transition states are of roughly equal free energy. Perfect symmetry would mean that the binding energy of cytochrome *c* to the electrode is independent of its oxidation state. We have suggested[31] that the simplest mechanism to facilitate an enzyme-catalyzed reaction is for the enzyme to bind the substrate and product in a nondiscriminatory fashion and that equality in the free energies of the different transition states may be a feature of efficient enzymes reacting with their natural substates. Finally, it is interesting that, by use of the rotating-disk and ring–disk techniques, it is possible to obtain a free-energy profile for an electrochemical reaction including the kinetics of the adsorption and desorption steps.

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Conclusions

The free-energy profile for the electron-transfer reaction of cytochrome *c* at the 4,4′-bipyridyl surface-modified gold electrode emphasizes the importance of the preceding binding step in the electron-transfer kinetics of cytochrome *c*. Furthermore, it suggests that, with the development of other, suitably modified electrode surfaces, it should prove possible to enhance the rates of electron transfer between electrodes and metalloproteins in general. Such surface-modified electrodes would be of use not only in the study of the electron-transfer mechanisms of metalloproteins but also in the exploitation of enzymes as electrocatalysts.

Acknowledgment. This is a contribution from the Oxford Imperial Energy Group. We thank the S.R.C., N.R.D.C., and Johnson Matthey Ltd. for financial assistance.

Model Studies for a Molecular Mechanism of Action of Oral Anticoagulants

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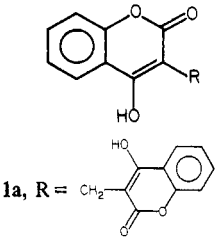
Contribution from the Department of Chemistry, Northwestern University, Evanston, Illinois 60201. *Received August 18, 1980*

Abstract: Warfarin [3-(α-acetonylbenzyl)-4-hydroxycoumarin], a potent oral anticoagulant agent, is known to inhibit the enzyme vitamin K epoxide reductase (Whitlon et al., ref 18 b). The molecular mechanism of inhibition, however, is not known. It is proposed that the two major classes of oral anticoagulants, the 3-substituted-4-hydroxycoumarins and the 2-substituted-1,3-indandiones, are mechanism-based inactivators of this enzyme. The proposed mechanism of inactivation involves enzyme-catalyzed activation of the oral anticoagulants by tautomerization to the hypothetically reactive diketo forms which then undergo attack by active-site nucleophiles. In order to test the chemistry of this proposal, it is shown that the two classes of oral anticoagulants are unreactive toward bases and nucleophiles (except for deprotonation), until they are electrophilically substituted at the 3 position of the coumarins or at the 2 position of the indandiones. These model compounds for the proposed enzyme-generated reactive intermediates, then, are shown to be highly reactive toward a variety of nucleophiles and support the hypothesis that the oral anticoagulants are converted by vitamin K epoxide reductase into reactive compounds which can acylate an active-site nucleophile and thereby inactivate the enzyme.

The oral anticoagulants, i.e., 3-substituted-4-hydroxycoumarins (e.g., warfarin, **1b**) and 2-substituted-1,3-indandiones (e.g., phenindione, **2a**), are some of the most important drugs for the prevention and treatment of a variety of venous and, to a lesser extent, arterial thromboembolic disorders.[1] Fifty percent of hospitalized patients that die show evidence of antemortem thromboembolism. The first oral anticoagulant, Dicumarol (**1a**),

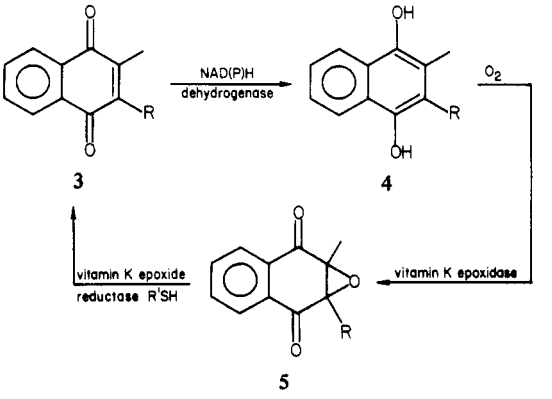


1a, R =

1b, R = $CH(Ph)CH_2COCH_3$
1c, R = CH_3

was isolated from spoiled sweet clover and was shown to be responsible for the hemorrhagic sweet clover disease of cattle.[2] That same year its structure was identified,[3] it was chemically synthesized[4] and was shown to be a clinically useful anticoagulant drug.[5] Numerous analogues of Dicumarol and of 4-hydroxycoumarins were synthesized and tested for anticoagulant activity.[6] Warfarin, 3-(α-acetonylbenzyl)-4-hydroxycoumarin (**1b**), origi-

