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PRO-DRUGS AS DRUG DELIVERY SYSTEMS VIII. BIOREVERSIBLE DERIVATIZATION OF HYDANTOINS BY N-HYDROXYMETHYLATION *

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SUMMARY

The kinetics and mechanism of decomposition of the N_3-hydroxymethyl derivatives of various hydantoins (phenytoin, nitrofurantoin and 5,5-dimethylhydantoin) in aqueous solution at 37°C was studied to assess their potential utility as pro-drugs for the parent substances. The derivatives were found to undergo an apparent hydroxide ion-catalyzed decomposition in the pH range 3.3–6.1, the specific rate constants being 7.1×10^7, 6.2×10^8 and 1.0×10^7 M^{-1} min^{-1} for the phenytoin, nitrofurantoin and dimethylhydantoin derivative, respectively. This difference in decomposition rates was correlated with the difference in the acidity of the parent hydantoins and the rate data for the hydantoins were shown to fit well with a rate–acidity relationship previously derived for a number of N-hydroxymethylated amides and imides. The N-hydroxymethyl hydantoins are very rapidly cleaved to formaldehyde and the parent compounds at pH 7.4 and 37°C (half-lives calculated to range from 0.1 to 6.9 s). The derivatives were shown to possess higher water solubilities than the parent compounds and it is suggested that N-hydroxymethylation may be a potentially useful means of obtaining pro-drug forms of hydantoins. In addition, it was demonstrated by determination of the hydrolysis kinetics of the acetate ester of 3-(hydroxymethyl)phenytoin that the N-hydroxymethylated hydantoins are amenable to further bioreversible derivatization.

INTRODUCTION

In a previous study (Johansen and Bundgaard, 1979), a number of N-hydroxymethyl derivatives of various amides and imides were shown to decompose quantitatively to

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formaldehyde and parent compound in neutral and basic aqueous solution. The decomposition showed a first-order dependence on hydroxide ion concentration up to pH about 12, and the rates increased sharply with increasing acidity of the parent compound. For amides and imides with a pK_a of less than 13.1, a half-life for the conversion of less than one hour at pH 7.4 and 37°C was predicted. The N-hydroxymethyl derivatives were found to be more water-soluble than the parent compounds, suggesting the potential utility of N-hydroxymethylation as a means of obtaining pro-drug forms of various amides or imides with, e.g., improved solubility and dissolution characteristics.

This study has been extended to include various hydantoins and the present paper reports on the kinetics and mechanism of decomposition of N_3-hydroxymethyl derivatives of phenytoin, 5,5-dimethylhydantoin and nitrofurantoin in aqueous solution at 37°C as well as on the aqueous solubilities of the derivatives. To explore further the possibilities of N-hydroxymethylation in making pro-drugs, the hydrolysis kinetics for the acetate ester of N_3-(hydroxymethyl)phenytoin was also studied.

MATERIALS AND METHODS

Apparatus

Ultraviolet and visible spectral measurements were performed with a Zeiss PMQ II spectrophotometer and a Perkin-Elmer 124 recording spectrophotometer, using 1 cm cuvettes. 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 LiChrosorb RP-8 (5 μm particles).

Chemicals

The N_3-hydroxymethyl derivatives of phenytoin and 5,5-dimethylhydantoin were prepared by refluxing a mixture of 0.02 mole of the hydantoins, 20 ml of 96% ethanol and 0.06 mole of formaldehyde (in form of a 37% aqueous solution) for 30 min as described by Zejc (1968). The products crystallized after partial removal of the solvent in a hood, m.p. 199–200°C (3-(hydroxymethyl)phenytoin), rep. m.p. 198–199°C (Zejc, 1968); m.p. 121–122°C (3-hydroxymethyl-5,5-dimethylhydantoin), rep. m.p. 117–119°C (Winstead et al., 1965). The N_3-hydroxymethyl derivative of nitrofurantoin was made according to the procedure described by Spencer and Michels (1964) and the acetate ester derivative of 3-(hydroxymethyl)phenytoin, 3-acetoxymethyl-5,5-diphenylhydantoin, as described by Vida et al. (1971), m.p. 160–161°C, rep. m.p. 162–163°C. Buffer substances and all other chemicals and solvents used were of reagent grade.

