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PRO-DRUGS AS DRUG DELIVERY SYSTEMS XVII. ESTERS OF 4-HYDROXYBUTYRIC ACIDS AS POTENTIAL PRO-DRUG TYPES FOR γ-LACTONES *

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SUMMARY

The methyl and ethyl ester of 4-hydroxybutyric acid were prepared and evaluated as pro-drug models for the γ-lactone moiety occurring in several drugs such as pilocarpine. The esters were found to undergo a quantitative cyclization in neutral and alkaline aqueous solution at 37°C to γ-butyrolactone. The rates of lactonization were directly proportional to the hydroxide ion activity over the pH range studied (7.4–9.8) and the reactions appear to proceed by an intramolecular nucleophilic attack of alkoxide ion on the ester carbonyl moiety. Lactone formation proceeded rather slowly at pH 7.4 but was greatly accelerated in case of the corresponding phenyl ester according to a previous report. The ester derivatives are more lipophilic than the parent γ-butyrolactone as determined by partition experiments in a L-octanol–water system. It is suggested that esters, in particular aryl esters, of 4-hydroxybutyric acids may be potentially useful pro-drug candidates for agents containing a γ-lactone moiety, e.g. to facilitate biomembrane penetration through increased lipophilicity of the transport forms.

INTRODUCTION

A major problem for the general application of the pro-drug principle to achieve improvements in the delivery characteristics of drug substances is the limited possibilities available for making bioreversible derivatives of many drugs. For a large number of drugs no apparently readily derivatizable functional groups or entities are present in the mole-

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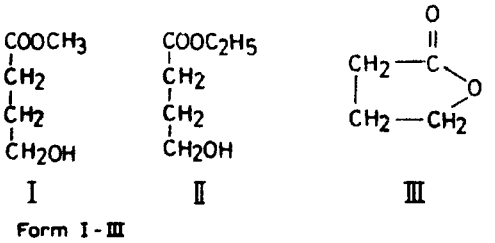
cules and for these none or only few pro-drug forms have been explored.

As a continuation of studies aiming to identify potentially useful transport forms of such not easily derivatizable drug molecules, an investigation was carried out to obtain pro-drug candidates for the γ-lactone moiety. There are several drugs containing this group which exhibit delivery problems, e.g. due to unfavourable solubility or lipophilicity characteristics. Examples of drugs containing a γ-lactone moiety include pilocarpine, spironolactone, canrenone, digoxin and a number of α-methylene-γ-lactone derivatives and sesquiterpene lactones. The basis of the present work was the findings of Capon et al. (1973) that aryl esters of various hydroxy-acids are capable of undergoing a facile lactonization in aqueous solution. Thus, the phenyl ester of 4-hydroxybutyric acid was found to lactonize with a half-time of about 5 min at pH 7.47 and 30°C, suggesting that esters of hydroxy-acids are possible candidates as pro-drug forms of the corresponding γ-lactones. In this paper this concept has been explored further by an examination of the kinetics of cyclization of the methyl (I) and ethyl ester (II) of 4-hydroxybutyric acid to γ-butyrolactone (III) in neutral and basic aqueous solution. Furthermore, the lipophilicity of γ-butyrolactone and its precursors have been determined.



MATERIALS AND METHODS

Apparatus

Ultraviolet spectral measurements were performed with a Zeiss PMQ II spectrophotometer and a Perkin-Elmer 124 recording spectrophotometer, using 1-cm quartz cuvettes. PMR spectra were run on a JEOL C-60-HL instrument using tetramethylsilane as an internal standard. Readings of pH were carried out on a Radiometer Type PHM 26 meter at the temperature of study. High-performance liquid chromatography (HPLC) was done with a Spectra-Physics Model 3500 B instrument equipped with a variable-wavelength UV detector (8-μl 1-cm flow cells) and a 10-μl loop injection valve. The detector was connected to a Servogor RE 541 potentiometric recorder. The column used, 10 cm long and 4.7 mm i.d., was packed with LiChorsorb RP-8 (5 μm particles). Thin-layer chromatography (TLC) was done on precoated 0.25 mm silica gel 60 F_{254} glass plates (E. Merck, G.F.R.). Microanalysis was carried out at the Microanalytical Department of Chemical Laboratory II, University of Copenhagen.

