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Albuterol prodrugs for ocular administration: synthesis and evaluation of the physico-chemical and IOP-depressant properties of three albuterol triesters

P. Chetoni [a], P. Crotti [b], M.F. Saettone [a,*]

[a] *Laboratory of Pharmaceutical Technology / Biopharmaceutics, Institute of Pharmaceutical Chemistry,*
[b] *Department of Bioorganic Chemistry, University of Pisa, Via Bonanno 6, I-56100 Pisa, Italy*

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Abstract

Three albuterol (salbutamol) new triesters (acetyl, isobutyryl and pivalyl) were prepared and evaluated in vitro (rate of chemical hydrolysis at different pH values, relative lipophilicity) and in vivo (depression of intraocular pressure, IOP, in a rabbit model of ocular hypertension). The three esters underwent quantitative hydrolysis in vitro to give the parent compound: first-order kinetics were observed, for several half-lives, for the disappearance of the compounds from solution at different pH values. The degradation mechanism, presumably involving a sequence of hydrolytic steps, was not investigated in detail. The rate constants for disappearance of the triesters and for formation of albuterol were in the order acetyl > isobutyryl > pivalyl; the relative lipophilicities of the compounds, as estimated by the corresponding reversed phase HPLC 'capacity factors', were in the order albuterol < acetyl < isobutyryl < pivalyl. When tested for reduction of IOP, all three ester solutions proved significantly more active than albuterol at several times after administration. The tripivalyl ester, in particular, after 5 h appeared more active than the other two esters, and, together with the triisobutyryl ester, was significantly more active than the triacetate after 8 h. These findings confirm the important influence of (pro)drug lipophilicity on transcorneal penetration. The in vivo tests also indicated that the ocular irritant properties of the parent drug were still present, albeit to a smaller degree, in the triester derivatives.

Key words: Albuterol; Prodrug; Albuterol triester; Stability; Hydrolysis kinetics; Relative lipophilicity; Ocular delivery; IOP depression; Rabbit test

1. Introduction

Conversion of a drug into a bioreversible, more lipophilic derivative (prodrug) is a well-known approach to improve absorption through biological membranes. In the ophthalmic field, prodrugs are aimed at improving transcorneal penetration, thus enhancing the intensity and duration of the pharmacological effect, while decreasing the systemic as well as the local side effects through a reduction of the instilled dose (Lee, 1993).

* Corresponding author.

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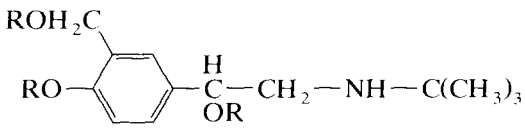
Dipivalyl epinephrine (McClure, 1975) was the first ophthalmic prodrug successfully introduced into clinical practice. The prodrug approach has been experimented on other ophthalmic drugs, such as vidarabine (Pavan-Langston et al., 1976), phenylephrine (Mindel et al., 1980), nadolol (Duzman et al., 1982), acyclovir (Maudgal et al., 1984), pilocarpine (Bundgaard et al., 1985; Mikkelson, 1985; Mosher et al., 1987; Järvinen et al., 1991), timolol (Bundgaard et al., 1986; Buur and Lee, 1987; Chang et al., 1987, 1988; Chien et al., 1988), prostaglandin $F_{2\alpha}$ (Camber et al., 1986; Bito and Baroody, 1987), terbutaline (Phipps et al., 1986), L-643,799, a carbonic anhydrase inhibitor (Bar-Ilan et al., 1986), idoxuridine (Narurkar and Mitra, 1986), and L-652,698, a β-blocker (Sugrue et al., 1988).

Albuterol (salbutamol) [2-*t*-butylamino-1-(4-hydroxy-3-hydroxy-methyl)phenylethanol; **1**], a β-receptor stimulant which appears to possess a selective effect on β_2 (bronchial and arterial) receptors, has been investigated for its ocular hypotensive action (Hopkins, 1984). A pilot study in normal subjects indicated that albuterol lowered the intraocular pressure (IOP) without affecting the heart rate, in a way which was similar to its specificity of action on bronchial muscle. However, in a considerable portion of the patients symptoms of conjunctival hyperhaemia, irritation and tachyphylaxis were observed (Paterson and Paterson, 1971).

