In Vivo Assay. Evaluation of compounds in conscious guinea pigs was carried out as described in the accompanying paper.[9a]

Acknowledgment. We warmly thank Dr. F. J. Brown and Dr. Y. K. Yee for discussions and exchanges of information during the course of this work. It is a pleasure to acknowledge the contributions of the NMR and MS groups, under the direction of Dr. C. L. Lerman and Dr. R. C. Spreen, respectively, and of J. M. Hulsizer and G. L. Moore (Large-Scale Laboratory) to the work reported. J. L. Morse and Dr. A. F. Heald are thanked for permission to quote the bioavailability of ICI 204,219.

Registry No. 1, 6146-52-7; 2, 70264-94-7; 3, 107786-36-7; 4a (R′ = CH_3), 107754-15-4; 4b, 126502-40-7; 5 (R′ = CH_3), 107754-14-3; 5 (R′ = CH_3, isocyanate), 126502-41-8; 6 (R′ = CH_3, R = cyclopentyloxy), 107754-19-8; 6 (R′ = CH_3, R = cyclopentylmethyl), 107786-49-2; 6 (R′ = CH_3, R = cyclopentylamino), 107753-65-1; 8 (R = cyclopentyloxy), 107786-92-5; 9 (R = cyclopentyloxy), 107786-93-6; 10 (R = cyclopentyloxy), 107786-91-4; 11a, 107786-90-3; 11b, 126644-52-8; 12, 126502-21-4; 13 (R′ = Me), 126502-22-5; 14, 126502-23-6; 15, 107786-85-6; 16, 126541-14-8; 17, 126541-14-8; 18, 107754-21-2; 19, 107786-37-8; 20, 107754-25-6; 21, 107754-26-7; 22, 107753-99-1; 23, 126541-15-9; 24, 107754-20-1; 25, 107786-57-2; 26, 107753-66-2; 27, 107754-23-4; 28, 107786-51-6; 29, 107803-03-2; 30, 107754-39-2; 31, 107786-56-1; 32, 107786-88-9; 33, 107786-58-3; 34, 126502-24-7; 35, 107753-76-4; 36, 126502-25-8; 37, 107786-63-0; 38, 107754-31-4; 39, 107786-65-2; 40, 107786-60-7; 41, 107786-67-4; 42, 107786-68-5; 43, 126502-26-9; 44, 126502-27-0; 45, 107753-78-6; 46, 126541-16-0; 47, 126502-28-1; 48, 126502-29-2; 49, 126502-30-5; 50, 107754-08-5; 51, 126502-31-6; 52, 107753-53-7; 53, 126502-32-7; 54, 126502-33-8; 55, 126502-34-9; 56, 126502-35-0; 57, 126541-17-1; 58, 126502-36-1; 59, 126502-37-2; 60, 126502-38-3; 61, 126502-39-4; LTE4, 75715-89-8; LTD4, 73836-78-9; trichloromethyl chloroformate, 503-38-8; cyclopentylamine, 1003-03-8; methyl 4-[(5-amino-1-methylindazol-3-yl)methyl]-3-methoxybenzoate, 126502-42-9; benzenesulfonamide, 98-10-2; cyclopentyl chloroformate, 50715-28-1; cyclopentylacetic acid, 1123-00-8.

Substituted Vitamin K Epoxide Analogues. New Competitive Inhibitors and Substrates of Vitamin K_1 Epoxide Reductase

Robert P. Ryall, Dhirendra L. Nandi,† and Richard B. Silverman*

Department of Chemistry and Department of Biochemistry, Molecular Biology, and Cell Biology, Northwestern University, Evanston, Illinois 60208-3113. *Received November 27, 1989*

2- and 3-substituted vitamin K 2,3-epoxide analogues were synthesized and tested as inactivators, inhibitors, and substrates for beef liver microsomal vitamin K_1 epoxide reductase. 2-(X)-3-phytyl-1,4-naphthoquinone 2,3-epoxides, where X is hydroxymethyl, chloromethyl, fluoromethyl, difluoromethyl, and formyl were all competitive inhibitors, but none was an inactivator. Only the 2-hydroxymethyl analogue was reduced to a quinone that was stable enough under the conditions of the experiment to be detected. Vitamin K_1 epoxide analogues with modified phytyl chains (1′-hydroxy, 3′-fluoro with isomerized double bond, 1′-hydroxy and 1′-fluoro with saturated double bond, and the corresponding unsubstituted chains) were synthesized. All of the analogues were competitive inhibitors of vitamin K_1 epoxide reductase. The nonfluorinated analogues also were shown to be substrates, being reduced to the corresponding quinone without enzyme inactivation. At least one other enzyme besides vitamin K_1 epoxide reductase in beef liver microsomes also metabolizes all of these analogues.

The biosynthesis of plasma clotting factors II, VII, IX, X, and various other proteins is dependent on vitamin K_1 (2-methyl-3-phytyl-1,4-naphthoquinone).[1,2] The initial event in the utilization of vitamin K_1 is its conversion to the corresponding hydroquinone catalyzed by a dithiol-dependent reductase that is sensitive to the oral anticoagulant warfarin.[3,4] Reduced vitamin K_1 is involved in an oxygen and carbon dioxide dependent reaction that converts the cofactor to vitamin K_1 epoxide and concomitant γ-carboxylation of specific glutamyl residues of the clotting proteins.[5] These two apparently concomitant events, which result in the activation of the clotting proteins, appear to be catalyzed by the same enzyme.[6,7] Vitamin K_1 epoxide then is converted back to vitamin K_1 by a dithiol-dependent enzyme, vitamin K_1 epoxide reductase,[3,8] which is thought to be the principal site of action of oral anticoagulants such as warfarin.[9-11] Although the mechanism of inhibition of vitamin K_1 epoxide reductase by warfarin is unknown, we have found that warfarin is noncompetitive against vitamin K_1 epoxide for the beef liver microsomal enzyme, but is competitive with the reducing agent dithiothreitol.[12] This suggests that warfarin interacts with a site other than the substrate binding site.

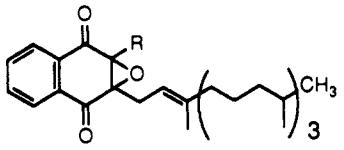
Recently, we reported the synthesis of 2-(fluoromethyl)-3-phytyl-1,4-naphthoquinone 2,3-epoxide (1, R = CH_2F) and showed that it was a potent *competitive* inhibitor of vitamin K_1 epoxide reductase from beef liver.[13] It was designed, however, as a potential mechanism-based inactivator[14] (Scheme I) in order to gain support for a

* To whom correspondence should be addressed at the Department of Chemistry.

† Dr. Nandi only carried out the experiments to test the effects of warfarin and inhibitors of epoxide hydrase on the metabolism on the analogues.

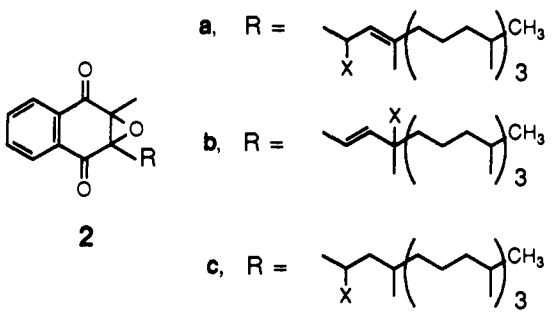
(1) Suttie, J. W. In *Fat-Soluble Vitamins*; Diplock, A. T., Ed.; William Heinemann Ltd.: London, 1985; p 225.
(2) Suttie, J. W. *CRC Crit. Rev. Biochem.* **1980**, *8*, 191–223.
(3) Whitlon, D. S.; Sadowski, J. A.; Suttie, J. W. *Biochemistry* **1978**, *17*, 1371–1377.
(4) Fasco, M. J.; Principe, L. M. *J. Biol. Chem.* **1982**, *257*, 4894–4901.
(5) Suttie, J. W. *Biofactors* **1988**, *1*, 55–60.
(6) Larson, A. E.; Friedman, P. A.; Suttie, J. W. *J. Biol. Chem.* **1981**, *256*, 11032–11035.
(7) McTigue, J. J.; Suttie, J. W. *FEBS Lett.* **1986**, *200*, 71–75.
(8) Zimmermann, A.; Matschiner, J. T. *Biochem. Pharmacol.* **1974**, *23*, 1033–1040.
(9) Bell, R. G. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1978**, *37*, 2599–2604.
(10) Sadowski, J. A.; Suttie, J. W. *Biochemistry* **1974**, *13*, 3696–3699.
(11) Hildebrandt, E. F.; Suttie, J. W. *Biochemistry* **1982**, *21*, 2406–2411.
(12) Silverman, R. B.; Mukharji, I.; Nandi, D. L. In *Current Advances in Vitamin K Research*; Suttie, J. W., Ed.; Elsevier: New York, 1988; p 65.
(13) Silverman, R. B.; Oliver, J. S. *J. Med. Chem.* **1989**, *32*, 2138–2141.