Chemicals

γ-Butyrolactone and sodium 4-hydroxybutyrate were purchased from Fluka AG, Switzerland. All other chemicals and solvents used were of reagent grade.

Preparation of methyl 4-hydroxybutyrate

This ester was prepared by acid-catalyzed alcoholysis of γ-butyrolactone, cf. Brown and Keblys (1966). A solution of γ-butyrolactone (20.0 g, 0.23 mol) in 100 ml of methanol was treated with 0.3 ml of concentrated sulphuric acid and stirred for 16 h at room temperature. At this time no further disappearance of γ-butyrolactone was observed as determined by TLC (silica gel; dichloromethane – ethyl acetate 1 : 2, visualization : iodine vapours). Then 3 g of calcium carbonate was added, and stirring was continued for 1 h. After filtration, excess methanol was evaporated in vacuo. The remaining solution was diluted with 100 ml of ether, washed with 15 ml of 1% sodium carbonate solution, 15 ml of water, and 15 ml of saturated sodium chloride solution. After drying over anhydrous sodium sulphate the ether was evaporated in vacuo. The remaining material consisting of a mixture of γ-butyrolactone and methyl 4-hydroxybutyrate as observed by TLC was diluted with 5 ml of a dichloromethane–ethyl acetate (1 : 2) mixture and transferred onto a column containing 400 g of silica gel (Silica Woelm 63–100 μm) pre-equilibrated with the solvent. The sample was eluted through the column with dichloromethane–ethyl acetate mixture at a rate of about 2 ml min^{-1}. The eluate fractions which contained pure methyl 4-hydroxybutyrate as determined by TLC were pooled and evaporated in vacuo leaving a liquid which was purified by vacuum distillation to give 21 g (80% yield) of ester: bp 53–54°C at 0.3 mm. The PMR-spectrum (in $CdCl_3$) was in agreement with the structure of the ester. Anal.: calcd. for $C_5H_{10}O_3$: C, 50.85; H, 8.54 – found: C, 51.02; H, 8.61.

Preparation of ethyl 4-hydroxybutyrate

This ester was prepared from γ-butyrolactone and ethanol by the procedure described above and isolated in 76% yield: bp 56–57°C at 0.4 mm. Anal.: calcd. for $C_6H_{12}O_3$: C, 54.33; H, 9.15 – found: C, 54.68; H, 9.29.

Kinetic measurements

All kinetic experiments were carried out in aqueous solutions at a constant pH and at 37.0 ± 0.1°C. The rates of lactonization of the esters were determined using either HPLC or UV-spectrophotometry.

Reactions followed by direct UV-spectrophotometry were performed in 2.5 ml aliquot portions of borate buffer solutions (pH 8.8–9.8) in a thermostatted quartz cuvette and were initiated by adding 5–8 μl of the esters to give a final concentration of about 0.02 M. The reaction progress was followed by recording the decrease in absorbance at 210 nm as a function of time. Pseudo-first-order rate constants were determined from linear plots of log $(A_t - A_\infty)$ against time, where A_t and A_∞ are the absorbance readings at time t and at infinity, respectively.

For HPLC, a solvent system of 10% (v/v) methanol in water was used. The flow rate was 1.6 ml min^{-1} and the column effluent was monitored at 215 nm. Under these conditions the methyl ester, the ethyl ester and γ-butyrolactone had an elution time of 3.1, 7.7 and 1.5 min, respectively, while sodium 4-hydroxybutyrate was eluted with the solvent front. Quantitation of the esters and the lactone was done from measurement of the peak heights in relation to those of standards chromatographed under the same conditions. In the kinetic runs an accurately weighed sample of 4-hydroxybutyrate ester (about 100 mg)

was dissolved in 40 ml of water pre-equilibrated at 37°C. The solution was kept at 37°C in a pH-stat to maintain constant pH and aliquots were removed at suitable intervals and chromatographed. First-order rate constants for the lactonization were determined from the slopes of linear plots of the logarithm of residual ester against time.