The purpose of this study was to prepare and to submit to a series of physico-chemical and preliminary pharmacological tests a series of albuterol esters, in order to verify their suitability as ocular prodrugs. It was speculated that conversion of albuterol into labile, more lipophilic ester derivatives might: (a) increase the therapeutic index (i.e., the aqueous humour-to-plasma absorption ratio), thus improving the IOP-depressant effect while decreasing potential systemic effects; and (b) reduce or eliminate undesirable side-effects (irritation and tachyphylaxis).



1, R = H (sulphate); **2**, R = acetyl (fumarate); **3**, R = isobutyryl (tartrate); **4**, R = pivalyl (tartrate).

2. Materials and methods

2.1. Chemicals

Albuterol sulphate (**1**) was kindly provided by Glaxo S.p.A., Verona, Italy. Acetyl, isobutyryl and pivalyl chloride were obtained commercially (Fluka Chemie AG, Switzerland). All other chemicals (solvents, buffer salts, etc.) used for synthesis, analysis, kinetic and biological studies were of reagent grade.

2.2. Apparatus

^{1}H-NMR spectra were run in $CDCl_3$ solution on a Bruker AC 200 instrument using tetramethylsilane as internal reference. IR spectra were recorded a Perkin Elmer Infracord Model 137 spectrophotometer. Melting points were taken on a Kofler hot stage and are uncorrected.

High-performance liquid chromatography (HPLC) was performed with a system consisting of a Shimadzu apparatus with LC6A pump, Rheodyne model 7125 sample injector with 20 μl loop, SPDM 6A photodiode-array detector and computer integrating system (Shimadzu Corp., Kyoto, Japan). The column (30 cm × 3.8 mm) was packed with μ-Bondpack C_{18}, pore size 10 μm (Waters Chromatography Div., Millipore Corp., Milford, MA, U.S.A.).

2.3. Synthesis of the albuterol ester derivatives

Compounds **2**–**4** were prepared using the following typical procedures:

2.3.1. 1-Acetoxy-1-(4-acetoxy-3-acetoxy-methyl)-phenyl-2-t-butylaminoethane (2)

A solution of albuterol sulphate (**1**) (1.68 g, 5.0 mmol) and triethylamine (52.3 ml, 16.5 mmol) in acetonitrile (40 ml) was treated at 0°C under stirring with a solution of acetyl chloride (8.47 ml, 0.15 mol) in acetonitrile (20 ml). When the addition was complete (20 min), the reaction mixture was refluxed with stirring for 15 h at 95–100°C.

After cooling, the solvent was evaporated under reduced pressure to yield an oily residue (5.46 g) which was repeatedly extracted with water (5 × 20-ml portions). Alkalinisation of the washed (ethyl acetate, 3 × 40-ml portions) aqueous solution with 10% aqueous sodium hydroxide, extraction with ethyl acetate, and evaporation of the dried (anhydrous sodium sulphate) ethyl acetate extracts afforded an oily residue (3.1 g), mostly consisting (^{1}H-NMR) of 1-acetoxy-1-(4-acetoxy-3-acetoxy-methyl)phenyl-2-*t*-butylamino ethane (**2**) (66% yield). The fumarate salt of **2** was crystallised from 2-propanol/diethyl ether.

IR, λ 5.75 μ (C=O); ^{1}H-NMR δ 7.19–7.36 (m, 3H, aromatic protons), 5.73 (unresolved m, 1H, ArCHO), 4.96 (d, 2H, $J = 3.7$ Hz, $ArC\underline{H}_2O$), 2.77–2.96 (m, 2H, CH_2N), 2.21, 2.02 and 1.97 (3s, 3H each, $-OCOC\underline{H}_3$), 1.06 [s, $C(CH_3)_3$]. Fumarate salt: ($C_{19}H_{27}NO_6 \cdot C_4H_4O_4 \cdot H_2O$); m.p. 140–144°C. Anal.: Calcd. for $C_{23}H_{33}NO_{11}$: C, 57.3; H, 6.4; N, 2.9. Found: C, 57.0; H, 6.5; N, 2.9.