0022-2623/90/1833-1790$02.50/0



1

proposed mechanism of the enzyme[15,16] as well as to develop a new class of potential anticoagulant agents that acts as competitive inactivators of vitamin K_1 epoxide reductase. No inactivation occurred. The rationale given for the lack of inactivation was that either the inactivation mechanism was not valid or elimination of fluoride ion (Scheme I, pathway b) does not compete with elimination of water (pathway a) and, therefore, generates only the normal reduction product. Another explanation could be that initial enzyme thiolate attack on the epoxide occurs at the 3-position, not at the 2-position as assumed previously on steric grounds. If that is the case, then the leaving group needs to be placed at the 1′-position of the phytyl chain for inactivation to occur (Scheme II). In order to test these hypotheses other vitamin K_1 epoxide analogues substituted at the 2-position (1, R = CH_2OH, CH_2Cl, CHF_2, CHO) and at the 3-position (**2a** and **2c** (X = OH, F) and **2b** (X = F)) were synthesized and tested as substrates, inhibitors, and inactivators of vitamin K_1 epoxide reductase. The results of these studies with the first 2- and 3-substituted analogues of vitamin K_1 epoxide are reported here.



2

Results and Discussion

Syntheses. The syntheses of 1 (R = CH_2OH, CH_2F, CH_2Cl, CHF_2, and CHO) were reported recently.[17] Hydroxymethylation of 2-phytyl-1,4-naphthohydroquinone was followed by epoxidation and transformation of the hydroxymethyl group to the other desired substituents.

The syntheses of **2a** (X = OH) (7 in Scheme III) and **2b** (X = F) (8 in Scheme III) were adapted from a literature procedure for the preparation of 1′-oxymenaquinones.[18] 3-Bromo-2-methyl-1,4-dimethoxynaphthalene (3) was metalated with *n*-butyllithium and condensed with phytal (prepared by pyridinium chlorochromate oxidation of phytyl alcohol) to give 4. Demethylation with either acidic silver(II) oxide[18] or ceric ammonium nitrate[19] led to a mixture of **5** and the rearranged alcohol **6**, which were separated by silica gel chromatography. Epoxidation of **5** produced **7** (which is the same as **2a**, X = OH). Treatment of **7** with (diethylamido)sulfur trifluoride, however, gave none of the corresponding fluoride (**2a**, X = F); instead, allylic rearrangement to **8** (the same as **2b** (X = F)) occurred. Rearrangement of allylic alcohols is a common problem with this fluorinating reagent.[20] Attempts were made to avert this rearrangement with use of alternative (alkylamido)sulfur fluorides, e.g., (dimethylamido)sulfur trifluoride and bis(diethylamido)sulfur difluoride,[20] and by changing the polarity of the reaction solvent. However, none of these modifications resulted in a detectable amount of unrearranged fluoride (**2a**, X = F).

Since the rearrangement during fluorination was the result of having an allylic alcohol in **7**, the approach taken to circumvent this problem was to hydrogenate the double bond on the phytyl chain prior to the reaction with the fluorinating agent (Scheme IV). Catalytic hydrogenation of **4** over PtO_2 led to the isolation of **9** in 68% yield, which was converted to **11** (the same as **2c** (X = OH)) in the usual way. When Pd/C was used as the catalyst, however, reductive deoxygenation occurred to give **14** (Scheme V), which was converted to **16** (the same as **2c** (X = H)). Although this was not the desired compound, it was an important compound to have as a control for the effect of saturation of the phytyl chain on its substrate activity relative to that of vitamin K_1 epoxide. Epoxidation of **10** went smoothly (Scheme IV) but treatment of **11** in *n*-hexane/isooctane (1:1) with (diethylamido)sulfur trifluoride gave almost exclusively the dehydration product **13**. When the reaction was carried out in fluorotrichloromethane as the solvent, a mixture of **12** (the same as **2c** (X = F)) and **13** was obtained in a 1:2.3 ratio; these compounds were separated by HPLC. The ^{19}F NMR spectrum of **12** showed two multiplets, indicating the presence of the two epimers. Compound **18** was synthesized from **9** (Scheme VI).

Enzymology. Table I summarizes the kinetic results for the series 1 (R = CH_2OH, CH_2Cl, CH_2F, CHF_2, CHO). None of the compounds shown in Table I was found to be a time-dependent inactivator of beef liver microsomal vitamin K_1 epoxide reductase, but all were competitive reversible inhibitors; 1 (R = CH_2F) also was found to be uncompetitive with DTT, as is the case for vitamin K_1 epoxide, suggesting that DTT binds prior to 1 (R = CH_2F). In the presence of DTT all of the compounds were metabolized linearly by the microsomes. However, the quinone reduction products were observed only for vitamin K_1 epoxide and for 1 (R = CH_2OH). None of the corresponding quinones of the halogenated analogues could be detected. In a control experiment it was found that 2-fluorovitamin K_1 underwent a rapid nonenzymatic reaction with DTT. Presumably, the other 2-substituted vitamin K_1 analogues also would react with the DTT in the assay solution. Consequently, it only can be inferred that the

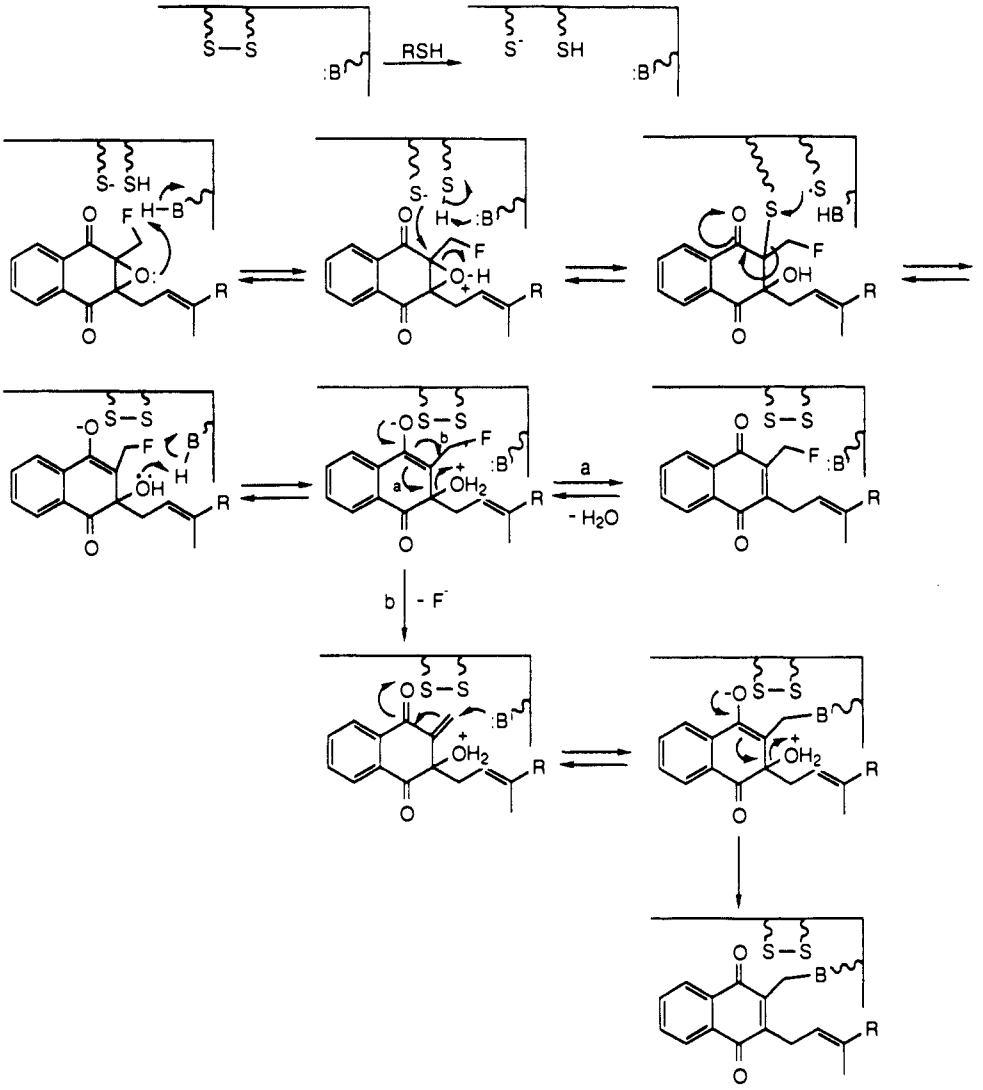
Table I. Apparent Kinetic Constants for 2-Substituted Vitamin K_1 Epoxide Analogues