Scheme I. Vitamin K–Vitamin K Epoxide Cycle (R = Phytyl)



3 4 5

(1) Levine, W. G. In "The Pharmacological Basis of Therapeutics", 5th ed., Goodman, L. S.; Gilman, A., Ed.; New York: MacMillan, 1975.
(2) Campbell, H. A.; Link, K. P. *J. Biol. Chem.* **1941**, *138*, 21.
(3) Stahmann, M. A.; Heubner, C. F.; Link, K. P. *J. Biol. Chem.* **1941**, *138*, 513.
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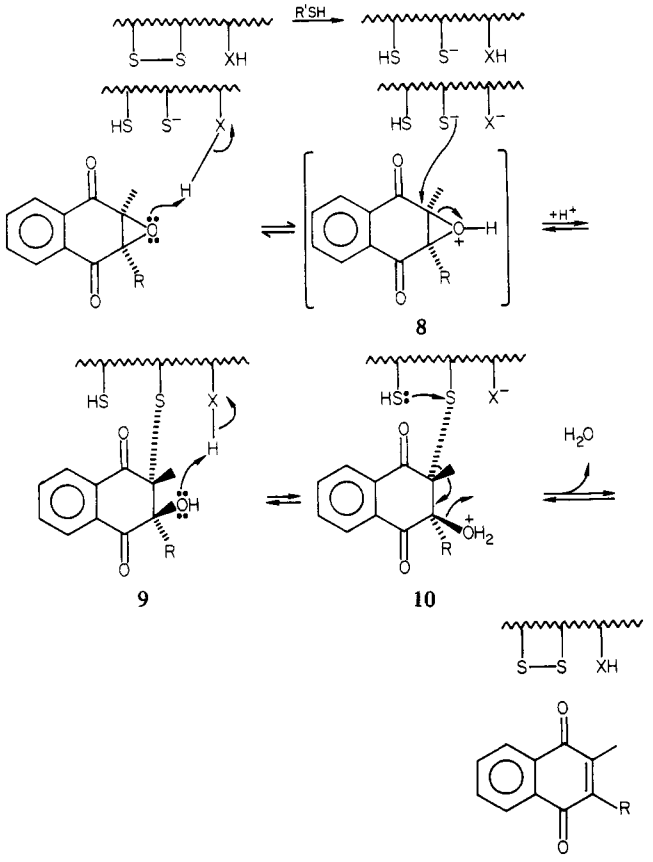
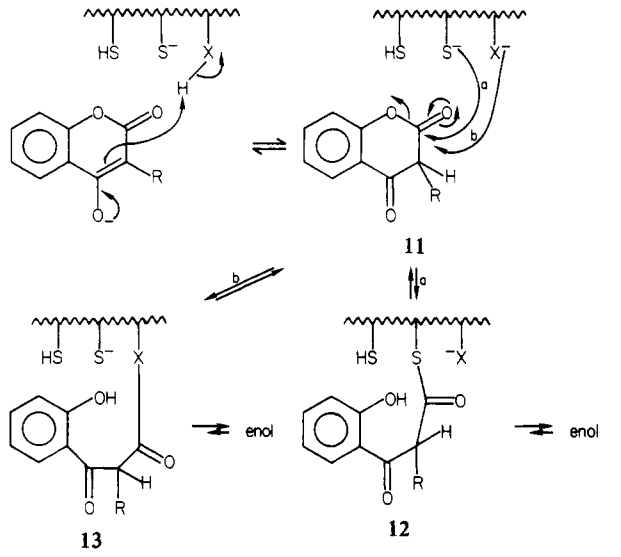
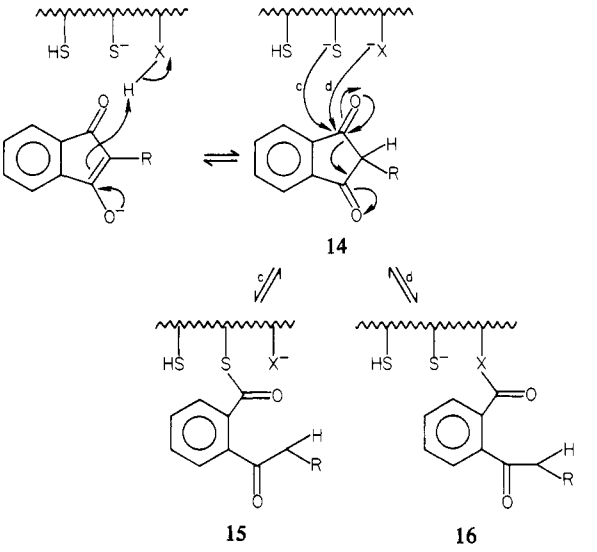
Scheme II. Proposed Mechanism of Action of Vitamin K Epoxide Reductase (R = Phytyl)



