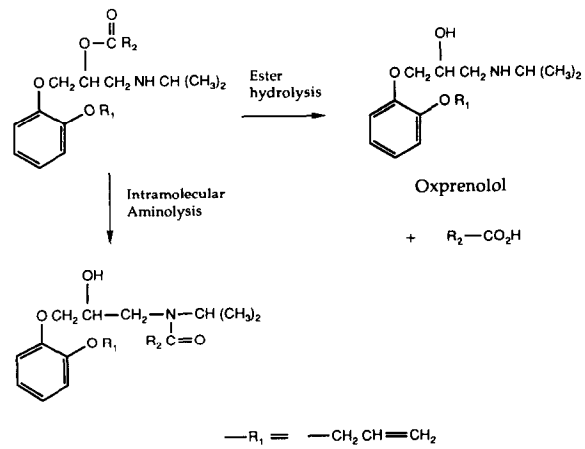


Fig. 5. Plot of log k_{OH} (at pH 6.20 and 7.40) vs the steric parameter (v) for various oxprenolol esters. The v values refer to the alkyl or cycloalkyl moiety in the acyl groups.

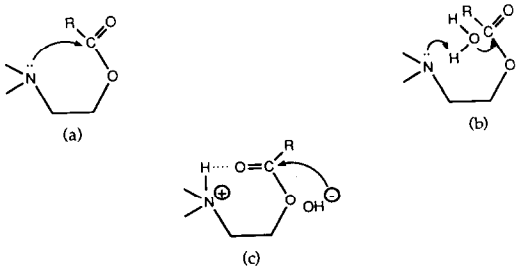
The contribution of the $k_{OH}^{|}$ term is less significant at pH 6.2.

Mechanism of degradation

From the HPLC data, it is clear that at pH < 6, ester hydrolysis to yield oxprenolol was the only reaction taking place. At pH values greater than 7, ester hydrolysis is accompanied by a competitive intramolecular rearrangement reaction (Scheme 2). The disappearance of all esters was accompanied by the appearance of a trace amount of a product along with free oxprenolol. This was confirmed by ^{1}H-NMR and mass spectroscopic



Scheme 2.



Scheme 3.

data to be the corresponding *N*-acyloxprenolol. These compounds were stable under the conditions of the reaction. Irwin and Belaid (1988) showed that the formation of *N*-acetylpropranolol from the *O*-acetyl compound indicated competitive first-order degradation. In contrast to the hydrolysis of propranolol esters, the amount of the *N*-acyloxprenolol derivative formed during neutral and alkaline hydrolysis is very small ($\approx$ 1%). This is presumably due to the steric effects exhibited by the $OCH_2CH=CH_2$ group in the *ortho* position of the benzene ring.

There are three possible kinetically indistinguishable mechanisms which may account for the shape of the pH-rate profiles in the alkaline region (Scheme 3). These are (a) intramolecular nucleophilic attack by the unprotonated amino group on the ester moiety; (b) intramolecular general base catalysis by the unprotonated amino group of the attack of a water molecule on the ester group; and (c) intramolecular general acid catalysis by the protonated amino group of the attack of hydroxide ion (Buur et al., 1988). The rearrangement reaction involves an intramolecular O $\rightarrow$ N migration (Scheme 3a), the transition state of which is rather susceptible to steric interactions. When larger substituents (R) are present it may be expected that the rearrangement process is less favoured. This is found to be the case. Approx. 5% of the *N*-acetyl derivative is formed, whereas only about 1% of the *N*-valeryl derivative is produced. The percentage recovery of the *N*-propionyl derivative is 2%. Less than 1% of the *N*-*p*-nitrobenzoyl derivative was found.

In the case of the *O*-pivaloyl ester (**X**) a simple hydrolysis to the parent compound, without the involvement of a competing intramolecular rearrangement, is observed. Hydrolytic reactions (Scheme 3b and c) result in the formation of oxprenolol alone. The inability of this compound to undergo intramolecular aminolysis is undoubtedly due to the presence of the bulky *tert*-butyl substituent in the ester side chain. This group prevents the close interaction between the carbonyl group and the amino residue in the side chain.