Kinetic measurements

All rate studies were performed in aqueous buffer solutions at 37.0 ± 0.2°C. The buffers used were formate, acetate, phosphate, borate, carbonate and sodium hydroxide solutions. A constant ionic strength (μ) of 0.5 was maintained for each buffer by adding a calculated amount of potassium chloride.

The rates of decomposition of the N-hydroxymethyl derivatives were measured by trapping the formaldehyde formed with semicarbazide and following the increase in absorbance of the semicarbazone at 235 nm. Semicarbazide hydrochloride was included in the buffer solutions at a concentration of 10^{-2} M and was used in the pH range 3.3–6.1. The concentration of the trapping reagent (and pH range) was such that there was no induction period in the observed pseudo-first-order rate plots, i.e. trapping of formaldehyde was fast relative to formaldehyde formation. It was checked that the semicarbazide had no influence on the reaction rate in the concentration range 0.5 to 2×10^{-2} M. The initial N-hydroxymethyl derivative concentrations were about 2×10^{-4} M and the reactions were performed either directly in a thermostated cuvette or in flasks kept in a water bath. In the last case aliquot portions were withdrawn at appropriate intervals and the absorbance was read at 235 nm. Pseudo-first-order rate constants were determined from plots of log $(A_\infty - A_t)$ against time, where A_∞ and A_t are the aborbance readings at infinity and at time t, respectively. This procedure involving trapping of formaldehyde was previously used to determine the rates of decomposition of the N-hydroxymethyl derivatives of succinimide and chlorzoxazone (Johansen and Bundgaard, 1979).

The rate of decomposition of N_3-(hydroxymethyl)phenytoin was also determined by using a reversed-phase high-performance liquid chromatographic procedure which enabled separation and simultaneous quantitation of the hydroxymethyl derivative and phenytoin. The chromatographic conditions are given in the legend to Fig. 1. An accurately weighed sample of N_3-(hydroxymethyl)phenytoin (about 30 mg) was dissolved in 10 ml of acetonitrile and an aliquot of 0.5 ml was added to 25 ml of aqueous buffer solution pre-equilibrated at 37°C. The solution was kept at 37°C and aliquots were removed at suitable intervals and chromatographed. The content of the hydroxymethyl derivative and phenytoin in the sample injected was determined by comparing their peak heights with those of standards chromatographed under similar conditions.

The rate of hydrolysis of 3-acetoxymethyl-5,5-diphenylhydantoin was determined in the pH range 7.5–12.5 using either HPLC or UV spectrophotometry.

For HPLC, a solvent system of 55% aqueous methanol containing 0.5% acetic acid was used. The flow rate was 1.2 ml min^{-1} and the column effluent was monitored at 230 nm. Under these conditions the acetate ester had an elution time of 4.7 min while that for phenytoin was 3.0 min. Quantitation of the two compounds was done from measurement of the peak heights in relation to those of standards chromatographed under the same conditions. The initial concentration of the acetate ester in the reaction solutions was about 0.1 mg ml^{-1}. First-order rate constants for the hydrolysis were determined from the slopes of linear plots of the logarithm of residual ester against time.

Reactions followed by direct UV-spectrophotometry were performed in 2.5 ml aliquot portions of buffer solution in a thermostated quartz cuvette and were initiated by adding 50 μl of a stock solution of the ester in acetonitrile to give a final concentration of about 0.1 mg ml^{-1}. The reaction progress was followed by recording the increase in absorbance at 240 nm as a function of time. Rate constants were determined from plots of log $(A_\infty - A_t)$ against time.

