Thermochemical Investigation of the Oxygenation of Vitamin K

Robert A. Flowers, II, Sriram Naganathan, Paul Dowd,* Edward M. Arnett,* and Seung Wook Ham

Contribution from the Departments of Chemistry, University of Pittsburgh, Pittsburgh, Pennsylvania 15260, and Duke University, Durham, North Carolina 27706

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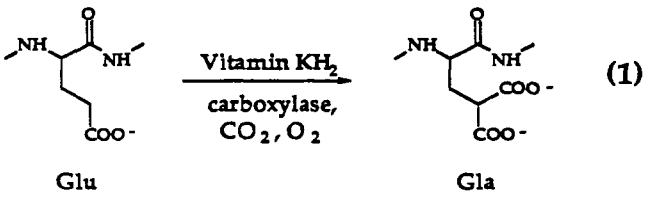
Abstract: Discovery of a new oxygenation reaction of naphthohydroquinone anions makes possible a determination of the heat of reaction (ΔH_{ox}) of oxygen with the potassium salt derived from deprotonation of the hydroquinone form of vitamin K. From that value (−33.52 ± 0.60 kcal/mol), the heat of deprotonation of vitamin KH_2 (−30.03 ± 1.20 kcal/mol), and the heat of deprotonation of water (−6.05 ± 0.3 kcal/mol), the enthalpy change for converting vitamin KH_2 to vitamin K oxide is established to be −57.5 kcal/mol, in reasonable agreement with our previous estimate of −62.4 kcal/mol for the oxygenation of the parent naphthohydroquinone. Indeed, in similar fashion the heat of oxygenation of the parent naphthohydroquinone was determined to be −58.47 kcal/mol, and this permits the assignment of a heat of formation to naphthoquinone epoxide of $\Delta H_f°$ = −47.6 kcal/mol. Heats of oxygenation and deprotonation of a variety of related phenols and naphthols provide perspective on cation and substitution effects. These data provide strong support for the base strength amplification mechanism for the biological action of vitamin K proposed by two of us (P.D. and S.W.H.).

Introduction

Vitamin K is essential for blood clotting.[1] It functions in this capacity as an obligatory cofactor for a membrane-bound carboxylase that, with vitamin K, is required to activate the zymogen precursors to the enzymes of the blood-clotting cascade.[2] In this instance, protein activation requires γ-carboxylation of the glutamate residues at the N-termini of factors II (prothrombin), VII, IX, and X and proteins C, S, and Z. In the critical carboxylation reaction, protein-bound glutamate (Glu) is transformed to γ-carboxyglutamate (Gla) (eq 1). Molecular oxygen,



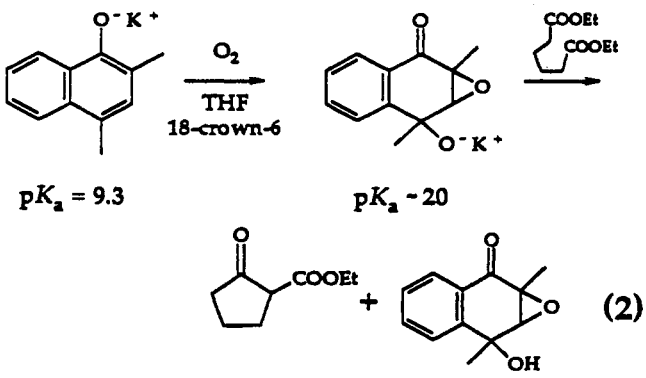
(1)

Glu Gla

the hydroquinone form of vitamin K (vitamin KH_2), and carbon dioxide are required for the carboxylation. In the course of the reaction, vitamin KH_2 is oxidized by molecular oxygen to vitamin K oxide (Scheme I).

A new mechanism for the carboxylation has recently been proposed, and a model has been devised to explain the capacity of vitamin K to abstract a weakly acidic proton to generate a carbanion at the γ-position of glutamate.[3] This model takes into account the enzymic juxtaposition of oxygenation and carboxylation and introduces a unique principle of *base strength amplification* to aid in understanding the proton abstraction from glutamate.[3a] It is proposed that oxygenation raises the pK_a of the biochemically accessible anion of vitamin KH_2 by transforming it from a weakly basic naphthoxide ion to a strongly basic alkoxide.

This is best illustrated by a discussion of the model reaction. In this model the course of the oxygenation can be mapped (eq 2), while the Dieckmann condensation of diethyl adipate is used to mimic the carbon–carbon bond forming capacity of the carboxylase (eq 2).



(2)

No reaction occurred when diethyl adipate was treated at ambient temperature with potassium 2,4-dimethylnaphthoxide in THF with 18-crown-6. The naphthoxide is not a sufficiently strong base to effect the Dieckmann condensation under these conditions.[3a,b] However, when 1.7 equiv of O_2 was bubbled into the solution, spontaneous oxygenation of the naphthoxide led to the production of a tertiary alkoxide (eq 2).[4] Production of the strong base enabled the Dieckmann condensation of diethyl

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(1) Review of early work on vitamin K: Wagner, A. F.; Folkers, K. *Vitamins and Coenzymes*; Interscience: New York, 1964; pp 407–434.

(2) Recent reviews: (a) Suttie, J. W. *Biofactors* **1988**, *1*, 55. (b) Suttie, J. W. *Annu. Rev. Biochem.* **1985**, *54*, 459. (c) Olson, R. E. *Annu. Rev. Nutr.* **1984**, *4*, 281. (d) Vermeer, C. *Biochem. J.* **1990**, *266*, 625.

(3) (a) Dowd, P.; Ham, S. W.; Geib, S. J. *J. Am. Chem. Soc.* **1991**, *113*, 7734. (b) Ham, S. W.; Dowd, P. *J. Am. Chem. Soc.* **1990**, *112*, 1660. (c) Dowd, P.; Ham, S. W.; Hershline, R. *J. Am. Chem. Soc.* **1992**, *114*, 7613. (d) Dowd, P.; Ham, S. W. *J. Am. Chem. Soc.* **1991**, *113*, 9403. (e) Naganathan, S.; Hershline, R.; Ham, S. W.; Dowd, P. *J. Am. Chem. Soc.* **1993**, *115*, 5839.

(4) The observation that the epoxide and hydroxyl groups are *cis* to one another in the product, together with ^{18}O-labeling studies, establishes that the model oxygenation is *strictly* intramolecular.[3a,d]

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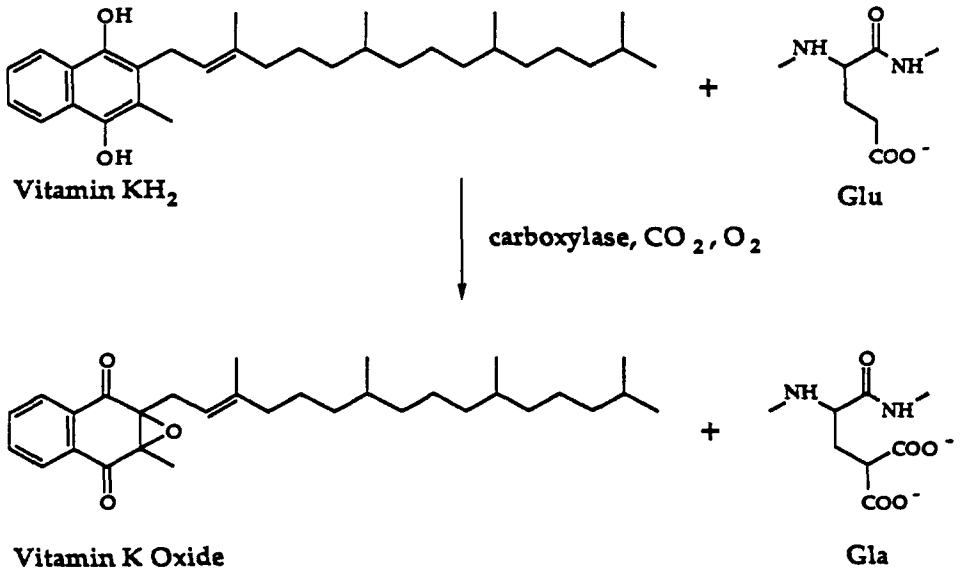
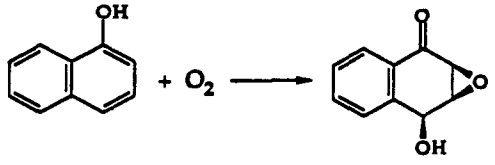
Scheme I



