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# DRUG-DELIVERY BY ION-EXCHANGE.

# PART I: ESTER PRO-DRUGS OF PROPRANOLOL.

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**Summary**

The syntheses of some ester pro-drugs of propranolol, suitable for complexing with cationic ion-exchange resins, are described. These include the n-acyl esters ranging from O-acetyl to O-decanoyl propranolol and the bulky pivaloyl ester. Conditions are presented which enable the isolation of the O-acyl compound, free from the competing N-acyl product, to be accomplished. Spectroscopic properties are described to confirm identity and the optimisation of high-performance liquid chromatographic separations, to enable resolution of mixed esters and degradation products, is described.

**Keywords**

High-performance liquid chromatography, ion-exchange resins, pro-drugs, propranolol, synthesis.

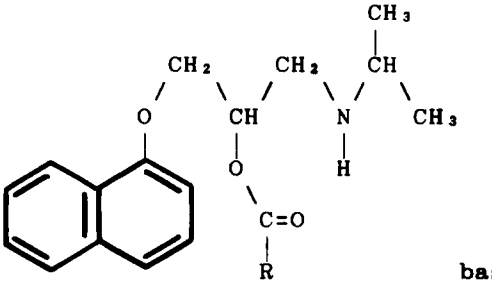
## INTRODUCTION

In the search for improved delivery systems for medicinal agents drug-delivery by ion-exchange has shown

certain advantages over competing technologies. These systems are of particular interest for oral products where a basic drug, in its cationic form, interacts with an cationic ion-exchange resin to form an insoluble drug-resinate complex. Drug release from the resin is initiated by an influx of competing cations from the bathing fluid which displace bound drug from the ionic binding sites. This specific activation process allows the preparation of liquid products which do not release the drug until the ions of the gastro-intestinal tract are encountered. Drug availability from drug-resinates is frequently too rapid to enable proper control of the release profile and to provide a further, diffusional barrier to the drug, coating of the resin particles has been undertaken. Coated resinates are claimed to show release rates which are independent of factors such as pH, gastro-intestinal fluid volume, enzyme levels and matrix composition.[1,2] Little of the work in this field has enjoyed the advantage of a congeric series of drugs which vary in properties in an incremental way. This may reveal possibilities of molecular and system design to control delivery rates without the requirement of particle coating. To investigate the potential of this approach to studying the factors which influence loading and release of mobile ions from ion-exchange resins a series of O-n-acyl ester pro-drug derivatives of propranolol (1-8) were prepared and their interaction with resin systems investigated.

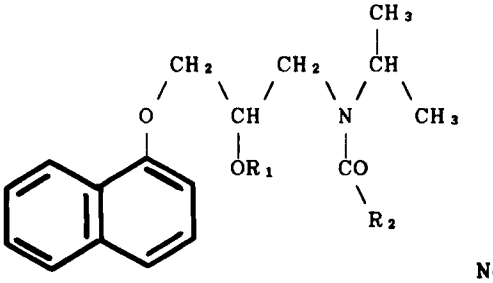
Propranolol has properties which make it suitable for controlled delivery and this was chosen as the model compound for this work. Molecular modifications of propranolol are many and varied with examples ranging from variations in the amino-substituent,[3,4] side-chain variants[5] and nuclear changes[6] including all mono ring-hydroxylated products.[7,8] Additionally, derivatives have been made for specific purposes. These include nitrogen mustard analogues,[9] N-hydroxy and N-nitroso derivatives[10] and spin-labelled compounds.[11] Comprehensive structure-activity relationships have been reported.[12] Few attempts have been made to prepare bio-reversible pro-drugs of propranolol although O-

acetylpropranolol and the oxazolidine derivative have been described.[3,13] The hemi-succinate has also been reported, initially as a means of attaching propranolol to bovine serum albumin to form an immunogen for radio-immuno assay.[13-15]



| | R |
|---|---|
| 1, | $CH_3$ |
| 2, | $C_2H_5$ |
| 3, | $C_3H_7$ |
| 4, | $C_4H_9$ |
| 5, | $C_5H_{11}$ |
| 6, | $C_7H_{15}$ |
| 7, | $C_9H_{19}$ |
| 8, | $(CH_3)_3C$ |

