data over a wider range of driving force (e.g., 2 eV).

It is interesting to compare values of the reorganization energy λ for the electron transfers in cytochrome *c* systems. It is seen from Table II that $\lambda = 0.70$ eV from our data for thermal back reactions is comparable with the values estimated by others for protein-protein systems. It is perhaps surprising that our value is consistent with those for the systems with two hemes (cytc/cytc and cytc/cyt b_5). Although these systems are close to identical from the view of reaction centers, the environment of the heme porphyrin is different from that of the Up moiety. The inner-sphere contribution λ_{in} to λ for these systems should be very similar. But the outer-sphere contribution (λ_{out}) of the solvent reorganization should be higher for Up/cytc systems since Up is more exposed to bulk solvent. The similarity in λ between cytc/cyt b_5 and Up/cytc probably arises from the compensation of the contribution of the reorganization of the protein matrix.[51]

We have calculated the solvent reorganization using the dielectric continuum model proposed by Brunschwig et al.[52] A value of 0.37 eV was obtained based on a model of a single sphere of 3.1-nm diameter, where the center is supposed to be that of the heme as is revealed by computer modeling for the Up/cytc complex. Taking account of the contribution of the reorganization of the protein matrix ($\lambda = 0.15$ eV[51]), $\lambda_{out} = 0.52$ eV is the predominant component in the nuclear reorganization energy for thermal backward electron-transfer reactions in Up/cytc systems.

In conclusion, our data indicate that the existence of an inverted region in electron-transfer reactions depends on the type of reaction under study. As predicted by electron-transfer theory, the rate constant for thermal electron-transfer reactions between charged species increase with the driving force and then undergo a decrease with further increase in driving force. The reorganization energy λ is 0.70 eV. In the case of photoinduced electron-transfer reactions, the variation of rate constants does not follow Marcus theory predictions. There is an apparent gating effect that can be conceptually explained by the contribution of the coordinated-solvent or by the two-step mechanism. Further work along this line is in progress in this laboratory.

Acknowledgment. This work is supported in part by NIH Grant GM31603 and by the Center for Photochemical Sciences at Bowling Green State University. We are grateful to Dr. G. McLendon for useful discussions.

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Mechanism of Action of Vitamin K†

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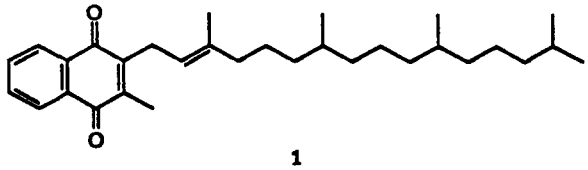
Abstract: Vitamin K is the blood clotting vitamin; it is important as an obligatory cofactor for the enzyme which carboxylates selected glutamate residues in the proteins of the blood coagulation cascade, including prothrombin (factor II), factors VII, IX, and X and proteins C, S, Z, and M. Molecular oxygen is required for the carboxylation. A new model, based on 2,4-dimethyl-1-naphthol demonstrates that spontaneous oxidation of the corresponding 2,4-dimethyl-1-naphthoxide anion **1** leads to the tertiary alkoxide **9**. The latter is a sufficiently strong base to effect the Dieckmann condensation of diethyl adipate (**2**) to ethyl cyclopentanonecarboxylate (**4**), a model for the carboxylation. In thermochemical terms this *base strength amplification* is driven by the oxidation to the extent of approximately −50 kcal/mol. It is proposed that the model oxidation proceeds through a dioxetane intermediate **8**, which demands that the epoxide be cis to the alcohol in the product **3**. This has been demonstrated by X-ray crystallography. Likewise, treatment of 2,3,4-trimethyl-1-naphthol (**16**) with oxygen in chloroform yielded the crystalline hydroperoxide **15**. The latter rearranged to the epoxy alcohol **18** upon treatment with potassium hydride. The structure of **17** was established by X-ray crystallography. Thermochemical analysis shows that the oxidation of vitamin K hydroquinone to vitamin K oxide is exothermic to the extent of −62 kcal/mol. The new mechanism suggests that a second atom of oxygen-18 might be incorporated in an $^{18}O_2$ labeling experiment. Analysis of mass spectral data in the literature indicates that this probably is the case, lending support to the new mechanistic proposal.

Introduction

Vitamin K (**1**) is an obligatory cofactor in the complex enzymic sequence known as the blood cotting cascade and is, therefore, a vital component of the chemistry governing blood coagulation.[1-6]



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The importance of vitamin K in blood coagulation was widely appreciated, but its specific function was not understood until γ-carboxyglutamic acid (Gla) was discovered independently by Stenflo,[7] Nelsestuen,[8] and Magnusson in 1974.[9,10] The critical observation that γ-carboxyglutamic acid was absent from bovine prothrombin circulating in the blood of animals administered the

† Dedicated with warmest best wishes to Professor Ronald Breslow on the occasion of his 60th birthday.

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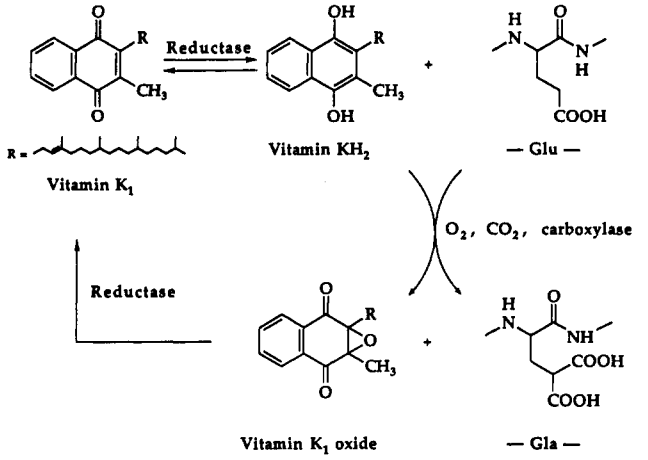
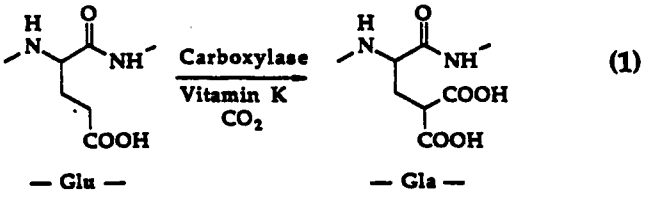
Scheme I



anticoagulant dicumarol led to the hypothesis that vitamin K is involved in the transformation of protein-bound glutamic acid to γ-carboxyglutamic acid (eq 1) in prothrombin or in a prothrombin precursor.



(1)

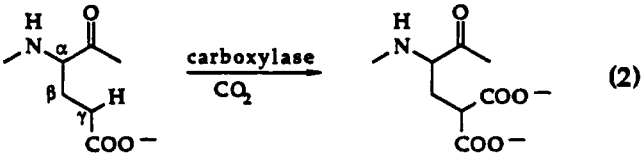
Carboxylation converts selected glutamates in the clotting cascade proteins to γ-carboxyglutamic acid (Gla) residues, employed by the protein to bind calcium. The bound calcium forms an ion bridge between the blood clotting enzymes and phospholipids on the membrane surfaces of blood platelets as well as endothelial and vascular cells. In addition to prothrombin (factor II), blood clotting factors VII, IX, and X, and proteins C, M, S, and Z all require calcium binding and depend upon vitamin K for their activation. All are highly homologous to prothrombin in residues 1–40. The bone protein osteocalcin,[11] which may play a role in bone calcification,[12] also contains γ-carboxyglutamate groups.

In carrying out its role in the carboxylation of glutamate, vitamin K is first reduced to the biologically active hydroquinone form, vitamin KH_2, by an NAD(P)H-dependent flavoprotein reductase (Scheme I). In the course of the carboxylation reaction, protein bound glutamate (–Glu–) is carboxylated to γ-carboxyglutamate (–Gla–), and vitamin KH_2 is transformed to vitamin K oxide.[13]

Molecular oxygen is required for the formation of vitamin K oxide and for the carboxylation of glutamate. A reductase-catalyzed deoxygenation step returns vitamin K oxide to vitamin K (Scheme I) and completes the catalytic cycle.[14] Dicumarol and warfarin exert their anticoagulant action by preventing completion of the cycle, inhibiting vitamin K oxide reductase, leading to a deficiency of vitamin K.[15]

Much effort has been devoted to learning whether the formation of vitamin K oxide occurs in concert with carboxylation. The literature contains both positive and negative views regarding this connection. At one extreme, it has been suggested that there may be two enzymes: one for carboxylation and the other for vitamin K oxide formation.[16] However, the experiments of Matschiner, Suttie, Friedman, and Vermeer, showing that oxide formation parallels carboxylation under a variety of circumstances, indicate that the two events are very probably linked.[17-19] The mechanism by which carboxylation and oxidation might be joined and the way that oxide formation might contribute to the carboxylation are not known. This paper will propose a new mechanistic hypothesis for the role of vitamin K.

