Vitamin K Prodrugs: 1. Synthesis of Amino Acid Esters of Menahydroquinone-4 and Enzymatic Reconversion to an Active Form

Jiro Takata,[1] Yoshiharu Karube,[1,4] Mitsunobu Hanada,[1] Kazuhisa Matsunaga,[1] Yoshikazu Matsushima,[2] Toshiaki Sendo,[3] and Toshinobu Aoyama[3]

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The efficacy and toxicity of vitamin K depends on the pathway and the extent of enzymatic reductive activation to vitamin K hydroquinone, which is an essential cofactor for the synthesis of clotting factors. Parenteral use of vitamin K is impaired by its water insolubility. With the aim to improve delivery problems associated with menahydroquinone-4 (MKH, **2**), an active form of menaquinone-4, *N,N*-dimethylglycine esters of **2** (1-mono, 4-mono, and 1,4-bis) were synthesized and assessed as potential water-soluble prodrugs for parenteral use. The esters can deliver the hydroquinone to its active site without a quinone reductive activation step. The hydrochloride salts of the esters were found to be quite soluble in water. The hydrolysis of the esters in 20% rat liver homogenate 9000 × g supernatant, rat plasma and phosphate buffer, pH 7.4, at 37°C was kinetically studied in the presence and absence of an esterase inhibitor. The hydrolysis was catalyzed by esterases located in the rat liver and rat plasma and quantitatively yielded **2**. These results suggest that esterification of **2** with *N,N*-dimethylglycine is a promising way for obtaining water-soluble prodrug forms of **2**. Based on the high susceptibility to liver esterase, the esters are potential prodrugs for achieving the site-specific delivery of **2**.

KEY WORDS: menaquinone-4; menahydroquinone-4; water-soluble prodrug; site-specific delivery; amino acid ester; enzymatic hydrolysis.

INTRODUCTION

Drugs containing a quinone function, such as the K vitamins and quinone-anticancer drugs, undergo reductive activation during effective processing (1-4), and their pharmacological efficacy and toxicity are expressed upon enzyme-dependent reduction of the quinone to hydroquinone. Vitamin K hydroquinone, the fully reduced form of vitamin K, is an essential cofactor for an enzyme to catalyze the post-translational modification of glutamyl residues (Glu) to γ-carboxyglutamyl residues (Gla) in precursors of vitamin K dependent proteins (Fig. 1) (reviewed in Ref. 1,2). The bioreductive activation step of vitamin K consists of one-electron and two-electron reductions. The two-electron reduction pathway has generally been considered to predominate in the vitamin K cycle.

Coumarin anticoagulants block the vitamin K cycle by inhibition of vitamin K epoxide reductase and the two-electron reduction. However, the anticoagulation can be overcome by administration of large doses of vitamin K (5,6). The antidote effect of the vitamin is due to the formation of vitamin K hydroquinone via the one-electron reduction pathway which is unaffected by coumarins (7). The one-electron reduction generates semiquinone which can readily react with molecular oxygen to produce active oxygen species and hence undergoes a futile redox cycling (8). The net result of this redox cycling is oxidative stress such as hepatotoxicity resulting from the generation of active oxygen species (7, 9-11). Based on these results, we proposed that the efficacy and toxicity of vitamin K might depend on the pathway and extent of reductive activation to vitamin K hydroquinone at the site of its action and especially the use of the quinone form of vitamin K in the treatment of coumarin anticoagulant poisoning which might cause oxidative toxicity. It would be the most effective way to selectively deliver the hydroquinone to its active site without the reductive activation step.

Another delivery problem associated with vitamin K hydroquinone arises from the fact that the most widely used form of vitamin K is practically insoluble in aqueous media. Intravenous dosing often produces an anaphylactoid reaction in certain individuals (12). These adverse reactions are believed to be related to the polyoxyethylene hydrogenated castor oil (HCO-60) used in the parenteral dosage form.

To overcome the delivery problems of vitamin K, we attempted to develop prodrugs of vitamin K hydroquinone for parenteral administration. A successful prodrug of vitamin K hydroquinone should possess sufficient aqueous solubility and should regenerate the hydroquinone selectively at the site of its action without a reductive activation process. Such a prodrug exhibiting systemic site-specific drug delivery would optimal if the reconversion is catalyzed by specific enzymes located at the active site (13).