2.3.2. *1-Isobutyryloxy-1-(4-isobutyryloxy-3-isobutyryloxy-methyl)phenyl-2-t-butylaminoethane (3)*

Analogous treatment of **1** (1.68 g, 5.0 mmol) with isobutyryl chloride (15.56 ml, 0.15 mol) afforded after 60 h refluxing with stirring a crude oily residue (3.75 g) mostly consisting (^{1}H-NMR) of 1-isobutyryloxy-1-(4-isobutyryloxy-3-isobutyryloxymethyl)phenyl-2-*t*-butylaminoethane (**3**) (60% yield). The tartrate salt of **3** was crystallised from 2-propanol/diethyl ether.

IR, l 5.72 μ (C=O); ^{1}H-NMR δ 7.23–7.41 (m, 3H, aromatic protons), 5.73 (dd, 1H, $J = 4.07$ and 2.2 Hz, $ArC\underline{H}O$), 5.07 (d, 2H, $J = 2.2$ Hz, $ArC\underline{H}_2O$), 2.72–2.90 (m, 2H, CH_2N), 2.46–2.60 (m, 3H, 3 OCOCH), 1.28 and 1.12 [2d, 18H, $J = 7.0$ and 7.1 Hz, 3 $CH(C\underline{H}_3)_2$], 1.04 [s, 9H, $C(CH_3)_3$]. Tartrate salt: ($C_{25}H_{39}NO_6 \cdot C_4H_6O_6 \cdot H_2O$); m.p. 143–146°C. Anal.: Calcd. for $C_{29}H_{47}NO_{13}$: C, 56.3; H, 7.3; N, 2.3. Found: C, 55.9; H, 6.5; N, 2.3.

2.3.3. *1-Trimethylacetoxy-1-(4-trimethylacetoxy-3-trimethylacetoxy-methyl)phenyl-2-t-butylaminoethane (4)*

Analogous treatment of **1** (1.68 g, 5.0 mmol) with trimethylacetyl pivalyl chloride (0.15 mol) afforded after 80 h refluxing with stirring a crude reaction product (4.48 g) mostly consisting (^{1}H-NMR) of 1-trimethylacetoxy-1-(4-trimethylacetoxy-3-trimethylacetoxy-methyl)phenyl-2-*t*-butylamino ethane (**4**) (55% yield). The tartrate salt of **4** was crystallised from 2-propanol/diethyl ether.

IR, l 5.72 μ (C=O); ^{1}H-NMR δ 7.10–7.60 (m, 3H, aromatic protons), 6.01–6.06 (three lines, 1H, $J = 8.93$, 11.9 and 1.9 Hz, ArCHO), 5.1 (d, 2H, $J = 3.3$ Hz, $ArC\underline{H}_2O$), 2.9 (m, 2H, CH_2N_2), 1.31, 1.29 and 1.14 [3s, 9H each, 3 $COC(CH_3)_3$], 1.08 [s, 9H, $C(CH_3)_3$]. Tartrate salt: ($C_{28}H_{45}NO_6 \cdot C_4H_6O_6 \cdot H_2O$); m.p. 162–165°C. Anal.: Calcd. for $C_{32}H_{53}NO_{13}$: C, 58.2; H, 7.8; N, 2.1. Found: C, 57.9; H, 7.7; N, 2.2.

2.4. *Kinetic measurements*

Hydrolysis of the albuterol esters **2–4** was investigated in aqueous solution at 37.0 ± 0.1°C, at different pH values. Hydrochloric acid, acetate, phosphate, borate and sodium hydroxide solutions were used as buffers. The total buffer concentration was generally 0.02 M and a constant ionic strength ($\mu = 0.5$) was secured for each buffer by adding a calculated amount of potassium chloride.