Carboxylation of protein-bound glutamate by the vitamin K-dependent carboxylase requires cleavage of a γ-CH bond and replacement with a carboxyl group at the γ-position of glutamate (eq 2). Arguments have been presented in favor of free-radical[20,21]



(2)

and anionic[22,23,24,25] reactive intermediates. The carbanion hypothesis is supported by the observation of tritium exchange into glutamate from tritiated water.[26] In spite of this, opinion seems now to be turning to favor a free-radical intermediate.[27]

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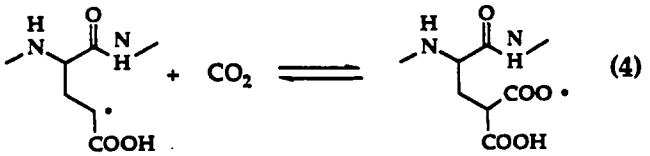
Thermochemical considerations do not support arguments favoring a free-radical pathway for carboxylation. Combination of the heat of formation of the acetoxy radical (ΔH_f° = −49.6 kcal/mol)[28] with those of the methyl radical (ΔH_f° = +35.1 kcal/mol)[28] and carbon dioxide (ΔH_f° = −94.051 kcal/mol)[34] leads to an endothermic heat of reaction, ΔH_r° = +9.35 kcal/mol for

$$\underset{\Delta H_f^\circ = +35.1}{CH_3\cdot} + \underset{\Delta H_f^\circ = -94.051}{CO_2} \rightleftharpoons \underset{\Delta H_f^\circ = -49.6}{CH_3COO\cdot} \quad (3)$$

$$\Delta H_r^\circ = -49.6 \text{ kcal/mole}$$

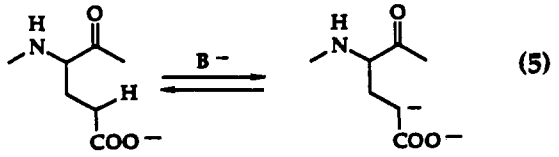
the carboxylation of the methyl radical (eq 3). Heats of formation are not available for the components of the glutamate carboxylation, but, since the carboxymethylene radical of glutamate (eq 4) will be stabilized, relative to the methyl radical, by as much



(4)

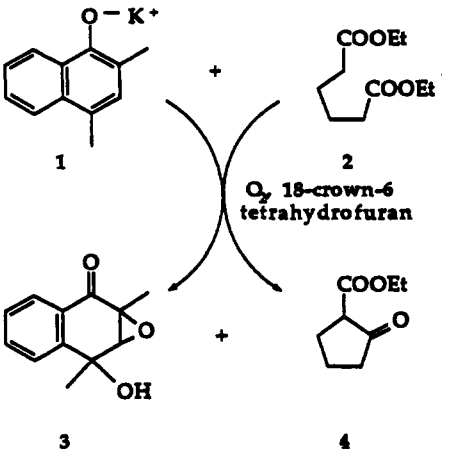
as 10 kcal/mol,[31] and the product carboxylate radicals in eq 3 and 4 have equivalent stability, free-radical carboxylation of a glutamate radical will be approximately 10 kcal/mol less favorable than carboxylation of the methyl radical, and a barrier of as much as 20 kcal/mol can be expected.

By contrast, carboxylation of a carbanion adjacent to a carboxyl group is favored by approximately 19–20 pK_a units. However, production of the carbanion intermediate demands a base strong enough to effect ionization of the γ-proton of glutamate. It is not obvious that vitamin K or its protein environment can support the production of such a strongly basic intermediate; a base with a pK_a in the neighborhood of 26–28 may be required to remove a proton adjacent to an ionized carboxyl group (eq 5).[32] This may account for the waning interest in the carbanion as the intermediate leading to carboxylation.

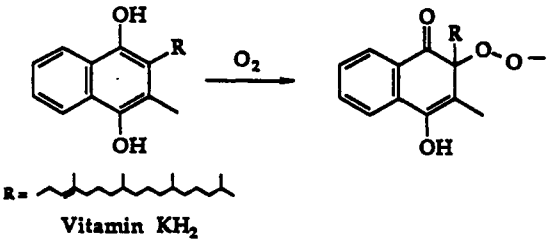


(5)

Scheme II



Molecular oxygen can react with the biologically active hydroquinone form of vitamin K, vitamin KH_2, in various ways. It has been suggested, for example, that molecular oxygen can add to the 2-position of vitamin KH_2 yielding a hydroperoxy anion which might then serve as a base (eq 6).[23]



(6)

Vitamin KH_2

The circumstances of the proton abstraction and carboxylation could be unusual insofar as the local environment and degree of solvation of the base are concerned. The action involving vitamin K appears to occur at membrane surfaces where the kind and extent of solvation can be quite different from those in solution. The phytyl side chain of vitamin K_1 is, in all probability, present to assist in drawing the vitamin into the membrane or into a hydrophobic pocket of the carboxylase. Even with these qualifications in mind, it still appears that with a pK_a ~ 13,[29] the peroxide anion is not sufficiently basic to abstract a proton from glutamate at an acceptable rate (vide infra).

Results

A New Model. A new model will demonstrate that vitamin K holds the potential for *base strength amplification*. Oxidation of vitamin KH_2 can be used to elevate the basicity from the hydroquinone level of vitamin KH_2 (pK_a ~ 9)[30] to that of a tertiary alkoxide, sufficiently strong to remove a proton from the α-position of a carboxylate group.

The vitamin K-dependent glutamate carboxylase catalyzes the formation of a new carbon–carbon bond between glutamate and carbon dioxide. In designing a nonenzymic model, it is important to mimic this carbon–carbon bond-forming reaction. There have been no models for the vitamin K-dependent carboxylation demonstrating that condensation with carbon dioxide can be promoted by transforming vitamin KH_2 to vitamin K oxide.[25] The new model shows how epoxide formation can drive the formation of strong base necessary for the carbon–carbon bond-forming condensation between glutamate and carbon dioxide.

In designing the model, the α-naphthoxide **1** was used in place of vitamin K, permitting the course of the oxygenation reaction to be mapped. For the condensation step, an intramolecular (Dieckmann) reaction was employed to mimic the capacity of the enzyme to hold the substrates of the enzymic condensation (eq 3) properly oriented and in close proximity to one another. The Dieckmann reaction demands a base of strength only marginally below that required for the glutamate proton abstraction, and, since it results in carbon–carbon bond formation, can be viewed

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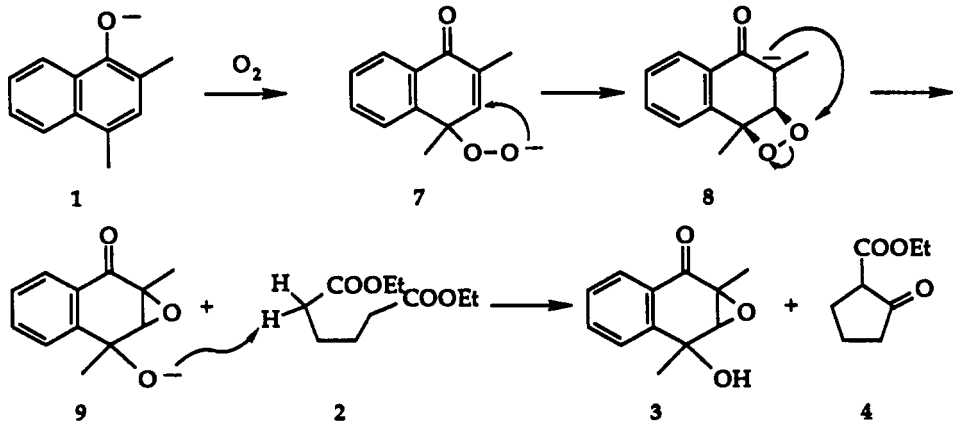
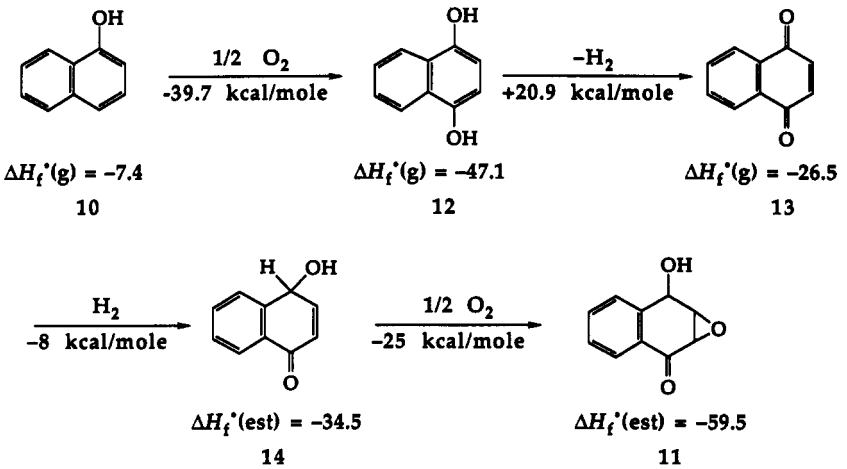
(36) (a) Kozina, M. P.; Timofeeva, L. P.; Lukyanova, V. A.; Pimenova, S. M.; Kasyan, L. I. *Russ. J. Phys. Chem.* **1988**, *62*, 609. (b) Podlogar, B. L.; Raber, D. J. *J. Org. Chem.* **1989**, *54*, 5032. (c) Timofeeva, L. P.; Luk'yanova, V. A.; Kozina, M. P.; Stepanova, N. V. *Kaiorim. Khim Thermadin. Dokl. Vses. Konf, 10th* **1984**, 1 (2), 193. *Chem. Abstr.* **1986**, *104*, 148234r.

