ammonia with ice-cooling, and the mixture was stirred for 1 h at this temperature. A deposited solid was collected and washed with water. Sublimation of the solid at 120–130 °C (5 mm) furnished 1.41 g of the (+)-amide **10** (71% yield): mp 237–239 °C; $[\alpha]^{27}_D$ +15.6° (*c* 0.205, acetone); IR (KBr) 1660, 1620, 1420 cm^{-1}.

Anal. Calcd for $C_9H_{13}ON$: C, 71.49; H, 8.67; N, 9.26. Found: C, 71.35; H, 8.60; N, 9.22.

Formation of 9-Noradamantanol (12) on the Demjanow Rearrangement of 2-(Aminomethyl)-D_{2d}-dinoradamantane (11). A solution of the (+)-amide **10** (1.20 g, 7.95 mmol) in dry THF (60 mL) was added to a stirred suspension of $LiAlH_4$ (500 mg, 13.2 mmol) in dry THF (20 mL), and the mixture was gently refluxed for 4 h. After cooling, saturated aqueous NH_4Cl (5 mL) was added to the mixture, and deposited solid was removed by filtration. Removal of the solvent gave crude 2-(aminomethyl)-D_{2d}-dinoradamantane (**11**) as an oil (1.08 g), which was dissolved in 5% aqueous acetic acid (18 mL). After addition of a solution of sodium nitrite (0.95 g) in water (7.5 mL), the mixture was heated at 100–110 °C for 2 h. Dilution with water was followed by extraction with ether, and the extract was washed with aqueous $NaHCO_3$ and water and dried ($MgSO_4$). The solvent was removed to afford a solid that was chromatographed over alumina. Elution with pentane–ether (1:1, v/v) gave 670 mg of 9-noradamantanol (**12**) (61% overall yield from **10**). An analytical sample was further purified by sublimation at 90 °C (20 mm): mp 243–245 °C (in a sealed tube) (lit.[5a] mp 244–247 °C); $[\alpha]^{27}_D$ 0° (*c* 0.458, EtOH); IR (KBr) 3320, 1015 cm^{-1}.

Anal. Calcd for $C_9H_{14}O$: C, 78.21; H, 10.21. Found: C, 77.96; H, 10.18.

9-Noradamantanone (13). An excess of Jones reagent (2 mL)[8] was added dropwise to a stirred and chilled (0 °C) solution of 9-noradamantanol (**12**) (340 mg, 2.46 mmol) in acetone (6 mL). After stirring for 2 h with ice cooling, sodium bisulfite was added until the brown color was gone. The mixture was diluted with water and extracted with ether. The extract was washed with aqueous $NaHCO_3$ and water and dried ($MgSO_4$). Concentration left a semisolid (270 mg) that was chromatographed over alumina, and elution with pentane provided 9-noradamantanone (**13**) (225 mg, 67% yield). An analytical sample was prepared by sublimation at 80 °C (20 mm): mp 191–192 °C (in a sealed tube) (lit.[5a] mp 193–195 °C); IR (KBr) 1720, 1460, 1220, 1045, 970 cm^{-1}; ^{13}C NMR ($CDCl_3$) δ 36.3, 44.1, 52.0, 213.7.

Anal. Calcd for $C_9H_{12}O$: C, 79.37; H, 8.88. Found: C, 79.31; H, 8.87.

Noradamantane (14). 9-Noradamantanone (**13**) (120 mg, 0.882 mmol) was mixed with 100% hydrazine hydrate (0.1 mL), KOH (52 mg), and triethylene glycol (1.5 mL). The mixture was heated for 1 h at 110–120 °C and for an additional 4 h at 190–200 °C. During this period, a white solid was observed to condense on the inner wall of the condenser. After cooling, the solid was dissolved in ether and the reaction mixture was diluted with water and extracted with ether. The ether extracts were combined, washed with water, dried ($MgSO_4$), and concentrated to give a solid that was sublimed at 80 °C (20 mm) to provide noradamantane (**14**) (65 mg, 60% yield): mp 198–199 °C (in a sealed tube) (lit.[5a] mp 203 °C). Spectral comparison with an authentic specimen confirmed its identity.

