The deuterated powder was suspended in deuterium oxide (9 mL) and an emulsion of squalene (105 mg) prepared with Triton X-100 (30 mg) in deuterium oxide (1 mL) was added. The mixture was incubated at ambient temperature for 24 h. The reaction was terminated by the addition of a mixture of ether–ethanol (1:3, 100 mL). The denatured proteins were removed by filtration and the filtrate was concentrated to leave an aqueous phase. The aqueous phase was saturated with NaCl and extracted with freshly distilled, peroxide-free hexane (3 × 100 mL). The hexane extract was washed with a small amount of a saturated saline solution, dried, and concentrated to a residue. The residue was fractionated by TLC (EA–hexane, 3:17) and the recovered tetrahymanol was chromatographed in the same system. The samples of tetrahymanol isolated from these incubations contained ca. 40–50% of $^2H_1$. The MS of a sample of tetrahymanol pooled from several incubations (11.9 mg) showed a deuterium content of 41.0% d_1.

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Specific Inclusion Catalysis by β-Cyclodextrin in the One-Step Preparation of Vitamin K_1 or K_2 Analogues

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Abstract: Electrophilic allylation at the C_3 position of 2-methylhydronaphthoquinone-1,4 (**5**) with allyl (**6a**), crotyl (**6b**), methallyl (**6c**), or prenyl bromide (**6d**) was successfully developed to give a highly selective one-step preparation of the corresponding vitamin K_1 (or K_2) analogue (**7a–d**) by the use of β-cyclodextrin in dilute aqueous alkaline solution. In order to elucidate the basis of this interesting inclusion catalysis which resembles "ligase and/or oxidase" reactions, mechanistic studies were carried out. The specific inclusion binding of the substrate, 2-methylhydronaphthoquinone (**5**), by β-cyclodextrin (K_a = 490 M^{-1}, pH 3.55) facilitated the proton dissociation of **5,** resulting a decrease in its pK_a value from 9.45 for uncomplexed **5** to 8.9 for complexed **5.** Based on kinetic results showing that the rate of allylation of α-naphthol at pH 10.4 was enhanced by 2.5 to 3.5 times in the presence of β-cyclodextrin, it was concluded that the nucleophilic reactivity of the partially charged carbanion increased in the hydrophobic cavity and, therefore, the allylation reactions were accelerated by β-cyclodextrin. Another noteworthy aspect of the mechanism was seen in the interesting observation that 2-methylnaphthosemiquinone anion radical (detected by ESR) which was produced by oxidation of the allylated hydronaphthoquinone by molecular oxygen was strongly bound by β-cyclodextrin. This indicated either the possibility that the oxidation proceeds predominantly through the complexed form of the allylated hydroquinone or that the lifetime of the semiquinone anion radical is prolonged by the inclusion binding. Vitamin K analogues **7** and **8,** which were products, were found to be highly susceptible to oxidative degradation due to the attack of hydrogen peroxide, which was shown to be another product of the oxidation step. β-Cyclodextrin effectively protected those quniones from the attack by hydrogen peroxide, and their oxidation rates were from $^1/_9$ to $^1/_{17}$ of that for the uncomplexed quinone.

In the past decade there has been considerable interest in cyclodextrin inclusion catalysis and a wealth of information has been collected on mechanistic details, mostly with respect to the use of cyclodextrin as hydrolase models.[2] However, no reported inclusion catalysis has been of practical use as a synthetic tool except for the interesting example of the exclu-

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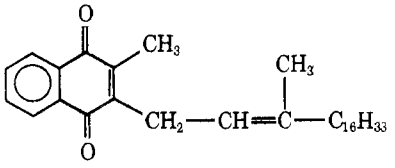
sive para chlorination of anisole bound in the cavity of α-cyclodextrin.[3].

The authors are currently attempting to develop the use of inclusion catalysis in the synthesis of bioactive compounds and now wish to report the first successful application of β-cyclodextrin to the one-step synthesis of vitamin K_1 (or K_2) analogues in dilute aqueous alkaline solution.

Vitamin K_1 (**1**), a factor important in blood clotting, was first synthesized from 2-methylhydronaphthoquinone-1,4 and phytol via the Friedel–Crafts reaction by Fieser,[4] which was followed by many modifications that used, e.g., phytyl halides and protected 2-methylhydronaphthoquinone.[5] However, these preparations have serious and inevitable disadvantages in the formation of undesirable products, 2-methyl-2-phytyltetralindione-1,4 (**2**), naphthotocopherol (**3**), and/or 3-methylheptadeca-1,3-diene (**4**).



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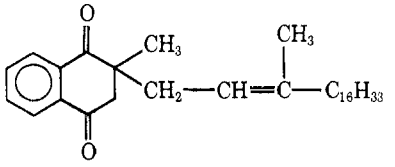

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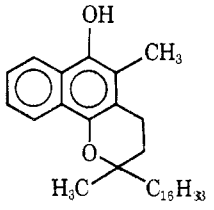

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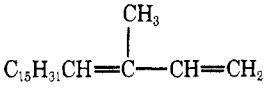

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Quite recently a new one-step route to vitamin K_1 (or K_2) analogues (**7a,b**) has been exploited by the authors,[6] where allyl or crotyl bromide was used as the electrophilic reagent to introduce allyl or crotyl residue regiospecifically into the C_3 position of 2-methylhydronaphthoquinone-1,4 (**5**). No undesired product was formed in the present preparation when β-cyclodextrin was used as the inclusion catalyst. This strongly suggests that β-cyclodextrin acts as an ideal "ligase and/or oxidase" model in the sense that it controls the reaction pathway, ensuring that it was a very favorable one.

In this article is presented the highly selective one-step preparation of vitamin K_1 (or K_2) analogues (**7**) making full use of the unique inclusion catalysis by β-cyclodextrin. Based on the results of a mechanistic study of the present "ligase and/or oxidase" model, we have succeeded in demonstrating the following four functions of β-cyclodextrin: (1) the specific inclusion binding of the substrate in aqueous solution; (2) the enhancement of the nucleophilicity of the partially charged carbanion of **5** in the hydrophobic cavity; (3) the inclusion binding of naphthosemiquinone anion radical, the productive intermediate in the oxidation process, through which the specific oxidation proceeds; and (4) the protection of products against further oxidative degradation.