The reactions were initiated by adding 300 μl of a stock solution of the compounds in water (1–1.5 mg/ml) to 3.0 ml of pre-heated (37.0°C) buffer solution in stoppered test tubes. The final ester concentration in the reaction mixture was in the range 0.18–0.20 mM. The solutions were kept in a water bath at constant temperature (37.0 ± 0.1°C). At appropriate times samples were withdrawn and immediately analysed.

The rates of hydrolysis were determined by means of a reversed-phase HPLC method capable of separating the esters from albuterol. The esters were analysed using a mobile phase consisting of 80% v/v acetonitrile and 20% v/v 0.02 M, pH 3.5 phosphate buffer. The flow rate was 1.0 ml/min and the column effluent was monitored in the 195–220 nm range. The retention times of the compounds varied from 2.7 min for **1** to 8.0 min for **4**.

Albuterol in the mixtures was determined more accurately in separate analyses, using a mobile phase consisting of 92% v/v aqueous 0.09 M NaH_2PO_4 (pH 3.5) and 8% v/v CH_3CN. The flow rate was 1.0 ml/min and the column effluent was monitored at 207 nm; the retention time was 5.0 min.

Quantitation of the compounds was performed by comparing the peak areas with those of standards, chromatographed under the same conditions.

Pseudo-first-order rate constants (K_{obs}) for the overall degradation of the triesters were determined using linear regression analysis (r value 0.97–0.99) from the slopes of the plots of the logarithm of residual albuterol ester vs time. The pseudo-first-order rate constants (K_f) for the formation of albuterol were determined from the slope of linear plots (r value 0.97–0.99) of the logarithm of unformed albuterol ($\log[\text{albuterol}_{max} - \text{albuterol}_t]$) vs time.

2.5. Lipophilicity of the albuterol esters

The relative lipophilicity of compounds **1–4** was evaluated by means of reversed-phase HPLC. In this method, the capacity factor (k') of a solute is taken as a measure of its relative lipophilicity (Hafkenscheid and Tomlinson, 1983):

$$k' = (t_r - t_o)/t_o$$

where t_r is the retention time of the solute and t_o denotes the elution time of the solvent. The k' values of compounds **1–4** were obtained using 80% v/v acetonitrile and 20% v/v pH 3.5, 0.02 M phosphate buffer as mobile phase.

2.6. Solutions used for the biological tests

The reference solution (A) and the solutions of esters utilized for the biological test (B–D) were prepared dissolving the appropriate amount of **1**, **2**, **3** or **4** in pH 8.6, 0.1 M borate buffer immediately before administration. The concentration of each triester (29.0 mM) was equivalent to that of a 1.0% w/w reference solution of **1**. The w/w concentrations of the solutions were:

(A) reference solution of **1**: 1.0%;
(B) test solution of **2**: 1.48%;
(C) test solution of **3**: 1.83%;
(D) test solution of **4**: 1.95%.

2.7. Biological tests

New Zealand male albino rabbits (weight range 3.0–4.0 kg) were individually housed under standard conditions (10 h dark/14 h light cycle, 20–21°C temperature, 40–70% relative air humidity), and had free access to water and standard laboratory diet. All experiments were performed in accordance with the ARVO resolution on the use of animals in research.

Ocular hypertension was induced according to the method of Bonomi et al. (1978). Briefly, three weekly subconjunctival injections of 0.8 ml of repository betamethasone (Bentelan Depot®, Glaxo, Verona, Italy) were given after topical anaesthesia (10 μl of 0.4% oxybuprocaine HCl, Novesine®, Sandoz, Basel, Switzerland), each time in a different sector of the eye. IOP measurements were carried out after topical anaesthesia (induced as indicated above) using a pneuma-tonometer (Digilab 30R, Bio-Rad, Cambridge, U.K.) previously calibrated by closed stopcock manometry. Baseline tonometries (from 10:0 a.m. to 18:00 p.m.) were obtained in hypertensive eyes during the third week of betamethasone treatment, when the ocular hypertension was sufficiently stabilised.