Anal. Calcd for C_9H_{14}: C, 88.45; H, 11.55. Found: C, 88.35; H, 11.52.

9-Noradamantanecarbonitrile (15). 9-Noradamantanone (**13**) (455 mg, 3.34 mmol) and TosMIC[9] (850 mg, 4.36 mmol) were dissolved in a mixture of absolute ethanol (0.33 mL) and dimethoxyethane (11.7 mL), and the mixture was cooled in an ice bath. After potassium *tert*-butoxide (938 mg, 8.36 mmol) was added to the stirred mixture at such a rate to keep the reaction temperature below 5 °C, the mixture was stirred at room temperature for 1 h and then at 35–40 °C for an additional 1 h. A deposited solid was collected by filtration and washed with ether. The washings were combined with the filtrate, and removal of the solvent left a residue that was chromatographed over alumina. Elution with pentane afforded 9-noradamantanecarbonitrile (**15**) (332 mg, 68% yield), which was further purified by sublimation in vacuo (80 °C (20 mm)): mp 110–113 °C; IR (KBr) 2230, 1460, 1320, 1100 cm^{-1}.

Anal. Calcd for $C_{10}H_{13}N$: C, 81.85; H, 8.90; N, 9.52. Found: C, 81.39; H, 8.88; N, 9.49.

Formation of 5-Protoadamantanol (24) on the Demjanow Rearrangement of 9-(Aminomethyl)noradamantane (16). A solution of the nitrile (**15**) (325 mg, 2.21 mmol) in dry ether (10 mL) was added to a chilled (0 °C) and stirred suspension of $LiAlH_4$ (127 mg, 3.36 mmol) in dry ether (20 mL), and the stirring at this temperature was continued for an additional 1 h. After 20% aqueous NaOH (0.2 mL) and water (0.5 mL) were successively added to the chilled mixture, a deposited solid was collected by filtration and washed with ether. The washings were combined with the filtrate, washed with water, and dried (Na_2SO_4). Removal of the solvent gave crude 9-(aminomethyl)noradamantane (**16**) as an oil, which was dissolved in 5% aqueous acetic acid (4.5 mL). After a solution of sodium nitrite (0.3 g) in water (2.5 mL) was added, the mixture was heated at 100–110 °C for 1 h. The procedure described for the preparation of 9-noradamantanol (**12**) furnished 5-protoadamantanol (**24**) as a semisolid (275 mg), which was oxidized with excess Jones reagent. The routine workup followed by alumina chromatography (pentane elution) and sublimation in vacuo (60 °C (5 mm)) gave 5-protoadamantanone (**25**) (175 mg, 53% overall yield from **15**): mp 218–220 °C (in a sealed tube) (lit.[10] mp 222–225 °C); IR (KBr) 1720, 1450 cm^{-1}.

Anal. Calcd for $C_{10}H_{14}O$: C, 79.95; H, 9.39. Found: C, 79.96; H, 9.37.

Protoadamantane (26). The Wolff–Kishner reduction of 5-protoadamantanone (**25**) (125 mg, 0.833 mmol) with 100% hydrazine hydrate (0.11 mL), KOH (60 mg), and triethylene glycol (1.5 mL) was carried out as described for the preparation of noradamantane (**14**). Sublimation (50 °C (20 mm)) of the product furnished protoadamantane (**26**) (70 mg, 62% yield): mp 214–215 °C (in a sealed tube) (lit.[7] mp 215–216 °C). Spectral comparison with an authentic specimen confirmed its identity.

Anal. Calcd for $C_{10}H_{16}$: C, 88.16; H, 11.84. Found: C, 88.10; H, 11.89.

Registry No. (+)-**9**, 64753-43-1; (+)-**9** acid chloride, 85798-29-4; (+)-**10**, 85736-27-2; **11**, 85736-28-3; **12**, 23691-64-7; **13**, 23691-62-5; **14**, 7075-86-7; **15**, 85736-29-4; **16**, 28224-46-6; **17**, 85736-30-7; **18**, 85736-31-8; **19**, 85736-32-9; **20**, 85736-33-0; **21**, 85736-34-1; **23**, 85736-35-2; **24**, 85798-30-7; **25**, 31517-40-5; **26**, 19026-94-9; TosMIC, 36635-61-7.