epoxide	V_{max}, pmol s^{-1} mg^{-1}	K_m, μM	K_i, μM
vitamin K_1 epoxide	2.9	18	–
1 (R = CH_2OH)	3.4	10	8
1 (R = CH_2Cl)	nd[a]	nd	74
1 (R = CH_2F)	4.0[b]	20[b]	17
1 (R = CHF_2)	nd	nd	63
1 (R = CHO)	nd	nd	26

[a] Not determined. [b] Determined from the rate of consumption of 1 (R = CH_2F).

(14) Silverman, R. B. *Mechanism-Based Enzyme Inactivation: Chemistry and Enzymology*; CRC Press: Boca Raton, FL, 1988; Vol. I and II.
(15) Silverman, R. B. *J. Am. Chem. Soc.* **1981**, *103*, 5939–5941.
(16) Preusch, P. C.; Suttie, J. W. *J. Org. Chem.* **1983**, *48*, 3301–3305.
(17) Ryall, R. P.; Silverman, R. B. *Synth. Commun.* **1990**, *20*, 431–438.
(18) Synder, C. D.; Bondinell, W. E.; Rapoport, H. *J. Org. Chem.* **1971**, *36*, 3951–3960.
(19) Jacob, P., III; Callery, P. S.; Schulgin, A. T.; Castagnoli, N., Jr. *J. Org. Chem.* **1976**, *41*, 3627–3629.
(20) Middleton, W. J. *J. Org. Chem.* **1975**, *40*, 574–578.

Scheme I. Hypothetical Mechanism of Inactivation of Vitamin K_1 Epoxide Reductase by 1 (R = CH_2F) and Reduction of 1 (R = CH_2F) [R′ = $[(CH_2)_3CHCH_3]_3CH_3$]



initial reduction products of these analogues were the corresponding quinones. When thioredoxin/thioredoxin reductase was used as the reducing agent in place of DTT,[21] the quinone of 1 (R = CH_2F) still could not be detected. Again, it was determined that this quinone was destroyed by thioredoxin/thioredoxin reductase in the absence of microsomes.

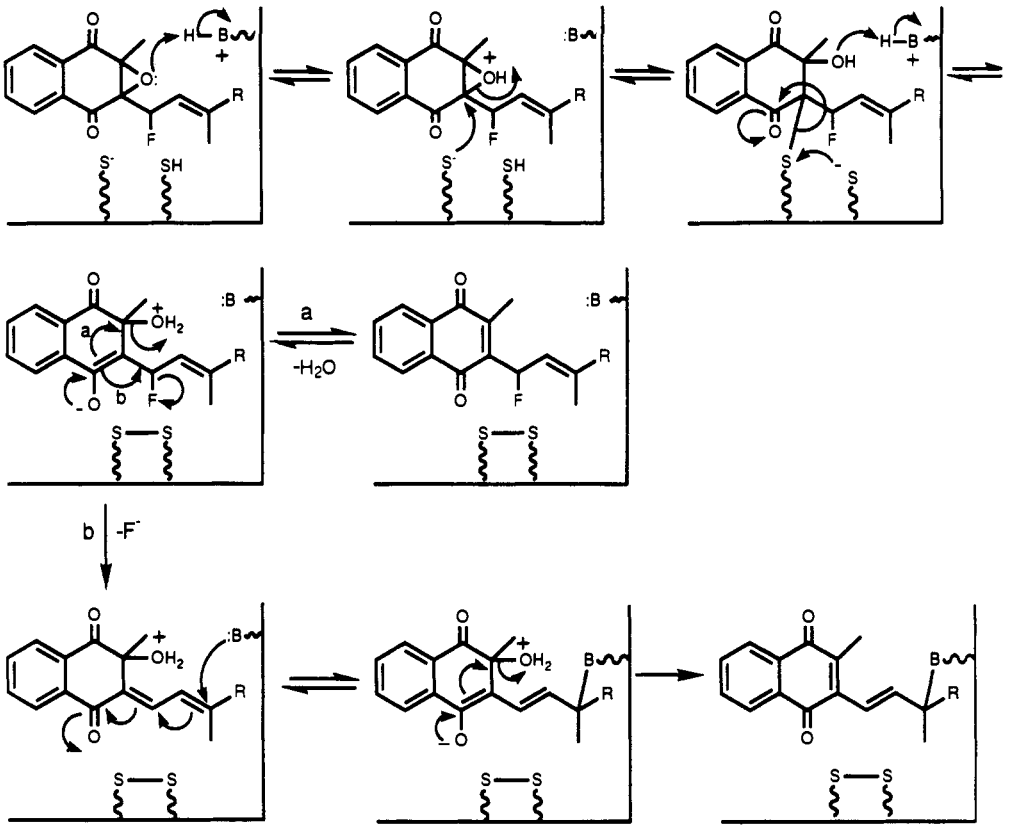
Since all of the compounds tested were metabolized by beef liver microsomes, K_m values for the analogues whose quinones could not be detected were determined initially by measuring the rates of consumption of the compounds. However, as previously reported for 1 (R = CH_2F),[13] when the microsomes were pretreated with warfarin, a known inactivator of vitamin K_1 epoxide reductase, there was virtually no change observed in the rate of metabolism of 1 (R = CH_2F), 1 (R = CH_2OH), or vitamin K_1 epoxide relative to that produced by unpretreated microsomes. For 1 (R = CH_2OH) and vitamin K_1 epoxide whose quinones could be detected, warfarin was found to shut down quinone formation; therefore, these compounds, and presumably all of the others, are metabolized by vitamin K_1 epoxide reductase. Further evidence that vitamin K_1 epoxide reductase is responsible for the metabolism of 1 (R = CH_2OH) is that thioredoxin/thioredoxin reductase could be substituted for DTT in the assay for this reaction.[21] Most of the metabolism of these compounds, however, occur as a result of the action of some other microsomal enzyme, and that enzyme or those enzymes do not reduce the epoxide to the corresponding quinone. The only reliable kinetic constants, therefore, are those obtained from rates of product formation, not from compound consumption. Consequently, K_m and V_{max} values in Table I are only listed for those compounds whose quinones were stable to DTT. It should be noted that none of the compounds was metabolized in the absence of microsomes or in the absence of DTT and in the presence of microsomes.

