Prodrugs of Vitamin E. 1. Preparation and Enzymatic Hydrolysis of Aminoalkanecarboxylic Acid Esters of *d*-α-Tocopherol

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Abstract □ Nine aminoalkanecarboxylic acid esters of *d*-α-tocopherol were synthesized and evaluated as potential water-soluble prodrugs suitable for parenteral administration. The hydrochloric acid salts of the esters were soluble in water. The kinetics of hydrolysis of the esters was studied in isotonic phosphate buffer, rat plasma, human plasma, and rat liver homogenate at 37 °C. The hydrolysis of the esters was proved to be catalyzed by liver esterases. The susceptibility of the esters to undergo liver esterase hydrolysis was affected by the structure of the amino functionality and size of the acyl moiety on the promoiety. The *N*-methylaminoacetyl and *N,N*-dimethylaminoacetyl esters of *d*-α-tocopherol were more rapidly hydrolyzed than *d*-α-tocopheryl acetate, a commercially available *d*-α-tocopheryl ester. These results suggested that the salts of the *N*-methylaminoacetyl and *N,N*-dimethylaminoacetyl esters are promising prodrug candidates of *d*-α-tocopheryl for parenteral use.

Introduction

α-Tocopherol (vitamin E), a biological antioxidant, is currently receiving attention concerning its efficacy in preventing and reducing the oxidative stresses resulting from ischemia and reperfusion,[1-4] and in the treatment of toxicant protection, malabsorption disorders, hematologic disorders, cardiovascular disease, and premature infants.[5]

α-Tocopherol is practically insoluble in water and is readily oxidized by atmospheric oxygen. Because of their high stability to oxidation, the acetate and acid succinate esters of the vitamin are commonly supplied for clinical use. When a rapid onset of action is required via parenteral administration, significant problems arise from the fact that the esters are characteristically as insoluble in water as α-tocopherol. This water insolublity of the esters may also be the predominant factor responsible for the much diminished absorption of the drug following oral administration.[6-8] α-Tocopheryl acetate is solubilized by large amounts of surfactant in the parenteral formulation and it has been confirmed that the hydrolysis of the acetate is the rate-limiting step in the course of the bioavailability of α-tocopherol.[9,10] The use of surfactants in parenteral dosage forms generally induces toxicity such as the anaphylactoid reaction.

These delivery problems can be primarily attributed to the low water solubility and bioreconversion rate of the ester to α-tocopherol. It is well-known that the prodrug approach is one useful method for improving the physicochemical properties.[11-13] The phenolic functional group in α-tocopherol is easily esterified and some of the ester derivatives are expected to provide the desired improvement in water solubility and stability to oxidation. In developing a prodrug, the bond between the parent drug and the promoiety should be stable in formulations but rapidly cleaved *in vivo*. In this regard, the most successful prodrugs of α-tocopherol are those that exhibit sufficient water solubility and in which the reconversion to the parent drug is catalyzed by enzymes encountered after administration.

We prepared a number of aminoalkanecarboxylic acid esters of *d*-α-tocopherol (**1**) with the aim to develop water-soluble prodrugs for parenteral use. In this paper, the synthesis of the esters and enzymatic reconversion of the esters to the parent drug are described. The results of *in vivo* studies of the prodrug candidates in rats will be reported in a future communication.

Results and Discussion

Nine aminoalkanecarboxylic acid esters of *d*-α-tocopherol (**2**–**10**) were prepared by procedures described in the Experimental Section and isolated as the hydrochloride salts (Chart 1). All compounds were characterized by ^{1}H-NMR as well as by elemental analysis.

Water Solubility of the Esters—The water solubility of the esters was determined at 25 °C. Most of the hydrochloride salts of the esters showed greatly improved aqueous solubility of more than 20 mM except **2** (Table 1). It was thought that the introduction of an ionizable amino group in the ester moiety of such derivatives would make it possible to obtain a water-soluble prodrug. The high water solubility of the prodrug candidates made it unnecessary to use any surfactant and cosolvent in order to solubilize **1** in water. The *d*-isomer of the aminoacetate **2** exhibited significantly lower solubility, whereas the racemate **2a** exhibited as high a solubility as the other *d*-isomer α-tocopherol esters. The high melting point of **2** compared to **2a** suggested that the difference in solubility might be the result of a difference in the crystal structure of the two isomers.

Enzymatic Hydrolysis of the *d*-α-Tocopheryl Esters—In developing a prodrug, selection of a promoiety should take the requirement into consideration that the bond between the parent drug and the promoiety should be stable in formulations but rapidly cleaved *in vivo*. In this regard, the most successful prodrug of **1** is that in which the reconversion of the esters to the parent drug must be catalyzed by any enzyme encountered after administration. Therefore, the kinetics of reconversion of the esters **2**–**10** to **1** was determined in isotonic phosphate buffer (pH 7.4), human plasma, rat plasma, and rat liver homogenate 9000*g* supernatant at 37 °C and were compared with that of TA.