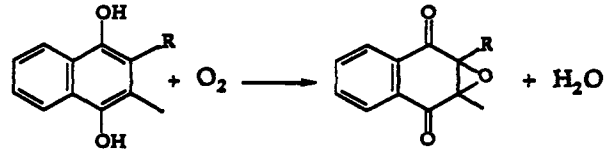
adipate to ethyl 2-oxocyclopentan-1-carboxylate (eq 2) and provided evidence for base strength amplification. Thus, oxygenation transformed the weak phenolic base of $pK_a = 9.3$ to the strong alkoxide base of $pK_a = 20$.[3a,b]

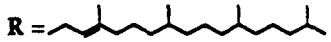
Enhancement of the base strength to this degree requires approximately 15 kcal/mol at 25 °C. Thermochemical analysis of the oxygenation model reaction, employing heats of formation available in the literature together with reasonable approximations to reaction enthalpies,[3a] suggests that the oxygenation of α-naphthol to the keto epoxy alcohol product will be exothermic to the extent of 52 kcal/mol (eq 3).[3a] This would provide ample energy to carry out the base strength amplification sequence.



$$\Delta H^\circ_r = -52 \text{ kcal/mole} \qquad (3)$$

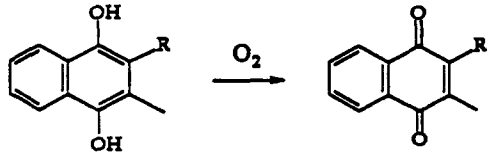
It was also estimated from thermochemical data available in the literature that the oxygenation leading from vitamin KH_2 to vitamin K oxide (eq 4) should be exothermic by approximately 62 kcal/mol.[3a]





$$\Delta H^\circ_{r(est)} = -62.4 \text{ kcal/mole} \qquad (4)$$

While it is well-known that the naphthohydroquinones are oxidized to naphthoquinones (eq 5), we discovered that when the hydroquinone is first transformed to the corresponding anion, the latter reaction is suppressed and vitamin K oxide is formed instead (eq 6).[3a] Since this is a quantitative reaction, it forms the basis of the thermochemical measurements reported here to



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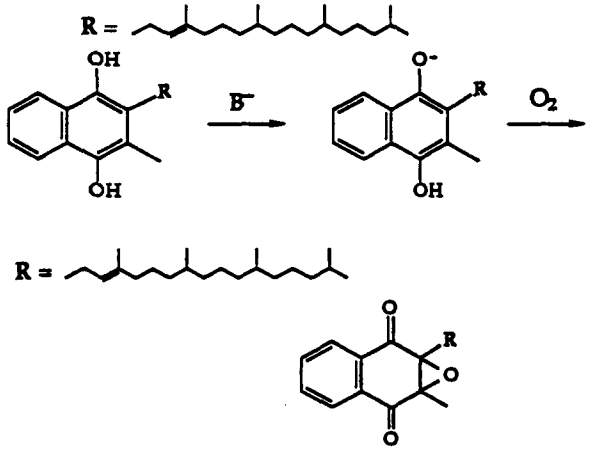

(6)

test the thermochemical prediction regarding the oxygenation of vitamin KH_2 to vitamin K oxide.

Experimental Section

Materials and General Procedures. All reactions were carried out in oven-dried glassware under magnetic stirring. All reactions involving moisture-sensitive material were conducted in flame-dried glassware, under argon. THF and ether were distilled from sodium benzophenone ketyl, under nitrogen. Solvents and solutions were degassed by several cycles of evacuating the vessel and refilling with argon. The drying agent employed was anhydrous magnesium sulfate, unless otherwise noted. Potassium hydride (Aldrich, 35% in mineral oil) was purified by washing repeatedly, under argon, with dry THF or dry benzene, and drying under vacuum. Purified potassium hydride was handled in a drybox or a glovebag, under argon.

For thermochemical studies, THF was distilled from sodium benzophenone ketyl and then refluxed over a column containing molecular sieves. The dry THF was then degassed using three freeze–thaw cycles and transferred to an argon-filled Vacuum Atmospheres drybox equipped with a VAC HE-493 purification system. The THF was tested for water content before each experiment by a Karl Fisher titration. THF containing more than 20 ppm of water was discarded. Lithium bis(trimethylsilyl)-amide, LiHMDS (Aldrich), and KHMDS were sublimed in vacuo at 80 and 100 °C, respectively, and transferred under argon to the drybox.

LiHMDS is available commercially as a 1.0 M solution in THF (Aldrich), and appropriate dilutions of this stock solution were also used. All reagents that were obtained from commercial sources were used without further purification, unless otherwise indicated. Melting points were recorded on a Mel-Temp apparatus and are uncorrected.

Flash chromatography was carried out using silica gel following the procedure of Still, Kahn, and Mitra.[7] Analytical TLC was performed on precoated silica gel plates (Merck Art. No. 5715) and was visualized by 254-nm UV and by staining with *p*-anisaldehyde. PCTLC refers to preparative centrifugal thin-layer chromatography and was performed on 4-mm silica gel layers (Merck Art. No. 7749) using the Model 7924T Chromatotron (Harrison Research, Palo Alto, CA).

HPLC analyses were performed using Waters 510 pumps and a Waters 410 detector at 254 nm. Reversed-phase (RP-HPLC) analysis employed a Rainin Microsorb-MV C-18 silica (4.6 × 250 mm) column, and normal-phase (Si-HPLC) analysis employed a Waters μ-Porasil silica gel (3.9 × 300 mm) column using HPLC grade solvents.

NMR spectra were recorded on Varian XL-300 (Duke University) or Bruker AC300 or AF300 (University of Pittsburgh) spectrometers operating at 300 MHz for proton and 75 MHz for carbon. The ^{1}H NMR spectra are referenced with respect to the residual $CHCl_3$ proton of the solvent $CDCl_3$ at 7.26 ppm. The ^{13}C NMR spectra are referenced with respect to the middle peak of the solvent $CDCl_3$ at 77.0 ppm. Infrared spectra were recorded on an IBM IR-32 Fourier transform spectrophotometer on NaCl plates. GC–MS were obtained on a Hewlett-Packard 5890 Series II gas chromatograph and a Hewlett-Packard 5970 mass selective detector, using an HP-1 capillary column (12 m × 0.2 mm, 0.33 μm film thickness) and helium as the carrier gas. High-resolution mass spectra (HRMS) were recorded on a VG 70-SE double focusing magnetic sector mass spectrometer.