Solubility determinations

The solubility of the hydroxymethyl derivatives of phenytoin and nitrofurantoin and the parent compounds in 0.1 M hydrochloric acid was determined by placing an excess of the compounds in 25 ml of the solvent. The mixtures were rotated for 24 h (where equilibrium was reached) at 22°C and filtered. For phenytoin and its hydroxymethyl derivative the absorbance of the filtrate was read at 255 nm. The saturated solutions of nitrofurantoin or its hydroxymethyl derivative were diluted with 0.1 M hydrochloric acid 10- and 20-fold, respectively, and the absorbances measured at 265 nm. The concentration of the compounds in the saturated solutions was finally calculated from the measured absorbances by reference to standard curves.

RESULTS AND DISCUSSION

Kinetics and mechanism of decomposition of N-hydroxymethylhydantoins

The kinetics of breakdown of the N-hydroxymethylated hydantoins were studied in aqueous solution at 37°C over the pH range 3.3–6.1. At constant pH and temperature the reactions displayed good first-order kinetics over more than 4 half-lives, and in all kinetic runs, formaldehyde was liberated in stoichiometric amounts as determined from the absorbance of the semicarbazone formed. Furthermore, as seen from Fig. 1, HPLC analysis of a reaction solution of N-(hydroxymethyl)phenytoin showed a continuous formation of phenytoin in quantitative amounts. The values of the pseudo-first-order rate constant (k_{obs}) derived using the HPLC method and the method of formaldehyde

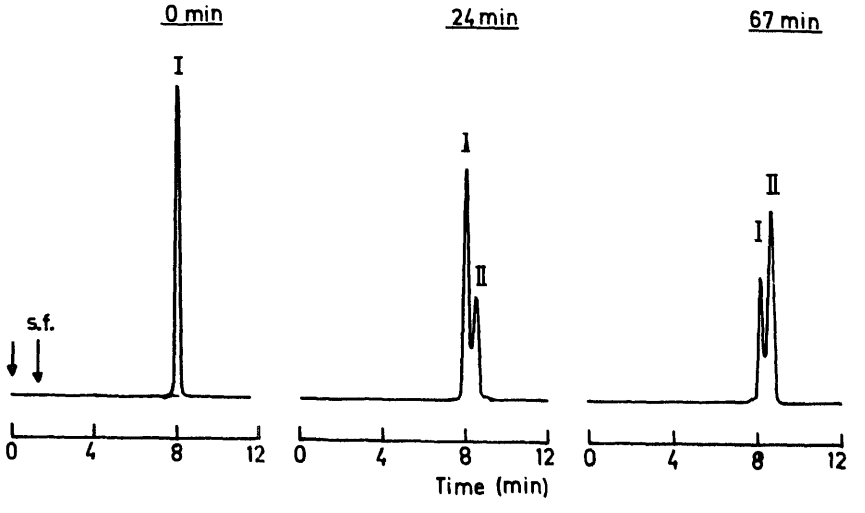


Fig. 1. High-performance liquid chromatographic traces of the degradation of N-(hydroxymethyl)-phenytoin (0.06 mg ml^{-1}) in 0.1 M acetate buffer solution pH 4.03 at 37°C. A 10 µl sample of the solution was chromatographed at the times indicated. Column: LiChrosorb RP-8; eluent: acetic acid–methanol–water (0.5 : 45 : 55); flow rate: 1.2 ml min^{-1}; temperature: ambient; detection: UV at 230 nm, 0.04 a.u.f.s. Peak identities: I, 3-(hydroxymethyl)phenytoin; II, phenytoin.

