30 min. The color of iodine disappeared after 5 min, and a heterogeneous pale yellow solution was obtained. The molar ratio of two products, *p*-chloroiodobenzene and *p*-nitroiodobenzene, was ca. 4.5:1 by GLC analysis after a correction for the concentration of the acids.

Acknowledgment. We thank Mr. Nanao Watanabe for the preparation of several pyridine derivatives. This research is supported by a grant-in-aid for scientific research, Ministry of Education, Japan.

Registry No. 1 (X = I), 1120-90-7; 1 (X = Br), 626-55-1; 2 (X = I; Y = NO_2), 636-98-6; 2 (X = I; Y = Cl), 637-87-6; 2 (X = I; Y = H), 591-50-4; 2 (X = I; Y = CH_3), 624-31-7; 2 (X = Br; Y = Cl), 106-39-8; 2 (X = Br; Y = H), 108-86-1; $Hg(PA)_2$, 86668-72-6; $Hg(NA)_2$, 41408-73-5; $Hg(INA)_2$, 41408-74-6; $Hg(NA\text{-}O)_2$, 86668-73-7; Tl(PA), 86668-74-8; Tl(NA), 86668-75-9; Tl(INA), 86668-76-0; $Tl(NA)_3$, 86668-77-1; HgO, 21908-53-2; nicotinic acid, 59-67-6; *p*-nitrobenzoic acid, 62-23-7; *p*-chlorobenzoic acid, 74-11-3; *p*-methylbenzoic acid, 99-94-5; benzoic acid, 65-85-0; 3-pyridinylmercury(II) chloride, 5428-90-0; bis(3-pyridyl)mercury, 20738-78-7.

A Chemical Model for the Mechanism of Vitamin K Epoxide Reductase[1]

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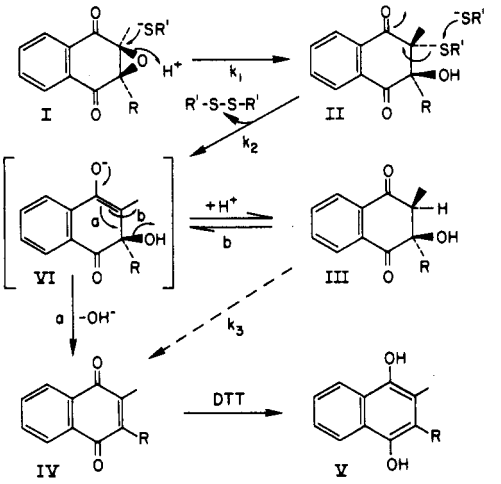
The reactions of vitamin K_1 epoxide and 2,3-dimethylnaphthoquinone epoxide with dithiothreitol and mercaptoethanol have been studied as a potential model for the mechanism of the enzyme vitamin K epoxide reductase. The reaction proceeds with thiol addition to open the oxirane ring, yielding preferentially the 2-thio-3-hydroxy adduct in the case of vitamin K_1 epoxide. Reaction with a second thiol group results in reductive cleavage of this adduct and elimination of water to yield the quinones. All steps are catalyzed by triethylamine. Evidence for a hydroxy-substituted 2,3-dihydronaphthoquinone enolate intermediate in the second step is found in the observation of the corresponding keto compounds as equilibrated side products in the reaction with dithiothreitol. With this reagent, intramolecular reaction to form the cyclic disulfide permits cleavage of the thiol adduct under mild conditions where protonation of the enolate is rapid relative to elimination of the hydroxyl. Isolation and characterization of the intermediates and their conversion to the quinones are described.

Vitamin K epoxide reductase is a key enzyme in the function of vitamin K to promote coagulation factor biosynthesis.[3-6] Vitamin K 2,3-epoxide is formed as a product of the vitamin K dependent microsomal carboxylation of peptide-bound glutamyl residues.[7-10] Vitamin K epoxide reductase is necessary to convert the epoxide back to quinone to permit continued carboxylation at physiologic concentrations of the vitamin. The clinically significant coumarin anticoagulants block this cycle by inhibiting the epoxide reductase.[11-14]

A mechanism for vitamin K epoxide reductase has recently been proposed, and a chemical model for the reaction of the epoxide with a reduced active site disulfide has been demonstrated.[15] Scheme I illustrates the proposed pathway of vitamin K epoxide reduction by thiols under alkaline conditions. Silverman[15] examined the reaction of dimethylnaphthoquinone epoxide (Ib) with ethanethiol and triethylamine and demonstrated the formation of the thiol adduct (II″b). This was converted to dimethylnaphthoquinone (IVb) by treatment with sodium ethanethiolate. The enolate (VI) was postulated as an intermediate in this reaction. We have now found evidence

Scheme I. Proposed Pathway of Vitamin K Epoxide Reduction[a]



[a] Ia-Va, R = phytyl. Ib-Vb, R = methyl. IIa,b, R′ = $CH_2(CHOH)_2CH_2SH$. II′b, R′ = CH_2CH_2OH. II″b, R = CH_2CH_3. Stereochemistry is shown to indicate the relationship between structures. All materials were racemic mixtures. The dashed line indicates the macroscopic rate constant.