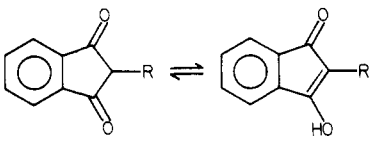
Scheme III. Proposed Mechanism of Inactivation of Vitamin K Epoxide Reductase by 3-Substituted-4-hydroxycoumarins



Scheme IV. Proposed Mechanism of Inactivation of Vitamin K Epoxide Reductase by 2-Substituted-1,3-indandiones



nally used as a rodenticide,[7] is now the most widely prescribed oral anticoagulant.[1] Another important oral anticoagulant, 2-phenyl-1,3-indandione (**2a**) was reported as having the same effect



2a, R = Ph

as Dicumarol but whose action was more rapid and less prolonged.[8] Numerous indandiones were synthesized[9] and were tested for anticoagulant activity. The nomenclature of these compounds (**2a**) is misleading since the equilibrium favors the enol when R is aryl.[9]

The vitamin K–vitamin K epoxide cycle (Scheme I) was shown to be inhibited by warfarin.[10] The structure of vitamin K (**3**), a fat-soluble vitamin which is required for coagulation of blood,[11] was determined about 40 years ago.[12] Its mode of action, however, was not suggested until about 7 years ago when it was found that vitamin K is required for the biosynthesis of active prothrombin and other plasma-clotting factors.[13] The active form of the vitamin is the reduced (hydroquinone) form[14] (**4**) and its conversion into vitamin K 2,3-epoxide (**5**) is catalyzed by the O_2-dependent enzyme vitamin K epoxidase. There is a direct correlation between the epoxidase activity and the activity of a vitamin K dependent carboxylase which is responsible for the activation of prothrombin and therefore coagulation of blood, but the mechanism of this dependency is unknown.[15] Vitamin K epoxide reductase activity has been observed,[16] and the enzyme has been solubilized;[17] vitamin K epoxide reductase catalyzes the conversion of the epoxide back to vitamin K, thereby completing the vitamin K–vitamin K epoxide cycle. Warfarin was shown to inhibit vitamin K epoxide reductase from rat liver microsomes,[17,18] even when vitamin K epoxidase activity was absent.[17] This suggests that the anticoagulant activity exhibited by warfarin arises from the blockage of the regeneration of vitamin K from the epoxide

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(8) Soulier, J. P.; Gueguen, J. *C.R. Seances Soc. Biol. Ses. Fil.* **1947**, *141*, 1007.
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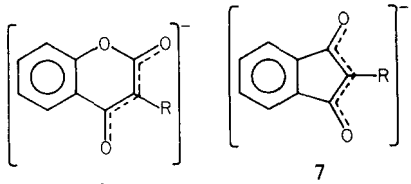
within the cell. Recently, a chemical model for a molecular mechanism of anticoagulant activity of 3-substituted-4-hydroxycoumarins was reported[19] in which it was proposed that these compounds may be mechanism-based inactivators[20] of vitamin K epoxide reductase (Schemes II and III). This chemical model has been extended to include the other major class of oral anticoagulants, the 2-substituted-1,3-indandiones (Scheme IV). A detailed discription of these model studies is reported here.