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Absolute Configuration of Vitamin K Epoxide[1]

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A hepatic microsomal enzyme forms vitamin K 2,3-epoxide from reduced vitamin K and molecular oxygen[2-4] in a reaction that appears to be coupled to a vitamin K de-

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pendent carboxylation of peptide-bound glutamyl residues.[2,5-7] The carboxylation reaction is a necessary posttranslational modification during biosynthesis of the vitamin K dependent coagulation factors. A second microsomal enzyme, vitamin K epoxide reductase, converts the epoxide to vitamin K quinone.[8,9] Inhibition of this enzyme is responsible for the accumulation[10-12] of vitamin K epoxide during coumarin anticoagulant treatment and is thought to be the pharmacologically important site of action of these drugs.[13,14] For reviews, see ref 15–19. Although first synthesized in 1940,[20] the chirality of vitamin K epoxide has only recently been recognized. Wynberg and co-workers[21-24] have synthesized a number of naphthoquinone epoxides in up to 45% enantiomeric excess, but optically active epoxides of the physiologically relevant 2-methyl-3-prenylnaphthoquinones have not been reported nor has the stereochemistry of the naturally occurring enantiomer been determined.

Results and Discussion

Vitamin K_1 epoxide was isolated from livers of Warfarin-pretreated rats 1 h after administration of vitamin K_1 by silicic acid chromatography and two passages of reverse-phase HPLC. The product was optically active, $[\theta]_{342}$ = +3800° cm^2 dmol^{-1}. Isolated hepatic microsomes formed epoxide with the same stereospecificity in vitro, $[\theta]_{342}$ = +3900° cm^2 dmol^{-1}.

The phase-transfer epoxidation method of Pluim and Wynberg[24] using benzylquininium chloride as catalyst was applied to both *cis*- and *trans*-vitamin K_1. Variable yields and optical purity were obtained due to side reactions resulting in the formation of an interfacial barrier. Reactions utilizing both isomers under several conditions gave products of the same sign of rotation as that generated biologically but of low optical purity ($[\theta]_{342}$ = 96–178° cm^2 dmol^{-1}). Attempts to determine the enantiomeric purity of the (+)-vitamin K epoxide product by using a chiral NMR shift reagent[25] were unsuccessful. No differential shifts in the resonances arising from the two enantiomers were observed at 280 MHz when Eu(TFC)$_3$ in chloroform was used over a wide range of total concentrations and shift reagent ratios. The (−)-menadione 2,3-epoxide (2-methylnaphthoquinone 2,3-epoxide) prepared[24] by using this method has been assigned the 2*R*,3*S* configuration by Snatzke et al.[26] The stereoselectivity of the reaction appears to be the same for all monosubstituted naphthoquinone derivatives, and the optical purity of the products as determined by using chiral NMR shift reagents increased with increasing substituent size. If the reaction of the disubstituted vitamin K_1 (2-methyl, 3-phytylnaphthoquinone) proceeded with the same stereoselectivity

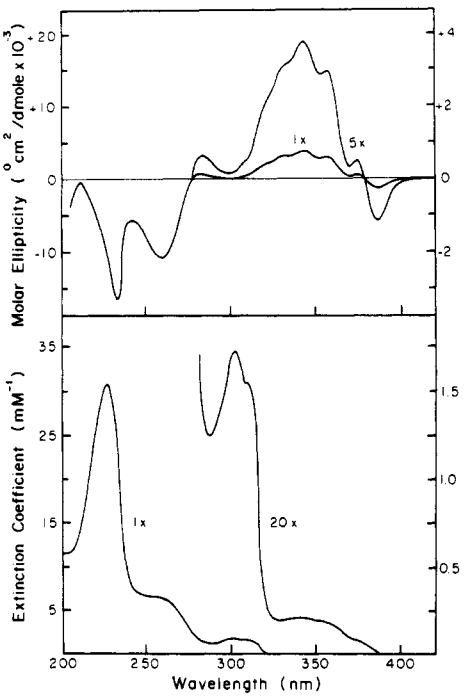

Figure 1. CD and absorbance spectra of (+)-vitamin K_1 epoxide in hexane.