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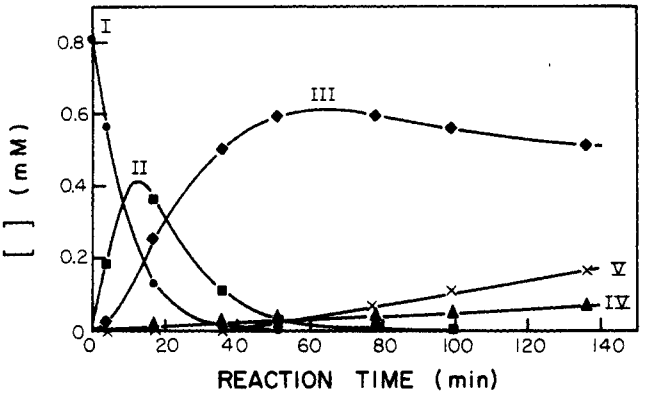

Figure 1. Kinetics of the reaction of Ia with 89 mM DTT and 92 mM TEA. Concentrations of I (●), II (■), III (♦), IV (▲), and V (×) are shown.

for this intermediate in the observation of its keto tautomer (3-hydroxy-2,3-dimethyl-2,3-dihydro-1,4-naphthoquinone)[16] (IIIb) and the equivalent 2- and 3-hydroxy derivatives of vitamin K_1 in the reactions of the corresponding epoxides with dithiothreitol or mercaptoethanol and triethylamine. These studies confirm the overall mechanism previously proposed[15] and are of particular interest in view of the recent observation of hydroxy vitamin K (IIIa) as a product of the genetically altered vitamin K epoxide reductase present in hepatic microsomes derived from warfarin-resistant rats.[17]

Results

Vitamin K_1 epoxide (Ia) reacts with dithiothreitol (DTT) and triethylamine (TEA) in ethanol to form vitamin K quinone (IVa). Hydroquinone (Va) formation is apparent in a second reduction step. Two additional materials formed during the reaction are separable by reverse-phase HPLC. The kinetics of the reaction (Figure 1) suggest that these are sequentially formed intermediates in the conversion of Ia to IVa. The decay of each species is first order with the rate constants $k_1 = 0.11$, $k_2 = 0.086$, and $k_3 = 0.023$ min^{-1} at 82 mM TEA and 89 mM DTT for the indicated steps shown in Scheme I.

Structure determination and the chemistry of product formation were simplified by studying the reaction of 2,3-dimethyl-1,4-naphthoquinone 2,3-epoxide (Ib). In addition to the analogous species formed from vitamin K_1 epoxide (II, III, and IV), two uncharacterized materials (X) are formed. These were isolated and shown to be stable under conditions for formation of III and IV, and thus are minor byproducts, not intermediates along the reaction pathway to quinone. The reaction of Ib with mercaptoethanol under similar conditions was also investigated. The first-order rate constant for reaction of Ib with mercaptoethanol is comparable to that for the reaction with DTT at similar thiol concentrations: $k_1 = 0.094$ min^{-1} at 200 mM mercaptoethanol and 5 mM TEA and $k_1 = 0.15$ min^{-1} at 94 mM DTT and 5 mM TEA in methanol. Because of differences in the rate constants for the second step, only the initial intermediate II'b was formed in the reaction with mercaptoethanol. Its conversion to III and IVb required more vigorous reaction conditions.

Sufficient material was prepared for structure determination under conditions giving maximum selectivity for IIb or IIIb. IIb was identified as the DTT adduct 2(*S*)-((2,3-*threo*-dihydroxy-4-mercaptobutyl)thio)-3-hydroxy-2,3-dimethyl-2,3-dihydro-1,4-naphthoquinone on the basis of its UV, IR, ^1H NMR, and mass spectra, analogous to those of the 2-ethylthio-3-hydroxy adduct previously demonstrated.[15] IIIb was found to be 3-hydroxy-2,3-dimethyl-2,3-dihydro-1,4-naphthoquinone by the same criteria. The UV spectra of II and III are similar to that of I, indicating structures which are isoelectronic with the epoxide for which the diketobenzenoid substructure is the principal chromophore.[18] In contrast, the spectrum of IV differs due to increased conjugation via the 2,3-double bond. The same conclusion may be reached from the carbonyl stretching frequencies in the IR spectra which are similar for I, II, and III but shifted by 45 cm^{-1} to lower frequency in IV. Additional bands in the IR characteristic of the hydroxyl, sulfhydryl, thioether, and epoxide functionalities, appear in the appropriate spectra of I–IVb.