After a 24 h free interval (to avoid interference from possible corneal damage), the rabbits were placed in restraint boxes to which they had been habituated, and 25.0 μl of the reference (A) or test solution (B–D) were administered into the lower conjunctival cul-de-sac using a micropipette. Measurements of IOP were taken immediately before drug administration, and 1.0, 2.0, 5.0, and 8.0 h afterwards. A comparison with the baseline values obtained in the same eyes 48 h before was then made. During and after each test, the eyes were also visually inspected for objective symptoms of irritation (conjunctival hyperhaemia, chemosis, etc.) Each vehicle was tested on at least six rabbits.

2.8. Statistical analysis of data

The statistical evaluation of data was performed using an ANOVA test (Statview® software, Abacus Concepts Inc., Berkeley, CA).

The statistical evaluation of data for biological tests included calculation of means and standard errors (S.E.), and comparisons using the Fisher PLSD level: one asterisk (*) and two asterisks (**) indicate a significant difference at the $p < 0.05$ and $p < 0.01$ levels, respectively.

3. Results and discussion

3.1. Synthetic procedure

Some preliminary attempts to prepare albuterol monoesters by selectively esterifying the primary (presumably more reactive) hydroxyl group failed to give the expected results. Among the different methods (Carpino et al., 1970; Bram, 1973; Tullar et al., 1976) which were tested in the attempt to obtain triesters of **1** without acylating the amino functionality, the direct reaction of an ammonium salt derivative of albuterol (sulphate) in the presence of a tertiary amine (triethylamine) in an aprotic solvent (acetonitrile) gave the best result (Bundgaard et al., 1986, 1988; Buur et al., 1988).

The esters thus obtained (**2–4**) were unequivocally identified by means of their ^{1}H-NMR spectra (free bases): a typical downfield shift of the signal of the benzylic-CHO and -CH_2O protons, from δ 4.60 and 4.90 (**1**) to δ 5.70–6.00 and 5.90, respectively, was observed when the starting material was converted into the esterified compounds. The introduction of the three esterifying groups was taken as a measure of the progress of the reaction.

3.2. Chemical hydrolysis of the triesters to albuterol

The hydrolysis of the albuterol triesters **2–4** was investigated in the pH range 2–11. As revealed by HPLC analysis, they were converted quantitatively to the parent compound. Fig. 1 illustrates, as an example, the disappearance of the triacetate **2** in pH 7.4 buffer and the progressive appearance of free albuterol.

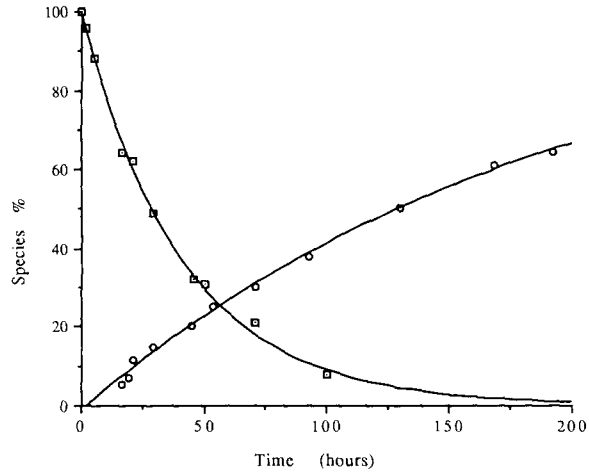


Fig. 1. Time courses for the disappearance of the triester **2** (□) and the appearance of albuterol (○) in pH 7.4, 0.02 M phosphate buffer.