TABLE 1

EFFECT OF VARYING BUFFER CONCENTRATION ON PSEUDO-FIRST-ORDER RATE CONSTANTS FOR THE DECOMPOSITION OF N_3-HYDROXYMETHYL DERIVATIVES OF SOME HYDANTOINS AT 37°C (μ = 0.5)

Buffer		pH	k_{obs} (min^{-1})	
			3-(hydroxymethyl)-phenytoin	3-(hydroxymethyl)-nitrofurantoin
Acetate	0.02 M	4.88	0.148	4.70
Acetate	0.05 M	4.88	0.150	4.60
Acetate	0.10 M	4.88	0.150	4.79
Phosphate	0.05 M	6.09	2.05	
Phosphate	0.10 M	6.09	2.02	

trapping were in favourable agreement (Fig. 2), thus confirming the utility of the latter for rate determinations.

The rates of decomposition were found to be independent of buffer concentration from 0.02–0.2 M at constant ionic strength (Table 1). Thus no general acid–base catalysis appears to be involved.

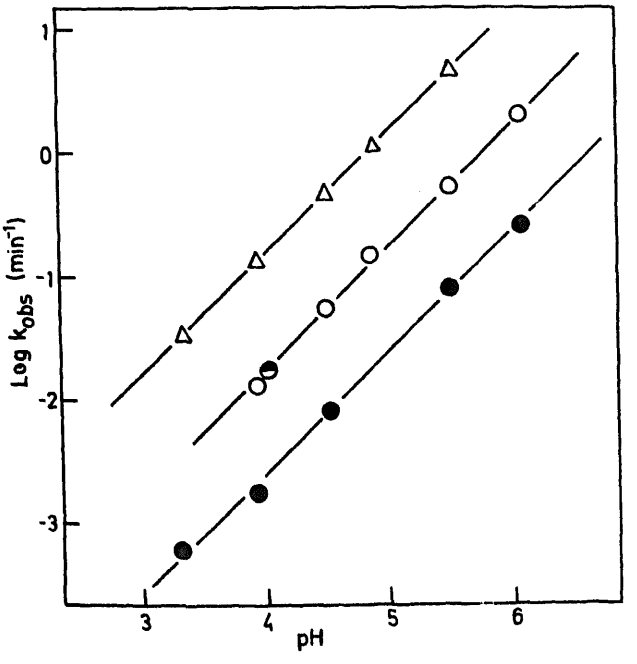


Fig. 2. Plots of the logarithm of the observed pseudo-first-order rate constants against pH for the decomposition of the N_3-hydroxymethyl derivatives of nitrofurantoin (△), phenytoin (○) and 5,5-dimethylhydantoin (●) in aqueous solution (μ = 0.5; 37°C). The symbol • represents rate data obtained by the HPLC method.

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:

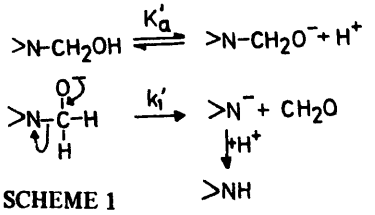
$$k_{obs} = k_1 a_{OH} \quad (1)$$

where a_{OH} refers to the hydroxide ion activity. This was calculated from the measured pH as described previously (Bundgaard et al., 1979). The values of the apparent hydroxide ion catalytic rate constants, k_1, for the decomposition of the N-hydroxymethyl hydantoins studied are listed in Table 2 together with half-times for the reactions at pH 7.4 and 37°C.

In a previous study on the decomposition of various N-hydroxymethylated amides and imides (Johansen and Bundgaard, 1979), a reaction mechanism involving a stepwise pathway with anionic N-hydroxymethyl amide or imide as an intermediate undergoing rate-determining N–C bond cleavage was proposed and shown to account for the levelling-off of the decomposition rates in strongly basic solutions (pH > 12.5) where ionization of the N-hydroxymethyl group becomes appreciable. According to this mechanism (Scheme 1) the rate law should be written as:

$$k_{obs} = k_1' \frac{K_a'}{a_H + K_a'} \quad (2)$$

where K_a' is the ionization constant of the N-hydroxymethyl derivatives and k_1' is a first-