The molecular ion is not observed in the mass spectrum of IIb, nor are any fragments greater than the molecular ion of IIIb. All peaks present in the spectrum of IIIb also appear in that of IIb in the same relative abundances. Additional peaks are present that arise from the DTT–adduct fragment. No fragments are observed that would arise from the IIb molecular ion directly. In contrast, the molecular ion and numerous fragments retaining portions of the thiol adduct are observed in the mass spectrum of II'b , the mercaptoethanol adduct. The fragment due to loss of the adduct by simple bond scission (203) is much more prominent than that for elimination with proton shift (204). No simple scission is evident in the mass spectrum of IIb. This result indicates that formation of the 204 fragment proceeds predominantly by intramolecular reaction with the distal sulfhydryl of the adduct to form IIIb and oxidized DTT. Many of the fragments of IIIb clearly arise from fragmentation of the IVb molecular ion. The same reaction pathway that occurs in solution, II to III to IV, takes place via thermal and fragmentation processes under mass spectra conditions.

Direct nucleophilic attack of the thiol at the oxirane ring should yield an initial adduct having the 2,3-threo configuration,[19] yet two resonances each of equal intensity are observed for the 2- and 3-methyl groups and for the 3-hydroxy proton in the NMR spectrum of IIb. These are diastereomeric differences in chemical shift due to the additional asymmetric centers in DTT, not threo and erythro isomers, as were observed for the analogous ethanethiol adduct formed under different reaction conditions in the previous model study.[15,20] In the spectrum of the mercaptoethanol adduct (II'b), which should be a racemic mixture, only one set of peaks was observed, indicating that threo/erythro interconversion did not occur under the conditions used here.

All resonances in the NMR spectrum of IIIb can be unambiguously assigned. Splitting of the 2-methyl doublet and the 2-hydrogen quartet was confirmed by decoupling. In contrast to the spectrum of IIb, only one set of resonances is observed for each group. The isomeric difference in the methyl group resonances of *threo*- and *erythro*-2,3-dimethyl-2-(ethylthio)-3-hydroxy-2,3-dihydro-1,4-naphthoquinone is approximately 0.25 ppm.[15,20] A difference of this magnitude may also be expected for the *cis*-

(16) Abbreviations used: (DTT) dithiothreitol, (TEA) triethylamine, (HPLC) high-pressure liquid chromatography. Naphthoquinone derivatives are abbreviated by Roman numerals and subscripts as indicated in the legend to Scheme I. The position numbering system for the predominant isomer of hydroxy vitamin K_1 (IIIa) has been applied for all structures.

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Table I. Kinetics of the Reaction of Ib with DTT: Pseudo-First-Order Rate Constants for Conversion of Ib

at 85 mM DTT		at 6 mM TEA	
[TEA], mM	k_1, min^{-1}	[DTT], mM	k_1, min^{-1}
2.3	0.10	8	0.6
5.0	0.15	21	0.10
7.7	0.25	42	0.13
46	1.06	83	0.17
92	2.16	208	0.24
		416	0.26

and *trans*-dimethyl configurations of IIIb and should have been readily detected. Thus apparently only one of the two possible isomers is formed. When the 3-methyl group was irradiated in a double resonance experiment, a small nuclear Overhauser effect was observed for both the 2-methyl and the 2-proton (1.1-fold enhancement each). This result is most consistent with the *trans*-dimethyl configuration with the diequatorial methyl chair conformation predominating.