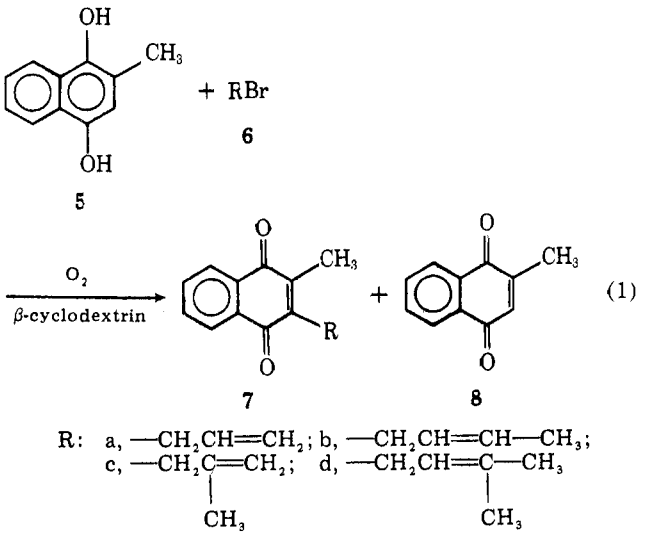
Results and Discussion

Reaction 1 illustrates the present one-step preparation of vitamin K_1 or K_2 analogues (**7**), which is successfully carried out by electrophilic allylation at the C_3 position of 2-methylhydronaphthoquinone-1,4 (**5**) by the use of 1–20 equiv of allyl (**6a**), crotyl (**6b**), methallyl (**6c**), or prenyl bromide (**6d**)

Table I. One-Step Preparation of Vitamin K_1 (or K_2) Analogues from 2-Methylhydronaphthoquinone (**5**) by Use of β-Cyclodextrin[a,b]

RBr	β-CD	yield, % 7	8
	10^{-2} M	43 (84)[c]	49
6a	none	12 (13)[c]	11
	10^{-2} M	60 (77)[c]	22
6b	none	20 (22)[c]	9
	10^{-2} M	47 (76)[c]	38
6c	none	22 (27)[c]	17
	10^{-2} M	40 (78)[c]	49
6d	none	15 (21)[c]	28

[a] RBr, 20×10^{-2} M; β-CD, 5×10^{-2} M; 2-methylhydronaphthoquinone (**5**), 10^{-2} M. [b] Phosphate buffer, pH 9.0 (30% MeOH by volume), room temperature, 9 h under nitrogen. [c] In parentheses are the yields measured after taking into account the amount of oxidized form (**8**) produced.



(1)

R: a, $-CH_2CH=CH_2$; b, $-CH_2CH=CH-CH_3$; c, $-CH_2C(CH_3)=CH_2$; d, $-CH_2CH=C(CH_3)-CH_3$

in the presence of 2–5 equiv of β-cyclodextrin in phosphate buffer (pH 9.0–12.0) containing 30% methanol by volume. The yield of the product is very sensitive to the presence of trace amounts of oxygen (vide infra), and therefore the reaction was carried out in the dark under nitrogen or argon atmosphere (carefully deoxygenated by passing through reduced copper).

After 9 h at room temperature, the vitamin K_1 (or K_2) analogue, **7**, was obtained in an excellent yield (Table I) together with 2-methylnaphthoquinone-1,4 (**8**). The products were purified by column chromatography on silica gel. The preparative yields of **7** and **8** shown in Table I were in good agreement with the yields independently determined based on the NMR analysis for vinyl (δ 4.93–5.20) and 3 proton (δ 6.80). The spectroscopic properties (NMR, IR) of the products were identical with those reported for the authentic compounds.[7]

The yield of allylated naphthoquinone (**7**) obtained in the control experiment (i.e., in the absence of β-cyclodextrin) was much lower than under the conditions of inclusion (Table I) even when the amount of the recovered starting material in the oxidized form (**8**) was taken into the consideration. This strongly indicates that β-cyclodextrin played a significant role in the present highly selective preparation of vitamin K_1 (or K_2) analogues as if it were a ligase and/or oxidase, "vitamin K_1 (or K_2) synthetase".

Mechanistic Studies on the "Ligase and/or Oxidase" Model. The strong interaction between β-cyclodextrin and the substrate, 2-methylhydronaphthoquinone-1,4 (**5**), in aqueous solution is suggested by several observations. Judging from the space-filling molecular model (CPK model) the present guest molecule, 2-methylhydronaphthoquinone (**5**), fits the cavity of β-cyclodextrin very nicely. The inclusion equilibrium between guest **5** and host, β-cyclodextrin, was investigated by the fluorescence technique. A blue shift of the fluorescence maximum from 432 nm for uncomplexed **5** to 423 nm for complexed **5** and a remarkable increase in the fluorescence intensity (Figure 1) were observed when 10^{-3} to 0.22×10^{-3} M concentration of β-cyclodextrin was added into the solution of **5** in phosphate buffer, showing that **5** is strongly bound by β-cyclodextrin in aqueous solution. The Benesi-Hildebrand plot[8] of the fluorescence change with the change in β-cyclodextrin's concentration afforded the association constant, K_{ass}, of 490 M^{-1} for the inclusion equilibrium, the value being comparable to K_{ass} values for the bindings of other guest compounds of the naphthalene type by β-cyclodextrin.[9]

The effect of inclusion binding on the equilibrium for proton dissociation of **5** was also investigated. Spectroscopic titration of 2-methylhydronaphthoquinone (**5**) (1.9×10^{-5} M) was carried out by monitoring the decrease in the absorption band of **5** at 245 or 266 nm with the addition of 0.1 N NaOH under a hydrogen atmosphere.[10] The pK_a value of **5** in the presence of 2×10^{-2} M β-cyclodextrin was thus determined to be 8.93 ± 0.03 at 25 °C. Since the titration was carried out under conditions where 91% of complexed **5** was in equilibrium with 9% of uncomplexed **5**, the proton dissociation of uncomplexed **5** (pK_a value of 9.45) was taken into account, thus providing the corrected pK_a value of 8.90 for complexed **5**.[11] The considerably smaller pK_a value for complexed **5** than that of uncomplexed **5** suggests that there is significant hydrogen bonding between the anion of **5** and β-cyclodextrin.[12]

As a result of the considerable decrease in the pK_a value of **5** bound by β-cyclodextrin, 52% of the added substrate exists in the form of complexed anion under the present condition (pH 9.0). This implies that the addition of β-cyclodextrin caused an increase in the amount of the monoanion (the active species), to an amount twice as much as that (26%) in the absence of β-cyclodextrin.

Accelerated Regiospecific Allylation of the Bound Substrate. Under conditions optimized for the one-step preparation of vitamin K analogues, the predominant amount (more than 90%) of the ionized form of **5** is strongly bound by β-cyclodextrin and this binding seems to be directly responsible for the unique ligase-like function, since the inclusion binding into the hydrophobic cavity seems to bring about dehydration of the partially charged carbanion in the activity, leading to the increase in its nucleophilic reactivity.