Synthesis

2-Methyl-1,4-naphthalenediol, 1-Acetate (1). A suspension of 2-methyl-1,4-naphthalenediol, diacetate (15.3 g, 60 mmol) in methanol (125 mL) was treated with aqueous ammonia (28%, 7.2 mL, 58 mmol), and heated to 45 °C, under argon. When all the solid had dissolved, after ca. 1 h, the brown solution was cooled to room temperature and stirring continued for 16 h. The solvent was evaporated in vacuo, and the oily residue was purified by flash chromatography using, sequentially, 10%, 20%, 33%, 50%, and 67% ethyl acetate in hexanes as eluent to yield 10.22 g (80%) of a brown solid: mp 120–122 °C (lit.[8] mp 123 °C); ^{1}H NMR ($CDCl_3$) δ 7.95 (d, J = 8.4 Hz, 1 H), 7.64 (d, J = 8.4 Hz, 1H), 7.49 (t, J = 8.4, Hz, 1H), 7.38 (t, J = 8.4 Hz, 1H), 6.32 (s, 1H), 5.85 (br s, 1H, exch with D_2O), 2.50 (s, 3H), 2.17 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 170.97 (s), 149.55 (s), 137.37 (s), 127.46 (s), 127.00 (d J = 161 Hz), 126.49 (s), 124.58 (d, J = 161 Hz), 123.96 (s), 122.28 (d, J = 158 Hz), 120.25 (d, J = 154 Hz), 111.05 (d, J = 156 Hz), 20.77 (q, J = 130 Hz), 16.03 (q, J = 127 Hz); IR (thin film) 3414 (s), 3067 (w), 1738 (s), 1640 (m), 1601 (s), 1582 (m), 1400 (s), 1368 (s), 1230 (s), 1159 (s), 1080 (s), 949 (m), 766 (s), 742 (s); MS (*m/z*) 216 (9), 174 (100), 145 (10), 131 (17), 105 (16), 77 (10).

1-Acetoxy-4-methoxy-2-methylnaphthalene (2). A solution of **1** (1.62 g, 7.5 mmol) in THF (10 mL) was added dropwise using a cannula to a suspension of potassium hydride (300 mg, 7.5 mmol) in THF (10 mL) at 0 °C, under argon. An additional 5 mL of THF was used to ensure complete transfer. After stirring for 30 min at 0 °C, the green solution was treated with iodomethane (1 mL, 16 mmol) and stirred overnight. A cream-colored precipitate resulted. The reaction mixture was cooled in ice, and saturated aqueous ammonium chloride (10 mL) was added carefully, followed by 10 mL of water and 30 mL of ether. The aqueous layer was extracted with ether (3 × 25 mL), and the combined ether layers were washed with water (25 mL), dried, filtered, and concentrated to yield 2.03 g of a brown oil. Purification by flash chromatography yielded 1.69 g (97%) of an orange oil, which solidified in the freezer. Recrystallization from pentane (charcoal) at −20 °C yielded colorless crystals: mp 58–59 °C; ^{1}H NMR ($CDCl_3$) δ 8.21 (d, J = 8 Hz, 1H), 7.67 (d, J = 8 Hz, 1H), 7.49 (m, 2H), 6.65 (s, 1H), 3.99 (s, 3H), 2.47 (s, 3H), 2.32 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 169.46 (s), 153.09 (s), 137.56 (s), 127.56 (s), 127.05 (d, J = 156 Hz), 126.11 (s), 124.78 (d, J = 160 Hz), 122.23 (d, J = 161 Hz), 120.35 (d, J = 160 Hz), 106.15 (d, J = 160 Hz), 55.55 (q, J = 144 Hz), 20.55 (q, J = 131 Hz), 16.80 (q, J = 129 Hz); IR (thin film) 2394 (w), 1759 (s), 1601 (m), 1462 (m), 1400 (m), 1363 (s), 1206 (s), 1163 (m), 1119 (m), 1088 (m), 767 (m); MS (*m/z*) 230 (14), 188, (100), 173 (79), 115 (27), 105 (23), 76 (9), 43 (20); high-resolution MS calcd for $C_{14}H_{14}O_3$ 230.0943, found 230.0940.

4-Methoxy-2-methyl-1-naphthalenol (3). Degassed 3 M aqueous sodium hydroxide (2 mL, 6 mmol) was added all at once to a degassed solution of **2** (594 mg, 2.58 mmol) in ethanol (5 mL), under argon. The resulting orange solution was stirred at room temperature for 45 min and then quenched by the addition of 5 mL of degassed 3 M hydrochloric acid. The cream-colored precipitate was dissolved in freshly distilled ether (10 mL), and the ether layer was washed, under argon, with degassed water (4 × 5 mL). The ether was evaporated under a stream of argon and then under vacuum to yield 427 mg (88%) of a tan solid: mp 96–98 °C dec. This substance is sensitive to oxygen and was not purified further: ^{1}H NMR ($CDCl_3$) δ 8.18 (d, J = 8 Hz, 1H), 8.06 (d, J = 8 Hz, 1H), 7.47 (m, 2H), 6.59 (s, 1H), 4.65 (s, 1H; exch with D_2O), 3.96 (s, 3H), 2.41 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 149.24 (s), 142.00 (s), 125.96 (d, J = 161 Hz), 125.40 (s), 124.85 (s), 124.66 (d, J = 160 Hz), 121.84 (d, J = 161 Hz), 120.70 (d, J = 154 Hz), 116.24 (s), 106.95 (d, J = 156 Hz), 55.72 (q, J = 144 Hz), 16.16 (q, J = 1261 Hz); IR (thin film) 3341 (m), 2928 (m), 1599 (m), 1389 (s), 1370 (s), 1281 (m), 1221 (s), 1169 (m), 1121 (s), 1092 (s), 841 (m), 760 (s), 644 (s); MS (*m/z*) 188 (73), 173 (100), 145 (16), 115 (34), 105 (76); high-resolution MS calcd for $C_{12}H_{12}O_2$ 188.0837, found 188.0833.

2-Methyl-1,4-naphthalenediol, 1-Benzoate (5). Degassed 3 M aqueous sodium hydroxide (6 mL, 18 mmol) was added all at once to a suspension of 2-methyl-1,4-naphthalenediol, dibenzoate (**4**)[9] (6 g, 15.7 mmol) in degassed ethanol (15 mL), under argon. The solids dissolved almost immediately. The resulting dark brown solution was stirred at room temperature for 30 min and then quenched by the addition of 20 mL of degassed 3 M hydrochloric acid and extracted with ether (4 × 100 mL). The ether extracts were combined, dried, and concentrated to yield 8.11 g of a dark brown oil. Purification by flash chromatography using, sequentially, 5%, 10%, and 20% ethyl acetate in hexanes as eluent gave 1.63 g (37%) of a gray solid. A 450-mg sample was recrystallized from ethyl acetate/hexanes (charcoal) to give 322 mg of colorless crystals: mp 171–172 °C (lit.[10] mp 171 °C); ^{1}H NMR ($CDCl_3$) δ 8.39 (d, J = 7.5 Hz, 2H), 7.93 (d, J = 8 Hz, 1H), 7.75–7.65 (m, 2H), 7.60 (t, J = 7.5 Hz, 2 H), 7.48–7.33 (m, 2H), 6.33 (s, 1H), 5.89 (br s, 1H, exch with D_2O), 2.23 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 166.22, 149.53, 137.33, 134.01, 130.44, 128.96, 128.79, 127.53, 126.92, 126.66, 124.52, 123.97, 122.19, 120.32, 111.10, 16.34; IR (thin film) 3412 (m), 1711 (s), 1601 (m), 1399 (m), 1252 (s), 1159 (m), 1111 (m), 1082 (m), 710 (s); MS (*m/z*) 278 (13), 105 (100), 77 (34), 51 (10).