The reaction of phylloquinone epoxide (Ia) is expected to be more complex than that of Ib. Attack at the 2- vs. 3-positions yields structural isomers. The intermediate IIa was not isolated, but selectivity of the initial thiol addition is reflected in the structure of the isolated IIIa. The NMR spectrum of IIIa indicates the presence of both the 2- and 3-hydroxy isomers in a ratio of 1:3.3. Separation of these isomers was not achieved. For the 3-hydroxy isomer, assignment of the 2-proton and the 2-methyl group and of the 2′-vinyl proton and the 1′-methylene protons was confirmed by decoupling. The pair of doublets of doublets arises from the inequality of the methylene protons adjacent to the asymmetric center. For the same reason, the 3-proton of the 3-hydroxy isomer appears as a doublet of doublets rather than as a triplet. The 2′-vinyl proton resonance is not a true triplet, but appears as one due to the fortuitous condition that $J_1 = J_2$. The eight lines expected for each of the methylene protons of the 2-hydroxy isomer overlap those of the 3-hydroxy isomer, and only a few are detected. Two sets of resonances are observed for the 2′-vinyl proton, 3′-vinyl methyl, and 4′-methylene on which the estimate of isomeric distribution is based.

The kinetics of these reactions were not studied in detail; however, some data are available from attempts to maximize yields of the intermediates. The disappearance of Ia or Ib is first order. As shown in Table I, the reaction of Ib with DTT is also first order in TEA at high DTT. The reaction shows saturation in DTT at low TEA consistent with formation of a TEA–DTT complex or ion pair as the active reagent.

When the isolated intermediates are used, the subsequent steps of the overall reaction could be demonstrated independently. The DTT adduct (IIb) contains a free sulfhydryl group and can undergo intramolecular reaction. Purified IIb decomposed slowly on storage. In the presence of 3.6 mM TEA, it decomposed rapidly by a first-order process, $k_2 = 0.043$ min^{-1}, comparable to its rate of decay in the reaction of Ib with DTT. The products IIIb and IVb initially appeared in a ratio of 2.3:1. The mercaptoethanol adduct (II′b) was stable in the presence of 200 mM mercaptoethanol and 5 mM TEA. At 720 mM TEA II′b reacted slowly, $k_2 = 0.0044$ min^{-1}, to yield a mixture of IIIb and IVb. Thus in the overall reaction of Ib with DTT, conversion of IIb to products proceeds predominately through intramolecular disulfide formation.

In the presence of TEA alone, IIIb is quantitatively converted to quinone (IVb) without the appearance of any isolable intermediates. Thus no redox chemistry is required for this step. As shown in Table II, the rate of IIIb conversion at low TEA concentrations is insufficient to account for the rate of IVb formation in the breakdown of IIb. The kinetics appear to be complex at low TEA but simple first order in base at high concentrations. The reaction could also be monitored spectrophotometrically as shown in Figure 2, which indicated that the concentration of any intermediates in the reaction at high TEA must be low. The conversion of the hydroxyphylloquinone derivative to quinone (IVa) was also investigated by using purified IIIa (a 3.3:1 mixture of 3-hydroxy and 2-hydroxy isomers). A single first-order reaction was observed, $k_3 = 0.013$ min^{-1} at 712 mM TEA, indicating the rates of reaction for the two isomers are nearly identical. Although this step occurs readily in methanol, no reaction of IIIb was observed after a 2-h treatment with 712 mM TEA in hexane, emphasizing the importance of solvent stabilization of a polar transition state during the reaction.

Table II. Kinetics for Conversion of Isolated IIIb to IVb: First-Order Rate Constants for Conversion of IIIb

[TEA], mM	k_1, min^{-1}
17	0.0012
28	0.0019
84	0.0045
170	0.0085
480	0.016
910	0.024