Scheme III



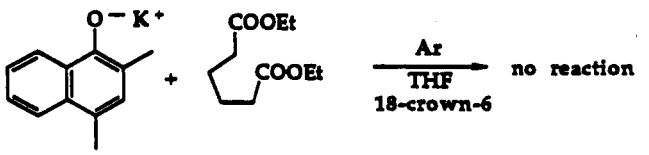
Scheme IV



as an intramolecular carboxylation.

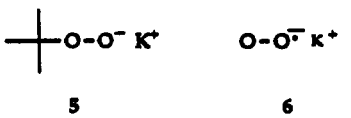
In the model experiment (Scheme II), a tetrahydrofuran solution containing the potassium α-naphthoxide **1** and diethyl adipate (**2**) was stirred at room temperature with 18-crown-6. Syringe pump infusion of 1.7 equiv of oxygen[33] into the solution yielded ethyl cyclopentanone-2-carboxylate (**4**), the product of Dieckmann condensation, and the keto epoxide **3**. Formation of the epoxide **3** *drives* the generation of the Dieckmann carbanion intermediate leading to carbon–carbon bond-forming cyclization.

The naphthoxide **1** is *not* a sufficiently strong base to effect the Dieckmann condensation of **2** under the reaction conditions. No cyclopentanone product **4** was observed in a control carried out under an oxygen-free atmosphere of argon (eq 7). Less than



(7)

1% of **4** would readily have been detected by the presence of the one-proton methine triplet of **4** at δ 3.14 in the 500 MHz NMR spectrum of the total crude reaction product. It was important to conduct the control reaction under scrupulously oxygen-free conditions; else trace amounts of cyclized product **4** were observed. Likewise, control reactions with potassium *tert*-butylperoxide (**5**) and potassium superoxide (**6**) showed that they are not sufficiently basic to effect condensation; no **4** was detected in either reaction.



Mechanism

Molecular oxygen reacts *spontaneously* at room temperature with the naphthoxide anion **1** (Scheme III). The peroxy anion adduct **7** is one of several possible first intermediates. The negatively charged peroxide can undergo internal nucleophilic addition to the unsaturated carbonyl system of **7** yielding the dioxetane enolate anion **8**. Dioxetane ring opening by internal nucleophilic displacement yields the epoxy alkoxide **9**, a strong base which can effect the condensation of diethyl adipate **2** to the cyclopentanonecarboxylate **4**.

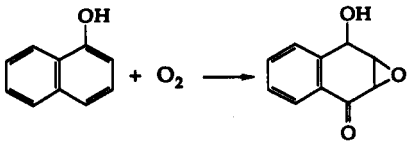
Molecular oxygen acts on the weakly basic, but biochemically accessible, naphthoxide anion **1** to create a path to the much stronger alkoxide base **9**, capable of carbon–carbon bond-forming condensation. This is a *base strength amplification* reaction. Formation of a base of the strength of alkoxide **9** would otherwise not be feasible in a biological milieu. We propose that the vitamin K-dependent carboxylation uses base strength amplification to effect the removal of the γ-proton of glutamate.

Thermochemistry

Energy is required to elevate the base strength in the model reaction leading to the tertiary alkoxide **9**. Thermochemical analysis of the sequence of events in the oxidation of **1** to **9** reveals that the model undergoes a partial *combustion* reaction and that a large amount of energy is made available to enhance the base strength.

The energetics of the model reaction (**1** → **3**, Scheme II) can be approximated by considering the oxidation of α-naphthol (**10**) to the hydroxy keto epoxide **11** with consumption of 1 mol of oxygen (eq 8), by means of the thermochemical cycle in Scheme IV.

The enthalpy of formation of α-naphthol (**10**) is $\Delta H_f°(g)$ = −7.4 kcal/mol.[34] Oxidation of **10** to 1,4-naphthalenediol **12** ($\Delta H_f°(g)$ = −47.1 kcal/mol)[34] is exothermic to the extent of −39.7



10 11

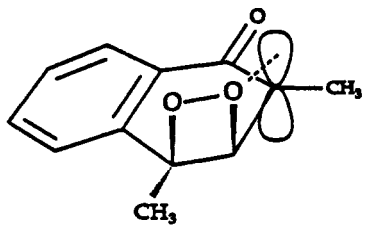
ΔH_r° = - 52 kcal/mole (8)

kcal/mol. Further oxidation of 1,4-naphthalenediol **12** to 1,4-naphthoquinone **13** ($\Delta H_f°$(g) = −26.5 kcal/mol)[34] is endothermic by +20.9 kcal/mol. When the naphthoquinone **13** is reduced to **14**, an approximation to the heat of formation of dihydronaphthalen-1-ol-4-one (**14**) ($\Delta H_f°$(est) = −34.5 kcal/mol) may be obtained from the heat of reaction of 1-tetralone ($\Delta H_f°$(c) = −50 kcal/mol)[34] to 1-tetralol ($\Delta H_f°$(c) = −57.6 kcal/mol)[34] leading to an enthalpy, $\Delta H_r°$ = −8 kcal/mol, for the reduction of **13** to **14**. This is a conservative estimate; most aromatic ketones have heats of reduction in the range −12 to −14 kcal/mol.[34] The heat of formation of the final epoxide **11** is also an estimate based on a survey of eight epoxidation reactions ranging in exothermicity from −25 to −35 kcal/mol with an average value of −31.3 ± 3.6 kcal/mol.[34,28] A conservative value of −25 kcal/mol was used to estimate the heat of epoxidation of **14** to **11** in Scheme V. Summing the values in Scheme V yields an estimate of the heat of oxidation $\Delta H_r°$ = −52 kcal/mol for eq 8. Raising the pK_a by ten orders of magnitude, from 9.3 for α-naphthol (**10**) to approximately 20 for a tertiary alkoxide, requires 14.6 kcal/mol at 25 °C. Thus, more than ample energy is available for the pK_a elevation, even with the approximations employed in evaluating the heats of reaction in the latter stages of the thermochemical cycle of Scheme IV.

Stereochemistry and Structure

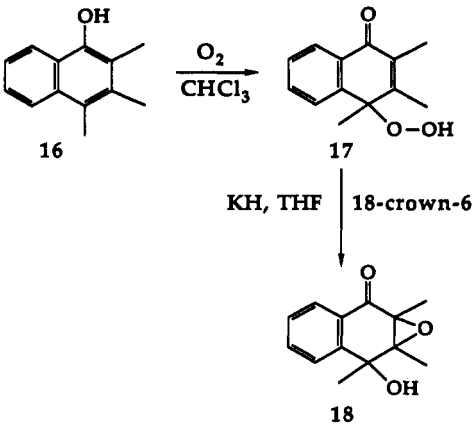
If epoxide formation leading to **3** proceeds through the dioxetane intermediate **8**, then the hydroxyl group in **3** (Scheme V) should be in the cis orientation with respect to the epoxide. The stereochemical prediction of the model regarding the structure of **3** was established using X-ray crystallography (Figure 1).

Molecular models demonstrate that, as indicated in **15**, the dioxetane ring is in a favorable orientation for stereoelectronic overlap, with *backside attack*[37] by the adjacent enolate anion on the dioxetane O–O bond, in the transition state leading to epoxide formation.



15

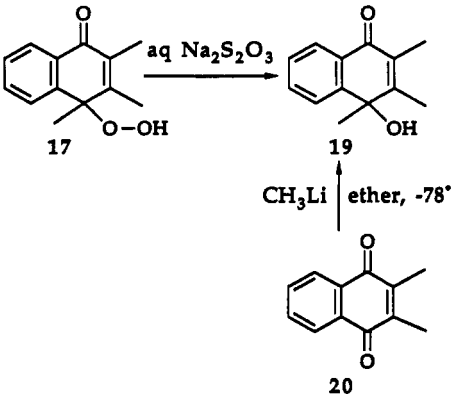
To explore the likelihood of the occurrence and chemical behavior of the peroxide intermediate **7** in our vitamin K model sequence, the trimethyl peroxide **17** was prepared by treatment of 2,3,4-trimethyl-1-naphthol **16** with oxygen in chloroform.[38] Hydroperoxide **17** is a stable, crystalline substance in contrast to its dimethyl analogue. When the trimethylhydroperoxide **17** was treated with potassium hydride in THF at 25 °C, rearrangement to the epoxy alcohol **18** occurred in 70% yield (eq 9).[39]



16 **17** **18**

KH, THF | 18-crown-6 (9)

Since either the 2- or the 4-hydroperoxide might have been produced in eq 9, it was important to establish the regiochemistry of the oxygenation reaction. The structure of the 4-hydroperoxide **17** was established by chemical means and by X-ray crystallography. Thus, treatment of the hydroperoxide **17** with aqueous sodium thiosulfate (eq 10) yielded the keto alcohol **19** (95%). An authentic sample of **19** was prepared in 94% yield by treatment of 2,3-dimethylnaphthoquinone (**20**) with methyllithium in ether at −78 °C (eq 10).



17 **19** **20**

CH_3Li | ether, -78° (10)

The structure of the trimethyl hydroperoxide **17** was also established by single-crystal X-ray structure determination (Figure 2), confirming the assignment of the structure of the 4-hydroperoxy adduct to **17**.