Various amino acid esters have been proposed as water-soluble prodrugs of drugs containing a hydroxyl group due to facile endogenous enzymatic cleavage, excellent solubility properties of their acid salts and ease of synthesis (14). We have already observed that primary (glycinate), secondary (sarcosinate) and tertiary aminoacetic acid (*N,N*-dimethylglycinate) esters of α-tocopherol, a phenolic compound containing a large lipophilic group as well as vitamin K hydroquinone, exhibit considerable water solubility and have a high susceptibility for hydrolysis catalyzed by liver esterase (15). Therefore, we designed esters with amino acids of the hydroxyl group(s) of menahydroquinone-4 (**2**). In the present study, several esters of **2** with an amino acid were prepared and assessed as possible prodrugs forms for parenteral administration.

MATERIALS AND METHODS

Menaquinone-4 (**1**) is extremely susceptible to photocatalyzed degradation, therefore, all experiments were per-

[1] Faculty of Pharmaceutical Sciences, Fukuoka University, 8-19-1 Nanakuma, Johnan-ku, Fukuoka 814-80, Japan.
[2] Kyoritsu College of Pharmacy, 1-5-30 Shibakoen, Minato-ku, Tokyo 105, Japan.
[3] Department of Hospital Pharmacy, Kyushu University Hospital, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812, Japan.
[4] To whom correspondence should be addressed.

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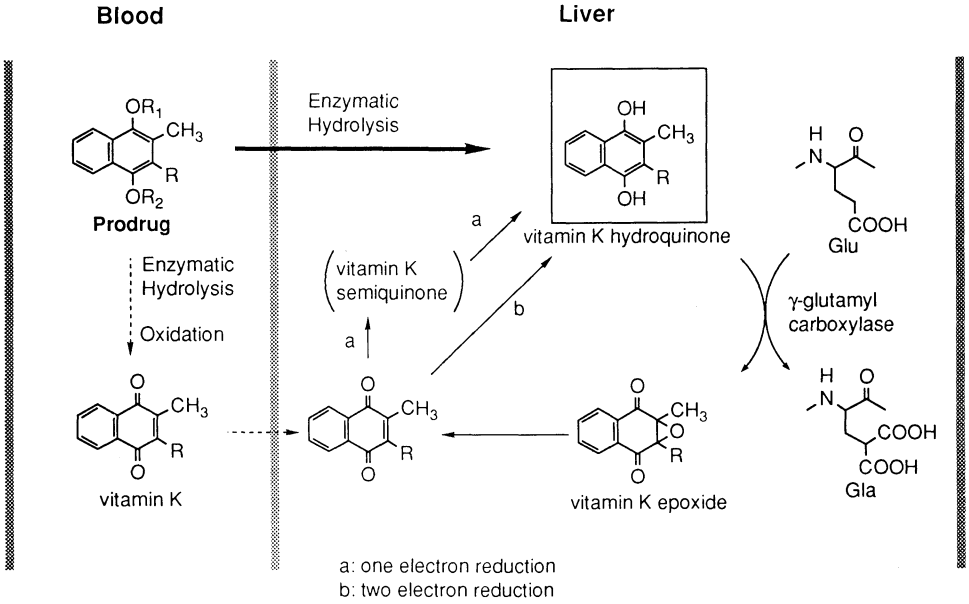


Fig. 1. Schematic illustration of the prodrug concept for systemic site-specific delivery of vitamin K hydroquinone. The prodrug can regenerate the hydroquinone without reductive activation steps.

formed in reduced light. All melting points were taken using a Yanagimoto micromelting point apparatus (Yanagimoto, Tokyo, Japan) and were uncorrected. Microanalyses, ^{1}H-NMR and mass spectra measurements were carried out at the Central Microanalytical Department of Pharmaceutical Science, Fukuoka University. The ^{1}H-NMR spectra were determined at 400 MHz using a JEOL GX-400 spectrometer (JEOL Ltd, Tokyo, Japan) in a solution of CD_3OD with tetramethylsilane as an internal standard. The following abbreviations are used: s = singlet, d = doublet, m = multiplet. Field desorption mass (FD-MS) spectra were obtained using a JEOL D-300 spectrometer (JEOL Ltd, Tokyo, Japan).