Significant acceleration of the hydrolytic rates of the esters were found in the rat liver homogenate preparation and produced **1** in quantitative amounts, as revealed by HPLC analysis. The kinetics of hydrolysis of the esters can be described by the Michaelis–Menten model. The kinetic data of the studies were analyzed using the Lineweaver–Burk equation,[14]

$$\frac{1}{v_0} = \frac{K_m}{V_{max}} \frac{1}{S_0} + \frac{1}{V_{max}} \quad (1)$$

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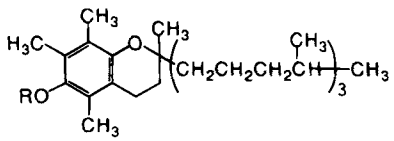

d-α-Tocopherol (**1**): R = H

TA: R = -$OCCH_3$

2: R = -$OCCH_2NH_2 \cdot HCl$

2a: racemate of **2**, R = -$OCCH_2NH_2 \cdot HCl$

3: R = -$COCH_2CH_2NH_2 \cdot HCl$

4: R = -$OCCH_2CH_2CH_2NH_2 \cdot HCl$

5: R = -$OCCH_2NHCH_3 \cdot HCl$

6: R = -$OCCH_2N(CH_3)_2 \cdot HCl$

7: R = -$OCCH_2CH_2N(CH_3)_2 \cdot HCl$

8: R = -$OCCH_2CH_2N(CH_2CH_3)_2 \cdot HCl$

9: R = -$OCCH_2CH_2CH_2N(CH_3)_2 \cdot HCl$

10: R = -$OCCH_2N^+(CH_3)_3$, Cl^-

Chart 1—The aminoalkanecarboxylic acid esters of *d*-α-tocopherol.

Table 1—Aqueous Solubilities of the Aminoalkanecarboxylic Acid Esters of *d*-α-Tocopherol at 25 °C

Compd	Solubility (mM)	Compd	Solubility (mM)
TA	ND[a]	**6**	>100
2	4.83×10^{-3} [b]	**7**	>100
2a	>100	**8**	>100
3	>100	**9**	>100
4	22.1[b]	**10**	>100
5	>100		

[a] Not detectable. [b] The adherence of the esters to the filter were ignored.

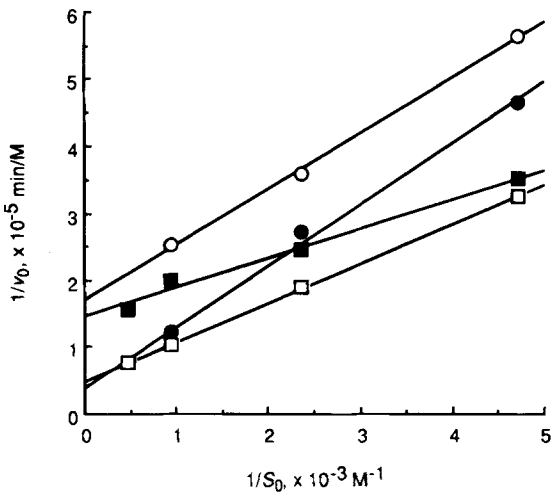

Figure 1—Lineweaver–Burk plots for initial rates of the hydrolysis of the *d*-α-tocopheryl esters in the rat liver homogenate 9000g supernatant at pH 7.4 and 37 °C. Key: (○) TA, (●) **2**, (□) **5**, (■) **6**. The lines represent the least-square regressions ($r > 0.98$).

where v_0 is the initial rate of the hydrolysis, S_0 is the initial concentration of the esters, K_m is the Michaelis constant, and V_{max} is the maximal hydrolytic rate for saturating substrate concentrations for a given enzyme concentration.

Representative Lineweaver–Burk plots are shown in Figure 1, which depict the hydrolysis of **2**, **5**, **6**, and TA. The kinetic parameters of V_{max} and K_m generated from the initial-rate data and a linear regression analysis of eq 1 are listed in Table 2 along with V_{max}/K_m. The value of V_{max}/K_m is the most meaningful kinetic parameter for comparing different substrates. A good substrate will have a large V_{max} and low K_m (tight binding) and, hence, a large V_{max}/K_m value.