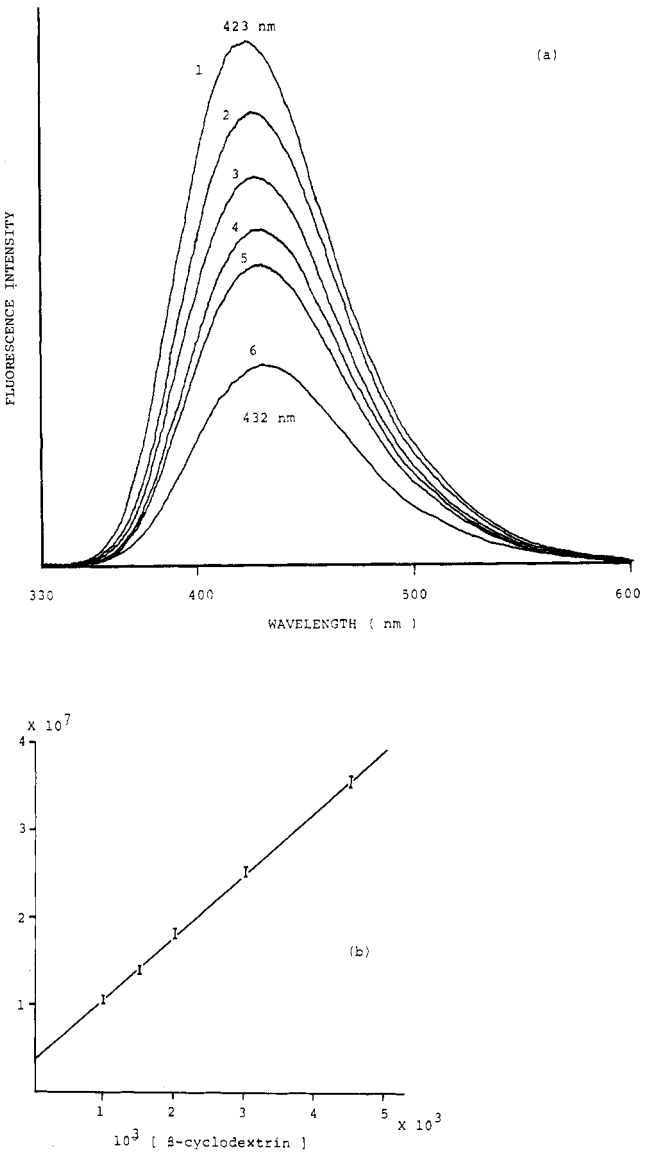
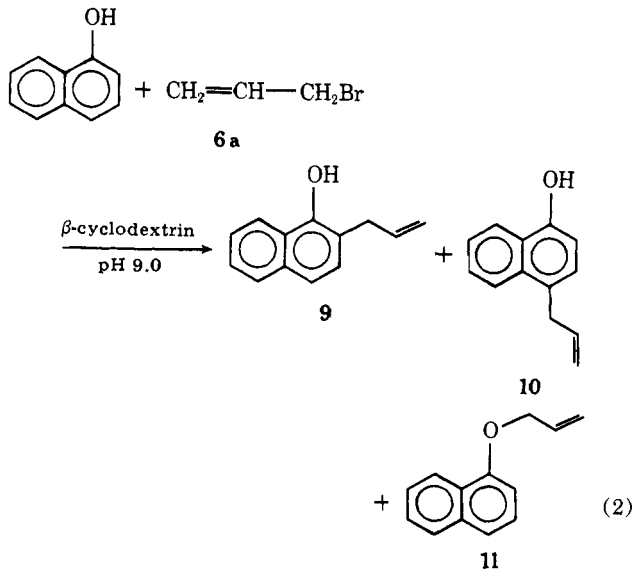


Figure 1. (a) Fluorescence spectrum of 2-methylhydronaphthoquinone-1,4 (1×10^{-5} M) in the presence of β-cyclodextrin: (1) 10^{-3} M, (2) $\frac{2}{3} \times 10^{-3}$ M, (3) 0.5×10^{-3} M, (4) $\frac{1}{3} \times 10^{-3}$ M, (5) $\frac{2}{9} \times 10^{-3}$ M, and (6) in the absence of β-cyclodextrin in phosphate buffer at pH 3.55 (30% methanol by volume). Excitation wavelength 312 nm. (b) Benesi-Hildebrand plot of the fluorescence intensities. K_{ass} = 490 M^{-1}.



(2)

Evidence for this enhanced nucleophilicity was obtained for the allylation of 1-naphthol included in β-cyclodextrin, rather than for the corresponding reaction of **5**, since, in this simplified system, any complexity due to the competitive oxidation should be avoided. The rate of allylation of 1-naphthol by allyl bromide (**6a**) in phosphate buffer at pH 10.4 (where more than 90% of naphthol should be dissociated) was measured by the appearance of products and the disappearance of 1-naphthol on GLC. The rate in the presence of β-cyclodextrin was found to be 2.5 to 3.5 times faster than that in the absence of β-cyclodextrin, and the three products isolated in a ratio of 13:4:11 were 2-allyl-1-naphthol (**9**), 4-allyl-1-naphthol (**10**), and allyl 1-naphthyl ether (**11**),[13] respectively (eq 2). The structures of the products were confirmed also by a separate experiment observing that upon acetylation with acetic anhydride only **11** remained unchanged on GLC with the same retention time,

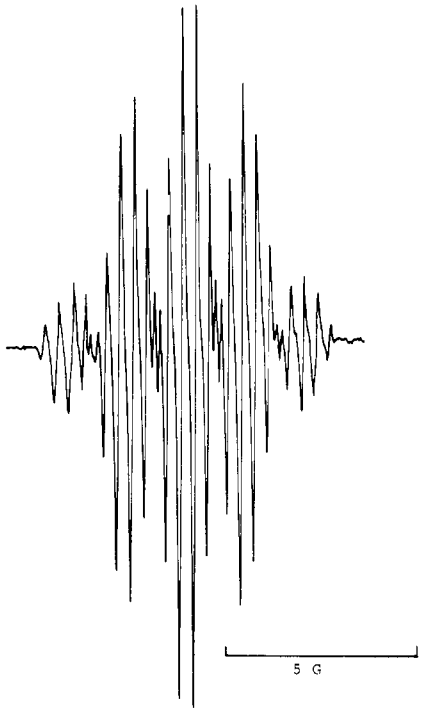
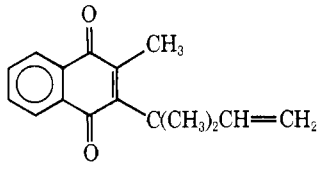


Figure 2. ESR spectrum of 2-methylnaphthosemiquinone radical anion complexed by β-cyclodextrin.

whereas the other two compounds were converted to the corresponding acetylated derivatives.