Compound 1 was a generous gift from Eisai Co. Ltd. (Tokyo, Japan) and used as received. *N,N*-Dimethylglycine hydrochloride was purchased from Tokyo Kasei Kogyo Co. Ltd. (Tokyo, Japan). Eserine (physostigmine sulfate) was obtained from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals were purchased from Wako Pure Chemical Ind. Ltd. (Osaka, Japan). Male Wistar rats 280-310 g were purchased from Charles River Japan (Atsugi, Japan) and were fasted in coprophagy-preventing cages for 16 hr before use.

Preparation of Menahydroquinone-4 (2)

A solution of 3.0 g (6.7 mmol) of 1 in 50 ml of isopropyl ether was added to 25 ml of methanol and 2.5 g (66 mmol) of sodium borohydride and stirred for 10 min in the dark under an argon atmosphere. The mixture was treated with 100 ml of deaerated water and extracted with isopropyl ether. The organic layer was dried over anhydrous sodium sulfate and evaporated *in vacuo*. The oily residue was treated with n-hexane to yield 2 as a white or grayish solid. [FD-MS (m/z) 446 (M^+)]. The obtained solid 2 was used in the following step without further purification.

Preparation of 1-*N,N*-dimethylglycyloxy-2-methyl-3-tetraprenyl-4-hydroxy-naphthalene hydrochloride (3) and 4-*N,N*-dimethylglycyloxy-2-methyl-3-tetraprenyl-1-hydroxy-naphthalene hydrochloride (4)

To a dry pyridine solution of 2, 0.94 g (6.7 mmol) of *N,N*-dimethylglycine hydrochloride and 1.38 g (6.7 mmol) of dicyclohexylcarbodiimide (DCC) were added. The reaction mixture was stirred at room temperature for 24 h and the dicyclohexylurea formed was removed by filtration. After the solvent was evaporated, the residue was treated with 100 ml of water and made alkaline by sodium bicarbonate. The solution was then extracted with ethyl acetate. The organic layer was dried over anhydrous sodium sulfate and evaporated. The residue was fractionated with a preparative HPLC column Wakosil-II 5SIL-100 (20 × 250 mm) (Wako Pure Chemical Ind. Ltd., Osaka, Japan) using isopropyl ether-ethyl acetate (7:3, v/v, flow rate 4 ml/min) as an eluant. The retention times of 4-mono- and 1-mono esters were 30.3 and 39.8 min, respectively. Since the free base of the monoesters are very unstable (hydrolyzed) under concentrated conditions, corresponding fractions were collected in isopropyl ether containing 3% HCl dioxane solution and isolated as hydrochloride salts 3 and 4. The esterified position of the monoester was confirmed by ^{1}H-NMR (nuclear Overhauser effect difference mode). The nuclear Overhauser effect between the 2-methyl protons and the methylene protons of the *N,N*- dimethylglycine moiety was observed in 3 but not in 4. The result indicated that 3 was an ester of 1-hydroxyl of 2 with *N,N*-dimethylglycine.

3: White solid yield (29.9%), mp 120–123°C. ^{1}H-NMR (MeOH-d_4) δ: menahydroquinone-4 moiety; 8.20 (1H, m, H-5), 7.71 (1H, m, H-8), 7.46 (2H, m, H-6, 7), 5.07 (4H, m, H-2′, 6′, 10′, 14′), 3.61 (2H, d, H-1′), 2.25 (3H, s, CH_3-2), 1.83 (3H, s, CH_3-3′), *N,N*-dimethylglycine moiety; 4.78 (2H, s, CH_2), 3.10 (6H, s, $N(CH_3)_2$). FD-MS (m/z); 531 (M^+-HCl). Anal. Calcd for $C_{35}H_{50}NO_3Cl+0.4\ H_2O$: C, 73.05%; H, 8.90%; N, 2.43%. Found: C, 73.09%; H, 8.75%, N, 2.65%.