As can be seen from the rate data given in Table 2, the esters show widely different rates of enzymatic hydrolysis. Compounds **5** and **6** were hydrolyzed more rapidly than TA. The rate of **2** is in the same range as that for TA. The rates of the other aminoalkanecarboxylic acid esters exhibited extremely slower rates in the liver preparation. The obtained results clearly demonstrated that the reconversion of the aminoalkanecarboxylic acid esters are catalyzed by enzyme(s) located in liver as well as TA, and that esters **5** and **6** are better substrates for the enzyme(s) than TA.

Further analysis of the data in Table 2 shows that the enzymatic reactivity of the aminoalkanecarboxylic acid esters appears to predominantly depend on the size of the acyl moiety and the structure of the amino functionality on the promoiety. Thus, the reactivity of a series of esters was found to decrease as the degree of the alkyl chain length of the acyl moiety increased: acetyl (**2**) > propionyl (**3**) > butyryl (**4**). This decreased rate was attributed to both an increased K_m (loose binding) and decreased V_{max} (small enzymatic capacity). The K_m values of the esters were found to decrease as the degree of methyl substitution at the nitrogen atom of the promoiety increased: tertiary amine (**6**) > secondary amine (**5**) > primary amine (**2**).

There was no significant acceleration of the hydrolytic rates in rat plasma and human plasma, whereas the esters appear to be relatively good substrates for the rat liver containing enzyme. These results agreed with a previous indication that the hydrolysis of *dl*-α-tocopheryl acetate is not catalyzed by the whole blood of human and dog[9] but it is significantly accelerated in a rat liver homogenate preparation.[14] On the other hand, human plasma enzyme catalytic hydrolysis of the aminoalkyl esters of metronidazol[15] and 1-(hydroxymethyl)-allopurinol[16] has been shown. These observations suggest that the susceptibilities to plasma enzymatic hydrolysis of ester prodrugs are not affected only by the structure of the promoieties but also by the structure of the parent drugs.

Effect of Esterase Inhibitor on the Hydrolysis of the *d*-α-Tocopheryl Esters—The aminoalkanecarboxylic acid esters studied here are clearly indicated as prodrugs of α-tocopherol with the anticipation that components of the liver will catalyze release of the parent drug from such derivatives. For assessing whether the observed catalytic release of **1** in the liver could be attributed to liver esterase, the effects of the presence of eserine, an esterase inhibitor, on the hydrolysis of esters **5**, **6**, and TA in the liver homogenate supernatant were studied.

The catalytic hydrolysis of the esters by liver components is inhibited by the addition of eserine (Figure 2). In case of **5** and **6**, a similar inhibition profile was observed and over 95% of the enzymatic activities was inhibited in the presence of 2.5 mM eserine. Under the same conditions, the enzymatic activity for TA remained at ca. 20%. Eserine is an inhibitor for cholinesterase (true and/or pseudo), carboxylesterase (ali-esterase), and acetylesterase. The latter two esterases are distributed in the liver but not in plasma.[18] The obtained results suggest that the reconversion of **5**, **6**, and TA is mainly catalyzed by these liver esterases. The remaining enzymatic activity for TA was attributed to different ester hydrolytic enzymes located in the liver. Furthermore, specification of the esterase is difficult because esterases show overlapping substrate specificities and a single substrate is often hydrolyzed by more than one enzyme, albeit at differing rates.[18,19]

In conclusion, the present results show that esterification of **1** with aminoalkyl carboxylic acid may be a potentially useful approach to obtain parenteral use prodrug forms of **1**. By appropriate selection of the aminoalkanecarboxylic acid moiety, it is possible to modify the aqueous solubility and

Table 2—Kinetic Parameters for the Hydrolysis of the Aminoalkanecarboxylic Acid Esters of *d*-α-Tocopherol and TA in Phosphate Buffer (pH 7.4) and Rat Liver Homogenate at 37 °C

Compd	Buffer:[a] k ($\times 10^{-2}$ h^{-1})	Liver Homogenate[b]		
		K_m[c] ($\times 10^{-3}$ M)	V_{max}[c] ($\times 10^{-5}$ M/min)	V_{max}/K_m ($\times 10^{-3}$ min^{-1})
TA	0.818	1.08	1.16	10.7
2	3.07	2.33	2.54	10.9
3	2.11	9.87	1.25	1.27
4	2.69	38.3	1.87	0.488
5	1.48	1.19	2.03	17.1
6	1.49	0.296	0.683	23.1
7	3.00	27.5	7.73	2.81
8	1.25	2.24	0.158	0.705
9	<0.521	—[d]	—[d]	—[d]
10	<0.521	—[d]	—[d]	—[d]

[a] Isotonic phosphate buffer (pH 7.4), rate was obtained from a first-order plot. [b] 20% rat liver homogenate 9000*g* supernatant. [c] Values are obtained from the Lineweaver–Burk plot. [d] Hydrolysis was not detectable during the time course of the experiment.