Bundgaard et al. (1986, 1988), in studying timolol ester hydrolysis, argue that the ester with the protonated amino group is much more reactive than the free base form. They found that $k_{OH} \gg k^{|}_{OH}$. Such reactivity is most likely ascribed to mechanism (c) above. The enhanced reactivity of protonated esters over the unprotonated form of other β-aminoalcohols has been observed (Zaslowsky and Fisher, 1963; Bruice and Mautner, 1973). It is noteworthy that even at high pH values, no intramolecular aminolysis was observed for timolol esters (Bundgaard et al., 1986). This can be ascribed to steric hindrance exhibited by the tertiary butylamino group. The behaviour of the oxprenolol esters would be more comparable with the propranolol esters (Buur et al., 1988) in this regard as both sets of derivatives contain a secondary isopropylamino group. These compounds are therefore more susceptible to intramolecular aminolysis than the timolol derivatives. The relative importance of hydrolysis and aminolysis is also determined by steric effects within the acyl moiety.

The shape of the pH-rate profiles indicates that ester hydrolysis accompanied by intramolecular aminolysis are the dominant reactions taking place in the neutral and alkaline regions. A base-catalysed hydrolytic reaction occurs in this region of the profile. All esters exhibit highest stability at pH < 5. In the case of the *O*-pivaloyl derivative (**X**), stability is also displayed at higher pH values. As the pH increased, the rate of amide formation increased slightly as indicated by the HPLC data. This is presumably the result of an increasing proportion of non-protonated base being available for anchimeric attack on the *O*-acyl group (Scheme 3a).

In the physiological range of pH values, direct hydrolysis of the *O*-valeryl derivative to yield ox-

prenolol was found to be much faster than the formation of the corresponding amide, while in the case of the *O*-acetyl derivative, the amide was formed almost as rapidly as the parent compound. This difference in the rate of amide formation is due to the larger size of the *O*-acyl group in the *O*-valeryl ester (**IX**).

Prediction of shelf-lives

Oxprenolol esters, due to their weak basicity, are readily soluble in aqueous solutions at pH 5.0. In order to predict the stability of these compounds under conditions similar for storage, the rate of hydrolysis was measured at pH 5.0 over the temperature range 37–70°C. Under these conditions, the predominant mechanism of hydrolysis is the hydroxide ion-catalysed hydrolysis of the protonated form of the esters (Scheme 1).

Intramolecular aminolysis did not usually occur at this pH value. However, with increasing temperature there was a significant increase in the rate of formation of the amide derivative.

The Arrhenius parameters, defined in Eqn 14, are given in Table 7.

$$\ln(k_{obs}) = \ln A - \frac{E_a}{RT} \qquad (14)$$

where A is the pre-exponential frequency factor while E_a denotes the activation energy of decomposition. The values quoted are those degradation rates at pH 5.0. In Fig. 6 the rate data obtained for all esters except **X** and **XIV** are plotted according to Eqn 14. The rates of degradation of the *O*-pivaloyl and *O*-*p*-nitrobenzoyl derivatives were determined at 70°C only and hence the Arrhenius parameters could not be determined. The activation energy of hydrolysis of the *O*-*n*-acyl esters (**VI–IX**) is of the order of 80 kJ mol^{-1}, which is typical of many reported values for drug decompositions (Kennon, 1964). The hydrolysis of the other sterically hindered esters (**XI–XIII**) is substantially retarded.

On the basis of these values, it is possible to predict the shelf-life of aqueous solutions of the