The finding that the isomer ratio was not changed in the earlier stages of the allylation reaction, together with the finding that structure **7d**, instead of structure **12** expected from



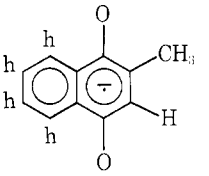
12

the Claisen-type rearrangement, was formed as a product, excludes the mechanism of the Claisen-type rearrangement.[13]

The exclusive selectivity of para over ortho (the ratio of para to ortho = 531:1) in the reported chlorination of anisole bound by α-cyclodextrin was interpreted as a result indicating that the approach to the ortho position was prohibited.[3] However, present inclusion catalysis exhibited a quite different and interesting aspect of ligase action, viz., the inclusion binding specifically activated the C_3 position of 2-methylhydronaphthoquinone (**5**). Based on the preliminary kinetic experiments on 1-naphthol as outlined above, the specific activation of the C_3 carbon of **5** was attributed to the increased nucleophilic reactivity of the partially charged carbanion in the hydrophobic cavity. This, therefore, accounts for absence of O-allylated products despite the easier access of the allylic bromides to the oxygen atoms. Furthermore, judging from the space-filling molecular model, there does not exist enough space in the cavity to include the transition state complex for allylation on C_2 carbon bearing a methyl group, and the C_2 allylation seems to be quite difficult, although it is one of the important side reactions in homogeneous acid allylations.[5]

Oxidation Process Simulating Oxidase Function. Molecular oxygen is responsible to converting the allylated hydroquinone **14** to the corresponding quinone, the vitamin K_1 (or K_2) analogue. However, use of nitrogen contaminated with substantial amounts of oxygen mostly resulted in increased amounts of the oxidized form of the substrate (**8**) and degradation products, **21** and **22** (vide infra), and the vitamin K analogue (**7**) was obtained only in much lower yield (0–10%). Thus, selective oxidation to **7** was achieved under nitrogen contaminated with a small amount of oxygen.

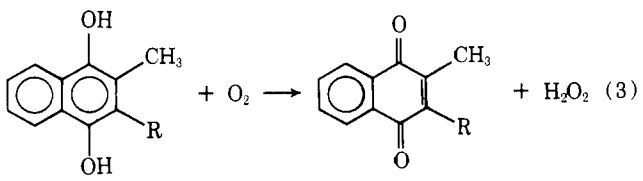
Table II. Hyperfine Coupling Constants of 2-Methylnaphthosemiquinone Radical Anion Bound by β-Cyclodextrin



	hfs constant, G
a_h	0.68
a_H	2.52
a_{CH_3}	3.24

The most plausible mechanism of the present oxidation step involves 2-methylnaphthosemiquinone anion radical as an intermediate. Figure 2 shows the ESR spectrum detected when to the solution of 2-methylhydronaphthoquinone (10^{-3} M) and β-cyclodextrin (10^{-2} M) in 0.5 mL of phosphate buffer at pH 9.0 were added a few drops of a 10^{-2} M solution of NaOH. The hfs constant (0.68 G, Table II) we assigned to the coupling with aromatic hydrogens on 2-methylnaphthosemiquinone anion radical was comparable in the magnitude to the values of 0.513 or 0.655 G reported for coupling with the aromatic hydrogens on naphthosemiquinone anion radical[14] (Table II). This agreement helped us to properly analyze the ESR spectrum and assign hfs constants (Table II). The presence of β-cyclodextrin resulted in much higher intensities of the ESR signals of 2-methylnaphthosemiquinone radical anion, viz., a 10- to 17-fold increase in the magnitude, although the hfs pattern observed was quite similar to that of uncomplexed radical anion. This indicates that the radical anion predominantly exists in the complexed form, although the complexation by cyclodextrin affects no environmentally dependent change of the hfs constant in the present system.[15] The fact that the anion radicals detected were those specifically bound by β-cyclodextrin rather than the uncomplexed ones provided strong evidence for the accelerated one-electron oxidation of the bound substrate by molecular oxygen and/or the prolonged lifetime of the anion radical in its bound state. The oxidase-type function of β-cyclodextrin, which consists of two one-electron oxidations and proton releases, is depicted in Scheme I.

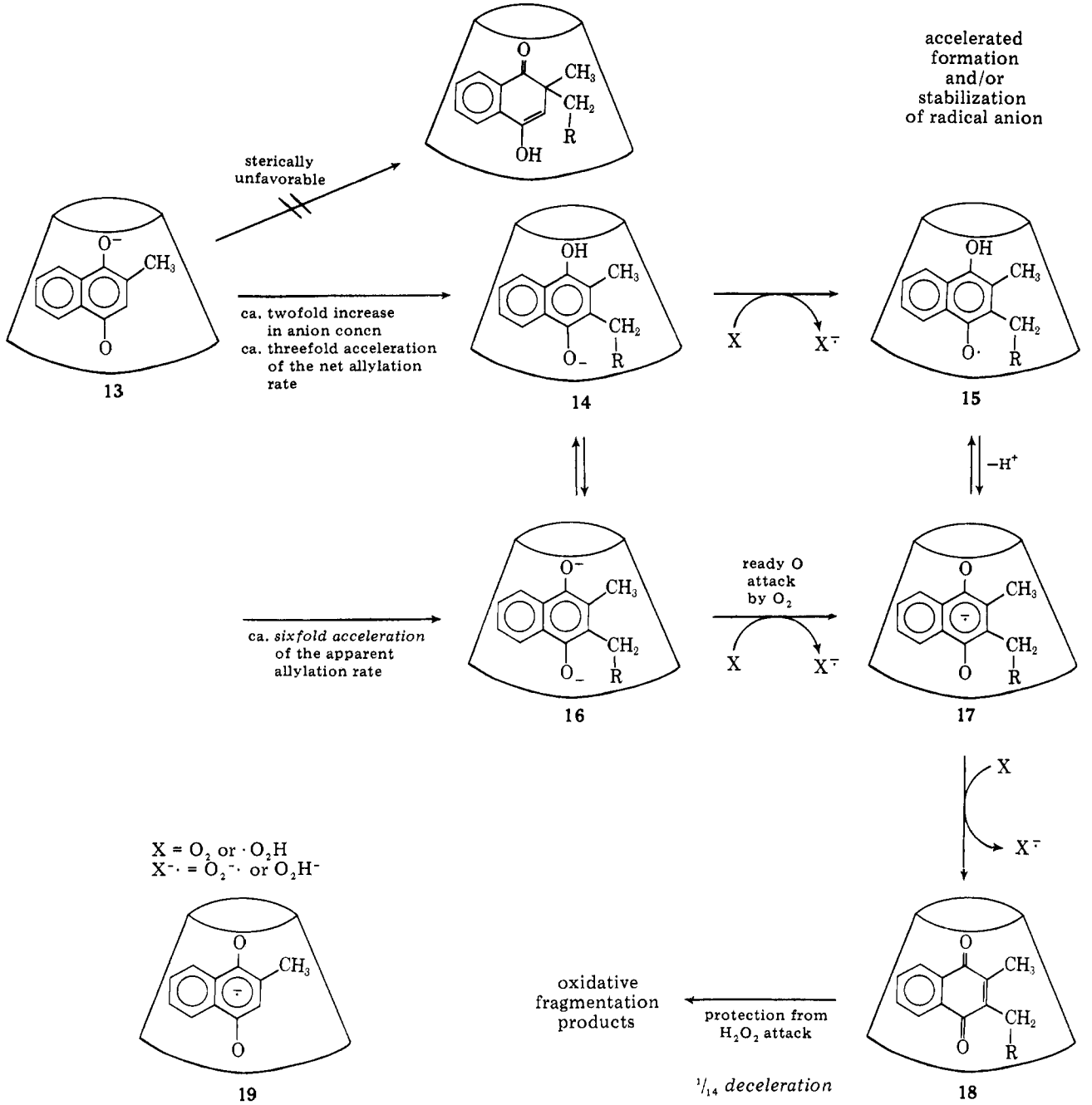
Further support for the oxidation mechanism suggested in Scheme I is provided by the quantitative determination of hydrogen peroxide formed (eq 3) which, as shown later, is