basic mono-acyl derivatives



| | $R_1$ | $R_2$ |
|---|---|---|
| 9, | H | $CH_3$ |
| 10, | H | $C_4H_9$ |
| 11, | $COCH_3$ | $CH_3$ |

Non-basic acyl derivatives

**Scheme 1.** Structures of Ester and Amide Pro-drugs of Propranolol.

## EXPERIMENTAL

### Apparatus

$^1H$ NMR spectra were recorded in deuteriochloroform solution, with tetramethylsilane as internal standard at 360 MHz by means of a Bruker Spectrospin spectrometer. Mass spectra were obtained with a VG Micromass MM12 mass spectrometer using direct insertion with an inlet temperature of 250°C, an ionisation energy of 70 eV, an accelerating voltage of 3 kV and a trap current of 100 μA. Hplc analyses were undertaken using a system constructed from an Altex 100A dual-piston reciprocating solvent-metering pump and a reversed-phase stainless steel Shandon-type column (10cm x 4.6 mm ID) packed with Hypersil-ODS (5 μm). Samples were introduced by means of a Rheodyne 7125 injection valve,

fitted with a 20 µL loop, and detection was accomplished with a Pye LC3 variable wavelength UV detector, fitted with an 8 µL flow cell, and operated at a wavelength of 290 nm with a sensitivity usually of 0.08 AUFS. The mobile phases consisted of aqueous acetonitrile, adjusted to pH=2.8 with phosphoric acid, containing diethylamine as moderator and were delivered at 1 mL $min^{-1}$.

## Methods

### Synthesis of O-Acylpropranolols (1-8)

Syntheses were adapted from the methods described by Crowther and Smith[9] and by Nelson and Walker.[13] Propranolol hydrochloride (1g, 3.4 mmoles), dissolved in chloroform (40 $cm^3$), was heated under reflux for three hours with the required acid chloride (acetyl, 1.18g; n-propanoyl, 1.39g; n-butanoyl, 1.60g; n-valeroyl, 1.81g; n-hexanoyl, 2.02g; n-octanoyl, 2.44g; n-decanoyl, 2.86g; pivaloyl, 1.81g; 15 mmoles). Excess acid chloride was removed under high vacuum and the residue for the lower homologues (acetyl to hexanoyl and pivaloyl) was evaporated twice under vacuum with benzene (50 $cm^3$) to complete the removal of acid chloride and yield solid products. Residues from the longer chain esters were triturated with dry diethyl ether (50 $cm^3$) and were stored at 0°C for a few hours until crystallisation occurred. The ester hydrochlorides were recrystallised from isopropanol with the exception of the decanoyl derivative which was isolated as an oil. Yields and melting points were as follows:

| Ester | Melting Point (°C) | Yield (%) |
|---|---|---|
| Acetyl | 171-173 | 80 |
| Propanoyl | 144-145 | 79 |
| Butanoyl | 135-137 | 60 |
| Valeroyl | 127-129 | 60 |
| Hexanoyl | 120-122 | 52 |
| Octanoyl | 101-102 | 28 |
| Decanoyl | - | 45 |
| Pivaloyl | 145-147 | 65 |

All products were shown to be homogeneous by hplc and structures were confirmed by spectroscopic analysis and by subsequent chemical conversions.

**Synthesis of N-acetyl and N-valeroyl propranolol (9,10)**

Propranolol hydrochloride (1.25g, 4.3 mmoles), together with the appropriate acid chloride (acetyl, 0.34g; n-valeroyl, 0.54g; 4.3 mmole) and triethylamine (2g, 19.7 mmole) dissolved in methylene dichloride (20 $cm^3$), was heated under reflux for one hour. The mixture was washed with water, aqueous hydrochloric acid (0.2M) to remove triethylamine, aqueous sodium carbonate (5%) to neutralise residual acid, and finally with water again. The organic phase was dried over anhydrous sodium sulphate and evaporated to yield the amides as yellow oils. Traces of unreacted propranolol were removed by dissolving the oil in freshly prepared dry ethereal HCl. Propranolol hydrochloride precipitated from solution leaving a clear organic layer which was separated and evaporated in vacuo to provide the N-acetyl (0.73g 56%) and N-valeroyl (0.88g, 60%) amides as uncrystallisable oils which were shown to be homogeneous by hplc and spectroscopic analysis.