The Mechanism of Action of Vitamin K

The vitamin K-dependent carboxylation differs from the model system of Scheme II in several important respects.

Carboxylation of protein-bound glutamate occurs in an aqueous environment in vivo, whereas the model reaction (Scheme II) was conducted in tetrahydrofuran. The vitamin K-dependent carboxylase appears to be strongly associated with the membrane-protein active site making it difficult, at this juncture, to know the nature of the reaction medium for the biological carboxylation. The observation of strong inhibition of tritium exchange[26] into glutamate in the presence of carbon dioxide implies that the glutamate γ-carbanion may be protected against protonation by water as a consequence of its hydrophobic environment at the active site. Thus, the use of tetrahydrofuran as the medium for the model (Scheme II) may not be entirely inappropriate.

The model of Scheme II and the enzymic system also differ in the use of the intramolecular Dieckmann condensation instead of the glutamate condensation with carbon dioxide—an intermolecular reaction. This clearly is a subject which must be addressed in future model research. However, it is a commonplace that an intermolecular reaction in an enzymic context will often possess the characteristics of an intramolecular reaction in solution.

(37) Cf.: Beak, P.; Li, J. *J. Am. Chem. Soc.* **1991**, *113*, 2796. It is a pleasure to acknowledge illuminating discussions with Professor Peter Beak on this point.

(38) Greenland, H.; Pinhey, J. T.; Sternhell, S. *Aust. J. Chem.* **1987**, *40*, 325.

(39) Griebhammer, R.; Schneider, H. P.; Winter, W.; Rieker, A. *Tetrahedron Lett.* **1979**, *20*, 3941. Nishinaga, A.; Itahara, T.; Shimizu, T.; Matsuura, T. *J. Am. Chem. Soc.* **1978**, *100*, 1820.

(40) Sadowski, J. A.; Schnoes, H. K.; Suttie, J. W. *Biochemistry* **1977**, *16*, 3856.

(41) Rêtey, J.; Umani-Ronchi, A.; Seibl, J.; Arigoni, D. *Experientia* **1966**, *22*, 502.

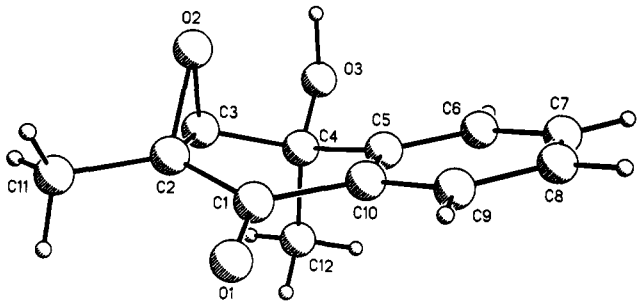


Figure 1. X-ray structure of **3**.

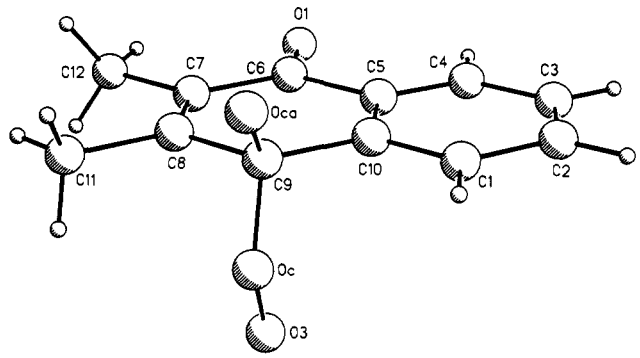
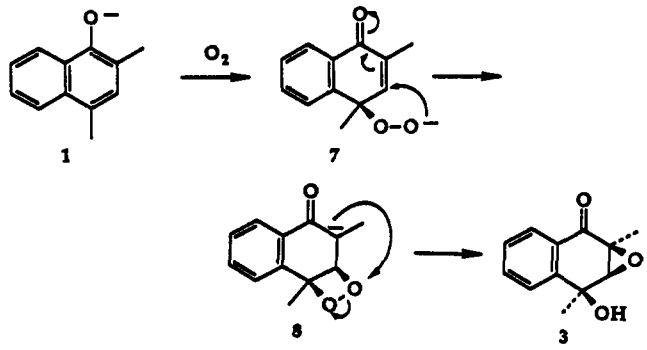


Figure 2. X-ray structure of **17**. The molecule resides on a crystallographic mirror plane which requires the hydroperoxide oxygen atoms and the 9-methyl carbon atom to be disordered. The crystallographically equivalent atoms Oc and Oca represent the hydroperoxide oxygen and methyl carbon, respectively.

Scheme V

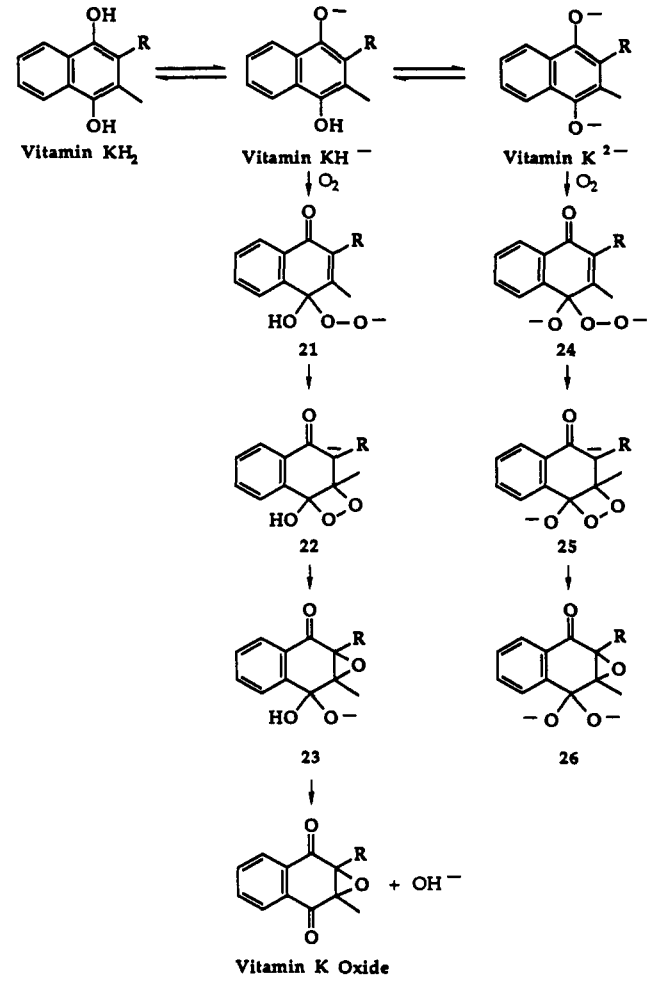


The third and most important difference between the model of Scheme IV and the vitamin K-dependent carboxylase resides in the structure of vitamin K. Vitamin K is a 1,4-dioxygenated naphthalene, whereas the model α-naphthol **1** carries only one oxygen. How can this difference be reconciled?

A sequence of oxygenation events parallel to those postulated in Scheme III might proceed as shown in Scheme VI starting from the readily produced anion, vitamin KH^-, or dianion, vitamin K^{2-}. If molecular oxygen reacts with vitamin KH^- yielding a peroxy anion adduct **21**, the latter can then rearrange to the ketone hydrate anion **23**, through the dioxetane **22**. Alkoxide **23** is a strong base. It may also eject a hydroxide ion that, in the proper hydrophobic environment, might be a sufficiently strong base to remove a proton from glutamate and promote carboxylation. Indeed, the excess energy produced in the oxygenation reaction (eq 8) may be needed to generate the hydroxide ion in a hydrophobic environment. Alternatively, if the doubly ionized form, vitamin K^{2-}, is oxygenated and follows the dioxetane path through **24** and **25**, the result will be the geminal dialkoxide **26**. Although geminal dialkoxides have not been explored in this regard, they should be very strong bases, fully adequate to the task of removing a proton from the γ-position of glutamate.

Scheme VI shows how oxygenation in the 1,4-position of vitamin K^{2-} can lead to generation of the strong base **26**. The model is

Scheme VI

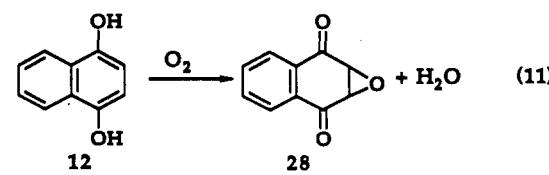


flexible; a 1,2-oxygenation (Scheme VII) can lead to the same intermediate. Thus, attachment of oxygen to the 2-position as in **27** followed by attack of the peroxy anion on the adjacent carbonyl group leads to the familiar dioxetane intermediate **25**, which may be in equilibrium with the peroxy anion **24** and which can then open to the dialkoxide **26**.

A 2-hydroperoxide also plays a role in a mechanism proposed by DeMetz et al. in which it is suggested that a hydroxide ion is generated by a decomposition pathway leading to vitamin K oxide.[20] Such a sequence might be differentiated from the dioxetane mechanism Scheme VI by ^{18}O labeling experiments (vide infra).