4-Acetoxy-3-methyl-2-phytyl-1-naphthaleneol (6).[8,11] Phytol (3.5 mL, 10 mmol) was added dropwise to a solution of **1** (2.16 g, 10 mmol) and BF_3·Et_2O (1.85 mL, 15 mmol) in dry dioxane (18 mL) and heated to 50 °C for 1 h. The brown reaction mixture was treated with water (10 mL) and extracted with ether (3 × 50 mL). The ether extracts were combined and washed with water (25 mL), dried, and concentrated to yield 7.81 g of a brown oil. Purification by flash chromatography using 12:1 hexanes/ethyl acetate as eluent afforded 2.70 g (55%) of a pale yellow oil: ^{1}H NMR ($CDCl_3$) δ 8.11 (dd, J = 6.8, 1 Hz, 1H), 7.63 (dd, J = 7, 1 Hz, 1H), 7.51–7.38 (m, 2H), 5.80 (br s, 1H, exch with D_2O), 5.24 (t, J = 6.5 Hz, 1H), 3.50 (d, J = 6.5 Hz, 2H), 2.48 (s, 3H), 2.28 (s, 3H), 2.03 (t, J = 6.5 Hz, 2H), 1.86 (s, 3H), 1.57–1.05 (m, 19H), 0.90–0.76 (m, 12H); ^{13}C NMR ($CDCl_3$) δ 169.78, 147.84, 139.69, 137.85, 126.32, 126.04, 124.91, 123.91, 121.74, 120.71, 120.51, 119.80, 119.73, 39.96, 39.35, 37.37, 37.28, 32.77, 32.65, 31.58, 27.96, 26.34, 25.27, 24.79, 24.46, 23.46, 22.72, 22.63, 20.65, 19.68, 16.35, 13.54; IR (thin film) 3467 (m), 2926 (s), 2865 (s), 1744 (s), 1599 (m), 1460 (s), 1364 (s), 1231 (s), 1183 (s), 1090 (m), 1057 (m), 760 (s); MS (*m/z*) 494 (8), 452 (100), 186 (63); high-resolution MS calcd for $C_{33}H_{50}O_3$ 494.3760, found 494.3762.

The *Z*-isomer is identified by a characteristic singlet at 1.77 ppm in the ^{1}H NMR spectrum. Other spectral characteristics for the two isomers are identical.

4-(Benzoyloxy)-3-methyl-2-phytyl-1-naphthalenol (7).[11] Phytol (0.56 mL, 1.60 mmol) was added dropwise to a solution of **5** (450 mg, 1.62

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mmol) and $BF_3{\cdot}Et_2O$ (0.30 mL, 2.4 mmol) in dry dioxane (5 mL) and heated to 50 °C for 1 h. The pale brown reaction mixture was treated with water (10 mL) and extracted with ether (3 × 25 mL). The ether extracts were combined and washed with water (2 × 10 mL), dried, and concentrated to yield 1.22 g of a pale yellow oil. Purification by flash chromatography using 9:1 hexanes/ethyl acetate as eluent afforded 573 mg (64%) of a pale yellow wax: ^{1}H NMR ($CDCl_3$) δ 8.39 (d, J = 7.7 Hz, 2H), 8.14 (dd, J = 6.5, 3 Hz, 1H), 7.75–7.65 (m, 2H), 7.60 (t, J = 7.6 Hz, 2H), 7.41 (dd, J = 6.4, 3 Hz, 2H), 5.94 (br s, 1H, exch with D_2O), 5.23 (t, J = 6 Hz, 1H), 3.52 (d, J = 6 Hz, 2H), 2.30 (s, 3H), 2.03 (t, J = 7.5 Hz, 2H), 1.85 (s, 3H), 1.58–1.11 (m, 19H), 1.10–0.84 (m, 12H); ^{13}C NMR ($CDCl_3$) δ 165.44, 147.78, 139.21, 137.88, 133.74, 130.37, 139.21, 128.72, 126.36, 126.28, 126.12, 124.85, 124.04, 121.77, 120.87, 120.60, 120.15, 39.96, 39.34, 37.34, 36.75, 36.65, 32.74, 32.62, 27.96, 26.18, 25.27, 24.79, 24.43, 22.72, 22.62, 19.65, 16.31, 13.56; IR (thin film) 3459 (m), 2953 (s), 2926 (s), 2865 (m), 1719 (s), 1599 (m), 1366 (m), 1264 (s), 1117 (m), 1111 (m), 1098 (m), 1062 (m), 758 (m), 710 (m); MS (m/z) 557 (20), 451 (18), 329 (48), 225 (13), 187 (11), 105 (100); high-resolution MS calcd for $C_{38}H_{52}O_3$ 556.3916, found 556.3890.

The E- and Z-isomers were separated by PCTLC using 8% ethyl acetate in hexanes as eluent. The 3-mL fractions that were collected were checked by Si-HPLC using 5% THF in hexanes as eluent at 1 mL/min. The E:Z ratios were determined by HPLC and confirmed by ^{1}H NMR spectroscopy. Samples with E/Z ratios of 31:1, 22:1, and 1:2.25 were thus prepared. The Z-isomer is identified by a characteristic singlet at 1.77 ppm in the ^{1}H NMR spectrum. Other spectral characteristics for the two isomers are identical.

1-Acetoxy-4-(benzoyloxy)-2-methyl-3-phytyl-1-naphthalene (8). A degassed solution of **6** (0.50 g, 1.01 mmol) in THF (5 mL) was added dropwise by cannula to a suspension of potassium hydride (45 mg, 1.13 mmol) in THF (5 mL) at 0 °C, under argon. An additional 3 mL of THF was used to ensure complete transfer. The dark green solution was stirred for 30 min at 0 °C, and benzoyl chloride (0.14 mL, 1.21 mmol) was added dropwise. The green color was discharged immediately, resulting in an orange solution. After 10 min, the reaction was quenched by addition of 10% aqueous ammonium chloride and treated with ether (25 mL). The aqueous layer was extracted with ether (3 × 25 mL), and the ether layers were combined, dried, and concentrated to yield 614 mg of an orange oil. Purification by flash chromatography, using 5% ethyl acetate in hexanes as eluent, yielded 444 mg (74%) of a pale yellow oil: ^{1}H NMR ($CDCl_3$) δ 8.39 (d, J = 8 Hz, 2H), 7.82–7.44 (m, 7H), 5.19 (t, J = 4 Hz, 1H), 3.54 (br d, 2H), 2.53 (s, 3H), 2.36 (s, 3H), 1.96 (t, J = 7.2 Hz, 2H), 1.66 (s, 3H), 1.61–0.98 (m, 19H), 0.97–0.83 (m, 12H); ^{13}C NMR ($CDCl_3$) δ 168.91, 164.99, 142.61, 142.48, 142.41, 136.88, 136.49, 133.71, 130.57, 130.32, 129.05, 128.64, 126.91, 126.29, 121.48, 121.38, 121.13, 120.83, 39.86, 39.32, 37.34, 37.26, 36.76, 36.66, 32.72, 32.62, 31.55, 27.93, 27.05, 26.79, 25.24, 24.76, 24.43, 23.20, 22.68, 22.59, 20.52, 19.65, 16.15, 13.08; IR (thin film) 2926 (s), 2863 (m), 1769 (s), 1744 (s), 1456 (m), 1358 (m), 1204 (s), 1173 (s), 1102 (m), 1065 (m), 708 (m); MS (m/z) 599 (8), 557 (27), 494 (15), 451 (10), 225 (8), 187 (8), 105 (100); high-resolution MS calcd for $C_{40}H_{54}O_4$ 598.4022, found 598.3980.