4: White solid yield (17.6%), mp 121°C (dec). ^{1}H-NMR (MeOH-d_4) δ: menahydroquinone-4 moiety; 8.22 (1H, m, H-5), 7.68 (1H, m, H-8), 7.45 (2H, m, H-6, 7), 5.04 (4H, m, H-2′, 6′, 10′, 14′), 3.42 (2H, d, H-1′), 2.34 (3H, s, CH_3-2), 1.81 (3H, s, CH_3-3′), *N,N*-dimethylglycine moiety; 4.76 (2H, s, CH_2), 3.09 (6H, s, $N(CH_3)_2$). FD-MS (m/z); 531 (M^+-HCl). Anal. Calcd for $C_{35}H_{50}NO_3Cl$: C, 73.98%; H, 8.87%; N, 2.46%. Found: C, 73.75%; H, 8.91%; N, 2.56%.

Preparation of 1,4-bis (*N,N*-dimethylglycyloxy)-2-methyl-3-tetraprenyl-naphthalene di-hydrochloride (5)

A dry pyridine solution of **2** (6.7 mmol) was added to 1.87 g (13.4 mmol) of *N,N*-dimethylglycine hydrochloride and 2.76 g (13.4 mmol) of DCC. The residue obtained by a procedure similar to that used for compounds **3** and **4** previously mentioned was purified by silica gel 60 column chromatography (isopropyl ether-ethyl acetate 1:1, v/v) to give the free base of **5**, a colorless oil, 1.98 g (47.6%). FD-MS (m/z): 616 (M^+). The ester was converted to the hydrochloride salt, **5**. White powder. mp 193°C. ^{1}H-NMR (MeOH-d_4) δ: menahydroquinone-4 moiety; 7.90 (2H, m, H-5, 8), 7.61 (1H, m, H-6, 7), 5.05 (4H, m, H-2′, 6′, 10′, 14′), 3.52 (2H, d, H-1′), 2.32 (3H, s, CH_3-2), 1.83 (3H, s, CH_3-3′), *N,N*-dimethylglycine moiety; 4.90 (4H, s, CH_2), 3.13 (6H, s, $N(CH_3)_2$ 1-ester), 3.12 (6H, s, $N(CH_3)_2$ 4-ester). FD-MS (m/z); 616 (M^+-2HCl). Anal. Calcd for $C_{39}H_{58}N_2O_4Cl_2+0.7H_2O$: C, 66.69%; H, 8.52%; N, 3.99%. Found: C, 66.62%; H, 8.50%; N, 3.98%.

In order to prepare esters containing primary and secondary amine groups, the coupling of protected amino acids with **2** was investigated and obtained the esters of **2** with *N*-t-butyloxycarbonyl (t-BOC)-glycine and t-BOC-sarcosine, but the corresponding mono and 1,4-bisesters resulted in very complex mixtures after deprotecting processes with HCl.

Aqueous Solubility

The aqueous solubilities of **3-5** were determined by adding 50 μmol of each compound to 1 ml of water in amber vials maintained at 25 ± 0.1°C in a constant-temperature water bath. The vials were shaken for 24 h and the contents were filtered using membrane filters (Columngard-LCR4, 0.5 μm, Nihon Millipore Kogyo K.K., Yonezawa, Japan), and the ester concentrations in the filtrates were determined by the HPLC method described below. During the entire procedure less than 1% of **1** was formed from **3** and **4** as determined HPLC.

Determination of Partition Coefficients

The apparent partition coefficient (P) of the esters were determined in an octanol-water system at 25°C. The aqueous phase was an isotonic phosphate buffer solution of pH 7.4. The buffer solution and octanol were mutually saturated at 25°C before use. The esters were firstly dissolved in distilled water and diluted with buffer solution and used as an aqueous phase. The volumes of each phase were chosen so that the solute concentration in the aqueous phase after distribution could readily be measured. The octanol-water mixtures were shaken for 20 min at 25°C to reach a distribution equilibrium. After centrifugation, concentration of the esters in both phases were measured using the HPLC method described below.