Attempts were made to determine the enzyme(s) other than vitamin K_1 epoxide reductase responsible for the metabolism of these compounds. One of the most likely candidates is epoxide hydrase, the microsomal enzyme that hydrolyzes epoxides to glycols. Several isozymes of this enzyme are known, and a variety of inactivators are effective for different isozymes.[22] Several of these inactivators were used in order to determine if pretreatment of the microsomes with these compounds slows down metabolism of the vitamin K_1 epoxide analogues. Pretreatment of microsomes with 1,1,1-trichloropropene oxide,[23]

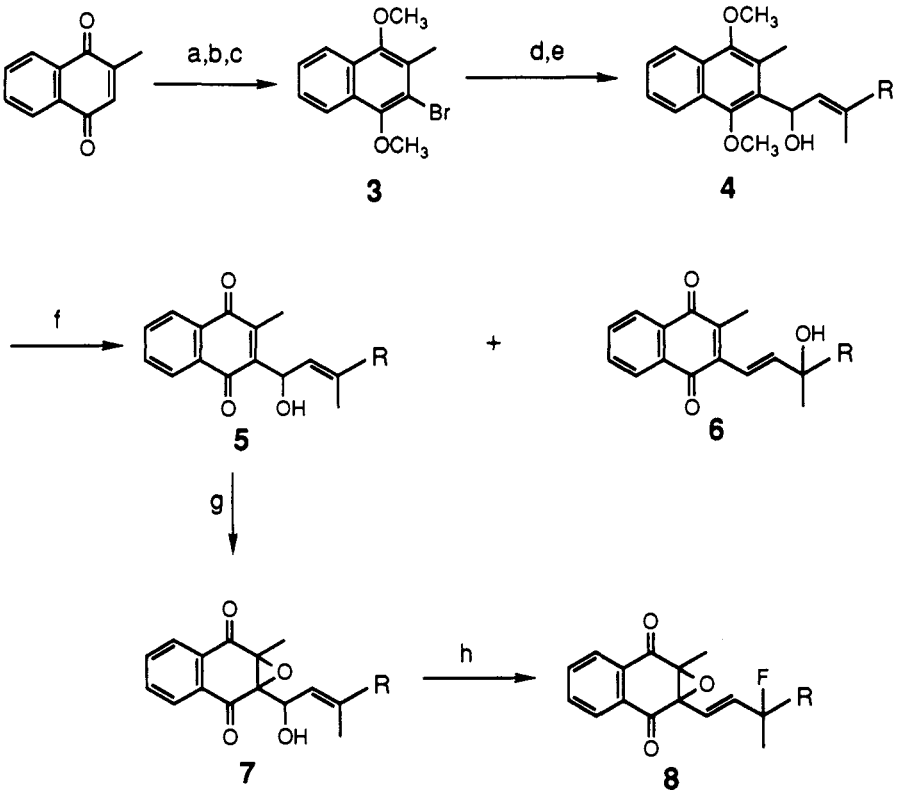
(21) Silverman, R. B.; Nandi, D. L. *Biochem. Biophys. Res. Commun.* **1988**, *155*, 1248–1254.

(22) (a) Miyamoto, T.; Silva, M.; Hammock *Arch. Biochem. Biophys.* **1987**, *254*, 203–213. (b) Guenthner, T. M. *Biochem. Pharmacol.* **1986**, *35*, 839–845.

Scheme II. Hypothetical Mechanism of Inactivation of Vitamin K_1 Epoxide Reductase by **2a** (X = F) and Reduction of **2a** (X = F) [R = $[(CH_2)_3CHCH_3]_3CH_3$]



Scheme III. Synthetic Route to **5**, **7**, and **8** [R = $[(CH_2)_3CHCH_3]_3CH_3$][a]

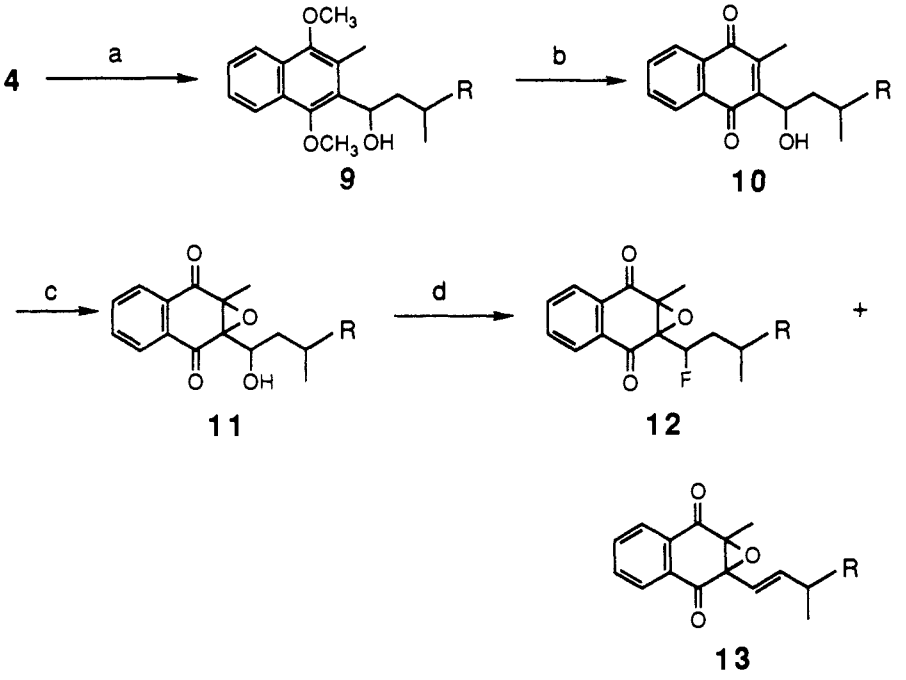


[a] (a) Br_2//HOAc; (b) $SnCl_2$/HCl; (c) $(CH_3)_2SO_4$/KOH; (d) *n*-BuLi; (e) phytal; (f) AgO/HNO_3; (g) H_2O_2/Na_2CO_3; (h) $(C_2H_5)_2NSF_3$.

trans-stilbene oxide, cyclohexene oxide, indomethacin (all at 1 mM) or tetrachloro-*p*-benzoquinone (40 μm) had no effect on the rate of metabolism of **1** (R = CH_2F), suggesting that epoxide hydrase is probably not involved. Glutathione *S*-transferase also is an unlikely candidate because these isozymes require glutathione, not DTT or thioredoxin, as the thiol transfer agent.[24] The most likely

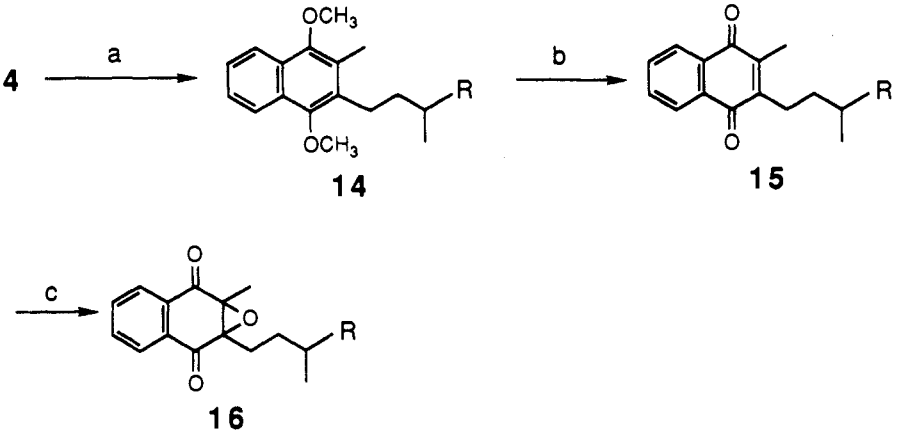
(23) Hanzlik, R. P.; Walsh, J. S. A. *Arch. Biochem. Biophys.* **1980**, *204*, 255–263.