At constant pH and temperature, first-order kinetics were observed for the hydrolysis of all esters for several half-lives. Typical first-order plots for disappearance of the triesters, and the corresponding plots for albuterol formation are shown in Figs. 2 and 3, respectively. The rate constants for disappearance of the triesters (K_d,

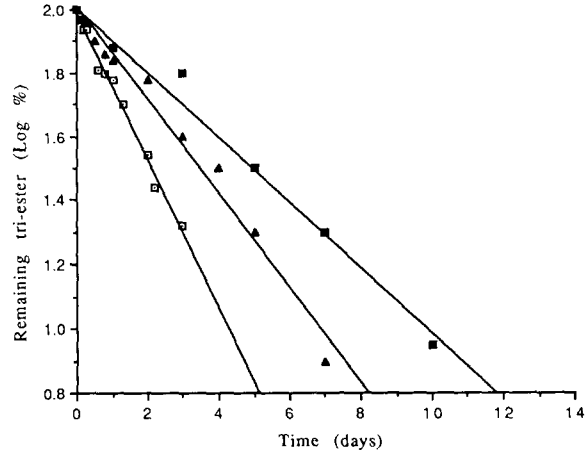


Fig. 2. First-order plots for disappearance (hydrolysis) of albuterol triesters **2** (□), **3** (▲) and **4** (■) in pH 7.4, 0.02 M phosphate buffer at 37°C.

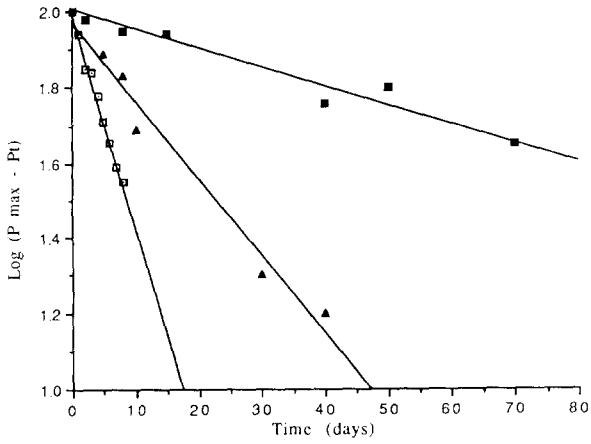


Fig. 3. First-order plots for formation of albuterol from triesters **2** (□), **3** (▲) and **4** (■) in pH 7.4, 0.02 M phosphate buffer at 37°C. P_{max} and P_t indicate the percent albuterol formed at t = infinity and at time t, respectively.

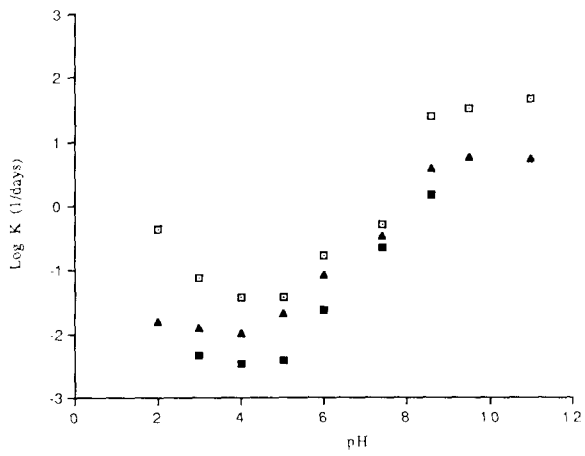


Fig. 4. pH-rate profiles for disappearance (hydrolysis) of albuterol triesters **2** (□), **3** (▲) and **4** (■) in aqueous solution at 37°C.

Table 1
Rate data for the disappearance of the triesters, and for formation of albuterol in aqueous buffer at pH 7.4 (37°C)

Compound no.	k_d (days^{-1})	$t_{d1/2}$ (days^{-1})	k_f (days)	t_{f50} (days)
2	0.530	1.310	0.130	5.23
3	0.339	2.044	0.047	13.17
4	0.235	2.950	0.025	61.14

k_d, $t_{d1/2}$: rate constant and half-time for disappearance of triesters, respectively; k_f: rate constant for formation of albuterol; t_{f50}: time at which 50% of total albuterol was formed.