The Z-isomer is identified by a characteristic singlet at 1.70 ppm and a triplet at 2.07 ppm (J = 7 Hz) in the ^{1}H NMR spectrum. Other spectral characteristics for the two isomers are identical.

4-(Benzoyloxy)-2-methyl-3-phytyl-1-naphthalenol (9). Degassed 3 M aqueous sodium hydroxide (0.7 mL, 2.10 mmol) was added all at once to a solution of **8** (1.2 g, 2 mmol) in degassed ethanol (5 mL), under argon. The resulting dark brown solution was stirred at room temperature for 40 min and then quenched by the addition of 3 mL of degassed 3 M hydrochloric acid and 5 mL of degassed water. The orange solution was extracted with ether (4 × 20 mL), and the ether extracts were combined, dried, and concentrated to yield 1.184 g of a dark brown oil. Purification of flash chromatography using 10% ethyl acetate in hexanes as eluent gave 631 and 259 mg of yellow waxy solids that were respectively 90% and 95% pure by Si-HPLC (80% combined yield): ^{1}H NMR ($CDCl_3$) δ 8.38 (dd, J = 8, 1 Hz, 2H), 8.02–7.97 (m, 1H), 7.73–7.57 (m 4H), 7.40–7.34 (m, 2H), 5.60 (br s, 1H), 5.10 (t, J = 6 Hz, 1H), 3.38 (br d, 2H), 2.09 (br s, 3H), 1.90 (t, J = 7.6 Hz, 2H), 1.61 (s, 3H), 1.52 (m, 2H), 1.41–1.01 (m, 17H), 1.00–0.79 (m, 12H); ^{13}C NMR ($CDCl_3$) δ 166.16, 147.01, 137.75, 137.69, 136.20, 133.84, 130.48, 129.18, 128.70, 126.01, 125.75, 124.91, 123.91, 123.51, 121.29, 120.80, 117.47, 39.93, 39.35, 37.38, 37.28, 36.82, 36.72, 32.77, 32.66, 27.96, 27.12, 25.31, 24.79, 24.47, 22.72, 22.62, 19.71, 16.21, 11.85; IR (thin film) 3449 (m), 2926 (s), 2864 (m), 1719, 1456 (m), 1264 (s), 1177 (m), 1102 (m), 1068 (s), 758 (m), 712 (m); MS (m/z) 557 (13), 451 (90), 225 (71), 198 (45), 186 (70), 105 (100), 57 (58); high-resolution MS calcd for $C_{38}H_{52}O_3$ 556.3916, found 556.4006.

The E:Z ratio was 6:1, as determined by HPLC and confirmed by ^{1}H NMR spectroscopy. The Z-isomer is identified by a characteristic singlet at 1.64 ppm in the ^{1}H NMR spectrum.

Separation of (*E*)- and (*Z*)-Vitamin K. A solution of 200 mg of vitamin K (from Sigma Chemical Co., 87% E-isomer) in hexanes (1 mL) was applied to a 4-mm PCTLC silica gel plate and eluted with 2% dibutyl ether in hexanes. The 3-mL fractions that were collected were analyzed by Si-HPLC using 2% dibutyl ether in hexanes as eluent at 1.5 mL/min. Fractions containing >98% (E)-vitamin K were combined and concentrated. The purified (E)-vitamin K from five chromatographic runs yielded 601 mg of 98.6% (E)-vitamin K.

2-Methyl-3-phytyl-1,4-naphthalenediol, Vitamin K Hydroquinone. A freshly prepared solution of sodium hydrosulfite (5 g, 28.7 mmol) in water (20 mL, degassed by bubbling Ar) was added rapidly to a refluxing solution of (E)-vitamin K (601 mg, 1.33 mmol) in ether (20 mL). After 6 h at reflux under argon, the ether layer was still yellow. The aqueous layer was removed by syringe and replaced by fresh sodium hydrosulfite solution. This procedure was repeated every 6 h until the ether layer was colorless. The reaction was cooled to room temperature, and the ether layer was washed, under argon, with degassed water (4 × 10 mL) and transferred by a cannula into a centrifuge tube. The ether was evaporated by argon stream to yield a yellowish paste. Degassed pentane (10 mL) was added and resulted in a white precipitate. The precipitate was collected by centrifugation and washed with 5-mL portions of degassed pentane until the washings were colorless. Then the sample was dried under vacuum, yielding 499 mg (83%) of a waxy white solid. A second crop (115 mg) was obtained by concentrating the pentane washings and repeating the precipitation and washing sequence: ^{1}H NMR (degassed $CDCl_3$) δ 8.12–8.07 (m, 1H), 8.03–7.99 (m, 1H), 7.47–7.41 (m, 2H), 5.29 (s, 1H), 5.20 (t, J = 6.3 Hz, 1H), 4.72 (s, 1H, exch with D_2O), 3.54 (d, J = 6.7 Hz, 2H), 2.37 (s, 3H), 2.02 (t, J = 7.4 Hz, 2H), 1.87 (s, 3H), 1.53–1.03 (m, 19H), 0.91–0.82 (m, 12H); ^{13}C NMR (degassed $CDCl_3$) δ 143.6, 142.3, 139.0, 125.2, 124.9, 124.1, 124.0, 121.5, 121.3, 120.9, 120.8, 117.4, 40.1, 39.4, 37.5, 27.4, 37.3, 36.7, 32.9, 32.7, 28.0, 26.4, 25.5, 24.9, 24.5, 23.8, 23.7, 19.9, 19.8, 16.3, 12.5; IR (mineral oil) 3300 (vs).

Oxygenation of the Monoanion of 1,4-Naphthalenediol. A solution of 1,4-naphthalenediol (128 mg, 0.80 mmol) in dry THF (3 mL) was added dropwise using a cannula to a suspension of potassium hydride (32 mg, 0.80 mmol) in THF (5 mL) at 0 °C, under argon. An additional 1 mL of THF was used to ensure complete transfer. The green solution was warmed to room temperature. After 30 min, the reaction mixture had turned into a greenish yellow suspension. A solution of 18-crown-6 (212 mg, 0.8 mmol) in THF (3 mL) was added by cannula. An additional 1 mL of THF was used to ensure complete transfer. The solids dissolved, resulting in a brown solution. After 30 min, the reaction flask was cooled to 0 °C and oxygen was bubbled through the reaction from a syringe, driven by a syringe pump, over 15 min. The wine-red reaction mixture was stirred for an additional 10 min, and saturated aqueous ammonium chloride (20 mL) was added, followed by water (20 mL) and ethyl acetate (25 mL). The aqueous layer was extracted with ethyl acetate (2 × 25 mL), and the organic layers were combined and washed with brine (2 × 25 mL), dried, filtered, and concentrated to yield 131 mg (94%) of a pale brown solid. Purification by flash chromatography on silica gel using 10% ethyl acetate in hexanes yielded 122 mg (87%) of 1,4-naphthoquinone oxide as a colorless solid: ^{1}H NMR ($CDCL_3$) δ 7.95–7.79 (m, 2H), 7.78–7.30 (m, 2H), 4.01 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 190.77 (s), 134.77 (dd, J = 166, 7 Hz), 131.79 (s), 127.27 (dd, J = 167, 5 Hz), 55.32 (d, J = 192 Hz); IR (neat) 1696 (s), 1595 (m), 1325 (m), 1294 (m), 857 (m), 722 (m); MS (m/z) 174 (69), 146 (44), 105 (100), 76 (53), 50 (40).

Spectral characteristics of 1,4-naphthoquinone oxide were identical to those of a sample prepared from 1,4-naphthoquinone by oxidation with basic hydrogen peroxide.[14] Recrystallization from ethanol gave colorless needles: mp 134–136 °C (lit.[15] mp 136 °C).