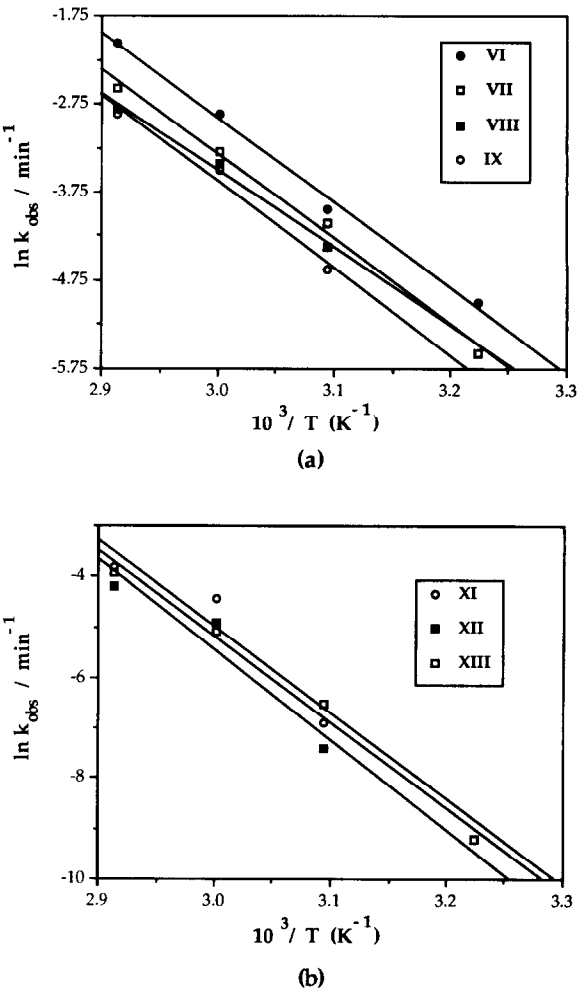


Fig. 6. Arrhenius plots of the rates of hydrolysis of *O*-acyl esters (a) **VI–IX** and (b) **XI–XIII** of oxprenolol ($\mu = 0.5$) at pH 5.0.

TABLE 7

Arrhenius parameters for the hydrolysis of various oxprenolol esters at pH 5.0 ($\mu = 0.5$)

Compound	ln A	E_a (kJ mol^{-1})	r [a]	n [b]
VI	26.06	80.26	0.997	4
VII	25.63	80.23	0.996	4
VIII	22.84	73.04	0.995	3
IX	25.86	81.76	0.990	3
XI	46.41	142.47	0.948	3
XII	48.26	148.85	0.957	3
XIII	46.06	142.00	0.994	4

[a] Correlation coefficient.

[b] Number of temperature values.

esters at lower temperatures (e.g., 10 or 25°C). At lower temperatures, the stability is much improved (Table 8). The most stable esters are the *O*-pivaloyl (**X**) and *O*-*p*-nitrobenzoyl (**XIV**) derivatives. The esters with branched (**XI**), cyclic (**XII**) or unsaturated substituents (**XIII**) are reasonably stable with shelf-lives of the order of days at 25°C.

Lipophilicity of the oxprenolol esters

The potentiometric titration method for the determination of partition coefficients provides for the accurate measurement of the ionization constant in aqueous solution (pK_a) and the apparent ionization constant [$(pK_a)_{app}$] in the presence of *n*-octanol (Table 5). The partition coefficients are determined using Eqn 1, while the distribution coefficients are calculated from these data using Eqn 2.

The lipophilicities of a number of β-adrenoceptor blocking agents have been determined by several investigators (Cruickshank, 1980; Woods and Robinson, 1981; Schoenwald and Huang, 1983; Betageri and Rogers, 1987; Mannhold et al., 1990). These values have been compared with theoretical estimates of log *P* (Recanatini, 1989; Mannhold et al., 1990). These compounds may be classified in terms of their lipophilicities. Three categories have been recognised (Schoenwald and Huang, 1983), namely, highly lipophilic [e.g., propranolol (**I**)], lipophilic [timolol (**II**) and oxprenolol (**V**)] and hydrophilic (e.g., atenolol). The thermodynamics of partitioning of these compounds in the *n*-octanol-water system have been studied (Betageri and Rogers, 1987; Burgot et al., 1990).