HPLC Analysis

Unless otherwise noted, a HPLC system (Shimadzu Co. Ltd., Kyoto, Japan) was used. The system consisted of a pump (LC-6A), an auto sample injector (SIL 9A), an UV detector (SPD-6AV), a spectrofluorometer (RF-540) equipped with a 12 μl LC flow cell and a two-channel peak integrator (C-R7A). A reversed-phase column CAPCELL PAK C18 (4.6 × 200 mm, Shiseido, Tokyo, Japan) with a guard column Guard-PAK (Japan Wates, Tokyo, Japan) and a mobile phase of methanol-acetonitrile (1:4, v/v) containing 0.02 M acetic acid and sodium acetate at a flow rate of 1.0 ml/min were used. The eluent was monitored spectrophotometrically at 275 nm and spectrofluorometrically at an excitation of 274 nm and an emission of 340 nm. With this system the compounds **1**, **3**, **4** and **5** showed retention times of 9.9, 6.1, 6.4 and 7.2 min, respectively. Quantitation of these compounds was achieved using linear calibration curves of peak area vs concentration.

Enzymatic Hydrolysis of the Esters

The hydrolysis of the esters was studied at 37°C in isotonic phosphate buffer (pH 7.4), and rat plasma as well as in the 20% rat liver homogenate 9000 × g supernatant fraction. The rat liver was homogenized with 4 volumes of isotonic phosphate buffer using a POLYTRON homogenizer (Kinematica, Switzerland) and centrifuged for 90 min at 9000 × g.

The reactions were initiated by adding 50 μl of an aqueous stock solution of the esters (dissolved in distilled water) and 50 μl of isotonic phosphate buffer to 900 μl of a preheated reaction medium in amber test tubes. The initial concentration of the esters was 5.6×10^{-5} M. The solutions were incubated at 37°C. At appropriate intervals, samples of 100 μl each were withdrawn and 350 μl of methanol-ethyl acetate (4:1, v/v) was added. After 2 min of vortex mixing and centrifugation at 3000 rpm for 5 min, 40 μl of the clear supernatant was analyzed by HPLC. The rates of hydrolysis were generally followed by monitoring the disappearance of the esters and the appearance of **1**. In the case of **5** the appearance of 1-mono and 4-mono esters were also monitored.

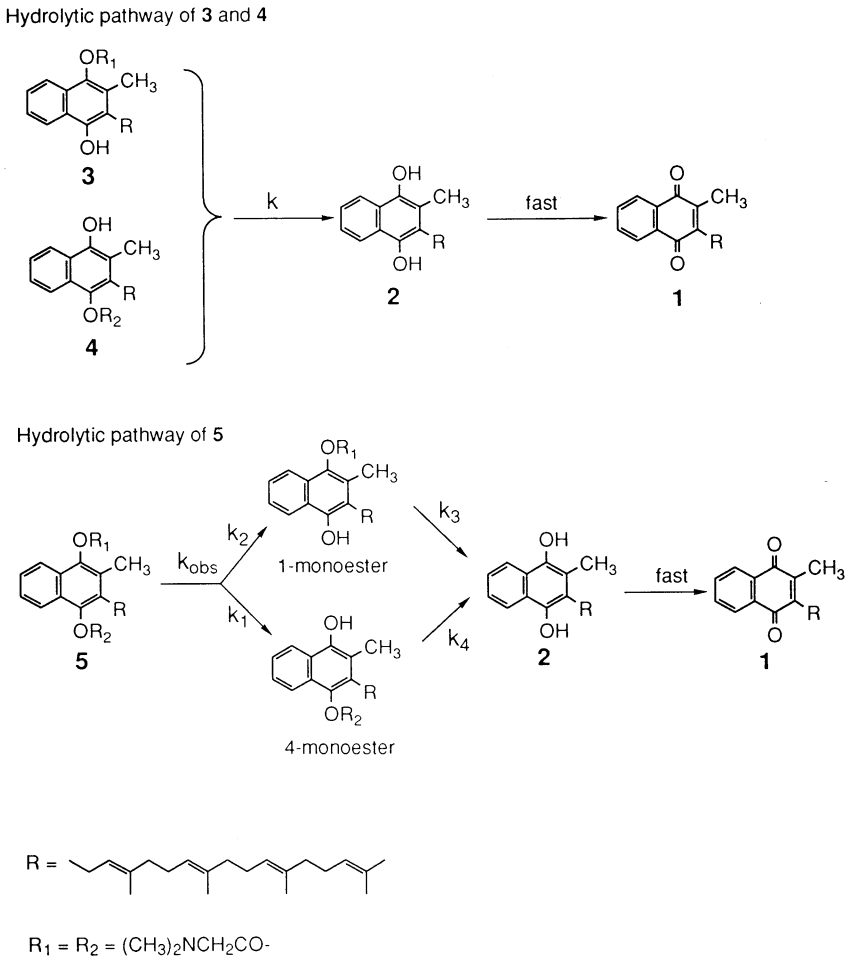
The effects of an esterase inhibitor (eserine, 1 mM) on the hydrolysis of the esters were also studied. The plasma and liver preparations (900 μl) were incubated with 50 μl of the inhibitor solution for 15 min at 37°C. Following the initial incubation, 50 μl of the stock solution of the esters was added to the mixture and the following experimental procedure was the same as that previously mentioned.