20 or 5 (R = H) **7 or 8 (R = H)**

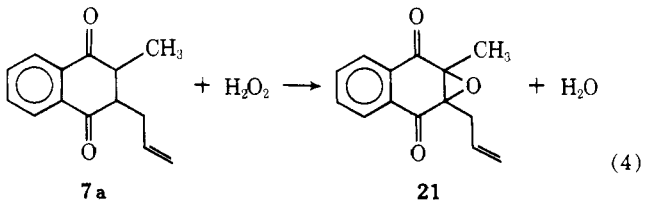
again consumed as the oxidant in the oxidative degradation of the quinones (eq 4 and 5). The mechanism (**14** to **18**), either via **15** or **16**, analogous to the reaction of **13** to **19**, accounts for the formation of hydrogen peroxide, another product in the present vitamin K syntheses. Thus a solution of 2-methylhydronaphthoquinone (**5**, 5×10^{-3} M) in phosphate buffer at pH 9.0 (30% MeOH) was subjected to the reaction condition and

Scheme I. β-Cyclodextrin Catalysis



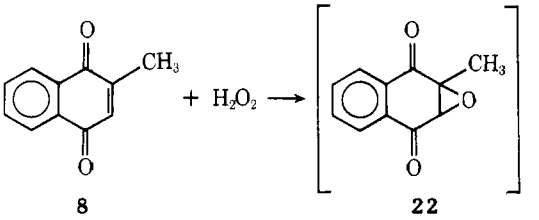
after a certain period of time each aliquot was taken and analyzed by iodometry.

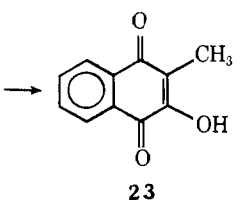
The time-dependent change in the amount of hydrogen peroxide titrated is shown in Table III and Figure 3, demonstrating that hydrogen peroxide was rapidly produced and within 2 min it amounted to 89 mol % of the 2-methylhydronaphthoquinone (**5**) used. The oxidation process (eq 3) was concluded to be very fast and practically quantitative, especially in the presence of β-cyclodextrin. However, in the absence of β-cyclodextrin the very rapid formation of hydrogen peroxide was followed by its relatively rapid disappearance due to further reaction (vide infra).



(4)

In conclusion, molecular oxygen easily attacks the oxygen atom (not carbon atoms) of the anion of allylated hydronaphthoquinone even in the bound state to generate the single





(5)

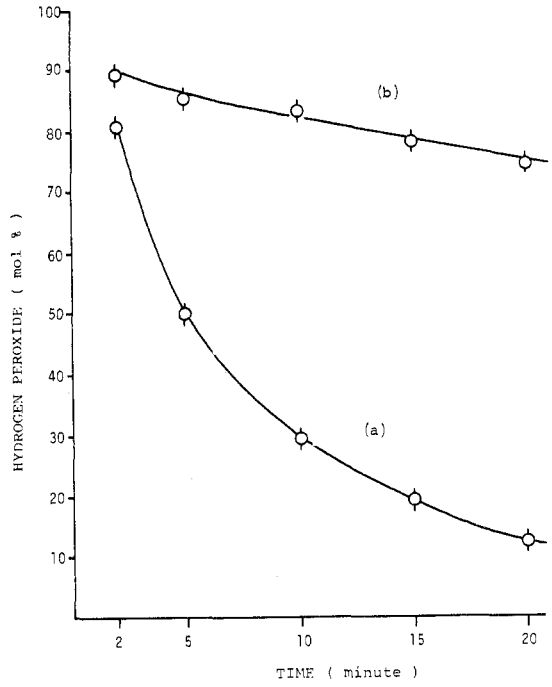


Figure 3. Quantitative determination of hydrogen peroxide produced in the oxidation of 2-methylhydronaphthoquinone (5) (0.005 M) in the absence (a) and presence (b) of β-cyclodextrin (0.01 M).

productive intermediate, **17**. In this aspect, therefore, the present catalysis by β-cyclodextrin appears to mimic oxidase action.

Protection of Vitamin K Analogues from Oxidative Fragmentations. As shown in Figure 3, the rate of the decrease in the amount of hydrogen peroxide after its rapid formation in the presence of β-cyclodextrin was much slower than that in the absence of β-cyclodextrin, and without β-cyclodextrin the yield of quinones also decreased with time. It was found that on treating with an equimolar amount of hydrogen peroxide for 1 h at room temperature in phosphate buffer at pH 9.0 (30% MeOH), 2-methyl-3-allylnaphthoquinone-1,4 (**7a**) was readily oxidized, affording 2,3-epoxy-2-methyl-3-allyl-1,4-tetralinedione (**21**) in 47% yield (98% based on the consumed **7a**). Furthermore, 2-methylnaphthoquinone (**8**) is well known to be very labile to hydrogen peroxide, giving 2-methyl-3-hydroxynaphthoquinone (**23**) followed by successive degradations.[16] Our further effort was devoted to clarifying the mechanism of protection of the quinone from oxidative degradation. The rate of epoxidation of 2-methylnaphthoquinone (**8**), 2-methyl-3-allylnaphthoquinone (**7a**), or 2-methyl-3-prenylnaphthoquinone (**7d**) with hydrogen peroxide was determined by measuring the decrease in the 340-nm absorption of the naphthoquinones at 32 °C in phosphate buffer at pH 9

Scheme II

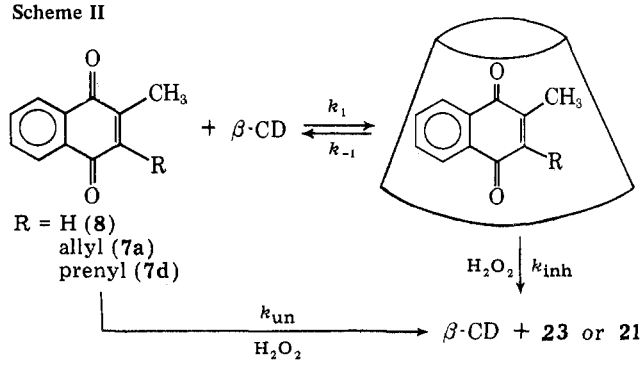


Table III. Mole Percentage of Hydrogen Peroxide Produced to 2-Methylhydronaphthoquinone (**5**)[a]

	β-CD	min 2	5	10	15	20
H_2O_2, %	10^{-2} M	89	85	83	78	74
H_2O_2, %	none	81	50	29	19	12

[a] 2-Methylhydronaphthoquinone (**5**), 5×10^{-3} M; phosphate buffer pH 9.0 containing 30% MeOH by volume, room temperature. An aliquot of 5 mL taken from the solution was analyzed by iodometry using 0.001 N sodium hyposulfite.