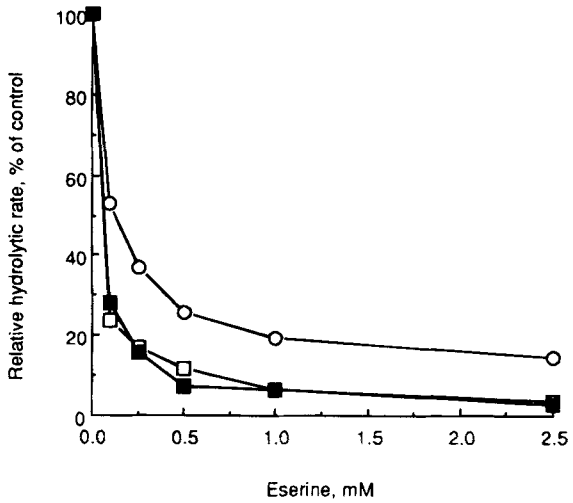

Figure 2—Effect of eserine on the rat liver enzymatic hydrolysis of the *d*-α-tocopheryl esters. Key: (○) TA, (□) **5**, (■) **6**.

reconversion rate. As has demonstrated, it is feasible to have the hydrochloride salts of the *N*-methylaminoacetate **5** and *N,N*-dimethylaminoacetate **6** of **1** display a high aqueous solubility and high susceptibility to enzymatic hydrolysis. Reconversion of these derivative to the parent drug is accelerated by liver esterase, but not by enzyme in plasma. The liver specificity of prodrug activation may be desirable in this case; the target organ for the action of **1** is mainly the liver. The results of *in vivo* studies will be the subject of a subsequent paper.

Experimental Section

All melting points were taken on a Yanagimoto micromelting point apparatus and were uncorrected. Microanalyses and measurements of ^{1}H-NMR and mass spectra were carried out at the Central Microanalytical Department of Pharmaceutical Science, Fukuoka University. The ^{1}H-NMR spectra were determined at 400 MHz in solutions of CD_3OD using a JEOL GX-400 spectrometer. The chemical shifts are expressed in δ (ppm) using tetramethylsilane as the internal standard. The following abbreviations are used: s = singlet, d = doublet, dd = double doublet, t = triplet. Field desorption mass spectra (MS) were obtained using a JEOL D-300 spectrometer.

d-α-Tocopherol (**1**) and *dl*-tocol were kindly supplied by Eisai Co., Ltd. (Tokyo, Japan) and used as received. *dl*-α-Tocopherol, *d*-α-tocopheryl acetate (TA), and eserine (physostigmine hemisulfate) were purchased from Sigma Chemical Co. (St. Louis, MO). *N-tert*-Butyloxycarbonyl (*t*-BOC)-protected aminoalkanecarboxylic acids were synthesized from di-*t*-BOC dicarbonate according to the conventional method.[20] *N,N*-Dimethylglycine hydrochloride was purchased from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan). *N,N*-Dimethyl-γ-aminobutyric acid hydrochloride and *N,N*-diethyl-β-aminopropionic acid hydrochloride were purchased from Aldrich Chemical Co. (Milwaukee, WI). *N,N*-Dimethyl-β-aminopropionic acid hydrochloride was synthesized from *N,N*-dimethylamine and 3-chloropropionic acid. All other chemicals were purchased from Wako Pure Chemical Ind., Ltd. (Osaka, Japan). Male Wistar rats were purchased from Charles River Japan (Atsugi, Japan) and used to obtain the rat plasma and rat liver homogenate.

Syntheses—*Primary and Secondary Aminoalkanecarboxylic Acid Esters of d-α-Tocopherol (compounds 2–5): General Procedure*—A mixture of 20 mmol of **1**, 20 mmol of *N-t*-BOC-aminoalkanecarboxylic acid, and 20 mmol of dicyclohexylcarbodiimide (DCC) in 70 mL of dry pyridine was stirred at room temperature for 24 h. After evaporation *in vacuo*, the residue was triturated with 100 mL of isopropyl ether and the dicyclohexylurea was removed by filtration. The filtrate was evaporated *in vacuo*, and the trituration was repeated twice. The *N-t*-BOC-aminoalkanecarboxylic acid ester of **1** was isolated by silica gel column chromatography (eluted by n-hexane–isopropyl ether). The acetone solution of the *N-t*-BOC-aminoalkanecarboxylic acid ester was added to HCl–dioxane solution (2 *N*) and the mixture was stirred for 30 min. The solvent was evaporated *in vacuo* and the residue was recrystallized from acetone–methanol to give the hydrochloride salt of **2**–**5**.