Oxygenation of the Monoanion of Vitamin K Hydroquinone. A solution of vitamin K hydroquinone (87 mg, 0.19 mmol) in dry THF (2.5 mL)

(14) Fieser, L. F. *Organic Experiments*, 2nd ed.; Raytheon Education Co.: Lexington, MA, 1968; Chapter 47, p 244.
(15) Mormor, S. *J. Org. Chem.* **1963**, *28*, 250.
(16) Arnett, E. M.; Moe, K. D. *J. Am. Chem. Soc.* **1991**, *113*, 7288.
(17) Arnett, E. M.; Moe, K. D. *J. Am. Chem. Soc.* **1991**, *113*, 7068.
(18) See, for example, for an extensive recent review of presumed radical reactions of O_2: Charron, M.; Juilliard, M.; Santamaria, J.; Charon, F. *New J. Chem.* **1992**, *16*, 171.

was added dropwise using a cannula to a suspension of potassium hydride (7.6 mg, 0.19 mmol) in THF (0.5 mL) at room temperature, under argon. The green solution was stirred at room temperature for 10 min. A solution of 18-crown-6 (33 mg, 0.13 mmol) in THF (1 mL) was added by cannula. The reaction mixture was stirred under oxygen for 20 min, and saturated aqueous ammonium chloride (20 mL) was added. The aqueuos layer was extracted with ether (2 × 20 mL), and the organic layers were combined and washed with brine (10 mL), dried, filtered, and concentrated to yield 108.7 mg of crude product. Purification by flash chromatography on silica gel using 2% ethyl acetate in hexanes yielded 78 mg (88%) of vitamin K oxide as a pale yellow oil: ^{1}H NMR ($CDCl_3$) δ 7.98–7.94 (m, 2H), 7.73–7.66 (m, 2H), 5.12 (t, J = 7 Hz), 3.24 (dd, J = 15, 7 Hz), 2.43 (dd, J = 15, 7 Hz), 1.93 (t, J = 7.7 Hz), 1.75 (s, 3H), 1.74 (s, 3H), 1.55–1.01 (m, 19H), 0.87–0.80 (m, 12H).

Preparation of the Potassium Monoanion of Vitamin K Hydroquinone. A solution of 150.0 mg (0.33 mmol) of vitamin K hydroquinone in 20 mL of dry THF was added slowly to a suspension of 12 mg (0.30 mmol) of potassium hydride in 10 mL of dry THF. Next, 79 mg (0.30 mmol) of 18-crown-6 was added to the mixture. The dark green solution was stirred for at least 0.5 h.

Calorimetry. All ΔH_{dep}s were determined with a Tronac 458 isoperibol solution calorimeter. The basic operation of the instrument has been described previously.[12,13] Solutions of LiHMDS and KHMDS (0.05–0.10 M) in THF were prepared in the drybox either by dissolving an appropriate amount of sublimed LiHMDS or KHMDS in THF or by diluting the commercially available stock solution of LiHMDS in THF. This solution was transferred to the Dewar calorimeter vessel. A known concentration of the vitamin K hydroquinone (or monosubstituted analog) in THF was prepared, and the motor-driven buret was used to introduce precise amounts into the base solution at a constant rate. The reactions were complete and instantaneous at 25 °C. In some cases a known molarity of the LiHMDS or KHMDS base was prepared and introduced into the Dewar calorimeter vessel containing the vitamin K hydroquinone in order to estimate the ΔH_{dep} of a single deprotonation of the hydroquinone, which contains two acidic sites. Each ΔH_{dep} presented here is the average of at least seven calorimetric measurements on two independently prepared solutions. Variation of the source of LiHMDS or KHMDS did not affect the magnitude of ΔH_{dep}.

The ΔH_{expt} for the reaction of oxygen with vitamin K hydroquinone monoanion was carried out by transferring the vitamin K solution contained in the drybox to the calorimeter Dewar. Next, successive 1-mL increments of 99.8% oxygen (Matheson) were injected instantaneously through a Teflon cannula from a 10-mL gas-tight syringe.[5] Clean, linear thermograms indicated that the exothermic reactions were complete and virtually instantaneous at 25 °C. Each ΔH_{expt} presented here is the average of at least five calorimetric measurements on two independently prepared solutions.

Results and Discussion

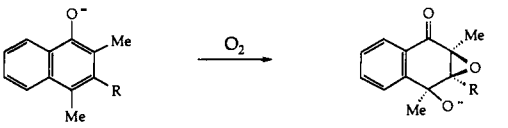
Thermochemistry in the Model Series. The thermochemical predictions of Dowd and Ham[3a] regarding the model reaction (eq 2) were confirmed by a calorimetric measurement of the heat of oxygenation of potassium 2,4-dimethylnaphthoxide (eq 2) in conjunction with a comparison of the heats of deprotonation of the resulting tertiary alcohol and that of the naphthol model (Table Ia).[5] Agreement between prediction and experiment was very good (within 10%). Indeed the measured value significantly exceeded the estimated exotherms for the model oxygenation of eq 2 (Figure 1).

Heat of Oxygenation of the Vitamin KH$^-$ Monoanion. We have now extended the previous measurements[5] to an investigation of vitamin K itself, to test the thermochemical predictions[3a] regarding the transformation of vitamin KH_2 to vitamin K oxide (eq 6).

Table Ib compares heats of oxygenation of the monopotassium salt of vitamin KH_2 in THF at 25 °C under several conditions with the previously reported data for the 2,4-dimethylnaphthoxide model (Table Ia).[5] Within experimental error neither the presence of the crown ether nor the stereochemistry at the double bond of the phytyl side chain has a significant effect on this property. However, in comparing the entries in Table Ia with those in Table Ib, it obviously matters a great deal whether a methyl or hydroxyl group is in the 4-position of the naphthoxide anion.

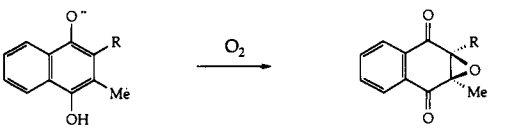
Table I. Heats of Reaction with Molecular Oxygen in Tetrahydrofuran at 25 °C

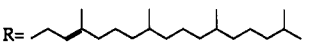
(a) Model Series



Conditions	ΔH (kcal/mol)
R= H, K^+	-47.75±0.60
R= H, 18-crown-6, K^+	-54.41±1.01
R= CH_3, 18-crown-6, K^+	-48.43±0.95

(b) Vitamin K Series





Conditions	ΔH_{ox} (kcal/mol)	Isomer
18-crown-6, K^+	-33.52±0.604	87 : 13 E : Z
no crown, K^+	-31.44±1.04	87 : 13 E : Z
18-crown-6, K^+	-32.40±0.76	E-isomer

Table II. Heats of Deprotonation of Vitamin K Hydroquinone in Tetrahydrofuran at 25 °C