Table IV. Protection of Vitamin K_1 (or K_2) Analogues by β-Cyclodextrin from Oxidative Degradation[a]

	k_{inh}, s^{-1} M^{-1}	k_{un}, s^{-1} M^{-1}	K_I, M^{-1}
8	5.3×10^{-2}	4.66×10^{-1}	310
7a	2.0×10^{-2}	2.76×10^{-1}	400
7d	1.17×10^{-2}	2.07×10^{-1}	412

[a] Substrate concentration 5×10^{-4} M, $[H_2O_2] = 5 \times 10^{-3}$ M, phosphate buffer pH 9.0 containing 30% MeOH by volume, $I = 0.1$, 32 °C.

(30% MeOH) (Table IV). The observed rate was analyzed by use of the Lineweaver-Burk type[17a] equation (eq 6). The ki-

$$1/(k_{un} - k_{obsd}) = \frac{1}{K_I(k_{un} - k_{inh})[\beta\text{-CD}]} + 1/(k_{un} - k_{inh}) \quad (6)$$

netic parameters determined are given in Table IV. The satisfactory Lineweaver-Burk type plot obtained over a moderately wide range of β-cyclodextrin concentration indicates the formation of a 1:1 complex as depicted in Scheme II.[17b] As readily seen from the values of k_{un} and k_{inh} listed in Table IV, naphthoquinones included by β-cyclodextrin are significantly resistant to the oxidation, the k_{inh} rate constant being only from 1/9 to 1/17 of the corresponding k_{un} rate constant. The strong inclusion binding of quinones by β-cyclodextrin was again demonstrated by the large inhibition constant observed, $K_I = (k_{-1} + k_{inh})/k_1$, 300–400 M^{-1}, consistent with the association constant of the methylhydronaphthoquinone-β-cyclodextrin complex.[9] Therefore it is concluded that another crucial factor that led to the successful one-step preparation of vitamin K analogues was the protection of the labile quinones from successive oxidative fragmentations.

In summary, the overall mechanism of cyclodextrin catalysis of ligase-oxidase activity is shown in Scheme I.

Experimental Section

General. Nuclear magnetic resonance spectra were recorded on JEOL 100-H and 60-H spectrometers (at the Faculty of Pharmaceutical Sciences, Kyushu University) and a Hitachi EM 360 spectrometer (at the Department of Synthetic Chemistry, Kyoto University). Infrared spectra were recorded on a Hitachi Model 215 spectrometer. Measurements of mass spectra were carried out at the Analytical Laboratory, Department of Synthetic Chemistry, Kyoto University. Analytical gas chromatography was performed on a Shimadzu GC-4BIT instrument with 3 m × 0.2 cm column packed with silicon oil DC 550 or polyethylene glycol 20M. pH measurements were performed on a Hitachi Horiba F-7SS pH meter. Fluorescence spectra were recorded on a Union FS-301 high-sensitivity fluorescence spectrophotometer.

Materials. 2-Methylhydronaphthoquinone-1,4 (**5**) was prepared by the reduction of 2-methylnaphthoquinone-1,4 (menadione) with $SnCl_2$ according to the reported procedure,[18] and was purified by recrystallization from water. Commercially available allyl bromide (**6a**) was distilled before use. Methallyl bromide (**6c**), crotyl bromide (**6b**), and prenyl bromide (**6d**) were prepared according to the reported procedures which appeared in ref 19 and 20, and were distilled in

vacuo just before use. Buffer solutions were made from distilled, deionized water, distilled methanol, and sodium dihydrogen phosphate or sodium borate (analytical grade). Analytical grade hydrogen peroxide (28% solution) was purchased and used without calibration for synthetic purposes. For kinetic purposes, the concentration of hydrogen peroxide was calibrated iodometrically.

Preparation of Vitamin K_1 or K_2 Analogues. 2-Allyl-3-methylnaphthoquinone-1,4 (7a). A solution of 8.505 g (7.5 mmol) of β-cyclodextrin, 3.630 g (30 mmol) of allyl bromide (**6a**), and 261 mg (1.5 mmol) of 2-methylhydronaphthoquinone-1,4 (**5**) in a mixture of 105 mL of borate buffer solution (pH 9.0) and 45 mL of methanol (30 vol %) was stirred at room temperature for 9 h under N_2 or argon atmosphere (carefully deoxygenated by passing through a reduced copper column) in the dark. After addition of 600 mL of H_2O, 30 mL of concentrated HCl, and 24 mL of cyclohexylamine to the solution with cooling by ice, the mixture was extracted eight times with 45 mL of chloroform. The chloroform extracts were combined, washed with two portions of 60 mL of 1 N HCl, and dried over anhydrous Na_2SO_4. Evaporation of chloroform, followed by column chromatography (silica gel, petroleum ether–ethyl acetate, 10:1), gave 216 mg (68% based on 2-methylhydronaphthoquinone-1,4 (**5**) used and 96% on the consumed starting material) of 2-allyl-3-methylnaphthoquinone-1,4 (**7a**) and 75.6 mg (29% of the starting material used) of 2-methylnaphthoquinone-1,4 (**8**). **7a:** NMR δ (CCl_4) 2.20 (3 H, singlet, CH_3), 3.35 (2 H, doublet, $-CH_2C=$, J = 6 Hz), 4.93 (2 H, doublet, $=CH_2$, J = 3 Hz), 5.20 (1 H, multiplet, $-CH=$), 7.57 and 7.90 (4 H, multiplet, aromatic); IR (KBr) 1665 ($\nu_{C=O}$), 995, 920 (vinyl) cm^{-1}; mass *m/e* (rel intensity) 212 (M, 100), 213 (M + 1, 18.6), 214 (M + 2, 3.5), 197 (M − 15, 115), 171 (M − 41, 56), 143 (M − 69, 37), 115 (M − 97, 65), 104 (M − 108, 19), 76 (M − 136, 68), 50 (M − 162, 32). Anal. Calcd for $C_{14}H_{12}O_2$: C, 79.22; H, 5.70. Found: C, 79.38; H, 5.90.