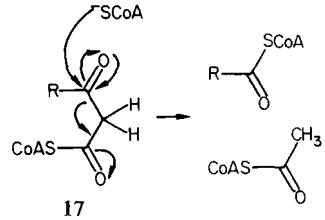
Results and Discussion

If the oral anticoagulants are mechanism-based inactivators of vitamin K epoxide reductase as proposed,[19] then they must have the following properties: they must (1) have a structural similarity to the enzyme substrates or products in order to assure E·I complex formation, (2) be unreactive toward enzyme active-site nucleophiles, (3) be converted into reactive compounds via the normal mechanism of action of the enzyme, and (4) form a covalent bond to an active-site nucleophile. The structures of 3-substituted-4-hydroxycoumarins (**1**) and of 2-substituted-1,3-indandiones (**2**) are quite similar to that of vitamin K, the product of the vitamin K epoxide reductase catalyzed reaction. Overman et at.[6a] prepared a series of 3-substituted-4-hydroxycoumarins and found that the potency of the anticoagulant effect was a function of the structure of the 3-substituent. This substituent may be important to the stability of the E·I complex but may not be involved in the chemistry of the inhibition of the enzyme. Consequently, for simplicity, 3-methyl-4-hydroxycoumarin (**1c**) was synthesized as the model for the 4-hydroxycoumarin class of oral anticoagulants. In the case of the indandiones, however, the potent anticoagulant phenindione (**2a**) was used. The enol form of **2**, which is the favored isomeric form when R is aryl,[9] strongly resembles the structure of the 4-hydroxycoumarins, suggesting a similar mechanism of action for these two classes of compounds. The stability of the 3-substituted-4-hydroxycoumarins and 2-substituted-1,3-indandiones toward bases and nucleophiles was demonstrated by treating **1c** and **2a** with aqueous sodium hydroxide, ethanolic sodium ethoxide, *n*-propylamine, and ethanol under vigorous conditions. Essentially quantitative yields of starting material were recovered under all conditions after reacidification. Presumably, the reason for this stability is that the most facile reaction is removal of the acidic proton to give the highly resonance-stabilized anions **6** and **7** which resist nucleophilic attack.



6 7

In fact, when any of the above-mentioned bases is added to a solution of **2a**, it turns deep red, suggesting a highly conjugated system. The mechanism by which vitamin K epoxide reductase acts is not known. It is known, though, that the isolated enzyme requires added thiol for activity and this requirement cannot be substituted by NAD(P)H.[17] A mechanism for this enzyme has been proposed,[19] which is shown in Scheme II. In accordance with this mechanism, the oxidized form of the enzyme is activated by thiol reduction. Physiologically, this activation could be carried out by glutathione. Once the enzyme is reduced, vitamin K epoxide could bind to the enzyme and be activated by protonation of the epoxide oxygen (**8**). For simplicity, a general acid is shown as the proton donor in Scheme II. Backside attack of **8** by a sulfhydryl group would lead to the β-hydroxy sulfide **9**. Most likely, protonation and sulfhydryl attack would be concerted. Epoxides are well-known to react with thiols, especially with acid catalysts, to give β-hydroxy sulfides.[21] Protonation of the β-hydroxyl group generates an active leaving group for reductive elimination which is promoted by another active-site sulfhydryl group (**10**). This yields vitamin K and regenerates the oxidized form of the enzyme. This step is analogous to the reductive elimination of β-keto sulfides to ketones, which occurs when they are treated with thiols.[22] With use of the mechanism shown in Scheme II as a working hypothesis, a mechanism of inactivation of vitamin K epoxide reductase by 3-substituted-4-hydroxycoumarins was proposed[19] and is shown in Scheme III. Analogous to the proposed enzyme-catalyzed protonation of the epoxide of the substrate, the double bond of the hydroxycoumarins could be protonated to give 3-substituted-2,4-chromandiones (**11**), the unstable tautomeric forms of **1**. These compounds, substituted *o*-ketophenyl lactones, should have reactivities intermediate between those of phenyl esters and *o*-nitrophenyl esters and would be expected to be susceptible to nucleophilic attack at the lactone carbonyl group. Although this does not occur in solution, as described above for the reactions of **1c** and **2a** with bases, nucleophiles at the active site of an enzyme, which may be free of solvent, may be capable of such a reaction. If the mechanism in Scheme II is valid, then the conditions necessary for acylation, as opposed to proton removal and enolization, would be optimal. This nucleophilic reaction could lead to **12** by pathway a or to **13** by pathway b (Scheme III). Similarly, the indandiones could acylate the enzyme by the mechanism shown in Scheme IV. Attack of **14** by an active-site nucleophile to give **15** or **16** would be an example of a retro-Claisen condensation, a common organic reaction[23] which also has precedence in enzyme systems. For example, the ubiquitous enzyme thiolase (β-ketoacyl thiolase) catalyzes the cleavage of β-ketoacyl-CoA substrates (**17**) by CoASH.[24] Note that in the thiolase reaction, the less stable keto form of the substrate also is the important isomer.