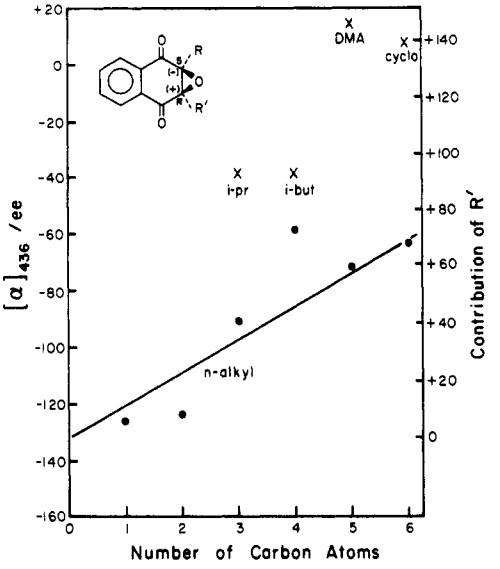

Figure 2. Correlation of specific rotation with size of the 2*R* substituent for several 2-substituted naphthoquinone epoxides. Data are derived from those of Pluim and Wynberg.[24] Right-hand ordinate and + and − signs refer to the effective contribution of R′ beyond the first methylene: i-pr = isopropyl, i-but = isobutyl, DMA = dimethylallyl. For all compounds R = H and R′ = methyl = (−)-(2*R*,3*S*)-menadione 2,3-epoxide; R = methyl and R′ = phytyl = (+)-(2*S*,3*R*)-vitamin K_1 2,3-epoxide.

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for the bulkier phytyl side chain, the product should have been enriched for the 2S,3R configuration, consistent with the following argument.

The CD and absorbance spectra of (+)-vitamin K_1 epoxide are shown in Figure 1. The spectra are identical for both cis and trans configurations of the phytyl side chain, suggesting that only the first isoprenoid unit need be considered when assessing the contribution of the phytyl group to the optical activity for which 2-(dimethylallyl)naphthoquinone epoxide[24] may be taken as a model. The maximum at 342 nm is characterized by fine structure spaced at approximately 1200 cm^{-1}, and high values of the ratio $\Delta\epsilon/\epsilon$ are typical of a carbonyl $n \rightarrow \pi^*$ transition. The configurational assignment for menadione epoxide was made from the sign of its corresponding transition on the basis of qualitative molecular orbital arguments.[26] Among the reported chiral naphthoquinone epoxides, the minimum at 386 nm is unique to vitamin K_1 epoxide. For the other compounds, the 342-nm transition gives rise to the lowest lying Cotton effect, and the CD corresponds in sign and magnitude to the rotation at 436 nm where both measurements are available. The specific rotation of the pure enantiomers, $[\alpha]_{436}$/ee, calculated from the reported data decreased with increasing size for the series of 2R-substituted naphthoquinone epoxides as shown in Figure 2. These results are consistent with a negative contribution to the rotational strength by the first methylene and an increasingly positive contribution by the remainder of the substituent. The relative contribution for different substituents may be taken as the difference between the observed rotation and the value for menadione epoxide. The positive rotation of 2(R)-dimethylallyl)-naphthoquinone epoxide is thus not an exception but a continuation of the series for which the positive contribution of the remainder of the substituent is greater than the negative contribution of the first methylene.

According to the sector rule developed for this transition,[26] the contributions of the 2-methyl and 3-methylene of the essentially planar disubstituted vitamin K_1 epoxide may be taken as equal and opposite. The problem is then treated as a perturbation of the dimethyl structure, and the optical activity is controlled by the remainder of the phytyl group. This contribution would be positive for a 3R substituent, and the configuration of (+)-vitamin K_1 epoxide must then be 2S,3R. The positive contribution evident for 2-(dimethylallyl)naphthoquinone epoxide is approximately equal to the specific rotation of menadione epoxide. The magnitude of the CD spectrum of vitamin K_1 epoxide ($[\theta]_{342}$ = 3900° cm^2 $dmol^{-1}$) should therefore be comparable to that of menadione epoxide ($[\theta]$ = 5088° cm^2 $dmol^{-1}$),[26] suggesting that the biologically derived material is optically pure.