**Synthesis of N,O-diacetylpropranolol (11)**

Propranolol hydrochloride (1.5g, 5.08 mmole), together with acetyl chloride (2.36g, 30 mmole) and triethylamine (10.1g, 0.1 mole) dissolved in chloroform (60 $cm^3$), was heated under reflux for four hours. The mixture was washed as described above for the N-acyl derivatives and evaporation yielded a yellow oil. Treatment with charcoal and crystallisation from benzene gave the N,O-diacetyl compound as colourless crystals (1.2g, 73%), melting at 100-102°C, which was shown to be homogeneous by hplc and spectroscopic analysis and by subsequent chemical conversions.

## RESULTS AND DISCUSSION

The major problem in preparing O-alkyl derivatives of propranolol and similar O,N-bifunctional compounds is to avoid the competing N-acylation reactions. Indeed, the previously reported hemi-succinate derivative of propranolol has been shown to have the N- rather than the O-acyl structure.[13] These workers also provided details of reaction

conditions which allowed control of the site of acylation and these were adopted for the synthesis of the n-acyl propranolol esters (1-8) prepared for this work. O-acylation was ensured by allowing reaction of propranolol hydrochloride with some 4-5 fold excess of the acid chloride without a catalyst. The protonation on nitrogen and the production of HCl during reaction ensured that the secondary nitrogen atom did not participate in the reaction. In contrast, N-acyl derivatives (9,10) could be obtained from equimolar amounts of propranolol hydrochloride and the required acid chloride when triethylamine, to liberated the nucleophilic nitrogen centre, was added. Diacylation, to give the O,N- ester amide (11), was also achieved under these conditions when excess acid chloride was present.

The compounds synthesised showed the anticipated spectral properties in accordance with the proposed structures. Of particular interest were the high field (360 MHz) $^1H$ NMR spectra, a representative example of which is shown in Figure 1 for O-hexanoylpropranolol, together with assignments. The presence of a pro-chiral centre at the $\alpha$-$CH_2$ of the acyl residue is clear with each component showing as a separate multiplet ($\delta$=2.44, 2.61 ppm). This pronounced differentiation of the signals, significantly greater than that observed for those protons adjacent to the asymmetric centre itself, suggests a conformational restriction in the acyl substituent. This probably results from a hydrogen bonding interaction between the protonated nitrogen atom of the amine and the carbonyl oxygen atom of the acyl substituent (C=O ... H–$N^+$) giving rise to a cyclic complex. Although the pro-chiral effect is also present in the N-acyl analogues, incomplete separation of the signals is observed in this case.

The 70 eV electron-impact mass spectra show an interesting fragmentation pattern. The typical fragment ions characteristic of both the ester (O-) and amide (N-) series are illustrated in Table 1 for the valeroyl derivatives. This shows the presence of the expected ions associated with the naphthyloxy and hydroxy-amino residues in propranolol.

In addition, a further decomposition was seen which resulted in the appearance of an ion at m/z 296 in all esters