d-α-*Tocopheryl Aminoacetate Hydrochloride (2)*—Yield 93%. mp 176-179 °C. MS *m/z*: 487 (M^+ − HCl). ^{1}H-NMR δ: 4.23 (2H, s, NCH_2-CO), 2.63 (2H, t, J = 7.0 Hz, 4-CH_2), 2.09 (3H, s, 7-CH_3), 2.03 (3H, s, 8-CH_3), 2.00 (3H, s, 5-CH_3), 1.82 (2H, m, 3-CH_2), 1.25 (3H, s, 2-CH_3), 0.88 (9H, d, J = 7.0 Hz, $CHCH_3$), 0.86 (3H, d, J = 7.5 Hz, $CHCH_3$). Anal. Calcd for $C_{31}H_{53}NO_3HCl$: C, 71.03; H, 10.38; N, 2.67. Found: C, 70.97; H, 10.54; N, 2.65.

d-α-*Tocopheryl β-Aminopropionate Hydrochloride (3)*—Yield 91%. mp 173–175 °C. MS *m/z*: 501 (M^+ − HCl). ^{1}H-NMR δ: 3.32 (2H, dd, J = 7.0, 6.5 Hz, NCH_2), 3.10 (2H, dd J = 6.5, 7.0 Hz, CH_2CO), 2.63 (2H, t, J = 7.0, 4-CH_2), 2.08 (3H, s, 7-CH_3), 2.01 (3H, s, 8-CH_3), 1.98 (3H, s, 5-CH_3), 1.81 (2H, m, 3-CH_2), 1.25 (3H, s, 2-CH_3), 0.88 (9H, d, J = 6.5 Hz, $CHCH_3$), 0.86 (3H, d, J = 8.0 Hz, $CHCH_3$). Anal. Calcd for $C_{32}H_{55}NO_3HCl$: C, 71.41; H, 10.49; N, 2.60. Found: C, 71.31; H, 10.48; N, 2.54.

d-α-*Tocopheryl γ-Aminobutyrate Hydrochloride (4)*—Yield 74%. mp 127–132 °C. MS *m/z*: 515 (M^+ − HCl). ^{1}H-NMR δ: 3.08 (2H, dd, J = 7.5, 8.0 Hz, NCH_2), 2.82 (2H, t, J = 7.5 Hz, CH_2CO), 2.62 (2H, t, J = 7.0 Hz, 4-CH_2), 2.09 (2H, m, CH_2CH_2CO), 2.08 (3H, s, 7-CH_3), 1.99 (3H, s, 8-CH_3), 1.96 (3H, s, 5-CH_3), 1.81 (2H, m, 3-CH_2), 1.24 (3H, s, 2-CH_3), 0.88 (9H, d, J = 7.0 Hz, $CHCH_3$), 0.86 (3H, d, J = 8.0 Hz, $CHCH_3$). Anal. Calcd for $C_{33}H_{57}NO_3HCl$: C, 71.77; H, 10.59; N, 2.54. Found: C, 71.49; H, 10.55; N, 2.43.

d-α-*Tocopheryl N-Methylaminoacetate Hydrochloride (5)*—Yield 47%. mp 208–209 °C. MS *m/z*: 501 (M^+ − HCl). ^{1}H-NMR δ: 4.38 (2H, s, NCH_2CO), 2.84 (3H, s, NCH_3), 2.62 (2H, t, J = 7.0 Hz, 4-CH_2), 2.09 (3H, s, 7-CH_3), 2.04 (3H, s, 8-CH_3), 2.00 (3H, s, 5-CH_3), 1.80 (2H, m, 3-CH_2), 1.24 (3H, s, 2-CH_3), 0.87 (9H, d, J = 6.5 Hz, $CHCH_3$), 0.86 (3H, d, J = 7.0 Hz, $CHCH_3$). Anal. Calcd for $C_{32}H_{55}NO_3HCl$: C, 71.41; H, 10.49; N, 2.60. Found: C, 70.91; H, 10.56; N, 2.63.

dl-α-Tocopheryl aminoacetate hydrochloride (**2a**) was obtained from *dl*-α-tocopherol by an analogous procedure for **2**.

dl-α-*Tocopheryl Aminoacetate Hydrochloride* (**2a**)—Yield 91%. mp 150–151 °C. MS *m/z*: 487 (M^+ − HCl). ^{1}H-NMR δ: 4.23 (2H, s, NCH_2CO), 2.62 (2H, t, J = 7.0 Hz, 4-CH_2), 2.09 (3H, s, 7-CH_3), 2.03 (3H, s, 8-CH_3), 2.00 (3H, s, 5-CH_3), 1.79 (2H, m, 3-CH_2), 1.23 (3H, s, 2-CH_3), 0.87 (9H, d, J = 6.8 Hz, $CHCH_3$), 0.86 (3H, d, J = 5.9 Hz, $CHCH_3$). Anal. Calcd for $C_{31}H_{53}NO_3HCl$: C, 71.03; H, 10.38; N, 2.67. Found: C, 71.27; H, 10.44; N, 2.62.