RESULTS AND DISCUSSION

All compounds (scheme 1) were characterized by FD-MS and ^{1}H-NMR spectroscopy as well as elemental analysis.

Water Solubility and Lipophilicity of the Esters

The hydrochloride salts of the esters showed greatly improved aqueous solubility. The solubilities of the



Scheme 1. Structures of the esters of menahydroquinone-4 and their hydrolytic pathways.

monoesters **3** and **4** in water were 24 and 5.7 mM, respectively. The diester **5** yielded a transparent solution up to about 50 mM concentration. The solubilities of **3** and **5** are greater than the concentration of a commercially available injection solution of **1** (22 mM), which was solubilized with HCO-60. The high water-solubility of the prodrug candidates make it unnecessary to use any surfactant and co-solvent in order to solubilize them in water.

The lipophilicity of the esters was evaluated in terms of partition coefficients (P) between octanol and phosphate buffer of pH 7.4. The log P of the esters found 4.56 (**3**), 4.67 (**4**) and 3.66 (**5**). The high lipophilic characteristics of the free base form of the esters in physiological pH are indicating sufficient distribution of the esters to tissues from systemic circulation after intravenous administration.

Enzymatic Hydrolysis of the Esters

The water soluble esters **3-5** must undergo cleavage to **2** under *in vivo* conditions to be true prodrugs. The susceptibilities of **3-5** for hydrolysis were studied in isotonic phosphate buffer (pH 7.4), the supernatant fraction of 20% rat liver homogenate and rat plasma to determine their *in vivo* behavior.

Hydrolysis of the Monoesters

Monoesters **3** and **4** underwent hydrolysis with quantitative formation of **1**, and the reaction followed apparent first order kinetics at an initial concentration of 5.6×10^{-5} M in all biological media. An attempt to monitor **2** was unsuccessful because of its high susceptibility for oxidation. The HPLC procedure was able to quantify **1**, and the monoesters **3** and **4**. The mass balance of these compounds in the reaction medium was well maintained (over 95% recovery) throughout a kinetic run, therefore, it was concluded that the monoesters were hydrolyzed to **2**, which was subsequently oxidized to **1** quantitatively as shown in Scheme 1. The apparent first order rate constants for the hydrolysis of the compounds in the biological media are listed in Table I along with the degradation rate constants of the esters in the phosphate buffer.

Rat liver and plasma components markedly accelerate the hydrolysis of the monoesters. The rate constants of the degradation for the esters in isotonic phosphate buffer were less than 5×10^{-4} min^{-1}, and the facts indicate that chemical instability cannot account for the rapid conversion in the biological media. In liver homogenate, **3** was more rapidly hydrolyzed than **4**. The hydrolytic rates dependent on the

Table I. Apparent First Order Rate Constants for the Hydrolysis of the Menahydroquinone-4 Esters and Regeneration Half-lives to 2 at 37°C in Phosphate Buffer (pH 7.4), Rat Liver Homogenate Supernatant and Rat Plasma with and without Enzyme Inhibitor

Compound	Without Eserine ($\times 10^{-2}$ min^{-1})	With Eserine ($\times 10^{-2}$ min^{-1})	Regeneration Half-life (min)
Liver homogenate[a]			
3	27.2	0.261	2.55
4	1.14	0.315	60.6
5	17.2[b]	0.328[b]	39.7[c]
Plasma			
3	23.0	0.351	3.01
4	23.9	4.70	2.90
5	6.40[b]	0.993[b]	15.0[c]
Buffer[d]			
3	0.0203		
4	0.0295		
5	0.0500[b]		

[a] 20% rat liver homogenate 9000 × g supernatant.
[b] disappearance of 5.
[c] calculated from eq. (4) (see text).
[d] isotonic phosphate buffer of pH 7.4.

esterified position of 2 was not seen in the hydrolysis catalyzed by rat plasma.