17

Since the tautomeric structures **11** and **14** do not acylate nucleophiles in solution because of facile proton removal, although they could acylate an active-site nucleophile as exemplified above for thiolase, it was necessary to synthesize model compounds. The models were designed to have the following characteristics: to be 2,4-chromandiones and 1,3-indandiones which could not enolize (i.e., contain no acidic protons) and which could be prepared by an electrophilic addition reaction in order to mimic the enzyme-catalyzed (electrophilic) proton addition step (**1** → **11**, Scheme III, and **2** → **14**, Scheme IV). Initially, methylation of **1c** was attempted, but all three possible methylation products were formed and there was difficulty in separating them without the desired isomer decomposing. That would have been the ideal reaction, but because of the technical difficulties, electrophilic chlorination, which rapidly gave only the C-chlorinated product **18** in high yield, was employed. The allyl analogue **21** also was synthesized from **1c** with allyl bromide bromide and triethylamine, a reaction which gives almost exclusive C-allylation, maybe because of the possibility of a [3,3] sigmatropic (Claisen) rearrangement of the *O*-allyl ether to the C-allyl isomer.[25] This

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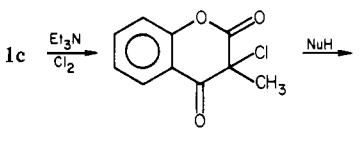

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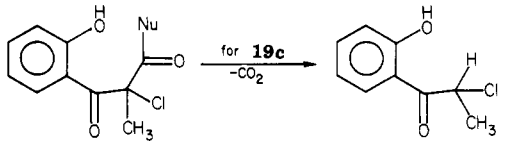
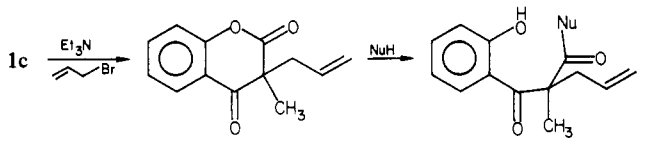


19a, Nu = NHPr
b, Nu = OEt
c, Nu = OH

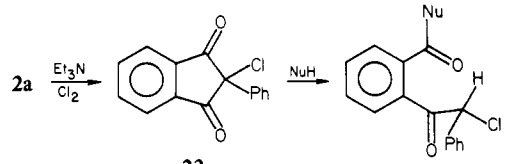
20



21

22a, Nu = NHPr
b, Nu = OEt

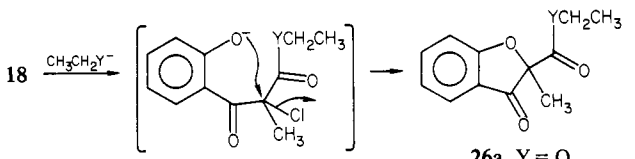
compound was prepared (after the studies on the chlorinated compounds were completed) as a model compound whose electronic properties would mimic more closely those of the proposed reactive acylating agent (**11**). It could be argued that **18** might be more reactive than **11** because of the electronegativity of the chlorine; however, small differences in solution reactivities should be insignificant relative to the catalytic capabilities of an enzyme. Nonetheless, compound **21** was prepared in order to demonstrate that it was capable of undergoing acylation reactions as well. The chlorinated indandione (**23**) was synthesized by the same route as **18**.