Measurement of partition coefficients

The partition coefficients of γ-butyrolactone and the 4-hydroxybutyrate esters were determined in a L-octanol–water system as previously described (Bundgaard et al., 1979). The initial concentrations of the compounds in the aqueous phase were 0.02–0.04 M. The solute concentrations in this phase were determined spectrophotometrically at 210 nm by reference to standard curves obtained with octanol-saturated water as solution medium.

RESULTS AND DISCUSSION

Lactonization of the methyl and ethyl 4-hydroxybutyrate esters

In neutral and basic aqueous solution the methyl and ethyl esters of 4-hydroxybutyric acid were found to undergo a quantitative cyclization to γ-butyrolactone as evidenced by the following experiments: (i) aqueous solutions of the esters (2.5 mg ml^{-1}) were kept at 37°C in a pH-stat (pH 7.4–9.2), and at appropriate times 10-μl portions were subjected to HPLC using the assay conditions described above. The disappearance of the peaks from the esters were observed to be accompanied by the progressive appearance of a peak with

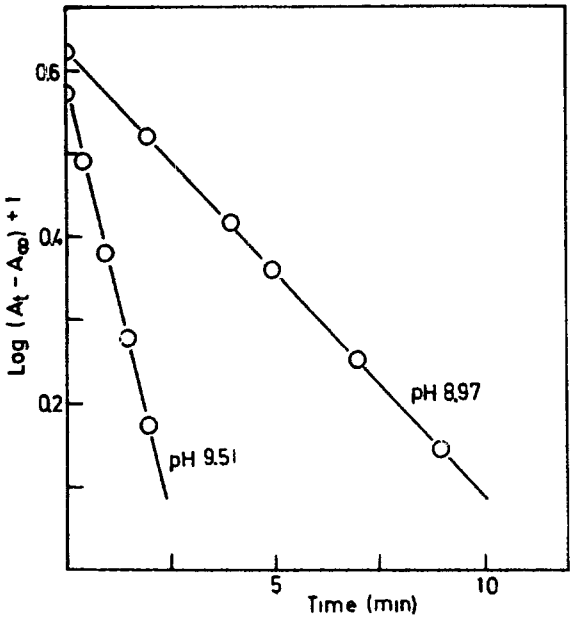


Fig. 1. First-order plots for the cyclization of methyl 4-hydroxybutyrate to γ-butyrolactone in aqueous buffer solutions at 37°C. The rate data were obtained by the direct UV-spectrophotometric method.

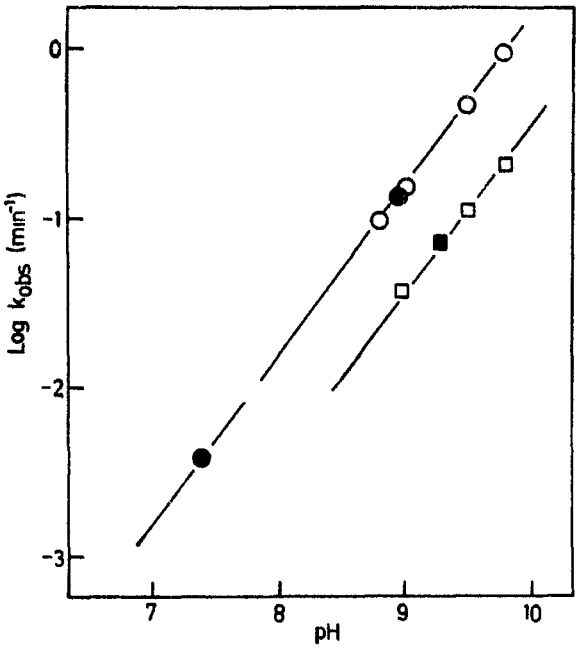


Fig. 2. pH–rate profiles for the cyclization of methyl 4-hydroxybutyrate (○) and ethyl 4-hydroxybutyrate (□) to γ-butyrolactone in aqueous solutions at 37°C. The filled symbols represent data obtained by the HPLC method and the open symbols data obtained by direct UV-spectrophotometry.

a retention time identical to that for γ-butyrolactone. No other peaks were observed and quantitative measurements indicated 100% conversion of the esters to the lactone; (ii) upon standing of aqueous borate buffer solutions (pH 8.8–9.8) of the esters (0.015 M) at 37°C, the UV-absorption at 205–210 nm gradually decreased, ending up to correspond to that of γ-butyrolactone in equimolar concentration.