Hydrolysis of the Bisester

The time courses of the degradation of 5 in the rat liver preparation and the rat plasma are shown in Fig. 2. The disappearance of 5 followed apparent first order kinetics under the experimental conditions (Table I) and was accompanied by formation of the monoester(s) and 1. At any given reaction time, the sum of the concentrations of 5, 3, 4 and 1 was over 95% of the initial concentration of 5.

The formation of 1 from 5 should proceed through the intermediates 4, 3 and 2, and the pseudo first order rate constants for the interconversion of the species are assumed as shown in Scheme 1. The concentrations of the species may be expressed by the following equations:

$$C_5 = C_0\, e^{-(k_1+k_2)t} \tag{1}$$

$$C_4 = C_0 \frac{k_1}{k_4 - (k_1 + k_2)} [e^{-(k_1+k_2)t} - e^{-k_4 t}] \tag{2}$$

$$C_3 = C_0 \frac{k_2}{k_3 - (k_1 + k_2)} [e^{-(k_1+k_2)t} - e^{-k_3 t}] \tag{3}$$

$$C_2 = C_0 \left\{ 1 - \frac{k_1}{k_4 - (k_1 + k_2)} [e^{-(k_1+k_2)t} - e^{-k_4 t}] - \frac{k_2}{k_3 - (k_1 + k_2)} [e^{-(k_1+k_2)t} - e^{-k_3 t}] - e^{-(k_1+k_2)t} \right\} \tag{4}$$

where C_0 represents the initial concentration of 5, and C_5, C_4, C_3 and C_2 are the concentrations of 5, intrinsic 4, intrinsic 3, and 2 at time t, respectively. Analysis of the progress curves according to the above equations, using the nonlinear regression analysis program MULTI (16) gave reasonable

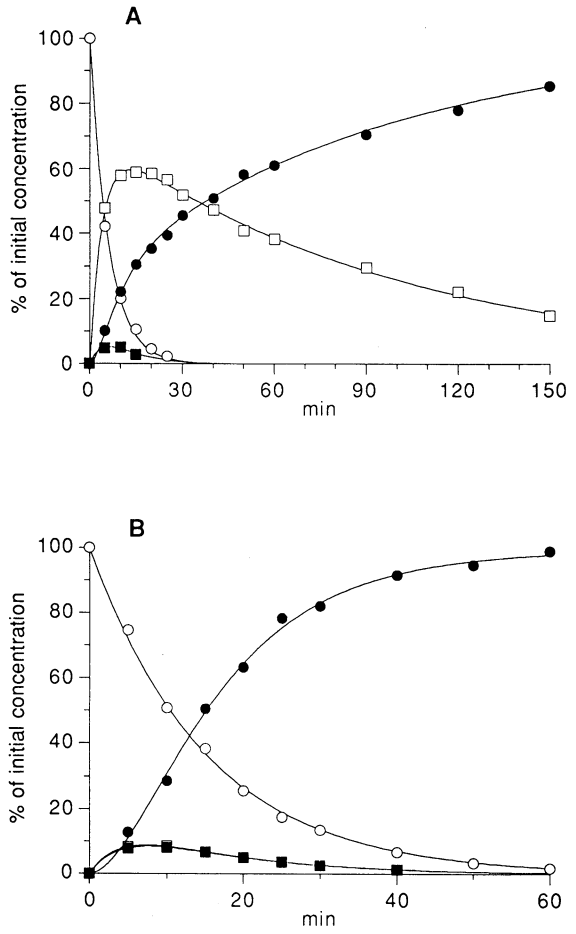


Fig. 2. Time course for the hydrolysis of 5 and concomitant formation of the monoesters and menaquinone-4 in the 20% rat liver homogenate supernatant (A) and in the rat plasma (B) at 37°C. Key: (○) 5; (□) 4; (■) 3; (●) 1. The curves are calculated from eqs. 1–4.