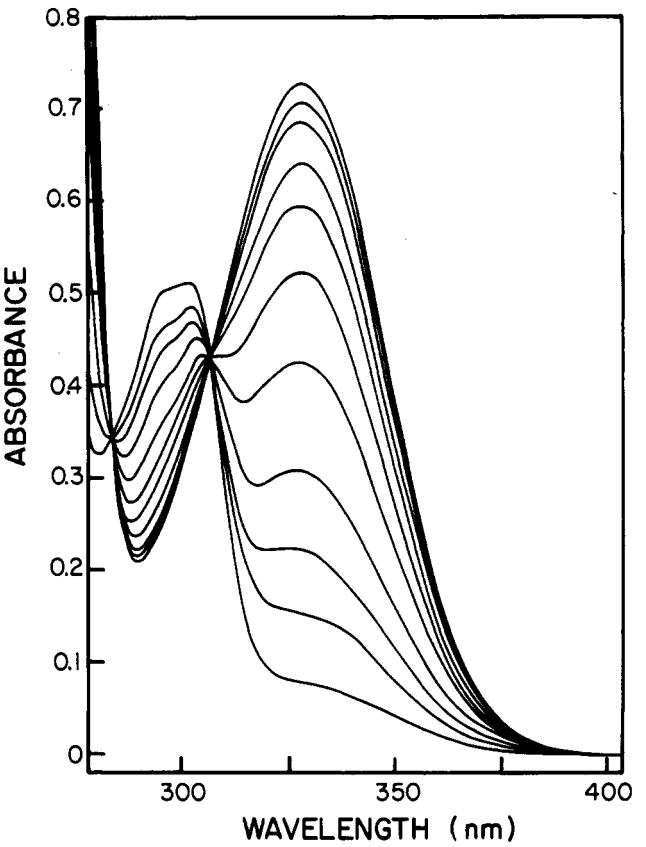

Figure 2. Reaction of IIIb to form IVb. Initial and final spectra are for the pure materials, λ_{max} IIIb = 302 nm, λ_{max} IVb = 330 nm. Intermediate spectra are shown for 1.0, 4.25, 9.0, 18, 30, 45, 60, and 121 min after initiation of the reaction with 0.46 M TEA.

Discussion

The enolate VI shown in Scheme I was postulated by Silverman[15] as an intermediate in the reaction of the ethanethiol adduct analogue of IIb with sodium ethanethiolate. Formation of the related keto compounds (IIIa,b)

Table III. HPLC Retention Times (min)

column	II	V	III	X	II′	I	IV
				Derivatives of IVa			
95% MeOH[a]	3.75	4.30	5.62			9.32	13.32
100% MeOH[a]			3.01				5.20
				Derivatives of IVb			
50% MeOH[a]	3.10	3.33	3.66	5.33	3.05	9.53	17.28
70% MeOH[a]			2.00				4.12
				Derivatives of IVa			
95% MeOH[b]	6.16		9.57			15.33	21.17
100% MeOH[b]						6.87	8.73
				Derivatives of IVb			
50% MeOH[b]	5.20		6.25	8.00		14.50	24.00

[a] Analytical column. [b] Preparative column.

and their conversion to the quinones (IVa and IVb) in this study may be taken as evidence for the occurrence of VI. Direct conversions of II to III and of III to IV are unfavorable because of unstabilized carbanion formation in the transition states. The pathway via VI is favored by resonance stabilization of the enolate. The observation of III is then dependent on the relative rates of hydroxyl elimination (pathway a) and of protonation of the enolate (pathway b) under the reaction conditions necessary for reductive elimination of the thiol adduct. Favorable approximation and six-membered ring formation in the disulfide product and neighboring group effects of the vicinyl hydroxyls facilitate the reaction of the DTT adducts (IIa and IIb) under mild conditions in the presence of low concentrations of TEA. The reaction of the mercaptoethanol adduct (II′b) with mercaptoethanol could only be observed at a higher TEA concentration at which the rate of the subsequent step is also enhanced. In the previous model study,[15] the ethanethiol adduct was shown to be even less reactive. No reaction with ethanethiol and triethylamine was observed. The reaction with sodium ethanethiolate occurred readily, but under those conditions protonation of the enolate should be slow and no accumulation of the keto tautomer III was observed.

Protonation of the enolate VI from both faces might be expected to yield a mixture of both *cis*- and *trans*-dimethyl isomers of IIIb. Formation of only a single isomer, however, is not inconsistent with the pathway shown in Scheme I, as stereospecific protonations of enolates are known.[21] The neighboring asymmetric center may provide some kinetic stereochemical steering, and the observed *trans*-dimethyl isomer should be more thermodynamically stable if equilibration can occur under these reaction conditions.

In general, the derivatives of Ib were more reactive than those of Ia. Isolation of IIb and IIIb was difficult due to conversion to the next sequential species during workup. This was overcome by the use of acetic acid to quench the reactions and emphasizes the importance of basic catalysis. Selectivity in the reaction of Ia is low owing to only a slight steric effect of the phytyl chain. Slight contrary steric and electronic effects yield little difference in the reactivity of the 2- and 3-hydroxy isomers in the subsequent steps.

The reactions described here have been useful in providing synthetic standards for use in the study of biological hydroxy vitamin K formation and in converting samples of vitamin K epoxide and hydroxy vitamin K to phylloquinone for analysis of the phytyl-chain cis/trans isomer composition.[17]

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Experimental Procedure

General Methods. ^{1}H NMR spectra were run on a Bruker WH 270-MHz instrument with Nicolet 1180E data system and 293A pulse programmer. All spectra were recorded in $CDCl_3$ and are reported as δ (ppm) from internal Me_4Si standard. Mass spectra were obtained on a Kratos MS-9/DS-50 mass spectrometer at 70 eV and 80 °C. NMR and mass spectra were run by Eric Peterson and Mel Micke of the Department of Biochemistry facilities. IR spectra were recorded with a Perkin-Elmer Model 567 IR spectrophotometer for thin films evaporated at room temperature from $CHCl_3$ onto sodium chloride blocks. UV spectra were measured in hexane (derivatives of IVa) or methanol (those of IVb) in 1-cm pathlength cells with a Varian Model 636 spectrophotometer.