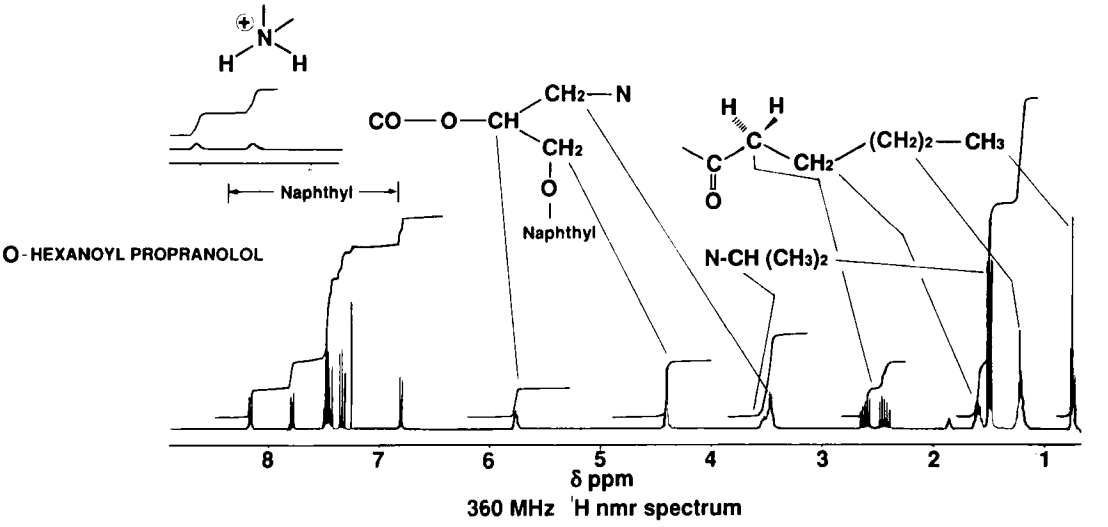
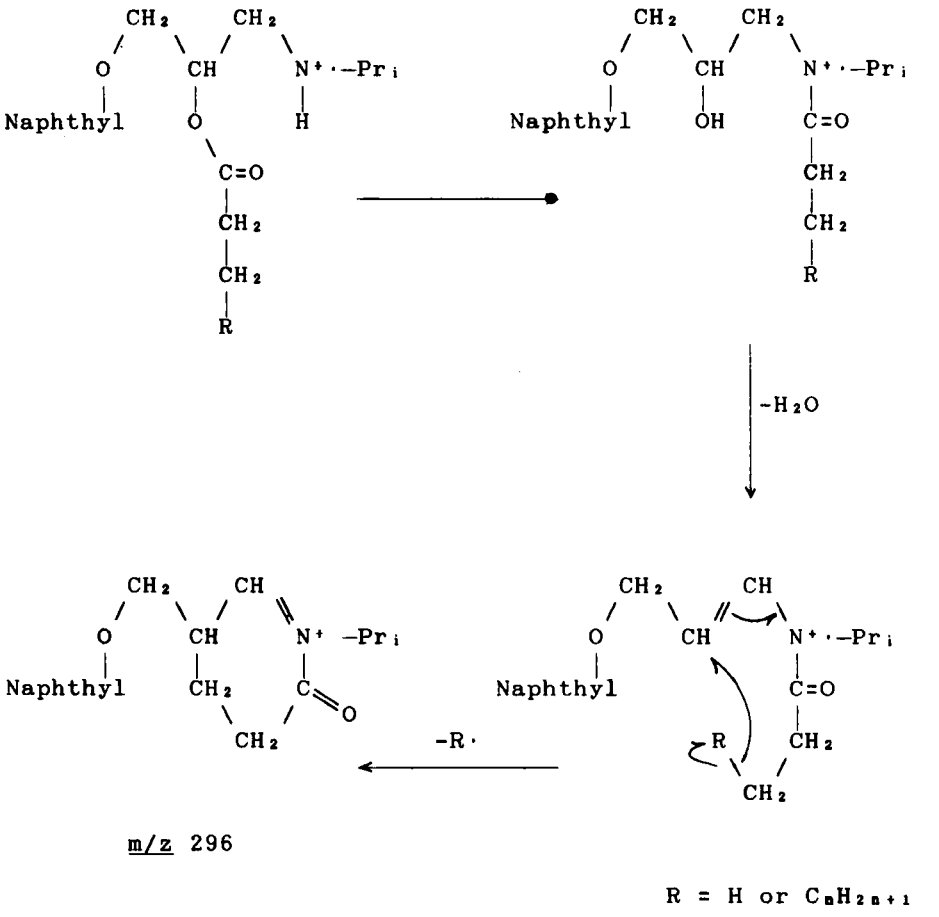



**Figure 1.** 360 MHz $^1H$ NMR spectrum of O-hexanoylpropranolol showing pro-chiral acyl methylene group (δ, 2.44, 2.61).