Scheme IV. Synthetic Route to 10–13 [R = $[(CH_2)_3CHCH_3]_3CH_3$][a]



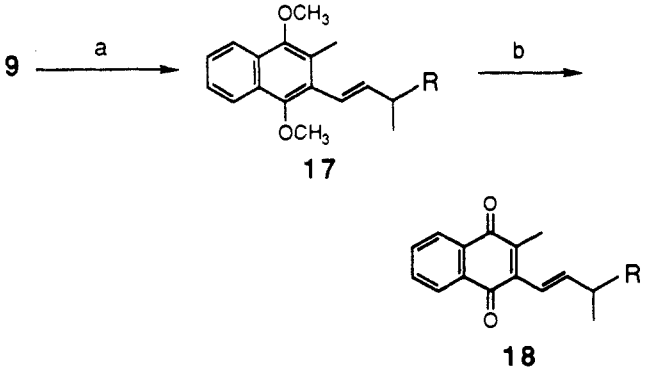
[a] (a) H_2/PtO_2; (b) $Ce(NH_4)_2(NO_3)_6$; (c) H_2O_2/Na_2CO_3; (d) $(CH_3)_2NSF_3$.

Scheme V. Synthetic Route to 15 and 16 [R = $[(CH_2)_3CHCH_3]_3CH_3$][a]



[a] (a) H_2/Pd–C; (b) AgO/HNO_3; (c) H_2O_2/Na_2CO_3.

Scheme VI. Synthetic Route to 18 [R = $[(CH_2)_3CHCH_3]_3CH_3$][a]



[a] (a) $(C_2H_5)_2NSF_3$; (b) $Ce(NH_4)_2(NO_3)_6$.

enzyme responsible for this metabolism is the warfarin-insensitive vitamin K epoxide reductase previously isolated from beef liver microsomes.[25]

The fact that none of the 2-halogenated analogues showed time-dependent inhibition could be rationalized in several ways. Previously,[13] we suggested that since 1 (R = CH_2F) did not inactivate the enzyme, the proposed enzyme mechanism[15,16] should be reconsidered or the elimination of water (Scheme I, pathway a) is a much more facile process than is elimination of fluoride ion (pathway b). In order to enhance the leaving group ability of the substituent, the corresponding chloro analogue (1, R = CH_2Cl) was prepared. This compound, however, also showed no time-dependent inhibition, but was a competitive reversible inhibitor. Another mechanistic explanation for the observance of no inactivation could be that the Michael acceptor generated upon halide elimination (Scheme I, pathway b) is not electrophilic enough. Consequently, the corresponding difluoro analogue (1, R = CHF_2) was prepared. Elimination of one fluoride ion would produce a β-fluoro-α,β-unsaturated ketone (the

(24) Armstrong, R. N. *CRC Crit. Rev. Biochem.* **1987**, *22*, 39–88.

(25) Mukharji, I.; Silverman, R. B. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 2713–2717.

Table II. Apparent Kinetic Constants for 3-Substituted Vitamin K Epoxide Analogues

epoxide	V_{max}, pmol s^{-1} mg^{-1}	K_m, μM	K_i, μM
vitamin K_1 epoxide	2.9	18	-
7	2.4	25	43
8	nd[a]	nd	nd
11	1.6	34	63
12	nd	nd	100
13	2.0	68	nd
16	2.1	30	nd

[a] Not determined.

product of pathway b with a fluorine at the β-position of the exocyclic double bond), which would be activated for nucleophilic attack.[26] This compound, however, also was not an inactivator, only a competitive reversible inhibitor.

Compound 1 (R = CHO) was designed as an affinity labeling agent to trap an active-site thiol; however, this compound also was only a competitive reverisble inhibitor with a K_i value slightly higher than that for 1 (R = CH_2F). Therefore, either there is no nucleophile at the appropriate position or any covalent interaction that occurs with the enzyme is highly reversible and favors the unbound form.

An alternative catalytic mechanism for vitamin K_1 epoxide reductase could involve initial enzymatic attack at the 3-position of substrate epoxide instead of attack at the 2-position as previously was suggested.[15,16] If that occurs, then leaving groups at the 2-position would be ineffective; however, a leaving group on the phytyl chain may be effective (Scheme II). 1′-Fluorovitamin K_1 epoxide (**2a**, X = F) could not be synthesized, but the rearranged isomer 8 (**2b** (X = F)) and the 1′-fluorodihydrovitamin K_1 epoxide (12) (**2c**, X = F) were prepared. As controls of the effect of the altered phytyl chain (isomerized double bond in 8 and reduced double bond in 12), the corresponding unsubstituted analogues 13 (Scheme IV) and 16 (Scheme V) also were synthesized and tested as substrates for vitamin K_1 epoxide reductase. As shown in Table II the V_{max} values for vitamin K_1 epoxide (i.e., **2a**, X = H), 13 (i.e., **2b**, X = H), and 16 (i.e., **2c**, X = H) are very similar; K_m values are only about 2 and 4 times higher for 16 and 13, respectively, relative to vitamin K_1 epoxide as determined by measuring the formation of the quinone products (15 and 18, respectively). Therefore, the phytyl chain modifications have little effect on substrate turnover. The fluorinated analogues, 8 (i.e., **2b** , X = F) and 12 (i.e., **2c**, X = F), however, still were not time-dependent inhibitors of vitamin K_1 epoxide reductase. This suggests that either this alternative hypothetical inactivation mechanism is incorrect or that fluoride ion elimination is much slower than elimination of water or that there is no nucleophile in the vicinity of the incipient Michael acceptor, or the Michael acceptor is not reactive enough.

The 1′-hydroxy analogues [**2a**, X = OH (7) and **2c**, X = OH (11)] also were good substrates for vitamin K_1 epoxide reductase as monitored by the corresponding quinone formation (5 and 10, respectively); both were competitive with vitamin K_1 epoxide. No quinone products could be detected when the fluoro analogues [**2b**, X = F (8) and **2c**, X = F (12)] were used as substrates, presumably because of the likely instability of the quinone products in the presence of thiol, as was observed for 1 (R = CH_2F). Both 8 and 12 also were relatively weak inhibitors; equimolar concentrations of either 8 or 12 with vitamin K_1 epoxide reductase resulted in less than 15% inhibition of the conversion of vitamin K_1 epoxide to vitamin K_1.

Both enantiomers of vitamin K_1 epoxide have been reported to be substrates for vitamin K_1 epoxide reductase.[27] Similar results were obtained with our analogues. Vitamin K_1 epoxide, 1 (R = CH_2OH), and 11 generated 62%, 65%, and 57%, respectively, of the theoretical amount of product after microsomal incubation for 22 h under the usual conditions. Therefore, both enantiomers are active.

Despite the lack of inactivation of vitamin K_1 epoxide reductase by these compounds, they are still important new potent competitive inhibitors. Since warfarin is a noncompetitive inhibitor of the enzyme, it will be interesting to determine the effect of competitive inhibition on coagulation. In particular, the effect of these compounds on warfarin-resistant rats should be a valuable endeavor.

Experimental Section

Analytical Methods. Proton magnetic resonance spectra were recorded on Varian EM-390 90 MHz and Varian XLA-400 MHz spectrometers. Chemical shifts are reported as δ values in parts per million downfield from internal standard tetramethylsilane. Fluorine magnetic resonance spectra were recorded on a Varian XLA-400 MHz spectrometer at 376 MHz. Chemical shifts are reported as δ values in ppm downfield from CCl_3F. IR spectra were recorded on a Perkin-Elmer Model 283 spectrophotometer. Low-resolution mass spectra were obtained with a Hewlett-Packard 5985 spectrometer. High-resolution mass spectra were obtained with a VG Instruments VG70-250SE mass spectrometer. Microanalyses were performed by Galbraith Laboratories (Knoxville, TN). Melting points were obtained on a Fisher-Johns melting point apparatus and are uncorrected. HPLC was performed with Beckman 110B pumps, 153 (254 nm) UV detector, 421A system controller, and a Hewlett-Packard Model 3390A integrator. HPLC columns were from Beckman and Alltech Associates. TLC analyses were done with Whatman 250μ PE SIL 6/UV plates. Column chromatography was performed with Merck silica gel 60 (230–400 mesh).