Assignment of the absolute configuration of vitamin K epoxide completes determination of the structure of this important metabolite and designates the active face of the planar vitamin K molecule with respect to the biological reaction with molecular oxygen. Proposed mechanisms for the epoxidation–carboxylation reaction must account for this observed stereospecificity, and a mechanism invoking conversion of reduced vitamin K to vitamin K quinone with generation of hydrogen peroxide followed by peroxide oxidation of vitamin K quinone as in the chemical synthesis of the epoxide[20,24] must be enzyme mediated. The nonenzymatic occurrence of this reaction, a real possibility in vitro, would lead to racemic product and can be discounted. A corollary of stereospecificity of epoxidation, the stereoselectivity of vitamin K epoxide reductase, has been investigated and the results are presented elsewhere.[27]

Experimental Section

Pure *cis*- and *trans*-phytyl isomers were prepared from vitamin K_1 (Sigma) by silica gel G column chromatography.[28] Racemic vitamin K epoxide standards were prepared according to Tischler et al.[20] Concentrations and purity of the isolated metabolites were determined from their absorbance spectra and by analytical HPLC. Absorbance spectra were recorded on a Varian Model 635 spectrophotometer, and CD spectra were recorded with a JASCO J41-C spectropolarimeter in HPLC-grade hexane by using 1-cm path length cells. Spectra recorded in isooctane were similar.

Isolation of Vitamin K_1 Epoxide from Rat Liver. Five male 350-g Holtzman rats were given Warfarin (5 mg/kg, intraperitoneally) 18 h and a second dose (10 mg/kg) 30 min before injection of *trans*-vitamin K_1 (100 μg each intracardially, as a suspension, 1 mg/mL in 5% Tween-80 saline). After 1 h, livers were excised, homogenates (30% w/v) in 0.25 M sucrose and 0.025 M imidazole HCl (pH 7.2) were prepared, and vitamin K epoxide was extracted with two volumes of 2-propanol–hexane (3:2). The extract was chromatographed on SilicicAR CC-4 Special (Mallinckrodt) as previously described.[4] The collected vitamin K epoxide fraction was concentrated and chromatographed twice on a μBondapak C18 10-μm semipreparative HPLC column (Waters) run at 4 mL/min by using first 100% methanol (t_R 6.87 min) and then 95% methanol–5% water (t_R 15.3 min). A yield of 2 μg/g liver vitamin K epoxide was recovered.

Formation of Vitamin K Epoxide in Vitro. Whole microsomes[4] prepared from five rats given Warfarin (5 mg/kg, i.p.) 18 h before being killed were incubated at 27 °C in a total volume of 130 mL of sucrose–imidazole buffer containing 20 mL of an ATP-generating mix, plus cycloheximide,[4] 50 μg/mL sodium Warfarin, 10 mM sodium cyanide, 1.4 mM NADH, and 6.5 μg/mL reduced vitamin K[4] added last dropwise in ethanol. After 2 h, vitamin K epoxide was extracted and purified as from liver to yield 6.5 μg/g liver.

Stereoselective Syntheses. The reactions were run essentially as described by Pluim and Wynberg[24] but at a lower concentration of quinone. Typically, sodium hydroxide (285 mg in 2 mL of water), 2 mL of 30% hydrogen peroxide, and 25 mg of benzylquininium chloride were added to a vigorously stirred solution of 10–100 mg of vitamin K_1 quinone in 4 mL of toluene, and the reaction was maintained at 40 °C for several hours. Occasionally further additions of hydrogen peroxide and benzylquininium chloride were made. The toluene layer was diluted with hexane and washed with saturated sodium chloride, and then dried over anhydrous sodium sulfate. The vitamin K epoxide was purified by preparative HPLC using 100% methanol. Yields ranged from 17% to 68%.

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