Reverse-phase high-pressure liquid chromatography (HPLC) was performed with Waters Model 6000A pump, 450 absorbance detector, UK-6 injector or M45 pump, 440 absorbance detector, 720 integrator, and 710 WISP automatic injector. Analytical separations were performed with a μBondapak C18 10-μm column (27324) run at 2 mL/min. Preparative chromatography was with a μBondapak C18 10-μm semipreparative column (84176) run at 4 mL/min. Solvents were the indicated v/v mixtures of methanol and water listed in Table III. Compounds were identified by their characteristic retention times for each column and solvent and by reference to the absorption spectra of standards. Quantitation was by peak height or total integrated area with reference to standard solutions. Concentrations of standards were determined from their UV spectra by using literature values: $\epsilon_{248\ nm}$ = 18 900 M^{-1} cm^{-1} for IVa,b[22] and $\epsilon_{226\ nm}$ = 30 800 M^{-1} cm^{-1} for Ia,b[23] The extinction coefficient for IIIa (34 600 M^{-1} cm^{-1} at 223 nm) was determined by its synthesis from ^{3}H-labeled IVa. This value has been assumed for the extinction coefficients of IIa,b, II′b, and IIIb as well. Where possible, these values were corroborated by the results of reactions giving quantitative conversion of an unknown to a standard. The response factor for hydroquinone Va was determined from its conversion to IVa by air oxidation.

Derivatives of IVa are oils. Small quantities of material were used in all experiments so no attempt was made to isolate derivatives of IVb in solid form. All samples were handled as solutions or evaporated residues. Concentration in vacuo or under nitrogen was at room temperature to avoid spontaneous breakdown of the intermediate products. All manipulations were carried out under yellow lights or in a darkened room. All reactions were at room temperature, 20 ± 2 °C, except as noted.

$CDCl_3$, Me_4Si, mercaptoethanol, and triethylamine were from Aldrich; HPLC grade solvents used throughout were from Fischer, dithiothreitol from Calbiochem, and vitamin K_1 from Sigma. 2,3-Dimethyl-1,4-naphthoquinone (IVb)[24] and 2,3-dimethyl-1,4-naphthoquinone 2,3-epoxide (Ib)[23] were prepared by Dr. J. Finnan,

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formerly of this group, according to literature methods. Radiolabeled vitamin K_1 (5,6,7,8-^{3}H) was a gift of Dr. Charles Siegfried, Department of Biochemistry, University of Nebraska College of Medicine, Omaha, NE.

2-Methyl-3-(*trans*-phytyl)-1,4-naphthoquinone (IVa). Pure *trans*-vitamin K_1 was separated from the contaminating *cis*-phytyl isomer by silica gel column chromatography with 7.5% *n*-butyl ether in hexane[25] and was used in all experiments. Analysis of the IVa recovered after conversion to the epoxide (Ia) and subsequent reduction to the quinone as described below showed that no *cis*/*trans* isomerization of the side chain occurred under any of the reaction conditions. Analysis of *cis*- and *trans*-vitamin K_1 was by HPLC with a Waters μPorasil 10-μm column (27477) run at 1 mL/min 7.5% $CHCl_3$ in hexane (cis 3.60, trans 4.07 min).

2-Methyl-3-phytyl-2,3-epoxy-2,3-dihydro-1,4-naphthoquinone (Ia). Vitamin K_1 was quantitatively converted to its 2,3-epoxide by alkaline hydrogen peroxide oxidation.[23] The product was freed of trace impurities by preparative HPLC using 100% methanol.

2,3-Dimethyl-1,4-naphthohydroquinone (Vb) and Vitamin K_1 Hydroquinone (Va)). The reduced forms of IVa,b were prepared by dithionite reduction of the corresponding quinones in ethereal solution.[26]

Reaction of Ia with DTT. Reactions were conducted in deoxygenated ethanol in septum-stoppered vials under nitrogen. Kinetic measurements were made by using WISP vials fitted with limited-volume inserts and resealable septa, permitting multiple injections directly from the reaction mixture with minimum oxygen contamination. Reactions were generally run under pseudo-first-order conditions with ca. 1.0 μmol of Ia and 10 μmol of DTT in 100 μL of ethanol. Following a preinitiation analysis to determine the zero time concentration, the reaction was started by addition of 10–100 μmol of TEA in a volume of 2–15 μL. At various times aliquots, ca. 10 μL, were injected and analyzed by HPLC. Rate constants for the indicated steps shown in Scheme I were determined from linear plots of log $(1 - x)$ vs. time.