Reagents. Unless otherwise noted, commercial chemicals were of reagent grade quality or better, and were used without further purification. Compounds 1 (R = CH_2OH, CH_2Cl, CH_2F, CHF_2, CHO) were synthesized as previously reported.[17]

2-(Fluoromethyl)-3-phytyl-1,4-naphthoquinone. 2-(Hydroxymethyl)-3-phytyl-1,4-naphthoquinone[17] (527 mg, 1.17 mmol) in dry CH_2Cl_2 (4 mL) was added dropwise over 1 h to a solution of (diethylamido)sulfur trifluoride (226 mg, 1.40 mmol) in dry CH_2Cl_2 (2 mL) at −78 °C under argon. After all of the alcohol was added, the reaction mixture was allowed to warm to room temperature and was stirred for 1.5 h. The solvent was removed by reduced pressure, and the product was purified by silica gel chromatography (5% ethyl acetate in *n*-hexane). The product was obtained in 65% yield as a yellow oil: ^1H NMR ($CDCl_3$) δ NMR 0.8–1.8 (m, 34 H), 1.95 (t, 2 H), 3.53 (d, 2 H), 5.04 (t, 1 H), 5.52 (d, 2 H, J = 47 Hz), 7.76 (m, 2 H), 8.14 (m, 2 H); IR (film) 1670, 1010 cm^{-1}; high-resolution mass spectrum calcd for $C_{31}H_{45}FO_2$ 468.3404, found 468.3400 (0.8 ppm deviation).

2-Methyl-3-(1′-hydroxyphytyl)-1,4-dimethoxynaphthalene (4). *n*-Butyllithium (2.5 mmol in pentane) was added dropwise to 2-bromo-3-methyl-1,4-dimethoxynaphthalene[28] (620 mg, 2.2 mmol) in dry ether (5 mL) under an argon atmosphere. The mixture was stirred for 8 min during which time the solution turned milky white. Phytal (2.5 mmol), freshly prepared by the oxidation of phytol with pyridinium chlorochromate,[29] in dry ether (5 mL) was added to the lithio compound. The reaction mixture, which turned yellow within 5 min, was stirred for a total of 30 min after which time it was diluted with ether (25 mL), washed with 4% aqueous ammonium chloride (2 × 10 mL), and then with water (10 mL). The product obtained after removal of the ether was purified on silica gel (15% ethyl acetate in *n*-hexane) to give

(26) Silverman, R. B.; Abeles, R. H. *Biochemistry* **1976**, *15*, 4718–4723.

(27) Preusch, P. C.; Suttie, J. W. *J. Biol. Chem.* **1983**, *258*, 714–716.

(28) Adams, R.; Geissman, T. A.; Baker, B. R.; Teeter, H. M. *J. Am. Chem. Soc.* **1941**, *63*, 528–534.

(29) Corey, E. J.; Suggs, J. W. *Tetrahedron Lett.* **1975**, 2647–2650.

a colorless oil in 84% yield: ^{1}H NMR ($CDCl_3$) δ 0.65–1.90 (m, 36 H), 2.53 (s, 3 H), 3.29 (d, 1 H), 3.90 (s, 3 H), 4.03 (s, 3 H), 5.67 (d, 1 H), 6.00 (t, 1 H), 7.51 (m, 2 H), 8.07 (m, 2 H); high-resolution mass spectrum calcd for $C_{33}H_{52}O_3$ 496.3917, found 496.3907 (1.0 ppm deviation).

2-Methyl-3-(1′-hydroxyphytyl)-1,4-naphthoquinone (5). Nitric acid (0.9 mmol) was added to a solution of **4** (225 mg, 0.45 mmol) and silver(I) oxide (134 mg, 1.08 mmol) in dioxane (7 mL) at 10 °C. After being stirred for 2 min, the reaction mixture was diluted with water (2 mL) and then extracted with CH_2Cl_2 (20 mL). The organic extract was washed with water (2 × 5 mL), and then after removal of the solvent, the product was purified by silica gel chromatography (6% ethyl acetate in *n*-hexane) to give **5** as a yellow oil in 69% yield: ^{1}H NMR ($CDCl_3$) δ 0.65–1.90 (m, 36 H), 2.33 (s, 3 H), 3.70 (d, 1 H), 5.47 (d, 1 H), 5.70 (t, 1 H), 7.81 (m, 2 H), 8.07 (m, 2 H); IR (film) 3600–3400, 1600 cm^{-1}; high-resolution mass spectrum calcd for $C_{31}H_{46}O_3$ 466.3447, found 466.3426 (2.1 ppm deviation).

2-Methyl-3-(1′-hydroxyphytyl)-1,4-naphthoquinone 2,3-Epoxide (7). To a solution of **5** (628 mg, 1.35 mmol) in ethanol (50 mL) was added sodium carbonate (0.3 g in 2 mL of H_2O) and 30% H_2O_2 (4 mL). The yellow mixture was stirred for 30 min at room temperature during which time it turned colorless. Water (50 mL) was added to precipitate the product which was extracted with dichloromethane (3 × 25 mL). After removal of the solvent, the colorless residue was purified by silica gel chromatography (10% ethyl acetate in *n*-hexane). The product was obtained as a colorless oil in a 73% yield (473 mg): ^{1}H NMR ($CDCl_3$) δ 0.8–2.1 (m, 39 H), 2.70 (d, 1 H), 4.80 (t, 1 H), 5.65 (d, 1 H), 7.74 (m, 2 H), 7.97 (m, 2 H); IR (film) 3600–3400, 1695 cm^{-1}; high-resolution mass spectrum calcd for $C_{31}H_{46}O_4$ 482.3396, found 482.3394 (0.4 ppm deviation). Anal. Calcd for $C_{31}H_{46}O_4$: C, 77.14; H, 9.60. Found: C, 77.06; H, 9.79.

2-Methyl-3-(3′-fluoro-3′,7′,11′,15′-tetramethyl-1-hexadecenyl)-1,4-naphthoquinone 2,3-Epoxide (8). Compound **7** (473 mg, 0.98 mmol) in *n*-hexane was added dropwise over 20 min at −78 °C to a solution of (diethylamido)sulfur trifluoride (0.12 g, 1.06 mmol) in 1:1 *n*-hexane/isooctane (8 mL) under argon. The reaction mixture was allowed to warm to room temperature and then was stirred for 30 min, diluted with *n*-hexane (10 mL), and washed with water (2 × 10 mL). Upon removal of the solvent, the product was purified by silica gel chromatography (5% ethyl acetate in *n*-hexane). The product, a colorless oil, was obtained in 47% yield: ^{1}H NMR ($CDCl_3$) δ 0.8–1.8 (m, 39 H) 5.97 (dd, 1 H), 6.29 (dd, 1 H), 7.75 (m, 2 H), 7.98 (m, 2 H); ^{19}F NMR ($CDCl_3$) δ −148; IR (film) 1695 cm^{-1}; high-resolution mass spectrum calcd for $C_{31}H_{45}FO_3$ 484.3353, found 484.3357 (0.8 ppm deviation). Anal. Calcd for $C_{31}H_{45}FO_3$: C, 76.82; H, 9.36. Found: C, 77.02; H, 9.62.

2-Methyl-3-(1′-hydroxyl-1′,2′-dihydrophytyl)-1,4-dimethoxynaphthalene (9). Catalytic hydrogenation of **4** (173 mg, 0.35 mmol), carried out with PtO_2 (40 mg) in ethyl acetate (20 mL) for 6 h, afforded **9** in a 68% yield: ^{1}H NMR ($CDCl_3$) δ 0.8–2.2 (m, 39 H), 2.45 (s, 3 H), 2.7 (d, 1 H), 3.85 (s, 3 H), 4.01 (s, 3 H), 5.21 (m, 1 H), 7.50 (m, 2 H), 8.03 (m, 2 H); high-resolution mass spectrum calcd for $C_{33}H_{54}O_3$ 498.4073, found 498.4072 (0.1 ppm deviation).