23

24a, Nu = NHPr
b, Nu = OEt

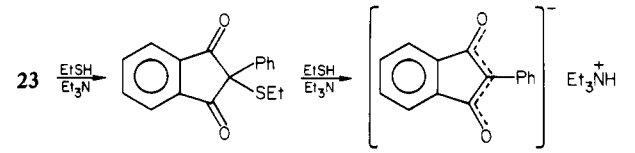
The reaction of compounds **18**, **21**, and **23** with *n*-propylamine (a model for the reaction of **11** and **14** with an active-site amino-containing residue) were all very fast and yielded the desired amides, **19a**, **22a**, and **24a**, in high yields. Since a kinetic study was not undertaken, the relative rates are not known. The important point, though, is that they all reacted rapidly and that the difference in reactivities between the allyl and chloro compounds is not that great. Therefore, it appears that these model reactions are consistent with the proposed enzyme acylation reactions (**11** → **13** and **14** → **16**). The reactions of **18** and **21** with absolute ethanol (a model for the reaction of **11** with an active-site hydroxyl-containing residue) seem to be reversible because of two observations. First, compound **19b** can be converted back to **18** with heat. Second, when a 0.3 M solution of **21** in absolute EtOH was refluxed for 28 h, the same ratio (1:1) of **21** and **22b** was obtained as when it was refluxed for only 13 h. This is, apparently, the equilibrium mixture. The indandione analogue **23** is not as reactive as the chromandione compound (**18**) as evidenced by the lack of reaction of **23** with refluxing EtOH for 12 h, whereas the reaction of **18** with EtOH was complete in less than 1 h. However, when triethylamine was added to the EtOH, the reaction with **23** also was complete in less than 1 h. Although the pK_a of triethylammonium is about 10^6 times lower than that of ethanol,[26] the low equilibrium concentration of ethoxide ion must be sufficient to effect the reaction. A small amount (~7%) of a byproduct, having the same R_f on silica gel TLC (CH_2Cl_2) as **23**, was obtained. Since approximately the same amount (6%) of Et_3N·HCl also was isolated in the reaction, this byproduct may be the S_N2 reaction product, 2-ethoxy-2-phenyl-1,3-indandione. Treatment of **18** with sodium ethoxide gave an immediate reaction which yielded not **19** but instead compound **26a**. This product may arise



25a, Y = O
b, Y = S

26a, Y = O
b, Y = S

from the desired reaction with ethoxide to give **25a** followed by an intramolecular cyclization via an S_N2 displacement of chloride ion. When triethylammonium thioethoxide was used as the nucleophile (a model for the reaction of **11** with an active-site cysteine residue), compound **26b** was obtained, presumably via the desired intermediate **25b**. Recently, Donnelly et al.[27] proposed a similar intramolecular cyclization for an analogous system. This conversion of **25** → **26** has no analogy in the proposed enzyme inactivation mechanisms because the substituent in **11**, a proton, is not a leaving group. Nonetheless, it was helpful in identifying the structures of the desired initial reaction products (**25**). When sodium ethoxide was used as the base with **23**, an immediate reaction ensued. However, a mixture of products was obtained, presumably as a results of some ethanolysis and some S_N2 displacement of chloride ion. These products were not identified. However, when ethanethiol and triethylamine were added to **23**, 1 equiv of triethylamine·HCl was obtained. The other product in the reaction was found to be 2-phenyl-2-(ethylthio)-1,3-indandione (**27**), the product of an S_N2 displacement of chloride ion. Treatment of this compound with one more equivalent each of ethanethiol and triethylamine gave a product whose NMR and IR spectra were identical with the triethylammonium salt of 2-phenyl-1,3-indandione (**28**). Presumably, the byproduct would



27 **28**

be diethyl disulfide. This sequence of reactions is another example of a thiol-promoted reductive elimination of a β-keto sulfide to give a ketone[22] and can be considered as a related model for the conversion of **10** to vitamin K in the proposed mechanism of action of vitamin K epoxide reductase (Scheme II). Reacidification of **28** gave 2-phenyl-1,3-indandione. Similar S_N2 reactions occurred with benzyl mercaptan and thiophenol and, therefore, because of this undersired side reaction, it was not possible to carry out a model for the reaction of **14** with a cysteine residue. The fact that **23** reacted with an amine and an alcohol at the carbonyl and since the anticoagulants do not contain a halogen, this thiol model reaction can be considered as an unrelated anomaly. Compound **18** hydrolyzes and decarboxylates in 50% aqueous dioxane over several hours. When the reaction was carried out in 0.6 M Hepes buffer in 1:1 dioxane/D_2O, pD 7.4 at 35 °C, the half-life was about 3 min as determined by NMR. A singlet, 0.1 ppm upfield of the methyl protons singlet of starting material, begins to appear. While that peak increases in size, the methyl proton doublet of the hydrolysis and decarboxylation product (**20**) appears. Ultimately, the starting material peak and the upfield singlet disappear and only the doublet remains. Although the compound responsible for the upfield singlet has not been stable enough to isolate under these conditions, it is presumed to be the hydrolysis product,

(26) Hendrickson, J. B.; Cram, D. J.; Hammond, G. S. "Organic Chemisty", 3rd ed.; McGraw-Hill: New York, **1970**; p 304.

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p-(2-carboxy-2-chloropropionyl)phenol (**19c**).