**Table 1.** Intensities (%) of fragment ions from O- and N-valeroylpropranolols on 70 eV Electron-impact mass spectral analysis. {Np = 1-naphthyl, ($C_{10}H_7$–); [], indicate loss from molecular ion}.

| m/z | O-valeroyl | N-valeroyl | Assignment |
|---|---|---|---|
| 343 | 4 | 3 | $M^{+\cdot}$ |
| 328 | 5 | 4 | $M[-CH_3]^+$ |
| 296 | 6 | 21 | $C_{19}H_{22}NO_2^+$ (see Scheme 1) |
| 244 | 3 | | $Np-O-CH_2-CH(OH)-CH_2-NH^+=CH-CH_3$ |
| 215 | 3 | | $M[-Np-H]^{+\cdot}$ |
| 200 | 6 | 59 | $M[-O-Np]^{\bullet}$ |
| 183 | 8 | 11 | $Np-O-CH_2-CH=CH^+$ |
| 144 | 15 | 31 | $Np-OH^{+\cdot}$ |
| 127 | 7 | 11 | $Np^{+\cdot}$ |
| 116 | 5 | 13 | $C_6H_{14}NO^+$ |
| 115 | 16 | 28 | $CH_2=C(OH)-CH_2-NH^{+\cdot}-CH(CH_3)_2$ |
| 98 | 28 | 15 | $CH_2=CH-CH=NH^+-CH(CH_3)_2$ |
| 72 | 100 | 100 | $CH_2=NH^+-CH(CH_3)_2$ |
| 57 | 31 | | $C_3H_7N^{+\cdot}$ |
| 43 | 15 | | $(CH_3)_2CH^+$ |




**Scheme 2.** Origin of the m/z 296 ion in the mass spectra of O-acylpropranolols.

from propanoyl and above (Scheme 2). The intermediacy of the N-acyl derivative is supported by the increased intensity of the m/z 296 ion in the amide spectrum.

Several useful hplc methods are available for the assay of propranolol and its metabolites using reversed-phase systems.[16-22] The use of aqueous buffered acetonitrile has proven satisfactory in several of these methods and was chosen as the basis for the separation of the ester pro-drugs. All derivatives showed a maximum absorption in the

**Table 2.** Effect of diethylamine concentration in the mobile phase on the hplc retention times of O-acylpropranolols

| Diethyl-amine (%) | Propranolol Ester Retention Time (min) | | | | | |
|---|---|---|---|---|---|---|
| | Propranolol | $CH_3$ (1) | $C_2H_5$ (2) | $C_3H_7$ (3) | $C_4H_9$ (4) | $C_5H_{11}$ (5) |
| 0.04 | 5.5 | 8.0 | 10.8 | 14.3 | 19.3 | 26.3 |
| 0.08 | 3.8 | 5.5 | 7.0 | 9.0 | 12.0 | 17.0 |
| 0.12 | 2.9 | 4.0 | 5.0 | 6.5 | 8.8 | 12.0 |
| 0.16 | 2.5 | 3.5 | 4.3 | 5.6 | 7.5 | 10.0 |
| 0.20 | 2.3 | 2.9 | 3.3 | 4.6 | 6.3 | 8.5 |
| 0.24 | 2.0 | 2.5 | 3.0 | 4.3 | 5.6 | 7.5 |

288-293 nm range and 290 nm was therefore chosen as the analytical wavelength. The proportion of acetonitrile in the mobile phase, the pH, and the addition of modifiers were important in optimising retention and resolution of the propranolols. In the absence of a modifier in the mobile phase acceptable chromatography was obtained for only the N-acyl derivatives. Long retention times coupled with peak-broadening were apparent for all compounds showing basic properties. Such behaviour is perhaps due to adsorption onto residual silanol sites. The incorporation of diethylamine as a modifier into the mobile phase effectively overcame this problem and allowed the development of systems to separate several of the pro-drugs simultaneously. The effect on retention times is shown in Table 2 and is illustrated in Figure 2.