Kinetics and mechanism of the lactonization

The kinetics of the lactonization of the 4-hydroxyburyate esters to γ-butyrolactone was studied at 37°C in the pH range 7.4–9.8. At constant pH and temperature strict first-order kinetics was observed for more than 3–4 half-lives. Some typical first-order plots are shown in Fig. 1. The values of the pseudo-first-order rate constants (k_{obs}) derived using the HPLC-method and direct UV-spectrophotometry were in favourable agreement (Fig. 2). Using the latter method the rates of lactonization were found to be independent of borate buffer concentration from 0.04 to 0.12 M at constant ionic strength (0.5 with potassium chloride).

In the pH range investigated, the observed pseudo-first-order rate constants were found to be directly proportional to the hydroxide ion activity as demonstrated in Fig. 2 in which plots of log k_{obs} against pH produced straight lines with slopes of 1.0. Thus Eqn. 1 is valid:

$$k_{obs} = k_1 a_{OH} \quad (1)$$

where a_{OH} refers to the hydroxide ion activity. This was calculated from the measured pH

TABLE 1

SECOND-ORDER RATE CONSTANTS (k_1) FOR THE APPARENT SPECIFIC BASE-CATALYZED LACTONIZATION OF METHYL AND ETHYL 4-HYDROXYBUTYRATE IN AQUEOUS SOLUTION AT 37°C AND HALF-TIMES OF LACTONIZATION ($t_{1/2}$) AT pH 7.40 AND 37°C.

Compound	k_1 (M^{-1} min^{-1})	$t_{1/2}$ (h)
Methyl 4-hydroxybutyrate	6.0×10^3	3.2
Ethyl 4-hydroxybutyrate	1.4×10^3	13.7 *

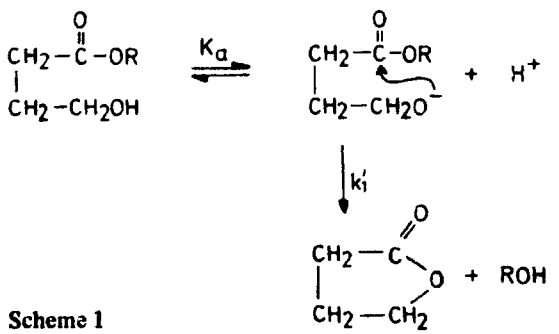
* Calculated from Eqn. 1 and the experimentally determined value of k_1.

(at 37°C) according to the following equation (Harned and Hamer, 1933):

$$\log a_{OH} = pH - 13.62 \quad (2)$$

The values of the apparent hydroxide ion catalytic rate constants, k_1, for the lactonization of the esters are listed in Table 1 together with half-times for the reactions at pH 7.4 and 37°C.

The lack of significant catalysis by borate ions along with the occurrence of the apparent hydroxide ion catalysis suggests that the lactonization of the alkyl esters proceeds via pre-equilibrium ionization of the hydroxy group as depicted in Scheme 1. The pK_a value of the hydroxy group might be expected to be about the same as that of ethanol, 15.9 (Ballinger and Long, 1960), which agrees with the observed linearity



Scheme 1

between k_{obs} and a_{OH} up to pH 9.8. According to the suggested mechanism, Eqn. 1 should be written as (for $a_H >> K_a$):

$$k_{obs} = k_1' \cdot \frac{K_a}{a_H} \quad (3)$$

where k_1' represents a first-order rate constant for the intramolecular attack of alkoxide ion on the ester carbonyl moiety. Assuming a value of $10^{-15.9}$ for the acid ionization constant of the hydroxy group, values of k_1' of 12×10^5 and 2.8×10^5 min^{-1} can be calculated for the methyl and ethyl ester, respectively.