The model studies described in this paper support the proposed molecular mechanism of action of the two major classes of oral anticoagulants as mechanism-based inactivators of vitamin K epoxide reductase (see Scheme II–IV). This hypothesis is consistent with certain physiological observations reported. Because of the relatively slow uptake of vitamin K into a cell, it has been suggested[15b] that the cell biosynthesizes vitamin K epoxide for intracellular storage of an inactive form of the vitamin. When the cell needs vitamin K rapidly, it would have ready access to it via reduction of the inactive epoxide, catalyzed by vitamin K epoxide reductase. This process of converting the epoxide into active vitamin K could be regulated by the cell just by controlling whether the enzyme was in the inactive disulfide form or the active dithiol form as proposed in Scheme II. If the epoxide were just the storage form of vitamin K, then *in the presence of vitamin K*, the cell temporarily would have no need for vitamin K epoxide reductase. Therefore, if an animal were treated with warfarin, which inactivates vitamin K epoxide reductase, the anticoagulant effect should be reversed and a coagulation response observed by administering vitamin K. This is, in fact, the case.[28] It also has been found that after treatment with warfarin, blood coagulatability rises *slowly* over several days until it reaches the normal level.[29] This slow recovery could arise from regeneration of the clotting factors as a result of new protein biosynthesis or could arise from regeneration of vitamin K epoxide reductase activity as a result of slow hydrolysis of the proposed acyl enzyme (**12**, **13**, **15**, or **16**) or both.

Experimental Section

General. For reactions requiring anhydrous conditions, the glassware was flame dried, and the reactions were run under dry nitrogen or argon. Tetrahydrofuran was dried by distillation from sodium benzophenone ketyl, dioxane was dried by stirring with KOH for several hours followed by distillation from sodium, and benzene was distilled from sodium. Triethylamine was purified by distillation from sodium. Chlorine gas was dried by bubbling through concentrated H_2SO_4. 2-Phenyl-1,3-indandione was purchased from Sigma Chemical Co., St. Louis, MO. All other reagents and solvents were of reagent grade and were used without further purification. Column chromatography was performed with silica gel, grade 923, 100–200 mesh from Davison Chemical, Baltimore, MD. E. Merck precoated silica gel 60F 254 sheets (thickness 0.20 mm) were used for thin-layer chromatography. Infrared spectra were recorded on a Perkin-Elmer 283 infrared spectrophotometer, and NMR spectra were obtained in $CDCl_3$ on a Perkin-Elmer–Hitachi R20B or Varian EM-360 60-MHz NMR spectrometer. Chemical shifts are reported in parts per million downfield from tetramethylsilane. Melting points were obtained on a Thomas–Hoover Unimelt apparatus in open capillary tubes and are uncorrected. Boiling points are uncorrected. Microanalyses were performed by Micro-Tech Laboratories, Skokie, IL, or by H. Beck, Northwestern University Analytical Services Department.

3-Methyl-4-hydroxycoumarin (1c). This was prepared by the method of Stahmann et al.[30] except that the conversion of methyl propionylsalicylate to **1c** was carried out by using NaH (2 equiv) as the base in refluxing dry benzene for 3 days. The product generally was obtained as off-white needles in 30–40% yield after recyrstallization from EtOH–H_2O.

Reactions of 3-Methyl-4-hydroxycoumarin (1c) and 2-Phenyl-1,3-indandione (2a) with Sodium Hydroxide. **1c** or **2a** (2.0 mmol) was dissolved in water (10 mL) containing NaOH (4.0 mmol) and was heated on a steam bath for 1 h. The solutions were cooled in an ice bath and acidified to below pH 2 with 6 N HCl. The precipitates were collected by filtration, washed with water, and dried under vacuum and were identified by IR as **1c** and **2a** in 95% and 98% yield recovery, respectively.

Reactions of 1c and 2a with Sodium Ethoxide. **1c** and **2a** (2.0 mmol) were dissolved in absolute ethanol (7.5 mL) containing sodium ethoxide (4.0 mmol) and were heated to reflux under a drying tube for 30 min. The solutions were cooled to room temperature, and the ethanol was removed by rotary evaporation. The residues were taken up in H_2O (10 mL) and acidified to below pH 2 with 6 N HCl. The precipitates were filtered, washed with H_2O, and dried under vacuum and were identified by IR as **1c** and **2a** in 98% yields.