fits of these equations to the curves shown in Fig. 2. The estimated kinetic parameters of k_1 and k_2 are listed in Table II. In these analyses, the hydrolytic rates of 3 and 4 under the conditions shown in Table I were used for k_3 and k_4, respectively, and the concentration of 1 was regarded as that of 2 (C_2). The sum of the rate constants ($k_1 + k_2$) obtained in this way agreed with k_{obs} determined in the hydrolysis experiment of 5 within ±10%.

As can be seen in Table II, the rate of hydrolysis of 5 at the 1-position (k_1) was about 2.6-fold faster than that at the 4-position (k_2) in the liver preparation, demonstrating that the cleavage of the 1-ester is the dominating hydrolytic

Table II. Rate Constants for the Hydrolysis of 5 at 37°C in Rat Liver Homogenate Supernatant and Rat Plasma at 37°C

Medium	k_{obs}[a] ($\times 10^{-2}$ min^{-1})	k_1 ($\times 10^{-2}$ min^{-1})	k_2 ($\times 10^{-2}$ min^{-1})
Liver[b]	17.2 ± 1.0	11.9 ± 0.39	4.39 ± 0.18
Plasma	6.40 ± 0.11	3.47 ± 0.28	3.24 ± 0.28

k_1 and k_2 accordance to Scheme 1.
[a] disappearance of 5.
[b] 20% rat liver homogenate 9000 × g supernatant.

route. In rat plasma, the values of k_1 and k_2 were almost equal and hence both routes contribute equally to the regeneration of the parent drug 2.

Effect of an Esterase Inhibitor

To assess whether the observed catalytic regeneration of 2 in the rat liver preparation and plasma can be attributed to the esterases, the effects of eserine on the hydrolysis were studied. Eserine is an inhibitor of liver carboxylesterase and plasma cholinesterase (true and/or pseudo) (17–19). The rates of 3, 4 and 5 in both biological media were significantly reduced in the presence of eserine (Table I). It is quite evident from the results that rat liver and rat plasma esterases significantly catalyze the hydrolysis of the esters.

A significant difference in the liver esterase catalyzed hydrolytic rates of ester bonds at positions 1 and 4 of 2 were observed in both of the monoesters and the bisester, indicating liver esterase catalytic hydrolysis is depend on the esterified position of 2. However, the structural dependence was not seen in the hydrolysis catalyzed by rat plasma esterase. The esterases in the liver and plasma seem to show different specificity against the esters of 2.

Since one of the aims of the present prodrug approach is to overcome the solubility problem in formulating the parenteral solution of 2, excellent solubility and the facile enzymatic reconversion of the esters should satisfy the criteria as prodrugs for parenteral use.

For achieving systemic site-specific delivery of the parent drug (another aim of this study), site-selective activation of the prodrug is an important criterion (13). As can be seen in Table I, the regeneration of 2 was catalyzed by both the liver and plasma esterases. The plasma esterase reconversion was competing with the liver reconversion and this characteristic was inconvenient for the specific delivery because it could be expected that resultant 2 might be easily oxidized to 1 in blood under oxidative conditions. Therefore, it is assumed that the esters 3 and 5 with excellent solubility and facile liver esterase triggering activation properties should be most promising candidate for the prodrug achieving systemic liver-specific delivery of active form of vitamin K. Especially in the treatment of coumarin anticoagulant poisoning, this ester prodrug approach might be useful for avoiding the oxidative toxicity induced by active oxygen species formed concurrent with one-electron reductive activation of vitamin K quinone.

In preliminary experiments, it was found that intravenous administration of the prodrugs to warfarin treated rats (under vitamin K cycle inhibited condition) afforded improved delivery of 2 to an active site compared to administration of an equivalent amount of an aqueous solution of 1 solubilized with HCO-60. The site-specificity of 2 was accorded to the enzymatic conversion characteristic of the prodrugs in liver preparation. The results of such bioavailability studies will be the subject of a subsequent paper.

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