SCHEME 1

TABLE 2

SECOND-ORDER RATE CONSTANTS FOR THE APPARENT SPECIFIC BASE-CATALYZED DECOMPOSITION OF N_3-HYDROXYMETHYL DERIVATIVES OF VARIOUS HYDANTOINS IN AQUEOUS SOLUTION (μ = 0.5) AT 37°C AND ESTIMATED HALF-LIVES AT pH 7.40

Compound	k_1 (M^{-1} min^{-1})	$t_{1/2}$ [a] (sec)
3-Hydroxymethyl-5, 5-dimethylhydantoin	1.0×10^7	6.9
3-(Hydroxymethyl)phenytoin	7.1×10^7	1.0
3-(Hydroxymethyl)nitrofurantoin	6.2×10^8	0.1

[a] At pH 7.40 and 37°C. The figures were calculated from Eqn. 1 and the experimentally determined values of k_1.

order rate constant for cleavage of ionized N-hydroxymethyl compound. In the investigated pH range, $a_H >> K'_a$ and Eqn. 2 is reduced to:

$$k_{obs} = k'_1 \frac{K'_a}{a_H} \tag{3}$$

or

$$k_{obs} = k'_1 \frac{K'_a}{K_W} a_{OH} \tag{4}$$

where K_W is the antoprotolysis constant of water. Eqn. 4 is of the same form as Eqn. 1 and the mechanism depicted in Scheme 1 may accordingly also be involved in the decomposition of N-hydroxymethyl hydantoins. The pK'_a of N-(hydroxymethyl)benzamide has been determined to be 13.1 at 37°C (Johansen and Bundgaard, 1979), and it is to be expected that the pK_a of the N-hydroxymethyl hydantoins is not more than a few units lower. As K'_a was not determinable, k'_1 cannot be calculated.

Structure–reactivity relationship

The major structural effect influencing the rate of decomposition of N-hydroxymethyl amides and imides has been shown to be the stability of the leaving group, e.g. expressed

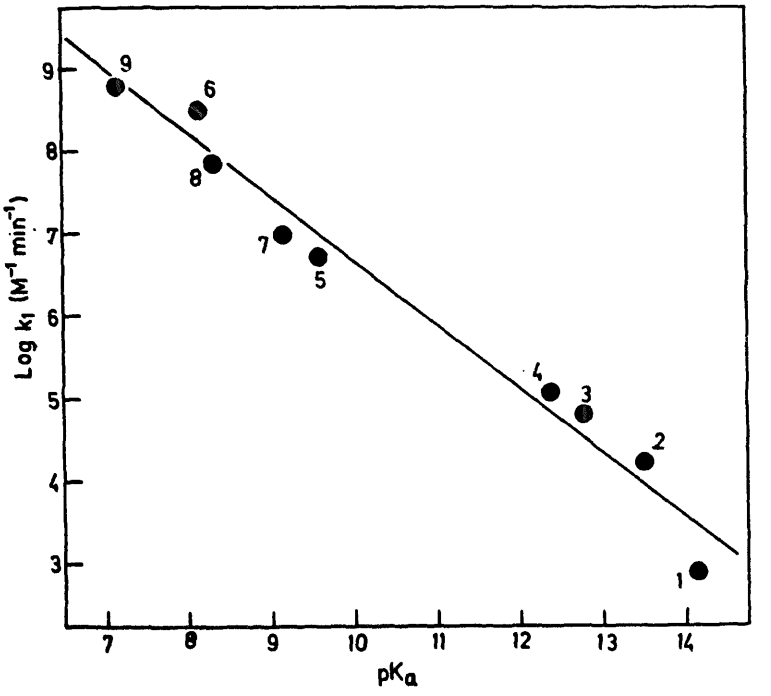


Fig. 3. Plot of the logarithm of the second-order rate consants, k_1, for decomposition of various N-hydroxymethyl derivatives against pK_a of the parent compounds. Key: 1, chloroacetamide; 2, dichloroacetamide; 3, thiobenzamide; 4, trichloroacetamide; 5, succinimide; 6, 5-chloro-2-benzoxazolinone (chlorzoxazone); 7, 5,5-dimethylhydantoin; 8, phenytoin; and 9, nitrofurantoin. The data for compounds 1–6 were from a previous study (Johansen and Bundgaard, 1979) while those for the hydantoins are from this study.