N,N-Dialkylaminoalkanecarboxylic Acid Esters (***6–9***): *General Procedure*—A mixture of 20 mmol of **1**, 20 mmol of (*N,N*-dialkylamino)-alkanecarboxylic acid hydrochloride, and 20 mmol of DCC in 70 mL of dry pyridine was stirred at room temperature for 24 h. After evaporation *in vacuo*, the residue was triturated with 100 mL of dichloromethane and the dicyclohexylurea was removed by filtration. The filtrate was evaporated *in vacuo*. The residue was dissolved in a minimum amount of dichloromethane and ethyl acetate was added until the solution became turbid. The precipitate was collected and recrystallized from acetone–methanol to give the hydrochloride salt of **6–9**.

d-α-*Tocopheryl N,N-Dimethylaminoacetate Hydrochloride* (***6***)—Yield 79%. mp 176–178 °C. MS *m/z*: 515 (M^+ − HCl). ^{1}H-NMR δ: 4.63 (2H, s, NCH_2CO), 3.08 (6H, s, $(CH_3)_2N$), 2.63 (2H, t, J = 7.0 Hz, 4-CH_2), 2.09 (3H, s, 7-CH_3), 2.04 (3H, s, 8-CH_3), 2.01 (3H, s, 5-CH_3), 1.80 (2H, m, 3-CH_2), 1.24 (3H, s, 2-CH_3), 0.87 (9H, d, J = 7.0 Hz, $CHCH_3$), 0.86 (3H, d, J = 6.5 Hz, $CHCH_3$). Anal. Calcd for $C_{33}H_{57}NO_3HCl{\cdot}0.2H_2O$: C, 71.30; H, 10.52; N, 2.52. Found: C, 70.99; H, 10.56; N, 2.58.

d-α-*Tocopheryl N,N-Dimethyl-β-aminopropionate Hydrochloride* (***7***)—Yield 88%. mp 173–176 °C. MS *m/z*: 529 (M^+ − HCl). ^{1}H-NMR δ: 3.55 (2H, dd, J = 7.0, 7.5 Hz, NCH_2), 3.27 (2H, t, J = 7.5 Hz, CH_2CO), 2.95 (6H, s, $(CH_3)_2N$), 2.61 (2H, t, J = 7.0 Hz, 4-CH_2), 2.08 (3H, s, 7-CH_3), 2.01 (3H, s, 8-CH_3), 1.98 (3H, s, 5-CH_3), 1.79 (2H, m, 3-CH_2), 1.23 (3H, s, 2-CH_3), 0.87 (9H, d, J = 6.5 Hz, $CHCH_3$), 0.86 (3H, d, J = 6.5 Hz, $CHCH_3$). Anal. Calcd for $C_{34}H_{59}NO_3HCl$: C, 72.11; H, 10.68; N, 2.47. Found: C, 71.47; H, 11.06; N, 2.56.

d-α-*Tocopheryl N,N-Diethyl-β-aminopropionate Hydrochloride* (***8***)—Yield 57%. mp 103 °C. MS *m/z*: 557 (M^+ − HCl). ^{1}H-NMR δ: 3.56 (2H, dd, J = 7.5, 7.0 Hz, NCH_2), 3.31 (4H, m, $(CH_3CH_2)_2N$), 3.25 (2H, t, J = 7.5 Hz, CH_2CO), 2.63 (2H, t, J = 7.0 Hz, 4-CH_2), 2.09 (3H, s, 7-CH_3), 2.02 (3H, s, 8-CH_3), 1.99 (3H, s, 5-CH_3), 1.82 (2H, m, 3-CH_2), 1.38 (6H, t, J = 7.5 Hz, $(CH_3CH_2)_2N$), 1.25 (3H, s, 2-CH_3), 0.88 (9H, d, J = 7.0 Hz, $CHCH_3$), 0.86 (3H, d, J = 8.0 Hz, $CHCH_3$). Anal. Calcd for $C_{36}H_{63}NO_3HCl{\cdot}0.5H_2O$: C, 71.66; H, 10.86; N, 2.32. Found: C, 71.62; H, 10.86; N, 2.20.