Thermochemistry of Vitamin K

The thermochemistry of the vitamin K system strongly favors oxide formation but differs somewhat from that of the model in Scheme IV. The thermochemistry of the vitamin K system can be modeled by the oxidation of naphthohydroquinone **12** to the corresponding quinone epoxide **28** (eq 11). In the model reaction

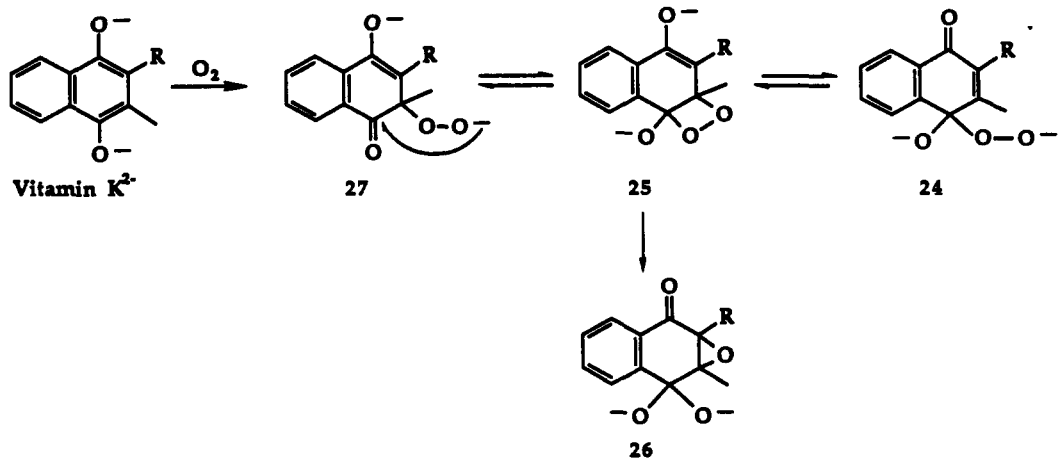


(11)

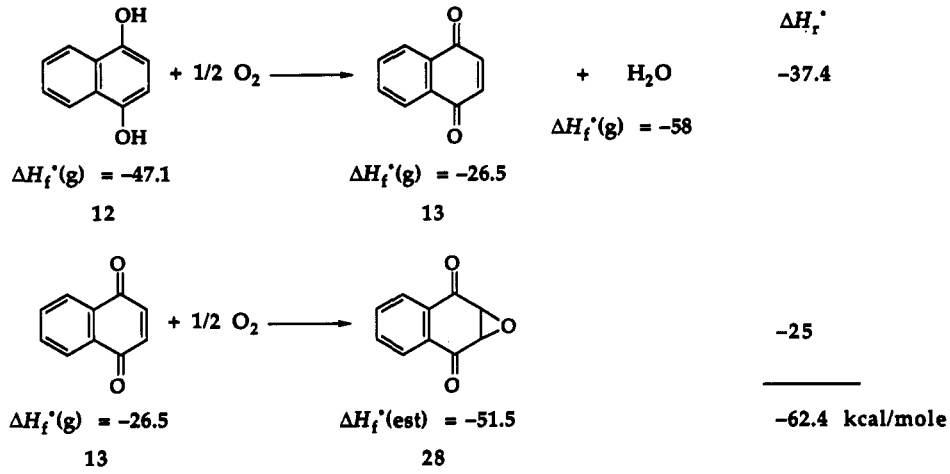
ΔH_r° = −62.4 kcal/mole

of Scheme IV, −40 kcal/mol is released in the first oxidation step of the cycle that produces the naphthohydroquinone **12**. Since the naphthohydroquinone **12** is the starting point of the vitamin K cycle of Scheme VIII, that energy increment is not available here. However, the energy difference is more than compensated

Scheme VII



Scheme VIII



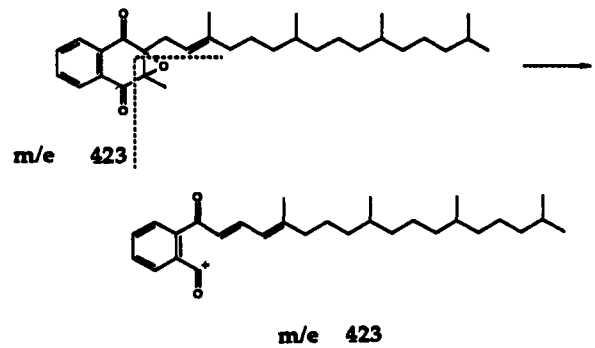
for by the production of water in the vitamin K cycle (Scheme VIII).

Starting from the naphthohydroquinone **12** ($\Delta H_f°(g)$ = −47.1 kcal/mol) and proceeding to naphthoquinone **13** ($\Delta H_f°(g)$ = −26.5 kcal/mol) and water ($\Delta H_f°(g)$ = −58 kcal/mol) yields $\Delta H_r°$ = −37.4 kcal/mol. In the second step, naphthoquinone **13** is oxidized to naphthoquinone epoxide **28** and, as in Scheme IV the heat of formation of the latter, may be approximated with the heats of the eight epoxidation reactions gleaned from the literature.[35] Again, as in Scheme IV, the least negative value, $\Delta H_r°$ = −25 kcal/mol, was used. The overall $\Delta H_r°$ = −62.4 kcal/mol. Thus, the reaction with oxygen leading to vitamin K oxide represents a partial *combustion* of vitamin K providing energy for the base strength enhancement reaction which, in our hypothesis, drives the carboxylation of glutamate.

Oxygen-18 Labeling Experiments

Were molecular oxygen labeled with ^{18}O to be used in the vitamin K-dependent carboxylation, the mechanisms in Schemes VI and VII predict that ^{18}O might be incorporated into the carbonyl group of the product, vitamin K oxide. This conclusion is stated tentatively because the two oxygens of the dioxygenated intermediate **26** are diastereotopic, and the oxygen lost could be that carrying the ^{18}O label, cis to the epoxide, or the unlabeled oxygen, trans to the epoxide (Scheme IX), depending upon enzymic preference and the degree of selectivity in the competing uncatalyzed dehydration.[42]

In fact, the ^{18}O labeling experiment with molecular oxygen has been carried out. The carboxylation of prothrombin in postmitochondrial supernatants was effected by Sadowski, Schnoes, and Suttie under an atmosphere of oxygen doubly labeled with ^{18}O.[40] The purpose of the experiment was to establish the origin of the epoxide oxygen in vitamin K oxide. The full mass spectra used in the product analysis were published in the Suttie paper[40] and are reproduced in Figure 3. The top spectrum (Figure 3a) shows the mass spectrum of unlabeled vitamin K oxide with its molecular ion at *m/e* 466. The $M^+ - 43$ peak at *m/e* 423 arises from fragmentation of the epoxide and loss of the $CH_3CO^•$ fragment. This fragmentation mode was established by determination of the exact mass [calcd for $C_{29}H_{43}O_2$ 423.3263, found 423.3240]. This fragmentation path is further confirmed by the ^{18}O labeling pattern

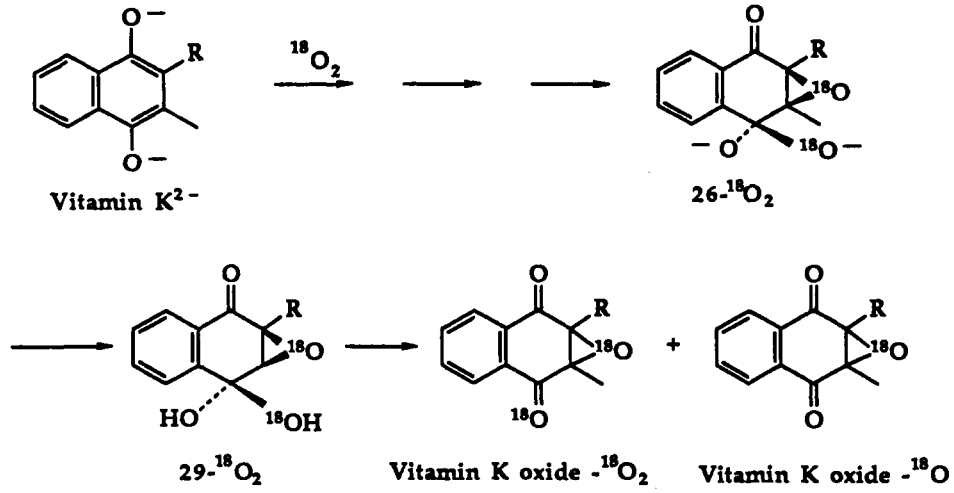


(12)

(vide infra). The *m/e* 306 fragment, corresponding to fragmentation of the epoxide in the alternative sense, is the isoprenoid side chain plus CO and minus H [calcd for $C_{21}H_{38}O$ 306.2923,

(42) Another possibility would involve the exchange of product ^{18}O-labeled water into the product vitamin K oxide following carboxylation. This path cannot be ruled out at present but might be detected by appropriate enzymic control reactions.