In terms of the acidity of the parent amide of imide (Johansen and Bundgaard, 1979). A linear correlation was found between log k_1 and pK_a for several carboxamides, thiobenzamide, succinimide and a carbamate (chlorzoxazone) and as shown in Fig. 3 the rate data for the N-hydroxymethyl hydantoins obtained in this study fit well with this correlation. The regression equation ($r = 0.986$) between log k_1 and pK_a including all the compounds is given by Eqn. 5:

$$\log k_1 = -0.77\ pK_a + 14.4\ (n = 9) \quad (5)$$

The pK_a values used for the hydantoins were 7.2 for nitrofurantoin (Chen et al., 1976), 8.33 for phenytoin (Agarwall and Blake, 1968) and 9.19 for 5,5-dimethylhydantoin (Zief and Edsall, 1937).

This structure–reactivity relationship is fully compatible with the proposed reaction mechanism since increasing acidity of the parent >NH compound affords an increase in the leaving ability of the nitrogen anion and hence a facilitation of the unimolecular N–C bond cleavage. Since the relationship apparently holds for a variety of nitrogen compounds amenable to N-hydroxymethylation it may be useful for the prediction of the reactivity of an N-hydroxymethyl derivative solely from a knowledge of the pK_a of the parent compound.

The solubility of N-hydroxymethyl hydantoins

The water solubility of the N-hydroxymethyl derivatives of phenytoin and nitrofurantoin were measured and compared to those of the parent hydantoins. The results given in Table 3 show that N-hydroxymethylation affords a 3–4-fold increase in solubility which is in the same range as observed for N-hydroxymethylated benzamides (Johansen and Bundgaard, 1979). The solubility of the hydroxymethyl derivative of nitrofurantoin in 1% formaldehyde solutions has previously been described to be about 4 times as great as that of the parent compound (Spencer and Michels, 1964). The increase in solubility by substitution of the acidic N–H protons in the hydantoins by CH_2OH may at least in part be attributed to decreased intermolecular hydrogen bonding in the crystal lattice. Thus for phenytoin the melting point drops from 295 to 200°C upon N_3-hydroxymethylation.

TABLE 3

WATER SOLUBILITY OF N_3-HYDROXYMETHYL HYDANTOINS AND THEIR PARENT COMPOUNDS AT 22°C

Compound	Solubility (mg/ml) [a]
Phenytoin	0.032
3-(Hydroxymethyl)phenytoin	0.14
Nitroflurantoin	0.11
3-(Hydroxymethyl)nitroflurantoin	0.33

[a] Determined in 0.1 M hydrochloric acid.

N-Hydroxymethyl derivatives as pro-drugs

The results of the present study show that the N-hydroxymethylated hydantoins are were rapidly cleaved to formaldehyde and the parent compounds at neutral pH and 37°C and accordingly, they fulfil the requirements for acting as pro-drugs of the parent drug substances. In fact, the N-hydroxymethyl derivative of 5,5-dimethylhydantoin has long been utilized as a preservative in various cosmetic formulations, the effect being attributed to the formation of formaldehyde upon decomposition (Cohen, 1957; Myddleton, 1960). Similarly the N-hydroxymethyl derivative of nitroflurantoin has been used clinically as an antibacterial agent for several years, but only very recently was the compound shown to be a pro-drug of nitrofurantoin (Sorel and Roseboom, 1979). Using an HPLC procedure, these investigators showed the rapid formation of nitrofurantoin from the derivative in aqueous solution to be in agreement with the results of the present study.