The efficiency of the intramolecular alcoholysis may be estimated by comparing the

rate constants k_1 with the rate constants (k_{OH}) for specific base catalyzed hydrolysis of the ester moiety. From data given by Blackburn and Dodds (1974) k_{OH} for hydrolysis of methyl acetate at 37°C is 34 M^{-1} min^{-1}. Therefore, k_1 for methyl 4-hydroxybutyrate is approximately 200 times greater, showing the large dominance of intramolecular reaction over intermolecular hydrolysis. The alkaline hydrolysis of γ-butyrolactone proceeds with a k_{OH} value of 148 M^{-1} min^{-1} at 37°C (Blackburn and Dodds, 1974) which is 10–40 times lower than the corresponding k_1 values for the hydroxybutyrate esters.

As expected aryl esters of 4-hydroxybutyrate are more reactive than the alkyl esters. For phenyl 4-hydroxybutyrate Capon et al. (1973) have reported a value of 6.4×10^5 M^{-1} min^{-1} for the apparent hydroxide ion-catalyzed lactonization at 50°C. At 30°C and pH 7.47 a half-life of 4.8 min was determined.

A few precedents exist of intramolecular nucleophilic displacement reactions by alcoholic hydroxy groups at the carbonyl moiety of esters with poor leaving groups. Thus, ethyl 2-hydroxymethylbenzoate has been described to undergo a base-catalyzed cyclization to phthalide in aqueous solution (Fife and Benjamin, 1973, 1976). Similarly, intramolecular alcoholysis by neighboring alkoxide ions of a carbamate ester has been observed for the ethyl esters of 2-hydroxymethylcarbanilic acid and 2-hydroxymethyl-N-methylcarbanilic acid (Hutchins and Fife, 1973). The efficiency of the intramolecular alcoholysis in these compounds are several orders greater than for the 4-hydroxymethyl butyrates which may be attributed to the greater degree of rigidity in the 2-hydroxymethyl derivatives.

The lipophilicity of γ-butyrolactone and its precursors

The partition coefficients (P) for γ-butyrolactone and the 4-hydroxybutyrate esters were measured using the widely used L-octanol–water system. The values found for log P were: –0.31 (γ-butyrolactone), –0.06 (methyl 4-hydroxybutyrate) and 0.43 (ethyl 4-hydroxybutyrate). The log P values for the esters are in excellent agreement with values calculated on basis of π substituent values (Leo et al., 1971). The results show that the alkyl esters are more lipophilic than γ-butyrolactone. From the values for the esters it may be possible to predict the lipophilicity of other ester derivatives. Thus, the phenyl ester of 4-hydroxybutyric acid should have an estimated log P value of 1.21 (calculated from $\pi = 0.5$ for a methylene group and $\pi = 1.77$ for the phenyl group).

Consideration of hydroxy-esters as pro-drugs for γ-lactone derivatives

The observed rates of lactonization of the methyl and ethyl esters of 4-hydroxybutyric acid at physiological conditions of pH and temperature (Table 1) appear to be too low for considering the esters as potentially useful pro-drug forms for γ-butyrolactone. According to the given mechanism of cyclization the rates might not be expected to change in vivo from the values described here. The present study shows, however, that in principle, esterification of the acid group in hydroxy-acids may be a method of obtaining pro-drug forms of γ-lactones. Esters more reactive than the methyl ester may be selected in order to increase the rate of lactonization, and as appearing from the rate data for cyclization of phenyl 4-hydroxybutyrate (Capon et al., 1973) phenyl esters should be a good choice for ensuring a rapid formation in vivo of the γ-lactone. It should be noted that steric effects exhibited by various substituents in the hydroxy-esters certainly may have an

influence on reaction rate although this remains to be investigated.

The observed increased lipophilicity of esters of 4-hydroxybutyric acid in comparison with the corresponding lactone may become advantageous in situations where delivery problems for lactone drugs are due to low lipophilicity. For example, the very low ocular bioavailability of topically applied pilocarpine has been attributed in part to resistance to corneal penetration and hence to the low lipophilicity of the drug (Lee and Robinson, 1979). As suggested by these authors a more lipophilic pro-drug derivative might be expected to result in improved bioavailability of topically applied pilocarpine. On basis of the present data a phenyl ester of pilocarpinic acid may possibly appear to be a suitable pro-drug candidate with enhanced delivery properties.

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