TABLE 8

Predicted values of the shelf life (t_{90}) for various oxprenolol esters in aqueous solution at pH 5.0 ($\mu = 0.5$)

Ester	t_{90} (h)	
	10°C	25°C
VI	5.4	1.0
VII	8.2	1.5
VIII	6.3	1.3
IX	12.5	2.2
X	–	–
XI	98.0 days	4.7 days
XII	231.6 days	9.6 days
XIII	113.9 days	5.5 days
XIV	–	–

TABLE 9

Partition coefficients (log P), apparent partition coefficients ($log\ P)_{app}$ and capacity factors ($log\ k^{|}$) of oxprenolol and its esters at 22°C

Compound	log *P*	log P_{app} [a]	log $k^{|}$ [b]
V	2.40; 2.62 [c]; 2.18 [d]; 2.29 [e]; 1.62 [f]; 2.37 [g]	0.32; 0.09 [e]; 2.28 [h]	0.22
VI	3.82	2.52	0.43
VII	3.76	2.58	0.58
VIII	3.95	3.29	0.74
IX	4.00	3.57	0.90
X	3.54	2.78	0.95
XI	3.77	2.61	0.88
XII	3.01	1.60	0.52
XIII	3.65	2.29	0.60
XIV	5.22	2.49	0.83

[a] pH 7.40 – calculated from log *P* using Eqn 4 except for XIV (Eqn 10).
[b] pH 7.40.
[c] Betageri and Rogers (1987) – molal partition coefficient.
[d] Cruickshank (1980).
[e] Mannhold et al. (1990) – 20°C.
[f] Recanatini (1989) – calculated value using MedChem Software.
[g] Schoenwald and Huang (1983) – 35°C.
[h] Woods and Robinson (1981) – 35°C.

Partition coefficients (log *P*) for the oxprenolol esters between *n*-octanol and water at 22°C are listed in Table 9. The results indicate that the esters (**VI–XIV**) are all more lipophilic than the parent compound (**V**). A similar trend was found in the case of propranolol (Irwin and Belaid, 1987) and timolol (Bundgaard et al., 1986, 1988) esters. Distribution coefficients (log P_{app}) and capacity factors (log $k^{|}$) determined at pH 7.4 are also included in Table 9. The capacity factor, $k^{|}$, of a solute is defined by the following equation

$$k^{|} = \frac{t_r - t_0}{t_0} \qquad (15)$$

where t_r is the retention time of the solute and t_0 represents the elution time of the solvent.

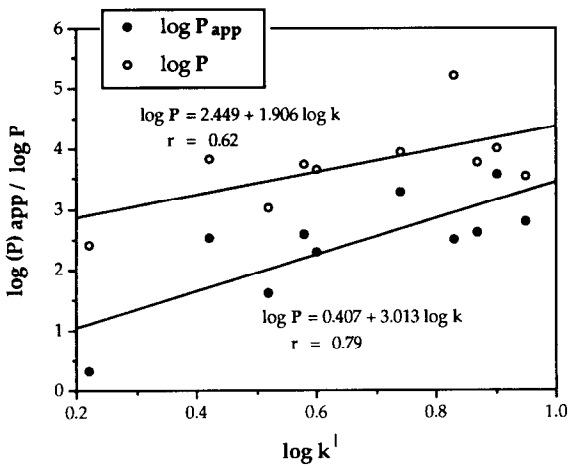


Fig. 7. Plot of both log P and log P_{app} (pH 7.40 and 22°C) against log k' for various oxprenolol esters. The values are taken from Table 9.

The increase in lipophilicity on esterification is partly due to the replacement of the hydroxyl group by an ester group. An important contribution to the observed change in lipophilicity is the observed decrease in the pK_a value on esterification (Table 5). Thus, a higher proportion of the lipophilic free base form is present at any given pH value. At pH 7.40, the value of $K_a/[a_H + K_a] = (1 - \alpha)$ for oxprenolol ($pK_a = 9.48$) is 8.25×10^{-3} (Fig. 4) while the corresponding values for the esters range between 0.048 (**VI, XII** and **XIII**) and 0.166 (**X** and **VIII**). The value for the *O-p*-nitrobenzoyl ester (**XIV**) is quite high (0.998), due to the particularly low value of the pK_a.

The relationship between both the partition (log P) and distribution coefficients (log P_{app}) and log k' is shown in Fig. 7. The relationship is not as linear as has been observed previously (Hafkenscheid and Tomlinson, 1983; Bundgaard et al., 1988).

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