Scheme IX



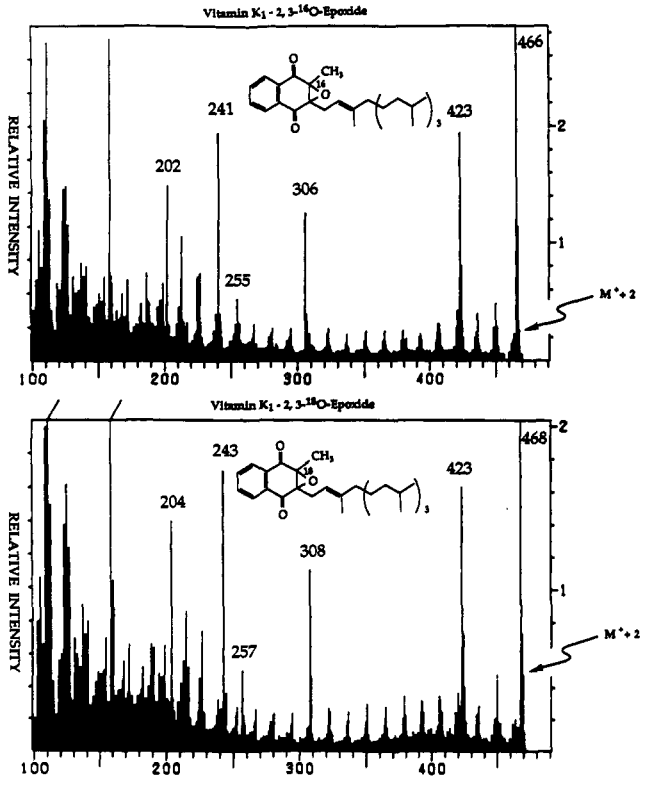
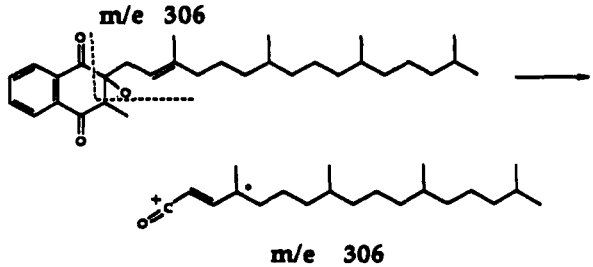


Figure 3. Mass spectra of vitamin K_1-2,3-epoxide. The epoxide was isolated from microsomal incubations under $^{16}O_2$ and $^{18}O_2$ atmospheres, as described by Sadowski, Schnoes, and Suttie:[40] (a) (top) incubation in an $^{16}O_2$ atmosphere and (b) (bottom) incubation in an $^{18}O_2$ atmosphere.

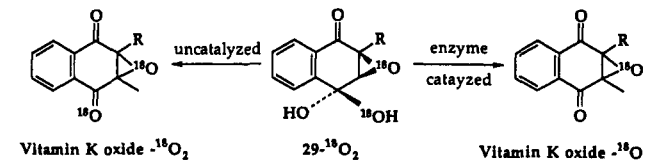
found 306.2947]. Plausible structures for the *m/e* 423 and 306 fragment ions are shown in equations 12 and 13.



(13)

Examination of the ^{18}O labeled vitamin K oxide from the $^{18}O_2$ experiment (Figure 3b) reveals that one atom of ^{18}O has been incorporated into the vitamin K oxide. The label could be located

Scheme X



at any of the three oxygen positions, but analysis of the mass spectrum is most consistent with the ^{18}O being located at the oxide position. Thus, the *m/e* 423 peak is not changed in comparing the unlabeled to the labeled spectrum, indicating that the ^{18}O label has been carried away with the *m/e* 43 acetyl fragment. By contrast, the *m/e* 306 peak becomes *m/e* 308 in the ^{18}O experiment, indicating retention of the ^{18}O label in the side-chain fragment. This fragmentation pattern is consistent with ^{18}O incorporation at the epoxide oxygen, as was assumed by Sadowski, Schnoes, and Suttie.[40]

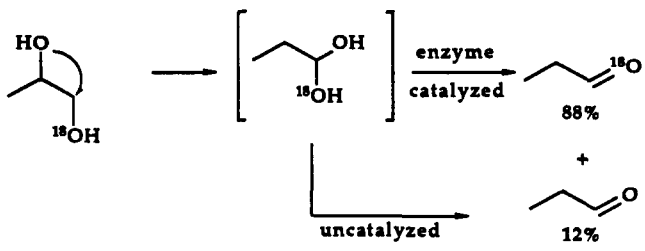
Further scrutiny of the mass spectra in Figure 3 reveals a most interesting feature. In the ^{16}O spectrum (Figure 3a) the *m/e* 466 peak is the base peak with intensity 100%. The $M^+ + 1$ peak at *m/e* 467 is 40% as intense as the *m/e* 466 peak. This value is slightly high; the calculated value is 34.4%, based on the natural isotopic constitution of the $M^+ + 1$ peak for $C_{31}H_{46}O_3$. Likewise, the intensity of the $M^+ + 2$ at peak *m/e* 468 in the ^{16}O spectrum (Figure 3a) is 4.5% of that of the *m/e* 466 peak; the calculated value is 6.3%.

In the ^{18}O spectrum (Figure 3b) the $M^+ + 1$ peak at *m/e* 469 is completely normal with intensity 36% of that of the *m/e* 468 peak. By contrast the intensity of the $M^+ + 2$ peak at *m/e* 470 is 24% of that of the parent peak at *m/e* 468. The $M^+ + 2$ peak is *four times larger than expected* on the basis of natural abundance isotopic constitution. *The presence of a second atom of ^{18}O in the vitamin K oxide from the labeling experiment is indicated.*

Could the too intense peak at *m/e* 470 be happenstance? Analysis of the fragments suggests otherwise. Thus, in the ^{16}O spectrum (Figure 3a), the *m/e* 423 cluster of peaks shows that the $M^+ + 1$ peak at *m/e* 424 is also normal at 39% of the intensity of the *m/e* 423 peak, while the $M^+ + 2$ peak at *m/e* 425 is normal at 3.5% of the intensity of the *m/e* 423 peak. By contrast, in the oxygen-18 spectrum (Figure 3b), the intensity of the $M^+ + 2$ peak at *m/e* 425 is 23% of the *m/e* 423 peak—*four times too intense*—while the $M^+ + 1$ peak at *m/e* 424 is a little higher than normal at 43% of the intensity of the *m/e* 423 peak. Clearly the *m/e* 423 peak contains an additional ^{18}O atom in accord with the proposed pattern of fragmentation (eq 12); the second oxygen-18 is incorporated in one of the carbonyl groups of vitamin K oxide and is retained in the *m/e* 423 fragment.

The peak at *m/e* 306 should show no extra oxygen-18 because that fragment contains only the side chain and the epoxide oxygen

Scheme XI



(eq 13). Indeed, inspection of the *m/e* 308 cluster in the oxygen-18 spectrum (Figure 3b) reveals that the $M^+ + 2$ peak at *m/e* 310 is completely normal in intensity; it is approximately 3% of the intensity of the *m/e* 308 peak; the calculated value is 2.8%.

How does the vitamin K model account for this result? There can be two competing courses for the ^{18}O labeled hydrate intermediate **29**-$^{18}O_2$ (Scheme X). The major path could be enzyme-catalyzed loss of the ^{18}O labeled hydroxyl group cis to the epoxide yielding vitamin K oxide-^{18}O. Since the vitamin K oxide hydrate is a reactive intermediate and will undergo spontaneous loss of water, a nonenzymic pathway will compete with the catalyzed path with loss of either oxygen. This would be a minor pathway leading to the observed presence of vitamin K oxide-$^{18}O_2$.

A useful comparison may be drawn between the vitamin K labeling pattern and that from the coenzyme B_{12}-dependent diol dehydrase experiments of Rêtey and Arigoni[41] (Scheme XI). Enzyme-controlled dehydration of the intermediate aldehyde hydrate from (*S*)-[1-^{18}O]propan-1,2-diol leads to stereospecific retention of label to the extent of 88%, accompanied by 12% of product in which the ^{18}O label is lost, presumably in a nonenzymic reaction. Rearrangement of (*R*)-[1-^{18}O]propan-1,2-diol results in 92% loss of ^{18}O and 8% retention of the label. In both instances the aldehyde hydrate is a reactive intermediate with its own rapid rate of decomposition, competitive with that catalyzed by the enzyme.

It may be possible to construct model reactions employing the reactive intermediates described in Scheme VII. In preliminary experiments, we have observed a simple and interesting change. It is well-known that naphthohydroquinones are unstable in air to the extent of approximately 0.2 v with respect to the quinone form. By contrast, when the mono- or dianion of 2,3-dimethylnaphthohydroquinone is exposed to oxygen, only minor amounts of the quinone can be detected (Scheme XII). The only product isolated (25–30% yield) is the corresponding epoxy quinone **31**. Although there are a number of possible pathways leading to such an epoxide, this spontaneous oxidation, occurring at room temperature, is clearly important to the ends we are pursuing.