2-Crotyl-3-methylnaphthoquinone-1,4 (7b). A solution of 87 mg (0.5 mmol) of 2-methylhydronaphthoquinone-1,4 (**5**), 2.835 g (2.5 mmol) of β-cyclodextrin, and 1.350 g (10 mmol) of crotyl bromide (**6b**) in a mixture of 35 mL of borate buffer of pH 9.0 and 15 mL of methanol was stirred at room temperature for 9 h under a N_2 or an argon atmosphere in the dark. The usual workup similar to that described for **7a**, followed by column chromatography (silica gel, petroleum ether–ethyl acetate, 10:1), gave 67.8 mg (60%, 77% based on the consumed starting material) of **7b** and 18.7 mg (22%) of **8**. **7b:** NMR δ (CCl_4) 1.60 (3 H, doublet, $=CH_3$, J = 5 Hz), 2.13 (3 H, singlet, CH_3), 3.27 (2 H, doublet, $-CH_2C=$, J = 4 Hz), 5.32 (2 H, multiplet, olefinic H), 7.60 and 8.00 (4 H, multiplet, aromatic H); IR (neat) 1650 ($\nu_{C=O}$), 1618, 1595, 965 ($=CH$) cm^{-1}; mass *m/e* (rel intensity) 226 (M, 100), 227 (M + 1, 21), 211 (M − 15, 191), 197 (M − 29, 61), 171 (M − 55, 7), 115 (M − 111, 76), 104 (M − 122, 59), 76 (M − 150, 196), 50 (M − 176, 79). Anal. Calcd for $C_{15}H_{14}O_2$: C, 79.62; H, 6.24. Found: C, 79.89; H, 6.43.

2-Methallyl-3-methylnaphthoquinone-1,4 (7c). A solution of 2.835 g (2.5 mmol) of β-cyclodextrin, 1.35 g (10 mmol) of methallyl bromide (**6c**), and 87 mg (0.5 mmol) of 2-methylhydronaphthoquinone-1,4 (**5**) in a mixture of 35 mL of borate buffer solution (pH 9.0) and 15 mL of methanol was stirred at room temperature for 9 h under N_2 or argon in the dark. Workup similar to that described for **7a**, followed by column chromatography (silica gel, petroleum ether–ethyl acetate, 19:1), gave 53 mg (46%, 76% based on the consumed starting material) of **7c** and 30 mg (35%) of **8**. **7c:** NMR δ (CCl_4) 1.78 (3 H, singlet, $=CCH_3$), 2.12 (3 H, singlet, CH_3), 3.32 (2 H, singlet, $-CH_2C=$), 4.65 (2 H, doublet, $=CH_2$, J = 10 Hz), 7.63 and 8.07 (4 H, multiplet, aromatic H); IR (neat) 1658 ($\nu_{C=O}$), 1620, 1595, 890 ($=CH_2$) cm^{-1}; mass *m/e* (rel intensity) 226 (M, 100), 227 (M + 1, 11.8), 211 (M − 15, 138), 171 (M − 55, 62), 143 (M − 83, 44), 115 (M − 111, 64), 104 (M − 122, 29), 76 (M − 150, 32), 50 (M − 162, 75). Anal. Calcd for $C_{15}H_{14}O_2$: C, 79.62; H, 6.24. Found: C, 79.13; H, 6.74.

2-Methyl-3-prenylnaphthoquinone-1,4 (7d). To a solution of 2.835 g (2.5 mmol) of β-cyclodextrin, 1.49 g (10 mmol) of prenyl bromide (**6d**), and 87 mg (0.5 mmol) of 2-methylhydronaphthoquinone-1,4 (**5**) in a mixture of 35 mL of borate buffer solution (pH 9.0) and 15 mL of methanol was passed through N_2 or argon gas for 2 h, and the mixture was stirred at toom temperature for 9 h under N_2 or argon atmosphere in the dark. Workup similar to that described for **7a**, followed by column chromatography (silica gel, petroleum ether–ethyl acetate, 10:1), gave 54.0 mg of **7d** (45%, 82% based on the consumed starting material) and 38.2 mg (45%) of **8**. **7d:** NMR δ (CCl_4) 1.77 (6 H, doublet, $=CCH_3$, J = 6 Hz), 2.19 (3 H singlet, CH_3), 340 (2 H, doublet, $-CH_2C=$, J = 7 Hz), 5.10 (1 H, triplet, $-CH=C$, J = 7 Hz), 7.75 and 8.15 (4 H, multiplet, aromatic H); IR (KBr) 1665 ($\nu_{C=O}$), 1620, 1595 cm^{-1}; mass *m/e* (rel intensity) 240 (M, 100), 241 (M + 1, 9.7), 225 (M − 15, 123), 197 (M − 43, 139), 169 (M − 71, 36), 141 (M − 99, 37), 128 (M − 112, 24), 104 (M − 136, 45), 76 (M − 164, 47), 50 (M − 190, 29). Anal. Calcd for $C_{16}H_{16}O_2$: C, 79.97; H, 6.71. Found: C, 79.99; H, 6.88.

Determination of K_{ass} Value. A solution of 1×10^{-5} M 2-methylhydronaphthoquinone-1,4 (**5**) in the presence of 10^{-3}, $2/3 \times 10^{-3}$, $1/2 \times 10^{-3}$, $1/3 \times 10^{-3}$, or $2/9 \times 10^{-3}$ M β-cyclodextrin and in the absence of β-cyclodextrin in a phosphate buffer solution (pH 3.55, 30% methanol by volume) was made up. The fluorescence spectrum was measured on a Union FS-301 high-sensitivity fluorescence spectrophotometer thermostated at 25.0 ± 0.1 °C by circulating thermostated water. Fluorescence spectra are shown in Figure 1a. The reciprocal of the increase in the fluorescence intensity at 423 nm was plotted against the reciprocal of the concentration of β-cyclodextrin (Benesi–Hildebrand plot, Figure 1b). The determination of the K_{ass} value was carried out using the least-square regression program for the nonlinear model on a Union System 77 computer.

Quantitative Determination of H_2O_2. A solution of 43.5 mg (0.25 mmol) of 2-methylhydronaphthoquinone (**5**) and 0.567 g (0.5 mmol) of β-cyclodextrin in a mixture of 35 mL of borate buffer solution (pH 9.0) and 15 mL of methanol was vigorously shaken. After treatment with a calculated amount of oxygen (air), an aliquot of 5 mL was taken from the mixture to which were added 0.5 mL of concentrated H_2SO_4 and 1 mL of tetrachloroethylene. The precipitate of the tetrachloroethylene–cyclodextrin inclusion complex was filtered off and the filtrate was titrated iodometrically with 0.001 N $Na_2S_2O_4$. The control experiment in the absence of β-cyclodextrin was carried out in a similar manner as described above. Results are given in Table III and Figure 3. It was ascertained by separate experiments that the correct titer was obtained in the presence of β-cyclodextrin or the substrate.

ESR Measurement. To a degassed 0.5-mL solution of 2-methylhydronaphthoquinone (**5**) (10^{-3} M in phosphate buffer of pH 9.0, 30% methanol by volume) and β-cyclodextrin (10^{-2} M) was added a few drops of degassed 10^{-2} M KOH solution under argon atmosphere. The resultant pink to slightly red colored solution was immediately introduced into a capillary tube under argon and the tube was sealed. The ESR spectra were recorded on a JEOL JES-ME ESR spectrometer (Faculty of Pharmaceutical Sciences, Kyushu University) and a JEOL JES-PE ESR spectrometer (Department of Petroleum Chemistry, Kyoto University). Results are given in Table II and Figure 2.