Optimum concentrations of diethylamine which gave satisfactory retention times, peak width and resolution were found to be : O-acetylpropranolol (1), 0.15%; O-propanoyl to O-hexanoylpropranolol (2-5), 0.2%; and for the O-octanoyl and O-decanoyl analogues (6,7) 0.4%.

The effect of acetonitrile concentration in the mobile phase also exerts a considerable influence on chromatographic efficiency. This is illustrated in Table 3 while examples of the chromatograms for O-acetyl- and O-propanoyl- propranolol,

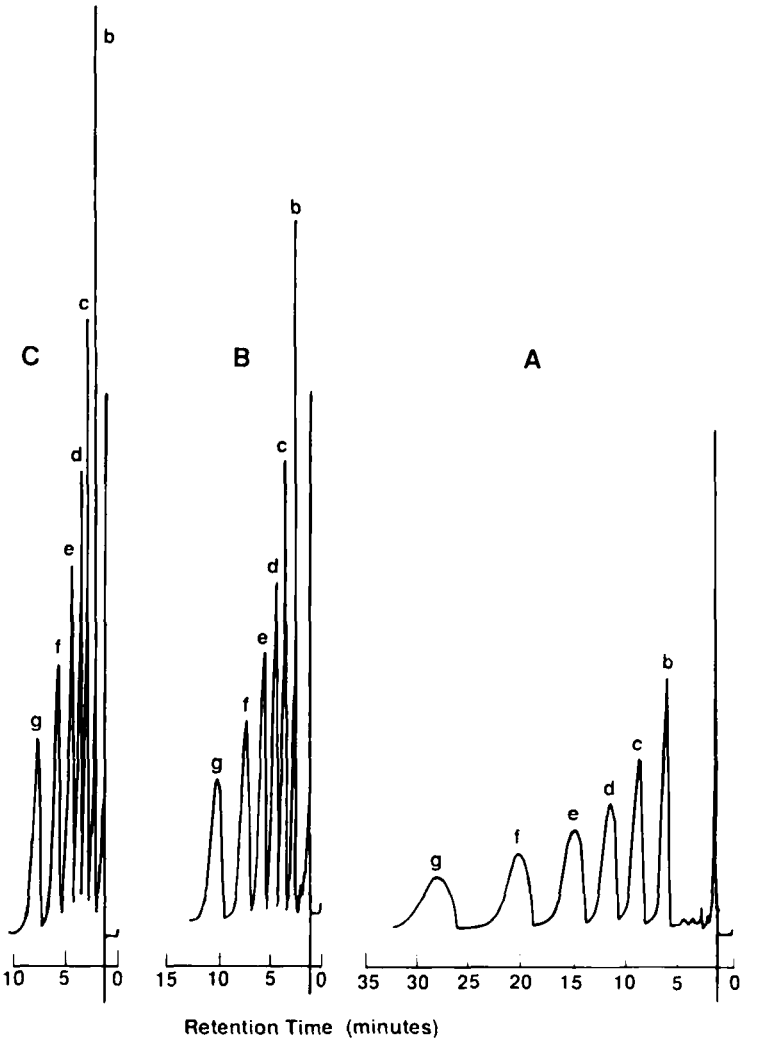



**Figure 2.**
Chromatograms showing the effect of diethylamine as moderator on the hplc of O-n-acylpropranolols.
[Identification of components: a, N-acetylpropranolol; b, propranolol; c, O-acetyl-; d, O-propanoyl-; e, O-butanoyl; f, O-valeroyl; g, O-hexanoyl-propranolol]
(Diethylamine concentration: A, 0.04%, B, 0.2%, C, 0.24%).
(Mobile phase: 65% acetonitrile with varying amounts of diethylamine adjusted to pH=2.5 with orthophosphoric acid)

**Table 3.** Effect of acetonitrile concentration in the mobile phase on hplc retention times of O-n-acylpropranolols.

| Aceto-nitrile (%) | Retention Time of Propranolol Derivative (min) | | | |
|---|---|---|---|---|
| | N-acetyl | Propranolol | O-acetyl | O-propanoyl |
| 45 | 8.0 | 8.0 | 11.0 | 17.2 |
| 50 | 5.0 | 6.0 | 8.8 | 13.0 |
| 55 | 4.0 | 5.0 | 7.3 | 10.6 |
| 60 | 3.0 | 4.0 | 6.0 | 7.7 |
| 65 | 2.5 | 3.6 | 4.5 | 6.0 |
| 70 | 2.3 | 3.1 | 4.2 | 5.0 |

together with N-acetylpropranolol and the parent drug, are recorded in Figure 3.