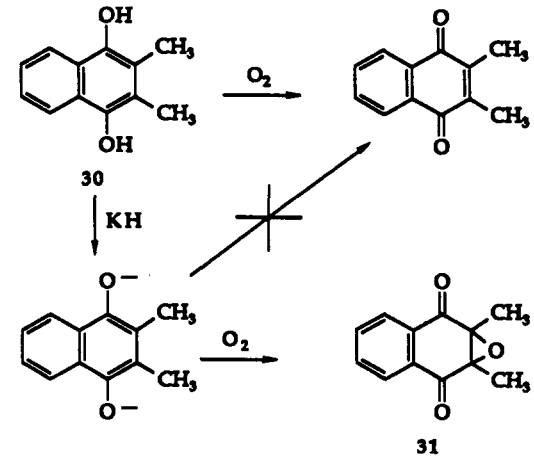
Summary

We have discovered a novel, spontaneous oxidation which leads to a powerful base capable of carrying out a carbon–carbon bond-forming condensation reaction analogous to that observed in the vitamin K-mediated carboxylation of glutamate.[43] The model sequence incorporates a novel base strength enhancement sequence, and it implicates molecular oxygen as the initiating factor in the vitamin K-dependent carboxylation.

Experimental Section

Materials and Methods. Tetrahydrofuran (THF) and benzene were distilled from blue or purple solutions of sodium benzophenone ketyl under nitrogen prior to use. Potassium hydride (35 wt % dispersion in mineral oil) from Aldrich was washed four times with dry benzene and dried under a flow of argon. Diethyl adipate, 18-crown-6, potassium superoxide, and a 3.0 M solution of *tert*-butyl hydroperoxide in 2,2,4-trimethylpentane (Aldrich) were used without further purification. 2,4-Dimethyl-1-naphthol was prepared according to a literature procedure,[44] stored at −78 °C, and dried under vacuum for 2 h before use.

Scheme XII



NMR spectra were obtained on a Brucker AF 300 or a Brucker AM 500 NMR spectrometer; chemical shifts are reported in units of δ (ppm) relative to tetramethylsilane as internal standard. Infrared spectra were recorded on a Mattson IR/32 FTIR spectrometer. Low-resolution mass spectra were recorded on a VG 70-SE double-focusing, magnetic sector mass spectrometer with samples inserted by direct probe and peaks reported in units of mass per charge ratio (*m/e*). High-resolution mass spectra were recorded on a Varian MAT CH-5DH mass spectrometer. Melting points were performed on a Fisher-Johns melting point apparatus and are not corrected.

Preparation of Potassium 2,4-Dimethyl-1-naphthoxide. A solution of 316.0 mg (1.83 mmol) of 2,4-dimethyl-1-naphthol in 5 mL of dry THF was slowly added to a suspension of 76.9 mg (1.92 mmol) of potassium hydride in 1 mL of dry THF, in a flame-dried, 10 mL, two-necked, round-bottomed flask, with a double-tipped needle under an atmosphere of argon at room temperature. The resulting yellow solution was stirred for 30 min and then divided into two equal portions for an oxygenation reaction and a control reaction to be carried out in the absence of oxygen.

Reaction with Oxygen. The potassium 2,4-dimethyl-1-naphthoxide solution (3 mL, 0.92 mmol) was transferred by annulation technique into a mixture of 0.18 mL (0.90 mmol) of diethyl adipate (**2**) and 200.0 mg (0.76 mmol) of 18-crown-6 in a flame-dried, two-necked, round-bottomed flask by means of a fine, fritted glass filter funnel sealed with a rubber septum. Into this reaction mixture was slowly bubbled 35.0 mL (1.56 mmol) of dry oxygen over a period of 3 h with a 50-mL gas-tight syringe driven by a syringe pump. After the addition was complete, the reaction mixture was stirred for 30 min, quenched with 10 mL of saturated NH_4Cl, and extracted three times with 10 mL of ether. The combined ether layers were washed with 15 mL of saturated potassium chloride solution and dried over sodium sulfate. Concentration in vacuo gave 326 mg of crude product. The ^1H NMR spectrum ($CDCl_3$, 500 MHz) of the crude product showed a 2:1 ratio of ethyl 2-oxocyclopentane carboxylate (**4**) to diethyl adipate (**2**), 90% of epoxy alcohol **3**, and 10% of starting 2,4-dimethyl-1-naphthol. Purification of the crude residue by flash chromatography with 9:1 hexane–ethyl acetate afforded 82.3 mg of a mixture of diethyl adipate and dimethylnaphthol, 43.2 mg (31%) of cyclic product **4** with $R_f = 0.37$ in 4:1 hexane-ethyl acetate and 94.4 mg (51%) of epoxy alcohol **3** as a white solid with $R_f = 0.18$ in the same developing solvent.

Recrystallization of the epoxy alcohol **3** from hexane gave colorless crystals, mp 82–83 °C: ^1H NMR (500 MHz, $CDCl_3$) δ 1.51 (s, 3 H), 1.64 (s, 3 H) 3.66 (s, 1 H), 7.40 (t, J = 7.3 Hz, 1 H), 7.62 (t, J = 7.8 Hz, 1 H), 7.70 (d, J = 7.9 Hz, 1 H), 7.86 (d, J = 7.8 Hz, 1 Hz); ^{13}C NMR spectrum (125 MHz, $CDCl_3$) δ 15.0 (q, J = 129 Hz), 28.9 (q, J = 125 Hz), 60.5 (s), 65.9 (d, J = 183 Hz), 69.9 (s), 125.9 (d, J = 164 Hz), 127.1 (d, J = 164 Hz), 127.8 (s), 128.1 (d, J = 164 Hz), 134.3 (d, J = 162 Hz), 145.4 (s), 194.4 (s). The IR spectrum (neat) showed bands at 3500 (br s), 1693 (s), 1688 (s). The mass spectrum showed peaks at *m/e* (rel intensity) 204 (M^+, 10), 189 (25, $M^+ - CH_3$), 175 (28, $M^+ - CHO$), 161 (100, $M^+ - CH_3CO$), 147 (50); exact mass calculated for $C_{12}H_{12}O_3$ 204.0787, found 204.0786.

Control Reaction without Oxygen. The potassium 2,4-dimethyl-1-naphthoxide solution (3 mL, 0.92 mmol) was transferred into 0.18 mL (0.90 mmol) of diethyl adipate (**2**) and 200 mg (0.76 mmol) of 18-crown-6 in a flame-dried, 10-mL, two-necked, round-bottomed flask with the method described above. The reaction mixture was stirred under

(43) See, also: Wu, S.-M.; Morris, D. P.; Stafford, D. W. *Proc. Nat. Acad. Sci. U.S.A.* **1991**, *88*, 2236. Hubbard, B. R.; Ulrich, M. M. W.; Jacobs, M.; Vermeer, C.; Walsh, C.; Furie, B.; Furie, B. C. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 6893.

(44) Buu-Hoi, N. P.; Lavit, D. *J. Chem. Soc.* **1955**, 2776.

argon for 3.5 h. The same workup procedure used for the oxygenation reaction gave 344 mg of crude product as a bright yellow oil. The ^1H NMR spectrum ($CDCl_3$, 500 MHz) showed only the two starting materials, diethyl adipate (**2**) and 2,4-dimethyl-1-naphthol. No cyclized product **4** was detected within the limits of the NMR method; it was estimated with standard mixtures that 1% of cyclic product **4** could have been observed by the presence of the methine triplet of **4** at δ 3.14.

Control Reaction with Potassium Superoxide (5). To a solution of 140.8 mg (2.0 mmol) of potassium superoxide (**5**) and 518 mg (1.96 mmol) of 18-crown-6 in 6 mL of dry THF was added 0.4 mL (2.0 mmol) of diethyl adipate (**2**) under an atmosphere of argon. The reaction mixture was stirred at room temperature for 3.5 h, quenched with 20 mL of saturated aqueous NH_4Cl solution, and extracted three times with 20 mL of ether. The combined ether layers were washed with 15 mL of saturated potassium chloride solution and dried over sodium sulfate yielding 398 mg of crude product after evaporation. After concentration, the ^1H NMR spectrum ($CDCl_3$, 500 MHz) of the total crude reaction product showed no cyclic product **4** within the limits (1%) of the NMR method.

Control Reaction with Potassium *tert*-Butylperoxide (6). To a suspension of 110.0 mg (2.7 mmol) of potassium hydride in 8 mL of dry THF was added 1 mL of 3.0 M *tert*-butyl hydroperoxide (**6**) in 2,2,4-trimethylpentane (Aldrich). The mixture was stirred for 30 min under an atmosphere of argon at room temperature and transferred into 0.55 mL (2.7 mmol) of diethyl adipate (**2**) and 712.0 mg (2.7 mmol) of 18-crown-6 in a flame-dried, 10-mL, round-bottomed flask. The reaction mixture was stirred at room temperature for 3.5 h, quenched with 20 mL of saturated NH_4Cl, and extracted three times with 20 mL of ether. The combined ether layers were washed with 15 mL of saturated potassium chloride solution and dried over sodium sulfate. The ^1H NMR spectrum ($CDCl_3$, 500 MHz) of the crude product (627 mg) showed only starting diethyl adipate (**2**). No cyclized product **4** was detected within the limits (1%) of the NMR method.