2-Methyl-3-(1′-hydroxydihydrophythyl)-1,4-naphthoquinone (10). Ceric(IV) ammonium nitrate (230 mg, 0.42 mmol) dissolved in water (0.8 mL) was added dropwise over 10 min to a solution of **9** (84 mg, 0.17 mmol) dissolved in acetonitrile (20 mL). This mixture was stirred for 0.5 h and then was diluted with water. The aqueous dioxane mixture was extracted with CH_2Cl_2 (2 × 10 mL), and the CH_2Cl_2 extracts were washed with water (15 mL). The product (**10**), a yellow oil, was purified by silica gel chromatography with 15% ethyl acetate/*n*-hexane: ^{1}H NMR ($CDCl_3$) δ 0.8–2.0 (m, 39 H), 2.22 (s, 3 H), 3.78 (dd, 1 H exchangeable with D_2O), 4.88 (m, 1 H), 7.72 (m, 2 H), 8.08 (m, 2 H); high-resolution mass spectrum calcd for $C_{31}H_{48}O_3$ 468.3603, found 468.3613 (−1.0 ppm deviation).

2-Methyl-3-(1′-hydroxy-1′,2′-dihydrophytyl)-1,4-naphthoquinone 2,3-Epoxide (11). Epoxidation of **10** was performed under the same conditions used to prepare **7**. The product was obtained as a colorless oil in 83% yield: ^{1}H NMR ($CDCl_3$) δ 0.8–1.6 (m, 39 H), 1.74 (s, 3 H), 3.27 (d, 1 H), 3.81 (t, 1 H), 7.77 (m, 2 H), 7.98 (m, 2 H); IR (film) 3600–3400, 1690 cm^{-1}. Anal. Calcd for $C_{31}H_{48}O_4$: C, 76.82; H, 9.98. Found: C, 76.52; H, 9.87.

2-Methyl-3-(1′-fluoro-1′,2′-dihydrophytyl)-1,4-naphthoquinone 2,3-Epoxide (12) and 2-Methyl-3-(3′,7′,11′,15′-tetramethyl-1-hexadecenyl)-1,4-naphthoquinone 2,3-Epoxide (13). A solution of **11** (130 mg, 0.26 mmol) dissolved in $CFCl_3$ (1 mL) was added dropwise under argon at −78 °C over a 15-min period to a solution of (dimethylamido)sulfur trifluoride (40 mg, 0.30 mmol) dissolved in $CFCl_3$ (1 mL). After all of **11** was added, the reaction was warmed to room temperature and stirred for 0.5 h. The reaction was then diluted with CH_2Cl_2 (20 mL) and washed with water (3 × 10 mL). The product mixture of **12** and **13** was isolated by silica gel chromatography with 2% ethyl acetate in *n*-hexane as eluent. Compounds **12** and **13** were then separated by C18 reversed-phase HPLC using 100% methanol. Both products were obtained as colorless oils in a 49% yield, in the ratio of 1:2.3 (**12**:**13**).

Compound **12**: ^{1}H NMR ($CDCl_3$) δ 0.8–1.6 (m, 39 H), 1.80 (s, 3 H), 5.37 (dm, 1 H, J_{HF} = 49 Hz), 7.75 (m, 2 H), 7.97 (m, 2 H); ^{19}F NMR (D_2O) −194.8 and −197.3; IR (film) 1700 cm^{-1}; high-resolution mass spectrum calcd for $C_{31}H_{47}FO_3$ 486.3509, found 486.3508 (0.2 ppm deviation). Anal. Calcd for $C_{31}H_{47}FO_3$: C, 76.50; H, 9.73. Found: C, 75.20; H, 9.50.

Compound **13**: ^{1}H NMR ($CDCl_3$) δ 0.8–1.7 (m, 39 H), 2.35 (m, 1 H), 5.80 (m, 1 H), 5.96 (m, 1 H), 7.72 (m, 2 H), 7.95 (m, 2 H); IR (film) 1690 cm^{-1}; high-resolution mass spectrum calcd for $C_{31}H_{46}O_3$ 466.3447, found 466.3442 (1.1 ppm deviation). Anal. Calcd for $C_{31}H_{46}O_3$: C, 79.78; H, 9.93. Found: C, 79.19; H, 10.28.

2-Methyl-3-(1′,2′-dihydrophytyl)-1,4-dimethoxynaphthalene (14). Catalytic hydrogenation of **4** (202 mg, 0.40 mmol) was carried out in ethyl acetate (20 mL) with 10% palladium on carbon (140 mg) for 20 h. The colorless oily product obtained after filtration and solvent evaporation was purified by silica gel chromatography (10% ethyl acetate/*n*-hexane), giving **14** in a 44% yield. No O–H stretch was detected in its IR spectrum.

2-Methyl-3-(1′,2′-dihydrophytyl)-1,4-naphthoquinone (15). Demethylation of **14** was performed under the same conditions used to prepare **5**: ^{1}H NMR ($CDCl_3$) δ 0.8–1.6 (m, 41 H), 2.20 (s, 3 H), 7.71 (m, 2 H), 8.09 (m, 2 H); high-resolution mass spectrum calcd for $C_{31}H_{48}O_2$ 452.3654, found 452.3654 (0 ppm deviation).

2-Methyl-3-(1′,2′-dihydrophytyl)-1,4-naphthoquinone 2,3-Epoxide (16). Epoxidation of compound **15** was performed under the same conditions used to prepare **7**, and an 83% yield of **16** was obtained: ^{1}H NMR ($CDCl_3$) δ 0.8–1.65 (m, 41 H), 1.75 (s, 3 H), 7.74 (m, 2 H), 7.99 (m, 2 H); IR (film) 1660 cm^{-1}; high-resolution mass spectrum calcd for $C_{31}H_{48}O_3$ 468.3604; found 468.3608 (0.8 ppm deviation). Anal. Calcd for $C_{31}H_{48}O_3$: C, 79.44; H, 10.32. Found: C, 79.34; H, 10.47.

2-Methyl-3-(3′,7′,11′,15′-tetramethyl-1-hexadecenyl)-1,4-dimethoxynaphthalene (17). Alcohol **9** (68 mg, 0.14 mmol) in dry CH_2Cl_2 (1 mL) was added dropwise, over 15 min at −78 °C under an argon atmosphere, to a solution of (diethylamido)sulfur trifluoride (40 mg, 0.24 mmol) in CH_2Cl_2 (1 mL). After all of **9** was added, the reaction mixture was warmed to room temperature and diluted with CH_2Cl_2 (20 mL). The mixture was washed with water (3 × 10 mL), the CH_2Cl_2 layer dried, and the solvent removed by rotary evaporation. The product was purified by silica gel with 1% ethyl acetate in *n*-hexane as eluent. The product was obtained as a colorless oil in 65% yield: ^{1}H NMR ($CDCl_3$) δ 0.8–1.6 (m, 37 H), 2.41 (s, 3 H), 3.80 (s, 3 H), 3.87 (s, 3 H), 6.08 (dd, 1 H), 6.44 (d, 1 H), 7.46 (m, 2 H), 8.06 (m, 2 H). There was no resonance in the ^{19}F NMR spectrum.

2-Methyl-3-(3′,7′,11′,15′-tetramethyl-1-hexadecenyl)-1,4-naphthoquinone (18). Demethylation of **17** was performed under the same conditions used to prepare **10**. The yellow quinone was obtained in 53% yield: ^{1}H NMR ($CDCl_3$) δ 0.8–1.6 (m, 37 H), 2.27 (s, 3 H), 6.40 (m, 2 H), 7.71 (m, 2 H), 8.09 (m, 2 H); high-resolution mass spectrum calcd for $C_{31}H_{46}O_2$ 450.3498, found 450.3500 (0.4 ppm deviation).

Enzyme and Assays. Beef liver microsomes were isolated and vitamin K_1 epoxide reductase activity was assayed as previously described[13] except the final concentration of Emulgen 911 detergent in the assay was 0.02%.