Optimum concentrations of 65% $CH_3CN$ were found suitable for all O-n-acyl compounds with the exception of the octanoyl and decanoyl derivatives were 85% $CH_3CN$ was more appropriate. In contrast, chromatographic efficiency was only marginally dependent upon the pH of the mobile phase in the acid region at values of pH≤5 (Table 4).

This is largely due to the presence of the moderator which exerts the major effect and although the bases may be expected to be effectively protonated at all pH values used (Propranolol pKa, 9.45), low pH values increase elution times sufficiently to threaten resolution. This is illustrated in Figure 4 for pH=2.2 and 4.4. The minimum acceptable value was pH=2.5 which was used for all analogues described in this work. The O-pivaloyl ester eluted satisfactorily using the same phase as the short chain esters (65% acetonitrile, 0.2% diethylamine and $H_3PO_4$ to pH=2.5) with a retention time of 7.4 min (cf O-hexanoylpropranolol, 9.7 min).

Ethyl p-hydroxybenzoate (ethyl paraben) was used as an internal standard for quantification purposes. Although

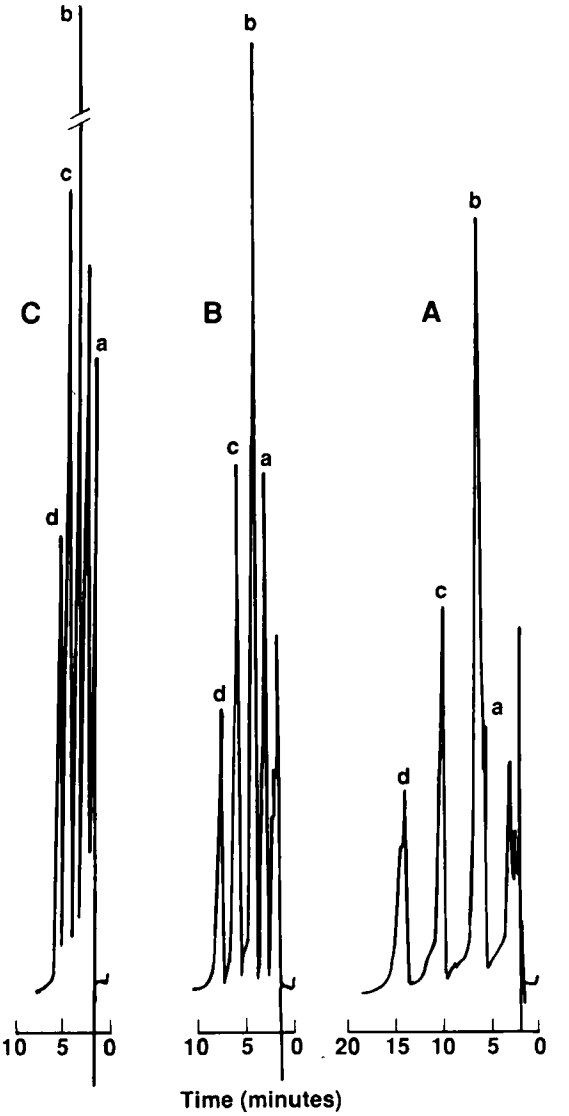



**Figure 3.**

Chromatograms showing the effect of acetonitrile concentration on the hplc of O-n-acylpropranolols.
[Identification of components: a, N-acetylpropranolol; b, propranolol; c, O-acetyl-; d, O-propanoyl-; e, O-butanoyl; f, O-valeroyl; g, O-hexanoyl-propranolol]
{Acetonitrile concentration: A, 45%; B, 65%; C, 70%}
(Mobile phase: aqueous acetonitrile containing diethylamine (0.1%) and 88% orthophosphoric acid (0.1%) adjusted to pH=2.5)