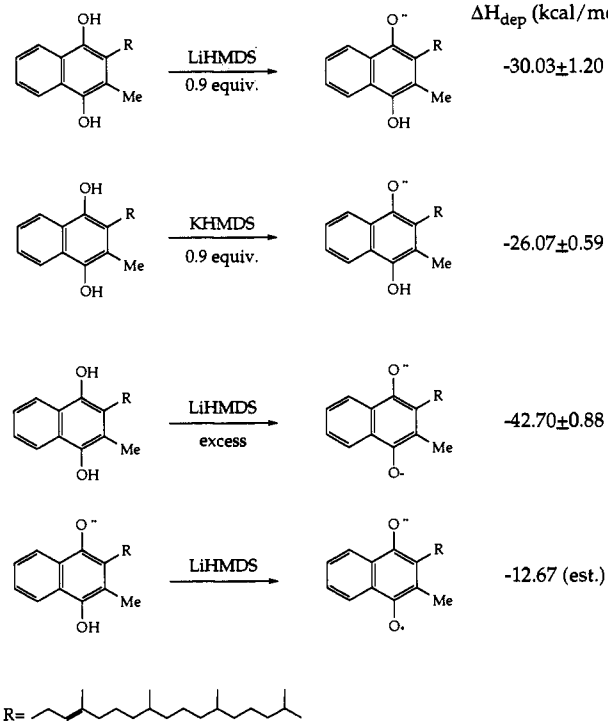


Table II explores the energetics of deprotonating vitamin KH_2 with 1 and 2 equiv of base and the effect of cation variation (Li^+ vs K^+). Both of these factors have sizable consequences, but it is especially noteworthy that removal of the second proton is

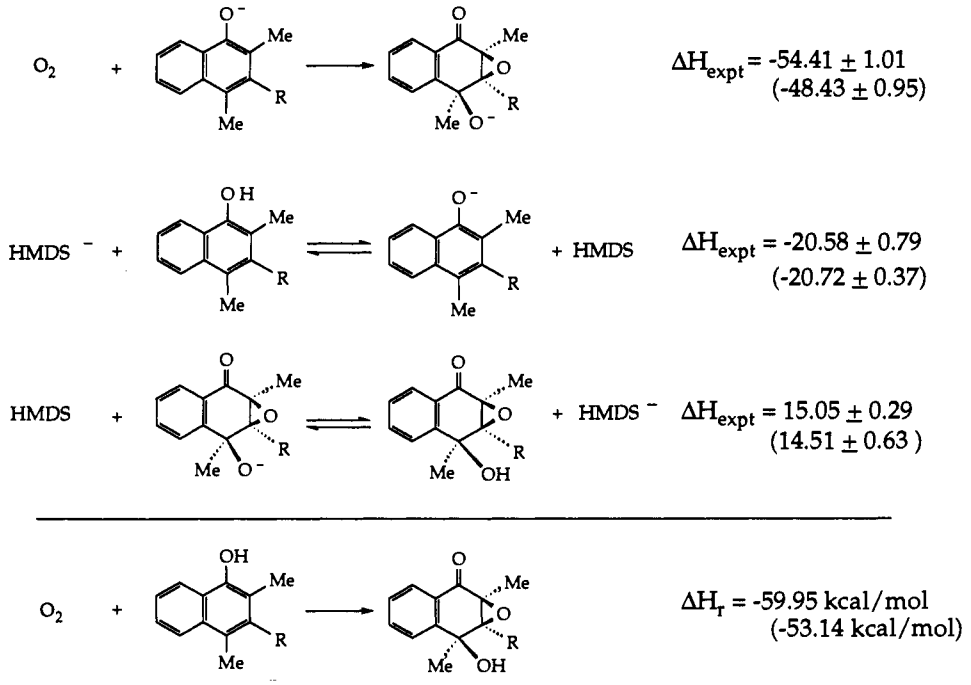


Figure 1. Experimental heats of conversion of the vitamin K models to their keto epoxy alcohols in tetrahydrofuran at 25 °C.[5] Numbers in parenthesis apply to the trimethyl compounds with R = Me; R = H for the dimethyl series.

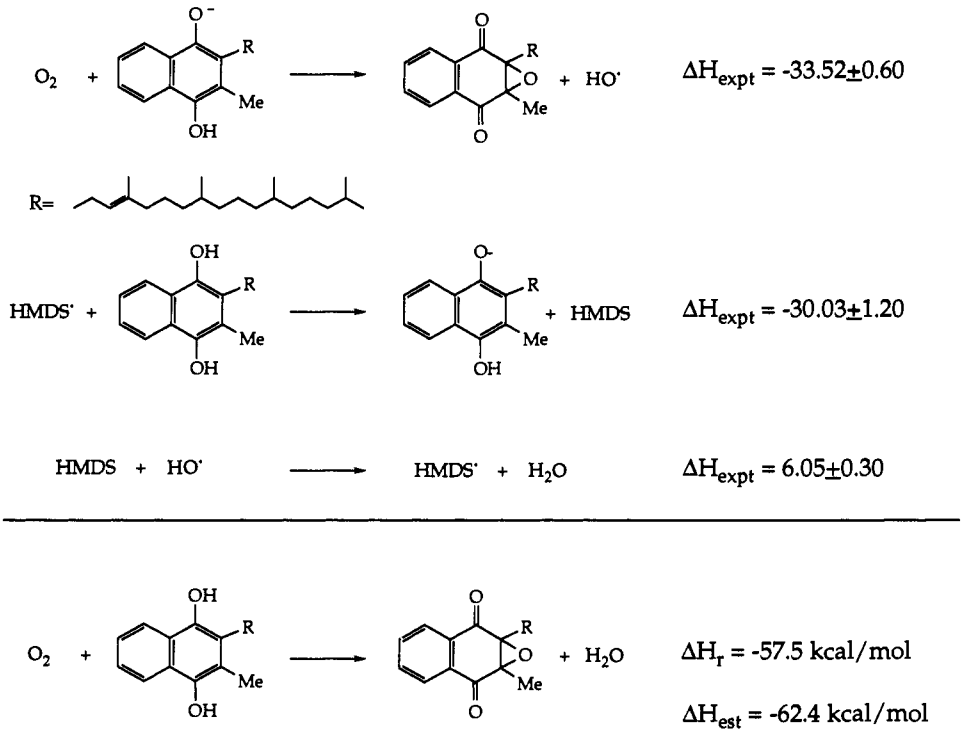


Figure 2. Experimental heats of conversion of vitamin K hydroquinone to vitamin K oxide in tetrahydrofuran at 25 °C.

considerably less exothermic than removal of the first, since the nominal pK_as (9.37 and 10.93)[19] are not very different.

In Figure 2 are combined the most relevant data from Tables I and II with our measurement of the heat of formation of hydroxide ion from water by LiHMDS in THF. This provides a determination of the overall enthalpy change for converting vitamin KH_2 to vitamin K oxide. The ΔH = −57.5 kcal/mol obtained with these experimental data may be compared with the estimate of −62.4 kcal/mol[3a] for the oxygenation of the parent naphthohydroquinone as an unsubstituted model.

As shown in Figure 3, it is also possible to apply this procedure to the parent naphthohydroquinone. This leads to a heat of oxygenation of ΔH_{ox} = −58.47 kcal/mol, to be compared with the −62.4 kcal/mol estimate. The discrepancy can now be attributed to an overestimate of the heat of epoxidation of naphthoquinone.[3a] If one assumes that the differential heat of solvation for the cycle in Figure 3 is small, the original estimate of ΔH_r = −25 kcal/mol for epoxidation of naphthoquinone can now be corrected to ΔH_r^{corr} = −21 kcal/mol. Indeed, one can also assign a heat of formation, $\Delta H_f°$ = −47.6 kcal/mol, to naphthoquinone epoxide. These conclusions are summarized in Figure 4. Using the corrected heat of epoxidation, the new estimate for oxygenation of vitamin

(19) *Lange's Handbook of Chemistry*, 13th ed.; John A. Dean, Ed.; McGraw-Hill: New York, 1985; pp 5–33.