Time-Dependent Inhibition. Beef liver microsomes (16 mg of protein) were incubated in a total volume of 800 μL in the presence (40 μM) and absence of the substrate analogues under

the same conditions as the assay except without vitamin K_1 epoxide. At various time intervals (0, 1, 2, 3, 4, 5 h) aliquots (40 μL) were withdrawn from the incubation mixtures, diluted into an assay mixture containing 40 μM vitamin K_1 epoxide, 3 mM DTT, and 0.02% Emulgen 911 (total volume 500 μL), and assayed for vitamin K_1 epoxide reductase activity as described above.

Competitive Inhibition of Vitamin K_1 Epoxide Reductase by Substrate Analogues. Vitamin K_1 epoxide reductase activity (0.8 mg of microsomes) was measured by varying concentrations (10, 12.5, 16.5, 25, and 40 μM) of vitamin K_1 epoxide and 5 mM DTT in the absence and presence of the analogues (0, 20, 40, and 60 μM). Enzyme assays were the same as described above. Double-reciprocal plots of enzyme activity versus vitamin K_1 epoxide concentration at various concentrations of the substrate analogues were constructed. Inhibition constants (K_i) were determined from replots of $K_{m,app}$ versus the concentration of the substrate analogues.

The experiment also was carried out at varying DTT concentrations (0.33, 0.50, 0.67, 1.0, 2.0 mM) and 40 μM vitamin K_1 epoxide in the presence of 1 (R = CH_2F) (0, 20, 40, 60 μM). Assays were done as described above.

Substrate Activity of 1 (R = CH_2OH), 7, 11, 13, and 16. The assay (minus vitamin K_1 epoxide) was carried out as above with the alternative substrates (1 (R = CH_2OH), 7, 11, 13, and 16) at 100, 66.7, 50, 40, 33, 25, 20, 16.5, 14.5, 12.5, 11, and 10 μM. The corresponding quinone products generated (2-hydroxyvitamin K_1,[17] 6, 10, 18, 15) were used as standards to quantitate the amount of enzyme-generated product. No epoxide reduction occurred under the same conditions when either microsomes or DTT was omitted.

Acknowledgment. We gratefully acknowledge the National Institutes of Health (Grant No. GM 35855) for financial support of this work.

Synthesis and Antiviral Activity of (*S*)-9-[4-Hydroxy-3-(phosphonomethoxy)butyl]guanine

Choung Un Kim,* Bing Yu Luh, and John C. Martin

Bristol-Meyers Squibb Company, Pharmaceutical Research and Development Division, 5 Research Parkway, Wallingford, Connecticut 06492. *Received November 2, 1989*

The synthesis of (*S*)-9-[4-hydroxy-3-(phosphonomethoxy)butyl]guanine (**3**), starting from (*S*)-4-(2-hydroxyethyl)-2,2-dimethyl-1,3-dioxolane (**4**), is described. Alkylation of trityl derivative **7** with (diethylphosphono)methyl triflate provided phosphonate **8**, which was readily converted to mesylate **12** in three steps. Nucleophilic substitution of the mesylate group of **12** by 2-amino-6-chloropurine sodium salt led to (*S*)-2-amino-6-chloro-9-[3-[(diethylphosphono)methoxy]-4-(tetrahydro-2*H*-pyran-2-yloxy)butyl]purine (**13**). Sequential treatment of **13** with trimethylsilyl bromide and then with 2 N HCl furnished **3**. Preliminary in vitro screening indicated that **3** exhibited a potent activity against human cytomegalovirus (HCMV) but was not active against herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2). The adenine and cytosine derivatives (**14** and **15**) did not exhibit activity against HSV-1 and -2 and HCMV.

The discovery of the potent selective antiherpes agents 9-[(2-hydroxyethoxy)methyl]guanine (acyclovir)[1] and 9-[(1,3-dihydroxy-2-propoxy)methyl]guanine (DHPG, ganciclovir, **1**)[2-4] has led to an extensive search for novel nucleoside analogues with improved properties. DHPG is an exceptionally potent agent against a broad range of herpesviruses including herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2),[2-4] human cytomegalovirus (HCMV),[3,4] varicella-zoster virus,[3] and Epstein–Barr virus.[3] Although the clinical use of DHPG for the treatment of cytomegalovirus retinitis has been approved, some adverse toxicological effects of DHPG in humans[5] prompted further search for a more effective agent against HCMV. Recently, HCMV has also been recognized as one of the most important pathogens in immunocompromised and aquired immune deficiency syndrome (AIDS) patients.[6] As the essential feature of the biochemical mechanism, DHPG is known to be converted to its monophosphate **2** by HSV thymidine kinases (TKs) or host kinases and then to the triphosphate by host cell kinases.[3] This triphosphate, in turn, acts as a viral DNA polymerase inhibitor.[7] Since HCMV is not known to code for a viral TK, a phosphonate

(1) (a) Schaeffer, H. J.; Beauchamp, L.; de Miranda, P.; Elion, G. B.; Bauer, D. J.; Collins, P. *Nature (London)* **1978**, *272*, 583. (b) Elion, G. B.; Furman, P. A.; Fyfe, J. A.; de Miranda, P.; Beauchamp, L.; Schaeffer, H. J. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, No. 12, 5716. (c) Collins, P.; Bauer, D. J. *J. Antimicrob. Agents Chemother.* **1979**, *5*, 431.

(2) (a) Smith, K. O.; Galloway, K. S.; Kennell, W. L.; Ogilvie, K. K.; Radatus, B. K. *Antimicrob. Agents Chemother.* **1982**, *22*, 55. (b) Ashton, W. T.; Karkas, J. D.; Field, A. K.; Tolman, R. L. *Biochem. Biophys. Res. Commun.* **1982**, *108*, 1716. (c) Martin, J. C.; Dvorak, C. A.; Smee, D. F.; Matthews, T. R.; Verheyden, J. P. H. *J. Med. Chem.* **1983**, *26*, 759. (d) Schaeffer, H. J. In Nucleosides, Nucleotides and their Biological Applications; Rideout, J. L., Henry, D. W., Beacham, L. M., Eds.; Academic Press: New York, 1983; pp 1–17.

(3) (a) Cheng, Y.-C.; Huang, E.-S.; Lin, J.-C.; Mar, E.-C.; Pagano, J. C.; Dutschman, G.; Grill, S. P. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 2767. (b) Field, A. K.; Davies, M. E.; Dewitt, C.; Perry, H. C.; Liou, R.; Germerschausen, J.; Karkas, J. D.; Ashton, W. T.; Johnston, D. B.; Tolman, R. L. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 4139.

(4) Smee, D. F.; Martin, J. C.; Verheyden, J. P. H.; Matthews, T. R. *Antimicrob. Agents Chemother.* **1983**, *23*, 676.

(5) Collaborative DHPG treatment study group *N. Engl. J. Med.* **1986**, *314*, 801.

(6) (a) Macher, A. M.; Reichert, C. M.; Straus, S. E.; Longo, D. L.; Parrillo, J.; Lane, H. C.; Fauci, A. S.; Rook, A. H.; Manischewitz, J. F.; Quinnan, G. V., Jr. *N. Engl. J. Med.* **1983**, *309*, 1454. (b) Quinnan, G. V., Jr.; Masur, H.; Rook, A. H.; Armstrong, G.; Frederick, W. R.; Epstein, J.; Manischewitz, J. F.; Macher, A. M.; Jackson, L.; Ames, J.; Smith, H. A.; Parker, M.; Pearson, G. R.; Parillo, J.; Mitchell, C.; Straus, S. E. *J. Am. Med. Assoc.* **1984**, *252*, 72. (c) Laskin, O. L.; Staht-bayliss, C. M.; Kalman, C. M.; Rosecan, L. R. *J. Infect. Dis.* **1987**, *155*, 323.

(7) (a) St. Clair, M. H.; Miller, W. H.; Miller, R. L.; Lambe, C. U.; Furman, P. A. *Antimicrob. Agents Chemother.* **1984**, *25*, 191. (b) Frank, K. B.; Chiou, J.-F.; Cheng, Y.-C. *J. Biol. Chem.* **1984**, *259*, 1566.

0022-2623/90/1833-1797$02.50/0