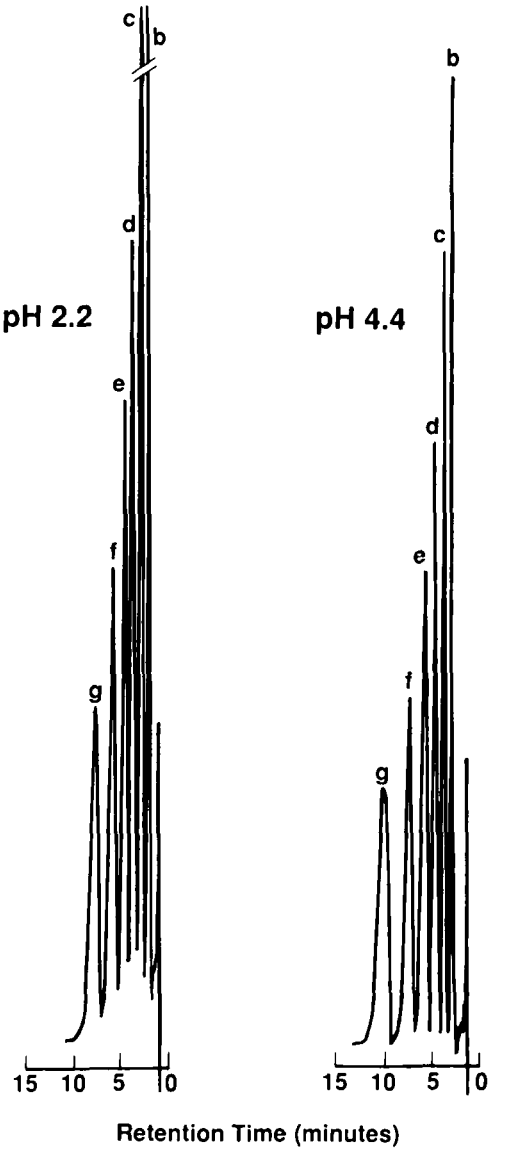



**Figure 4.**
Chromatograms showing the effect of pH on the on the hplc of O-n-acylpropranolols.
[Identification of components: a, N-acetylpropranolol; b, propranolol; c, O-acetyl-; d, O-propanoyl-; e, O-butanoyl; f, O-valeroyl; g, O-hexanoyl-propranolol]
(Mobile phase: 65% acetonitrile containing diethylamine (0.2%) and 88% orthophosphoric acid (0.02-0.2%) to provide pH control).

Table 4. Effect of pH of the mobile phase on the hplc retention times of O-n-acylpropranolols.

| pH | Propranolol Ester Retention Time (min) | | | | | |
|---|---|---|---|---|---|---|
| | Propranolol | $CH_3$ (1) | $C_2H_5$ (2) | $C_3H_7$ (3) | $C_4H_9$ (4) | $C_5H_{11}$ (5) |
| 2.2 | 1.5 | 2.1 | 2.8 | 3.6 | 5.0 | 7.0 |
| 2.7 | 1.8 | 2.5 | 3.1 | 4.1 | 5.8 | 8.0 |
| 3.0 | 1.8 | 2.7 | 3.4 | 4.4 | 6.0 | 8.3 |
| 4.2 | 1.8 | 2.9 | 3.7 | 4.9 | 6.5 | 9.0 |
| 4.6 | 1.8 | 3.0 | 3.8 | 5.0 | 6.6 | 9.3 |

satisfactory linear relationships were obtained for propranolol and the lower esters, deviations were found with longer chain compounds. This behaviour was traced to solubility problems in the sample solvent, even at acidic pH values for these compounds. For example, at pH=3.0, injection of O-pivaloyl- and O-hexanoyl- propranolol in the concentration range 0.06-0.6 mM failed to yield linear calibration curves. As an increasing proportion of dimethylformamide (DMF) was incorporated as a cosolvent into the sample solvent linearity was improved and at 30% DMF linear plots, suitable for assay purposes, were obtained.

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