days^{-1}) and for formation of albuterol (K_f, days^{-1}) at pH 7.4 are listed in Table 1. The t_{f50} values, also reported in Table 1, represent the time (days) after which 50% of the total albuterol was formed: they were calculated from the slopes of the linear plots representing formation of albuterol. The rate constants (k_d, days^{-1}) and half-times ($t_{1/2}$, days) for disappearance of triesters in aqueous solution at different pH values (2–11) at 37°C are reported in Table 2. The influence of pH on the rates of hydrolysis is shown in Fig. 4, where the logarithms of the

Table 2
Rate constants (k_d, days^{-1}) and half-times ($t_{1/2}$, days) for disappearance of triesters in aqueous solution at different pH values (37°C)

pH	Compound no. **2**		Compound no. **3**		Compound no. **4**	
	k_d (days^{-1})	$t_{1/2}$ (days)	k_d (days^{-1})	$t_{1/2}$ (days)	k_d (days^{-1})	$t_{1/2}$ (days)
2.0	0.435	1.59	0.0159	43.58	–	
3.0	0.075	9.24	0.0123	56.34	0.00616	112.5
4.0	0.037	18.58	0.0105	66.0	0.0031	223.5
5.0	0.037	18.60	0.0212	32.69	0.0039	177.7
6.0	0.170	4.07	0.085	8.150	0.024	28.87
7.4	0.530	1.310	0.339	2.044	0.235	2.950
8.6	25.65	0.027	3.950	0.175	1.515	0.460
9.5	33.35	0.021	5.810	0.0120	–	–
11.0	47.97	0.014	5.530	0.125	–	

observed pseudo-first-order rate constants (K_{obs}) are plotted vs pH.

The three esters showed a different tendency to hydrolysis, under both acidic and alkaline conditions, esters **3** and **4** being consistently more stable than **2**. All esters, and **4** in particular, appeared more stable under acidic conditions. This behaviour is in agreement with a hydrolytic process slowed down by a progressively increased crowding around the reactive ester carbonyl group, going from the small acetate ester group (**2**) to the sterically hindered 2,2-dimethyl acetate ester **4**. Inspection of the hydrolysis data shows that free albuterol appeared only after relatively long reaction times, even when the starting esters began disappearing at early times. This is undoubtedly due to the different reactivity of the three alkoxy moieties present in the molecule of albuterol, the phenolic hydroxyl being reasonably the faster *O*-deprotected group. In any case, no attempts were made to identify the intermediates (di- and monoesters) of the hydrolysis of the triesters: the hydrolytic sequence was probably complex, and was not investigated in detail since its full elucidation was not within the scope of the present study.

Formation of albuterol from the triesters at physiological pH (7.4) appeared rather slow: as shown in Table 1, the times for formation of 50% **1** from the triesters **2**–**4** were 5.23, 13.17 and 61.14 days, respectively. On consideration of the pharmacological results reported below, regeneration of **1** from the prodrugs due to enzymatic hydrolysis in vivo should be much faster. The high sensitivity of ester prodrugs to enzymatic hydrolysis is well documented (Bundgaard, 1985). A much greater susceptibility to hydrolysis in plasma, and/or in ocular tissue homogenates, than in buffer has been reported, e.g., by Bundgaard et al. (1986) for various timolol esters, and by Järvinen et al. (1991) for bispilocarpic acid esters.

3.3. Relative lipophilicity of compounds 1–4

The k' values (capacity factors, obtained by reversed-phase HPLC) of the compounds under study, indicative of their relative lipophilicity, are reported in Table 3. As expected, the order of increasing lipophilicity was **1** < **2** < **3** < **4**, and the relative lipophilicities of the triesters with respect to **1** were 2.33, 6.15 and 9.82, respectively.

Table 3
Capacity factors (k') and relative lipophilicity of albuterol and of the triesters

Compound no.	Capacity factor (k')	Relative lipophilicity
1	0.29	1.00
2	0.67	2.33
3	1.76	6.15
4	2.81	9.82

3.4. IOP-depressing activity of compounds 1–4

The results of the pharmacological tests, reported in Fig. 5, are expressed as the IOP differences, in mmHg ± S.E., from the values at $t = 0$ measured just before administration of the solutions (reference or test). Repeated subconjunctival injections of betamethasone in rabbits produced an IOP increase of 5.0 ± 0.8 mmHg, without important daily spontaneous fluctuations of pressure (cf. baseline graph in Fig. 5).