d-α-*Tocopheryl N,N-Dimethyl-γ-aminobutyrate Hydrochloride* (***9***)—Yield 75%. mp 121 °C. MS *m/z*: 543 (M^+ − HCl). ^{1}H-NMR δ: 3.26 (2H, t, J = 8.5 Hz, NCH_2), 2.91 (6H, s, $(CH_3)_2N$), 2.82 (2H, t, J = 7.5 Hz, CH_2CO), 2.61 (2H, t, J = 7.0 Hz, 4-CH_2), 2.15 (2H, dd, J = 8.0, 8.5 Hz, CH_2CH_2CO), 2.08 (3H, s, 7-CH_3), 1.99 (3H, s, 8-CH_3), 1.96 (3H, s, 5-CH_3), 1.79 (2H, m, 3-CH_2), 1.25 (3H, s, 2-CH_3), 0.87 (9H, d, J = 6.5 Hz, $CHCH_3$), 0.86 (3H, d, J = 7.0 Hz, $CHCH_3$). Anal. Calcd for $C_{35}H_{61}NO_3HCl$: C, 72.44; H, 10.77; N, 2.41. Found: C, 72.05; H, 10.97; N, 2.40.

d-α-Tocopheryl betaine chloride (**10**) was obtained from **1**, betaine hydrochloride, and DCC using an analogous procedure.

d-α-*Tocopheryl N,N,N-Trimethylaminoacetate Chloride* (***10***)—Yield 45%. mp 173 °C. MS *m/z*: 530 (M^+ − Cl). ^{1}H-NMR δ: 4.89 (2H, s, NCH_2), 3.42 (9H, s, $(CH_3)_3N$), 2.64 (2H, t, J = 7.0 Hz, 4-CH_2), 2.10 (3H, s, 7-CH_3), 2.04 (3H, s, 8-CH_3), 2.01 (3H, s, 5-CH_3), 1.82 (2H, m, 3-CH_2), 1.23 (3H, s, 2-CH_3), 0.88 (9H, d, J = 6.5 Hz, $CHCH_3$), 0.86 (3H, d, J = 7.5 Hz, $CHCH_3$). Anal. Calcd for $C_{34}H_{60}NO_3Cl{\cdot}0.5H_2O$: C, 70.98; H, 10.69; N, 2.43. Found: C, 71.09; H, 10.67; N, 2.69.

Water Solubility—A 1.0 mL aliquot of distilled water was added to 100 μmol of the ester placed in a screw-capped vial. The mixture was then sonicated for 10 min. The vials were protected from light by wrapping them with aluminum foil and then rotating them in a 25 °C constant temperature water bath for 5 h. The mixture was filtered through a 0.45 μm Millipore filter (HAWP 01300) and the ester in the filtrate was determined by means of HPLC. The adherence of the esters to the filter was ignored.

Hydrolytic Studies—The hydrolysis of the esters was studied in isotonic phosphate buffer (pH 7.4), human plasma, rat plasma, and rat liver homogenate 9000*g* supernatant at 37 °C. The *d*-α-tocopherol esters **2–10** and TA were dissolved in 1% Polysorbate 80 stock solution.

The reactions in phosphate buffer, rat plasma, and human plasma were initiated by adding 50 μL of stock solution of the compounds to 950 μL of preheated reaction medium. The initial concentration of the compounds was 2×10^{-4} M. The solutions were incubated at 37 °C. At appropriate intervals, samples were taken and analyzed for remaining ester as well as for α-tocopherol formed by the HPLC method described below. For the analysis of the plasma, 100 μL samples of the reaction solution were withdrawn and added to 350 μL of methanol containing *dl*-tocol as the internal standard in order to deproteinize them. After vortex mixing for 1 min and centrifuging for 5 min, the clear supernatant was analyzed for remaining ester derivatives and formed α-tocopherol using HPLC. In phosphate buffer, the apparent first-order rate constants for the hydrolysis were obtained by linear regression analysis of the natural logarithm of concentration vs time (correlation coefficient > 0.97).

Liver Enzymatic Hydrolysis of the Esters—The 20% rat liver homogenate was prepared in isotonic phosphate buffer (one part tissue to four parts buffer). The tissue was homogenized using a Polytron homogenizer (Kinematica) and the homogenate was centrifuged at 9000g for 90 min.

The reactions in the rat liver homogenate supernatant were initiated by adding 100 μL of stock solution of the esters and 100 μL of phosphate buffer to 800 μL of the preheated 20% liver homogenate supernatant and the procedure was identical to the reaction in rat plasma. The initial concentration of the esters was $2–0.1 \times 10^{-4}$ M. The initial hydrolytic rate in units of moles of α-tocopherol formed per liter of reaction medium volume was calculated from the initial slope of the formation plot of α-tocopherol vs time. No measureable chemical hydrolysis of the ester occurred during the time span of these hydrolysis studies as evident by the HPLC mentioned below.