As demonstrated by the solubility data, N-hydroxymethylation may be a useful approach to obtain hydantoin pro-drugs with improved aqueous solubility and hence with potentially improved absorption characteristics. The N-hydroxymethyl derivatives are reasonably stable in acidic aqueous solutions corresponding to conditions encountered in the stomach and are cleaved immediately at neutral pH.

Besides having possibilities as pro-drugs per se, the N-hydroxymethylated derivatives are readily amenable to bioreversible derivatization, e.g. by esterification of the hydroxy group introduced and hence making it possible to modify the physicochemical properties of the parent drugs further (cf. Stella, 1977). In the present study the hydrolysis kinetics for the acetate ester of N-(hydroxymethyl)phenytoin was studied in neutral and basic

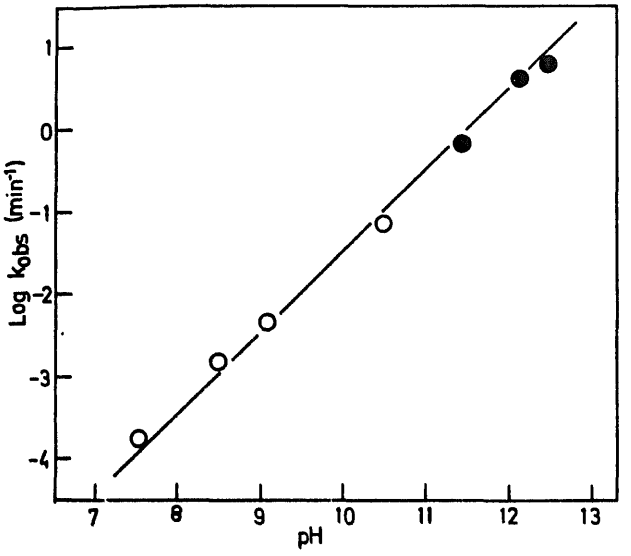
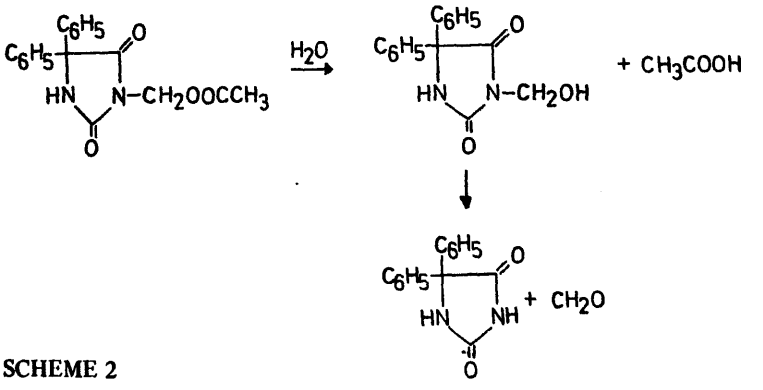


Fig. 4. pH-rate profile for the hydrolysis of 3-acetoxymethyl-5,5-diphenylhydantoin in aqueous solution (μ = 0.5; 37°C). The rate constants were determined by HPLC (○) and UV-spectrophotometry (●).



SCHEME 2

aqueous solution. Using an HPLC procedure, phenytoin was shown to be formed in quantitative amounts upon hydrolysis. The hydrolysis, passing through N-(hydroxymethyl)phenytoin as an intermediate (Scheme 2), showed specific base catalysis in the pH range 7.5–12.5 (Fig. 4):

$$k_{obs} = k_{OH}\, a_{OH} \qquad (6)$$

where the second-order specific base catalytic rate constant, k_{OH}, has a value of 140 M^{-1} min^{-1} at 37°C and $\mu = 0.5$. This rate of alkaline hydrolysis is of the same magnitude as that for the acetate ester of phenol (Bruice et al., 1967). Thus, esterification of the hydroxy group of an N-hydroxymethyl derivative affords a means to stabilize the derivative in vitro, and by appropriate selection of the acid moiety it may represent a useful approach to obtaining pro-drugs of hydantoins with varying physicochemical properties.

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