Oxidation of 2,3,4-trimethylnaphthalen-1-ol (16). A solution of 2,3,4-trimethylnaphthalen-1-ol (**16**) (350 mg, 1.88 mmol) in 15 mL of chloroform was stirred under oxygen for 2 h at room temperature. The resulting yellow solution was combined with 10 mL of chloroform, washed with 20 mL of saturated $FeSO_4$ solution, and dried over Na_2SO_4. The solvent was evaporated to furnish 426 mg of a brown oily solid. The crude product was recrystallized from carbon tetrachloride yielding light brown crystals of **17** (241 mg, 63%): mp 118–119 °C; lit.[38] mp 119.5–120.5 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 1.50 (s, 3 H), 2.04 (s, 3 H), 2.19 (s, 3 H), 7.46 (t, J = 7.3 Hz, 1 H), 7.63 (t, J = 7.3 Hz, 1 H), 7.69 (s, exchange 1 H), 7.70 (d, J = 7.1 Hz, 1 H), 8.12 (d, J = 7.9 Hz, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.9 (q, J = 128 Hz), 14.8 (q, J = 128 Hz, 27.2 (q, J = 129 Hz), 82.0 (s), 124.8 (dd, J = 160, 7 Hz), 127.1 (dd, J = 164, 7 Hz), 128.2 (dd, J = 161, 9 Hz), 131.0 (s), 132.7 (d, J = 163 Hz), 133.1 (s), 144.9 (s), 155.1 (s), 183.8 (s). The IR spectrum (neat) showed a band at 3400 (br s), 1641 (s). The mass spectrum (70 ev) showed peaks at m/e (rel intensity) 202 (M^+ – OH, 2.9), 184 (100), 157 (25), 142 (23); exact mass calculated for $C_{13}H_{14}O_2$ 202.0994, found 202.0994. Slow recrystallization of **17** from ethanol gave clear crystals suitable for X-ray analysis.

Formation of 4-Hydroxy-2,3-epoxy-2,3,4-trimethyl-3,4-dihydronaphthalen-1(2*H*)-one (18) from 4-Hydroperoxy-2,3,4-trimethylnaphthalen-1(4*H*)-one (17). A solution of 105.7 mg (0.48 mmol) of peroxide (**17**)[37] and 130 mg (0.49 mmol) of 18-crown-6 was added to a suspension of 18.6 mg (0.47 mmol) of potassium hydride in 1 mL of dry THF under an atmosphere of argon with a double-tipped needle. The mixture was stirred for 1 h. The resulting dark green reaction mixture was quenched with 10 mL of saturated NH_4Cl solution and extracted with three 10-mL portions of ether. The combined ether layers were washed with 10 mL of saturated potassium chloride solution, dried over $MgSO_4$, and concentrated. The crude product was purified by flash chromatography on silica gel with 9:1 hexane/ethyl acetate and yielded 70.2 mg (66%) of epoxy alcohol **18** as a white solid, recrystallized from THF/pentane: mp 127–128 °C; ^1H NMR (500 MHz, $CDCl_3$) δ 1.37 (s, 3 H), 1.59 (s, 3 H), 1.66 (s, 3 H), 2.96 (s, exchange 1 H), 7.34 (t, J = 7.5 Hz, 1 H), 7.54 (t, J = 7.6 Hz, 1 H), 7.65 (d, J = 7.8 Hz, 1 H), 7.75 (d, J = 7.7 Hz, 1 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 11.6 (q, J = 128 Hz) 13.4 (q, J = 129 Hz), 29.3 (qd, J = 125, 7 Hz), 64.9 (s), 67.5 (s), 73.0 (s), 125.4 (dd, J = 161, 7 Hz), 126.7 (dd, J = 161, 6 Hz), 128.0 (dd, J = 163, 8 Hz), 128.1 (t, J = 7 Hz), 134.1 (dd, J = 160, 7 Hz), 146.4 (s), 195.7 (s). The IR spectrum (neat) showed bands at 3500 (br s), and 1690 (s). The mass spectrum (70 ev) showed peaks at m/e (rel intensity) 218 (M^+, 0.4), 202 (2), 187 (10), 175 (100, $M^+ - CH_3CO$), 161 (12); exact mass calculated for $C_{13}H_{14}O_3$ 218.0943, found 218.0944. The more polar fractions afforded 3.4 mg (3%) of 4-hydroxy-2,3,4-trimethylnaphthalen-1-one (**19**) as a pale yellow solid, identified by comparison with an authentic sample.

Preparation of 4-Hydroxy-2,3,4-trimethylnaphthalen-1-one (19). A solution of 100 mg (0.54 mmol) of 2,3-dimethyl-1,4-naphthoquinone (**20**) in 10 mL of dry ether, cooled in a dry ice/acetone bath, was treated with 0.36 mL (0.54 mmol) of 1.5 M methyllithium for 5 min under an argon atmosphere. After 30 min, the reaction was quenched by adding 10 mL of saturated NH_4Cl at −78 °C. The flask was warmed to room temperature, the layers were separated, and the aqueous phase was extracted with three 10-mL portions of ether. The combined organic layers were dried with magnesium sulfate, filtered, and concentrated to 115 mg. Chromatography of the crude product on silica gel with 4:1 hexane/ethyl acetate as eluent provided 102 mg (94%) of the alcohol **19** as a pale yellow solid: mp 167–168 °C (from hexane), lit.[38] mp 162–163 °C; ^1H NMR (500 MHz, $CDCl_3$) δ 1.59 (s, 3 H), 1.89 (s, exchange 1 H), 2.00 (s, 3 H), 2.17 (s, 3 H), 7.42 (td, 1 H, J = 7.3, 0.9 Hz), 7.60 (td, 1 H, J = 7.7, 1.3 Hz), 7.81 (d, 1 H, J = 7.8 Hz), 8.10 (dd, 1 H, J = 7.8, 1.2 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 11.5 (q, J = 128 Hz), 15.3 (q, J = 127 Hz), 30.8 (qd, J = 129, 7 Hz), 70.5 (s), 126.0 (dd, J = 161, 7 Hz), 126.3 (dd, J = 163, 7 Hz), 127.7 (dd, J = 163, 7 Hz), 128.9 (s), 130.5 (s), 132.6 (dd, J = 161, 8 Hz), 148.6 (s), 157.2 (s), 184.0 (s); The IR (KBr) spectrum showed bands at 3450 (br s), 1639 (s), 1597 (s), 1381 (s), 1365 (s). The mass spectrum showed peaks at m/e (rel intensity), 202 (M^+, 20), 187 (76), 159 (43), 141 (11); exact mass calculated for $C_{13}H_{14}O_2$ 202.0994, found 202.0994. This hydroxy ketone (**19**) was identical with the sample obtained by the reduction of 4-hydroperoxy-2,3,4-trimethylnaphthalen-1-one (**17**) with aqueous sodium thiosulfate.

Collection of Diffraction Data. The parameters used during the collection of diffraction data for **3** and **17** are summarized in Table I. Colorless crystals of **3** and **17** were attached to fine glass fibers with epoxy cement.

On the basis of systematic absences, **3** was uniquely assignable to the space group $P2_1/c$, and **17** was found to crystallize in one of the orthorhombic space groups $Cmc2_1$, $C2cm$, or $Cmcm$. Noncentrosymmetric $Cmc2_1$ was chosen based on E values and the successful solution and refinement of the structure. Unit cell dimensions were derived from the least-squares fit of the angular settings of 25 reflections with $18° \leq 2\theta \geq 25°$. An empirical absorption correction was applied to both crystals with the program XEMP. No significant decay occurred in three standard reflections for either compound. A profile fitting procedure was applied to all intensity data to improve the measurement of weak reflections.

Solution and Refinement of Structure. The structures of **3** and **17** were solved with the direct methods program SOLV. All non-hydrogen atoms were refined anisotropically. Idealized atom positions were calculated for all hydrogen atoms (d(C–H) = 0.96 Å, thermal parameters 1.2 times the isotropic equivalent for the carbon atom to which it was attached), except for H(3o) in **3** which was located and refined isotropically.

The asymmetric unit of **3** contains one ordered molecule with no crystallographically imposed symmetry; **3** contains no unusual bond parameters.

The crystal structure of **17** has one-half molecule in the asymmetric unit with all atoms except the 4-methyl and 4-hydroperoxide atoms sitting on a mirror plane. This requires the 4-methyl carbon atom and the ring-coordinated hydroperoxide oxygen atom to be disordered. Thus atom OC (refined as a nitrogen atom) represents a half-occupancy methyl carbon and a half-occupancy hydroperoxy oxygen (Oc and Oca), respectively, in Figure 2. O3 has a site occupancy of one-half.

For both compounds, final difference Fourier syntheses showed only diffuse backgrounds. An inspection of F_o vs F_c values and trends based upon sin θ, Miller index, or parity group failed to reveal any systematic errors in the data for either structure. All computer programs used in the data collections and refinements are contained in the Nicolet program packages P3 and SHELXTL (version 5.1).

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Registry No. 1, 84-80-0; **2**, 141-28-6; **3**, 125541-07-3; **16**, 110871-52-8; **17**, 110871-53-9; **18**, 125637-05-0; **19**, 68437-77-4; **20**, 2197-57-1; O_2, 7782-44-7; 2,4-dimethyl-1-naphthol, 4709-20-0; potassium 2,4-dimethyl-1-naphthoxide, 125541-06-2; ethyl 2-oxocyclopentanecarboxylate, 611-10-9.

Supplementary Material Available: Crystallographic details for **3** and **17** including tables of atomic coordinates, thermal parameters, and bond lengths and angles, and ORTEP drawings (9 pages); tables of observed and calculated structure factors (10 pages). Ordering information is given on any current masthead page.