The effects of eserine on the hydrolysis of the esters in the liver preparation were also studied. The procedure for the experiment was the same as that mentioned above except that 100 μL of eserine solution was added in place of phosphate buffer to the supernatant fraction of liver homogenate at the beginning of the experiment. Eserine was used at a 0–2.5 mM concentration.

HPLC Analysis—A Shimadzu HPLC system (Kyoto, Japan) was used. The system consisted of a pump (LC6A), an auto sample injector (SIL 9A), a UV detector (SPD-6AV), a spectrofluorophotometer (RF-540) equipped with a 12 μL LC flow cell, and peak integrators (C-R7A). The eluent was monitored spectrophotometrically at 283 nm and spectrofluorometrically at an excitation of 298 nm and emission of 325 nm. For analysis of compounds TA and **2–4**, a reversed-phase column CAPCELL PAK C18 (4.6 × 200 mm, Shiseido, Tokyo, Japan) and a mobile phase of methanol–acetonitrile containing 0.02 M acetic acid and sodium acetate at a flow rate of 1.0 mL/min were used. For **5–10**, a reversed-phase column, Wakosil 5C4 4.6 × 150 mm (Wako Pure Chem. Ind., Osaka Japan), and a mobile phase of 90:10, v/v methanol–0.1 M acetate buffer (pH 5.0) at a flow rate of 1.0 mL/min were employed. Quantitation of these compounds was achieved using linear calibration curves of peak area vs concentration.

References and Notes

1. Weisel, R. D.; Mickle, D. A. G.; Finkle, C. D.; Tumiati, L. C.; Madonik, M. M.; Ivanov, J.; Burton, G. W.; Ingold, K. U. *Circulation* **1989**, *80*, III–14–18.
2. Massey, K. D.; Burton, K. P. *Am. J. Physiol.* **1989**, *256*, H1192–1199.
3. Mickle, D. A. G.; Ki, R. K.; Weisel, R. D.; Birnbaum, P. L.; Wu, T. W.; Jackowski, G.; Madonik, M. M.; Burton, G. W.; Ingold, K. U. *Ann. Thorac. Surg.* **1989**, *47*, 553–557.
4. Grisar, J. M.; Petty, M. A.; Bolkenius, F. N.; Dow, J.; Wagner, J.; Wagner, E. R.; Haegele, K. D.; Jong, W. D. *J. Med. Chem.* **1991**, *34*, 257–260.
5. Bieri, J. G.; Corash, L.; Hubbard, V. S. *N. Engl. J. Med.* **1983**, *308*, 1063–1071.
6. MacMahon, M. T.; Thompson, G. R. *Eur. J. Clin. Invest.* **1970**, *1*, 161–166.
7. Bell, E. F.; Brown, E. J.; Milner, R.; Sinclair, J. C.; Zipursky, A. *Pediatrics* **1979**, *63*, 830–832.
8. Morselli, P. L.; Franco-Morselli, R.; Bossi, L. *Clin. Pharmacokinet.* **1980**, *5*, 485–527.
9. Newmark H. L.; Pool, W.; Bauernfeind, J. C.; Ritter, E. D. *J. Pharm. Sci.* **1975**, *64*, 655–657.
10. Pedraz, J. L.; Calvo, B.; Bortolotti, A.; Celardo, A.; Bonati, M. *J. Pharm. Pharmacol.* **1989**, *41*, 415–417.
11. Stella, V. In Pro-drugs as Novel Drug Delivery Systems; Higuchi, T.; Stella, V., Ed.; American Chemical Society: Washington, DC, 1975; pp 1–115.

12. Sinkula, A. A.; Yalkowsky, S. H. *J. Pharm. Sci.* **1975**, *64*, 181–210.
13. Bundgaard, H. In Design of Prodrugs; Bundgaard, H., Ed.; Elsevier: Amsterdam, 1985; pp 1–92.
14. Lineweaver, H.; Burk, D. *J. Am. Chem. Soc.* **1934**, *56*, 658–666.
15. Nakamura, T.; Aoyama, Y.; Fujita, T.; Katsui, G. *Lipids* **1975**, *10*, 627–633.
16. Bundgaard, H.; Larsen, C.; Thorbek, P. *Int. J. Pharm.* **1984**, *18*, 67–77.
17. Bundgaard, H.; Falch, E. *Int. J. Pharm.* **1985**, *25*, 27–39.
18. La Du, B. N.; Snady, H. *Handb. Exp. Pharmacol.* **1971**, *28/2*, pp. 477–499.
19. Walker, C. H.; Machness, M. I. *Biochem. Pharmacol.* **1983**, *32*, 3265–3269.
20. Moroder, L.; Hallett, A.; Wunsch, E.; Keller, O.; Wersin, G. *Hoppe-Seyler's Z. Physiol. Chem.* **1976**, *357*, 1651–1653.

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