Albuterol sulphate (solution A) significantly decreased the IOP at 1 and 2 h ($p < 0.01$), and at 8 h ($p < 0.05$) with respect to the baseline value.

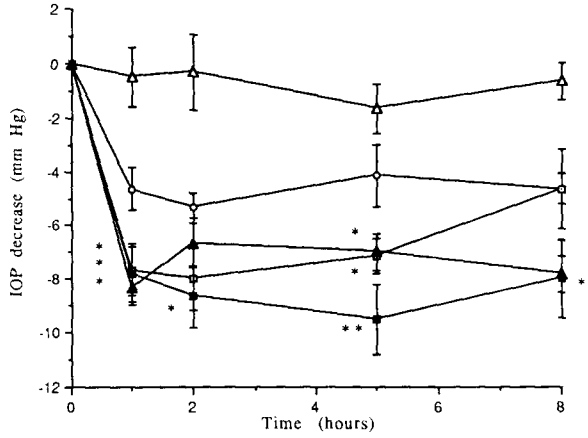


Fig. 5. Effect of albuterol (○) and of the triesters **2** (□), **3** (▲) and **4** (■) on the IOP of artificially hypertensive rabbits (betamethasone model). (△) Baseline values; error bars represent standard errors for an average of six eyes. * $p < 0.05$; ** $p < 0.01$.

When compared with the reference solution A, the test solutions B (acetate) and C (isobutyrate) induced a significantly ($p < 0.05$) greater hypotensive effect 1 and 5 h after administration. Solution D (pivalate) showed a greater hypotensive activity 1 h, 2 h ($p < 0.05$), 5 h ($p < 0.01$) and 8 h ($p < 0.05$) post administration. No significant activity differences were detected among the three *O*-ester solutions, with the exception of solution D, which produced a hypotensive effect at 8 h which was significantly greater ($p < 0.05$) than that produced by solution B.

The corneal drug penetration by prodrugs may be influenced by both lipophilicity and enzymatic lability, and branched-chain esters should be less susceptible to hydrolysis in the cornea than straight-chain ones. In the case of timolol *O*-esters, Chien et al. (1991) found, e.g., that penetration of the drug from the less lipophilic, but more hydrolysis-prone acetyl ester was greater when compared with that from the more lipophilic, but stabler isobutyryl and pivalyl esters. In the present case, the two more stable branched-chain esters **3** and **4** appeared to induce a longer duration of activity, and a greater activity 5 h after administration, when compared with the less lipophilic, but less stable ester **2**. Probably a third factor is involved in activity, i.e., formation of an enhanced depot in the ocular tissues, favoured by a greater lipophilicity. Similar effects have been reported for timolol and pilocarpine prodrugs (Mosher et al., 1987; Sasaki et al., 1988).

In conclusion, the preliminary results collected in the present investigation on a small series of albuterol ester prodrugs appear to indicate that the lipophilic character predominates over other parameters, such as chemical and enzymatic lability and corneal permeability, in determining the intensity and duration of activity. This indication should be supported by further studies on transcorneal penetration and enzymatic stability. The pivalyl triester **4**, in particular, in view of its relatively high chemical stability and its activity on ocular hypertension in the rabbit model, might warrant further investigation. However, the practical usefulness of these prodrugs is open to question, since they produced a slight irritation in rabbit eyes, lower than that produced by **1**, but nonetheless evident. This effect decreased in the order **1** > **2** > **3** > **4**.

Thus, although the prodrug technique allowed us to attain one of the two goals of this study, i.e., enhanced ocular penetration of albuterol, it did not apparently help us to achieve the other, i.e., elimination of the irritant properties of the drug. Since irritation was apparent immediately after instillation of the prodrug solutions, it is unlikely that it resulted from liberation of small amounts of **1** in the lacrimal fluid, and it is very probably an intrinsic property of the prodrug molecules.

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