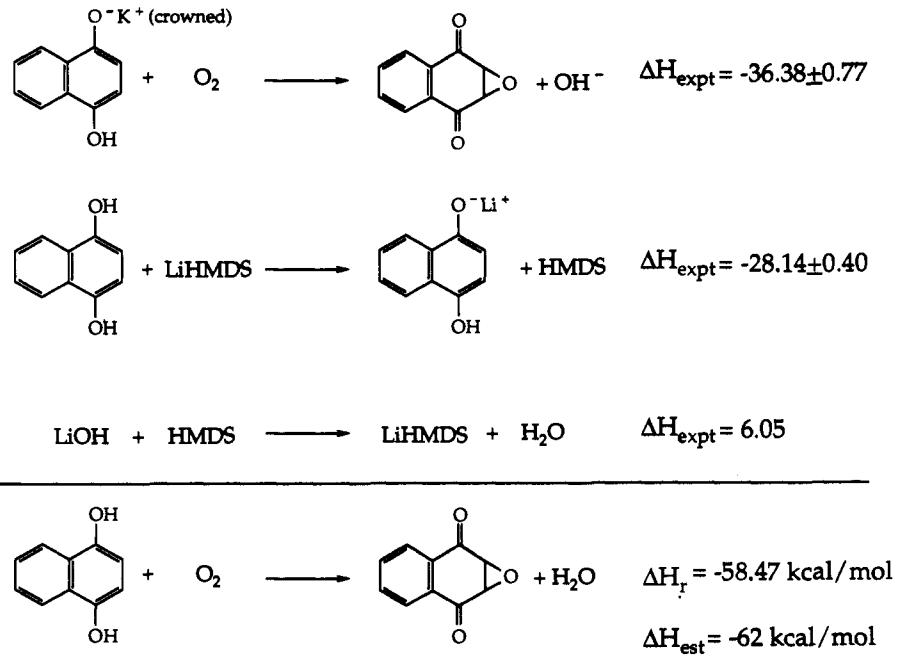
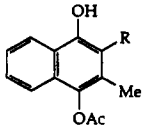
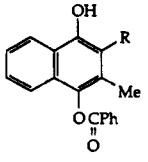
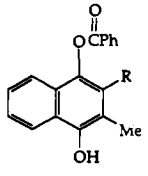


Figure 3. Heat of oxygenation of naphthohydroquinone.

Table III. Heats of Deprotonation of Vitamin K Esters in Tetrahydrofuran at 25 °C

	E/Z	ΔH_{dep} (kcal/mol) (LiHMDS)
 6	3 /1	-27.52±0.34
 7	E/Z	ΔH_{dep} (kcal/mol) (LiHMDS)
	2.2/1	-25.71±0.44
	31/1	-26.34+0.54
	1/2.25	-25.94±0.47
 9	E/Z	ΔH_{dep} (kcal/mol) (LiHMDS)
	6/1	-23.44±0.49

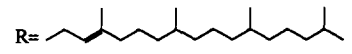
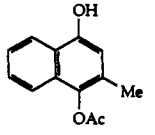
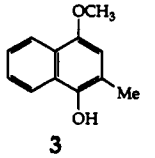
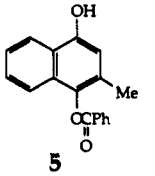
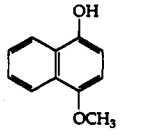


Table IV. Heats of Deprotonation of Substituted Naphthols in Tetrahydrofuran at 25 °C

	ΔH_{dep} (LiHMDS) (kcal/mol)
 1	-26.91±0.62
 3	-23.68±0.68
 5	-23.65±0.51
	-29.20±0.67
Hydroquinone —(LiHMDS, Excess)→	-41.93±0.24
Hydroquinone —(LiHMDS, 0.09 equiv.)→	-26.20±0.51
p-methoxyphenol —(LiHMDS)→	-24.14±0.61
H_2O —(LiHMDS)→	-6.05±0.30

K would be ΔH_{ox} = –58 kcal/mol, in good agreement with the measured value.

The experimental value of ΔH_{ox} = –58.47 kcal/mol obtained with naphthohydroquinone supports the evidence in our previous report[5] (Table Ia and Figure 1) that variation of substitution by hydrogen, methyl, or phytyl has only modest (0–6 kcal/mol) effects on heats of oxygenation and deprotonation in this series.

Which Hydroxyl Group Is Deprotonated? Dowd, Ham, and Geib[3a] assumed that deprotonation to form the monoanion derived from vitamin KH_2 occurred adjacent to the phytyl group so that oxygen attack occurred on the carbon bearing the hydroxyl group adjacent to methyl. Alternatively, deprotonation could occur at the OH adjacent to methyl and oxygen attack adjacent to phytyl.

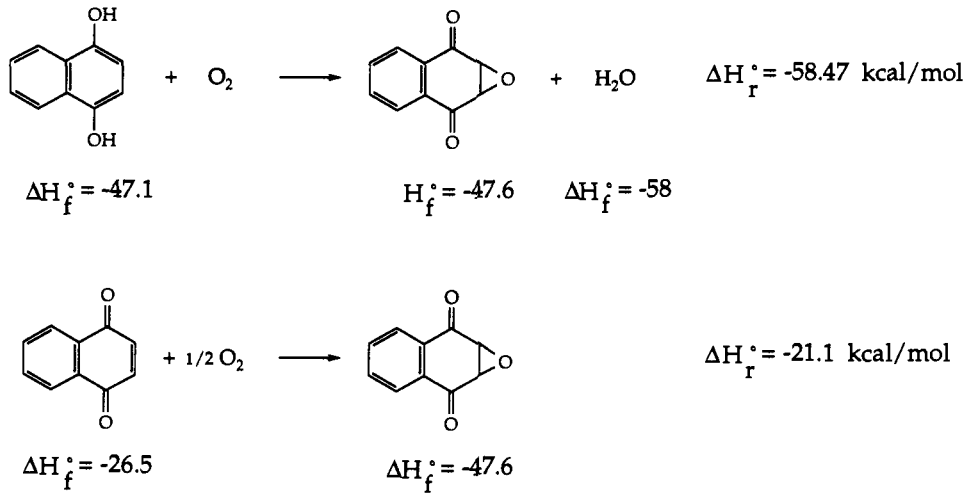


Figure 4. Heats of oxygenation of naphthoquinone and naphthohydroquinone.

The former supposition has now been established firmly by ^{18}O-labeling experiments.[3e]

This question can also be explored by varying the position of deprotonation[16] on well-authenticated compounds. Tables III and IV provide a partial answer to this question, especially when compared to Table II. Although ΔH_{dep}s of some isomers of the compounds shown could not be obtained because of insolubility of reactant or product, it is clear that within this series of phenols and naphthols the largest effects of substitution, ion-pairing, or solvation are no more than 6 kcal/mol and most of these factors contribute much less than that.

Significance of the Results. Although thermodynamics cannot provide a detailed probe of mechanism, it can test the limits of proposed mechanistic schemes. Specifically, thermochemistry provides an answer to the question of whether there is sufficient energy to effect the suggested chemical transformations. The thermochemical results assembled here and previously[3a,5] are completely consistent with the proposal that reaction with molecular oxygen converts the weakly basic anion of vitamin KH_2 into a strong base. In the model series (eq 2) this is sufficient to effect the Dieckmann condensation of diethyl adipate to ethyl 2-oxocyclopentan-1-carboxylate, a cogent model for the carboxylation step in the blood-clotting mechanism. This confirmation of the notion of "base strength amplification" is noteworthy beyond its relevance to the vitamin K problem.

It has become almost an article of faith that reactions with molecular oxygen are predestined to follow free radical pathways.[18] Although that tradition may well apply to the majority of organic reactions with dioxygen, the present study implies that radical pathways can no longer be taken as unexamined assumptions for such processes, It remains to be seen how many more mechanisms presently assumed to require free radicals will be revealed as preferring the ionic pathway of base strength amplification.

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