2,3-Epoxy-2-allyl-3-methyltetraline-1,4-dione (23). A solution of 20 mg (0.094 mmol) of 2-allyl-3-methylnaphthoquinone (**7a**) and 12 mg of 28% hydrogen peroxide (0.094 mmol) in 100 mL of phosphate buffer of pH 9.0 (30% methanol by volume) was stirred in the dark for 1 h. To the reaction mixture were added 4 mL of 1 N HCl and 4 mL of cyclohexylamine under ice cooling, and the solution was extracted four times with 20 mL of chloroform. After washing of the combined chloroform extracts with 10 mL of 1 N HCl, the solution was dried over anhydrous Na_2SO_4 overnight. Chloroform was distilled in vacuo under nitrogen. The product (**23**) was isolated by column chromatography on alumina eluting with petroleum ether–ethyl acetate (20:1). **23:** NMR δ ($CDCl_3$) 1.78 (3 H, singlet, CH_3), 3.10 (2 H, doublet, $-CH_2C=$, J = 6 Hz), 5.06–5.77 (3 H, multiplet, vinyl H), 7.8 (4 H, multiplet, aromatic H); IR (neat) 1685 ($\nu_{C=O}$), 1250, 945 ($=CH_2$) cm^{-1}.

Kinetic Measurements for Hydrogen Peroxide Attack on Vitamin K Analogues. A 2-mL solution of 1×10^{-3} M 2-methylnaphthoquinone-1,4 (**8**), 2-allyl-3-methylnaphthoquinone-1,4 (**7a**), or 2-allyl-3-prenylnaphthoquinone-1,4 (**7d**) in phosphate buffer of pH 9.0 (30% methanol by volume) was put into a quartz cuvette. The cuvette was placed in the cell holder of a Union High Speed UV Spectromonitor Model SM-303, whose cell chamber was thermostated at 32.0 ± 0.1 °C by circulating thermostated water. β-Cyclodextrin (2.5×10^{-3} to 1×10^{-2} M) was added to the above solution of quinones for the kinetic measurement of the inhibitory action of β-cyclodextrin on the H_2O_2 oxidation of the quinones. To the cuvette was added 2 mL of hydrogen peroxide solution (1×10^{-2} M) (also thermostated) to initiate the reaction. The reaction was followed spectrophotometrically by monitoring the decrease in the absorbance of quinones at 340 nm. Each kinetic run followed pseudo-first-order kinetics up to the second half-life; correlation coefficients of the lines obtained were

0.9998–0.9954 (15–20 points). The dependence of pseudo-first-order rate constants on the β-cyclodextrin concentration was analyzed by the use of eq 6. Data processing was carried out using a nonlinear least-square regression program on a Union System 77 computer.

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$$a(1-x)\mathrm{CD}\cdot\mathrm{AH} \overset{\mathrm{p}K_a}{\rightleftharpoons} ax\mathrm{CD}\cdot\mathrm{A}$$
$$K_{ass} = 490\ \mathrm{M}^{-1} \uparrow\downarrow \qquad \uparrow\downarrow K_{ass}'$$
$$b(1-y)\mathrm{AH} \overset{\mathrm{p}K_a' = 9.45}{\rightleftharpoons} by\mathrm{A}$$

respectively. At the titration point (pH 8.93), the amount of CD·AH plus AH should be equal to the amount of CD·A plus A. The corrected pK_a value of 8.90 and K_{ass}' value of 1737 were obtained based on the above assumptions.

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(20) 9,10-Anthraquinone, which is ca. 11 Å in width and, therefore, is too big to be inserted sideways into the cavity of β-cyclodextrin, exhibited no significant blue shift of the λ_{max} upon inclusion (332 nm in water, ca. 331 nm in the inclusion complex). Meanwhile, the inclusion of 2-methyl-1,4-naphthoquinone (**8**) by β-cyclodextrin showed a blue shift of the λ_{max} from 339 nm (in water or in 30% MeOH–water) to 336 nm, strongly suggesting that the mode of inclusion differs from that of anthraquinone. The different modes of inclusion of **8** and 9,10-anthraquinone are also reflected in the differences in the K_{ass} values: 310 M^{-1} for **8**; 45 ± 20 M^{-1} for 9,10-anthraquinone, pH 9, borate buffer (30% MeOH). These results strongly support the postulate that **8** is included sideways by β-cyclodextrin, as shown in Scheme I, in marked contrast to anthraquinone. The protection of **8** from attack by hydrogen peroxide also strongly supports the postulated mode of interaction of the naphthalene ring with β-cyclodextrin.

Superoxide Dismutase Activities of an Iron Porphyrin and Other Iron Complexes

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Abstract: The superoxide dismutase (SOD) activities of a number of metal complexes have been measured and a mathematical model is proposed which allows their rate constants to be calculated. $Fe(CN)_6^{4-}$ showed no activity whereas $Fe(CN)_6^{3-}$ appeared to react stoichiometrically with superoxide. True catalytic SOD activity appeared to be shown by chelates of iron with EDTA [$Fe(EDTA)^-$ and $Fe(EDTA)^{2-}$], with diethylenetriaminepentaacetic acid [$Fe(DTPA)^{2-}$ and $Fe(DTPA)^{3-}$], and with *o*-phenanthroline [$Fe(phen)_3^{2+}$]. The most efficient superoxide dismutase catalyst of the compounds tested here was found to be tetrakis(4-*N*-methylpyridyl)porphineiron(III) [$Fe^{III}TMpyP$], with a rate constant of 3×10^7 M^{-1} s^{-1} at pH 10.1. Exposure of this compound to H_2O_2 produced in a superoxide-generating system caused its degradation to a product with lower SOD activity, but it could be protected by catalase. H_2TMpyP itself showed no SOD activity, nor did its complexes with Zn(II) or Cu(II). $Co^{III}TMpyP$ showed much lower SOD activity than the Fe(III) complex.

That molecular oxygen is indispensable for most life forms has been known since the time of Priestly and Lavoisier. More recently, investigations have demonstrated that, as oxygen is utilized by organisms, highly toxic intermediates are produced on the pathway to the formation of water. Respiring organisms have evolved a system of defenses against these dangerous metabolites which allows them to enjoy the benefits of living in an oxygen-rich environment. As part of this protective arsenal, superoxide dismutases are present in aerobes to catalyze the conversion of superoxide ion into H_2O_2 and O_2, while catalase and the peroxidases function to scavenge peroxides. Not only are H_2O_2 and O_2^- potentially deleterious in their own right, but evidence exists that under appropriate conditions they can react together to form the even more lethal OH· radical.[2-5]

Peroxide eliminating enzymes as well as superoxide dis-

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