3(2)-Hydroxy-2-methyl-3-phytyl-2,3-dihydro-1,4-naphthoquinone (IIIa). Compound Ia (20 μmol) and 100 μmol of DTT were dissolved in 100 μL of ethanol, and 13 μL of TEA was added. After 15 min, the reaction was diluted with 3 mL of hexane, washed with saturated sodium chloride, and dried over anhydrous sodium sulfate, and the solvent was evaporated under nitrogen. The residue was redissolved in 1 mL of methanol and separated by preparative HPLC using 95% methanol as eluant. The collected IIIa containing fraction was evaporated in vacuo to yield 10 μmol (50%) of IIIa as a mixture 3.3:1 by NMR of the 3-hydroxy and 2-hydroxy isomers: UV (hexane), λ_{max} nm (log ε) 223 (4.54), 250 (4.04), 294 (3.32), sh325 (1.62); ^{1}H NMR ($Me_4Si/CDCl_3$) 3-hydroxy isomer δ 0.80–0.94 (12 H), 0.94–1.44 (19 H), 1.48 (d, J = 6.6 Hz, 3 H), 1.55 (s, 3 H), 1.80 (t, J = 7.0 Hz, 2 H), 2.13 (dd, J_1 = 7.9, J_2 = 13.9 Hz, 1 H), 2.57 (dd, J_1 = 6.9, J_2 = 14.6 Hz, 1 H), 3.18 (q, J = 6.6 Hz, 1 H), 4.07 (s, 1 H), 4.86 (t, J = 7.5 Hz, 1 H), 7.78 (m, 2 H), 8.08 (m, 2 H); 2-hydroxy isomer δ 1.53 (s, 3 H), 1.70 (s, 3 H), 1.95 (t, J = 7.0 Hz, 2 H), 3.05 (dd, J_1 = 2.6, J_2 = 8.5 Hz, 1 H), 4.03 (s, 1 H), 5.36 (t, J = 7.5 Hz, 1 H); MS (70 eV), m/e 468.

Conversion of IIIa to IVa. IIIa (0.09 μmol) was dissolved in 90 μL of methanol and 10 μL of TEA was added. At various times, 10 μL was injected and analyzed by HPLC using 100% methanol as eluant.

Reaction of Ib with DTT. The reactions of Ib were conducted as described for Ia, but in deoxygenated methanol. Concentrations of Ib and DTT were similar, but typically 0.2–10 μmol of TEA in a reaction volume of 100 μL was used.

2(*S*)-((2,3-*threo*-Dihydroxy-4-mercaptobutyl)thio)-3-hydroxy-2,3-dimethyl-2,3-dihydro-1,4-naphthoquinone (IIb). Ib (80 μmol) and DTT (400 μmol) were dissolved in 2 mL of methanol, and 1.3 μL of TEA was added. After 30 min, the reaction was quenched with 4 μL of acetic acid and diluted with 2 mL of H_2O. The reaction mixture was separated by preparative HPLC in four 1-mL injections using 50% methanol as eluant. The collected IIb-containing fractions were concentrated in vacuo, shell frozen, and lyophilized to yield 53 μmol (66%) of IIb as an equal mixture of diastereomers: UV (methanol), λ_{max} nm (log ε) 225 (4.54), 254 (4.18), 297 (3.23), sh 337 (2.74). IR (film) 3480 (sb), 1690 (s), 1590 (s), 1450 (m), 1380 (w), 1265 (s sh), 1065 (m), 990 (mb), 790 (w), 750 (m), 710 (s) cm^{-1}; ^{1}H NMR ($Me_4Si/CDCl_3$) δ 1.46 (dd, 1 H), 1.58 (s, 1.5 H), 1.59 (s, 1.5 H), 1.66 (s, 1.5 H), 1.67 (s, 1.5 H), 2.6–3.0 (mm, 6 H), 3.38 (br s, 0.5 H), 3.47 (br s, 0.5 H), 3.56 (br s, 1 H), 3.71 (br s, 1 H), 7.83 (m, 2 H), 8.16 (m, 2 H). MS (70 eV), m/e (relative intensity) 204 (11), 189 (32), 186 (13), 171 (1.0), 161 (79), 152 (35), 147 (31), 143 (42), 134 (4.0), 133 (12), 115 (29), 108 (41), 105 (34), 104 (100), 87 (11), 77 (48), 76 (86), 70 (13), 64 (18), 59 (15).

2(*S*)-((2-Hydroxyethyl)thio)-3-hydroxy-2,3-dimethyl-2,3-dihydro-1,4-naphthoquinone (II′b). Ib (20 μmol) and 2-mercaptoethanol (400 μmol) were dissolved in 2 mL of methanol and 1.4 μL of TEA was added. After 30 min, 50 μL of acetic acid was added, and the reaction was partitioned between diethyl ether and saturated sodium chloride. The ether layer was dried with magnesium sulfate, filtrated, and the solvent was evaporated to yield 10 μmol (50%) of II′b: UV (methanol), λ_{max} nm (log ε) 225 (4.54), 254 (4.21), 297 (3.35), sh 337 (2.83); ^{1}H NMR ($Me_4Si/CDCl_3$) δ 1.62 (s, 3 H), 1.69 (s, 3 H), 2.70–2.93 (mm, 4 H), 3.69 (br s, 1 H), 3.93 (t, J = 5.6 Hz, 1 H), 7.83 (m, 2 H), 8.16 (m, 2 H); MS (70 eV), m/e (relative intensity) 280 (13), 262 (12), 237 (78), 219 (47), 204 (13), 203 (68), 202 (44), 191 (62), 161 (83), 160 (69), 159 (28), 147 (11), 143 (20), 133 (10), 132 (19), 131 (26), 115 (15), 113 (29), 105 (44), 104 (30), 87 (100), 77 (58), 76 (30).

3-Hydroxy-2,3-dimethyl-2,3-dihydro-1,4-naphthoquinone (IIIb). Ib (80 μmol) and DTT (400 μmol) were dissolved in 2 mL of methanol, and 26 μL of TEA was added. After 30 min, the reaction was quenched with 40 μL of acetic acid and diluted with 2 mL of water. The mixture was chromatographed and the collected IIIb solution evaporated as in the preparation of IIb to yield 56 μmol (70%) of IIIb: UV (methanol), λ_{max} nm (log ε) 223 (4.54), 252 (4.15), 302 (3.23), sh 330 (2.42). IR (film) 3480 (sb), 1690 (s), 1595 (s), 1450 (m), 1380 (m), 1270 (s), 1260 (s), 972 (s), 930 (m), 800 (m), 733 (m) cm^{-1}; ^{1}H NMR ($Me_4Si/CDCl_3$) δ 1.29 (s, 3 H), 1.46 (d, J = 6.7 Hz, 3 H), 3.18 (q, J = 6.7 Hz, 1 H), 4.0 (br s, 1 H), 7.81 (m, 2 H), 8.15 (m, 2 H); MS (70 eV), m/e (relative intensity) 204 (8.5), 189 (37), 186 (9.0), 171 (1.5), 161 (83), 147 (34), 143 (43), 133 (14), 115 (25), 105 (35), 104 (100), 77 (39), 76 (76).

Conversion of IIb to IIIb. IIb (0.15 μmol) was dissolved in 90 μL of methanol, and 10 μL of TEA (diluted 1:200) was added. At various times 10 μL was injected directly and analyzed by HPLC using 50% methanol as eluant.

Conversion of II′b to IIIb. II′b (0.14 μmol) was dissolved in 90 μL of 0.2 M mercaptoethanol in methanol, and 10 μL of TEA was added. The reaction was analyzed as above.

Conversion of IIIb to IVb. IIIb (0.4 μmol) was dissolved in 100 μL of methanol, and varying amounts of TEA (up to 13 μL) were added in each reaction. At various times 10 μL aliquots were injected directly and analyzed by HPLC using 70% methanol.

Conversion of IIIb to IVb (Spectral Measurement). IIIb (3.0 μmol) was dissolved in 0.935 mL of methanol, and 64 μL of TEA was added. Spectra were recorded at a scan rate of 50 nm/min leading to little kinetic distortion in the observed spectra. The spectra of 3.0 μmol of IIIb and of 3.0 μmol of IVb in 1 mL of methanol were recorded for comparison as the initial and final spectra. Cuvette temperature was constant at 28 °C.

Registry No. Ia, 25486-55-9; Ib, 86595-14-4; IIb, 86595-15-5; II′b, 86595-16-6; IIIa (3-hydroxy isomer), 86631-62-1; IIIa (2-hydroxy isomer), 85873-38-7; IIIb, 86595-17-7; IVa, 81818-54-4; IVb, 2197-57-1; Va, 16869-68-4; Vb, 38262-43-0; vitamin K epoxide reductase, 55963-40-1.

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