Reactions of 1c and 2a with *n*-Propylamine. **1c** (1.0 mmol) and **2a** (2.0 mmol) were dissolved in dry dioxane (5 mL) containing *n*-propylamine (2.0 and 4.0 mmol, respectively) and heated under N_2 in a 90 °C oil bath for 30 min. The solutions were cooled to room temperature, and the solvent was rotary evaporated. The residues were taken up in 10 mL of H_2O (in the case of **2a**, not all dissolved) and acidified to below pH 2 with 6 N HCl. The precipitates were filtered, washed with H_2O, and dried under vacuum and were identified by IR as **1c** and **2a** in 93% and 98% yield recoveries, respectively.

Reactions of 1c and 2a with Ethanol. **1c** and **2a** (1.0 mol) were heated in refluxing absolute ethanol for 12 h. The solvent was rotary evaporated and the residues, identified as **1c** and **2a**, respectively, were recovered in quantitative yield.

3-Chloro-3-methyl-2,4-chromandione (18). Triethylamine (5.2 mL, 37 mmol) was syringed into a stirred solution of **1c** (6.16 g, 35 mmol) in dry THF (200 mL) under N_2. The pale-yellow solution was cooled in an ice bath and dry Cl_2 was bubbled slowly into it for 30 min. Excess Cl_2 was removed by bubbling N_2 into the reaction mixture for 15 min in an ice bath and then for 10 min in a room-temperature water bath. The solvent was removed by rotary evaporation (a drying tube was placed in the line from the aspirator). The resulting off-white semisolid was triturated with anhydrous ether (3 × 200 mL) and filtered. The insoluble solid was identified as triethylamine·HCl by IR and NMR. The combined filtrates were evaporated to a semisolid, which was recrystallized from *n*-hexane to give 6.3 g (85%) of **18** as off-white shiny needles. An additional recrystallization from *n*-hexane gave the product as shiny white needles: mp 140–140.5 °C; NMR ($CDCl_3$) δ 1.99 (s, 3 H), 7.1–8.1 (m, 4 H); IR (KBr), 1780 (s), 1705 (s), 1607 (s), 1455 (s), 1320 (s), 1215 (s), 1090 (s) cm^{-1}. Anal. Calcd for $C_{10}H_7ClO_3$; C, H, Cl.

***o*-[2-(*N*-Propylcarboxamido)-2-chloropropionyl]phenol (19a).** *n*-Propylamine (83 μL, 1.0 mmol) was added dropwise to a stirred solution of **18** (210 mg, 1.0 mmol) in dry dioxane (1.0 mmol). An immediate flash of yellow color appeared and disappeared with each drop. When the addition was complete, the reaction solution was rotary evaporated to a pale-yellow oil, which slowly solidified. The off-white solid (270 mg, quantitative) was recrystallized from *n*-hexane to give **19a** as shiny fine white needles: mp 88.5–89 °C, NMR ($CDCl_3$) δ 0.95 (t, 3 H), 1.52 (m, 2 H), 1.96 (s, 3 H), 3.28 (m, 2 H), 6.6–7.8 (m, 5 H), 11.6 (s, 1 H); IR (KBr) 3320 (s), 3200 (b), 1640 (s), 1610 (m), 1525 (m), 1435 (m) cm^{-1}. Anal. Calcd for $C_{13}H_{16}ClNO_3$; C, H, Cl, N.

***o*-(2-Carbethoxy-2-chloropropionyl)phenol (19b).** Compound **18** (2.1 g, 10 mmol) was heated in refluxing absolute ethanol (50 mL) for 1 h. The solvent was removed by rotary evaporation and the residual solvent removed in vacuo to give 2.5 g (97%) of **19b** as a yellow oil: NMR ($CDCl_3$) δ 1.11 (t, 3 H), 1.98 (s, 3 H), 4.22 (q, 2 H), 6.6–7.85 (m, 4 H), 11.55 (s, 1 H); IR (film) 3100 (b), 2990 (s), 1750 (s), 1720 (s), 1640 (s), 1610 (m), 1575 (m), 1480 (m), 1445 (s) cm^{-1}.

Thermolysis of 19b. Compound **19b** (2.5 g) was distilled at 102–103 °C (0.05 mm) to give a pale-yellow oil, which on standing crystallized. NMR showed that this was a 60:40 mixture of **19b** and **18**. Upon heating this product at 120–130 °C for 33 h, **18** was obtained and identified by NMR and IR.

Reaction of 18 with Sodium Ethoxide (26a). A 10-mL (10 mmol) solution of sodium ethoxide (prepared from 757 mg of Na in 32.9 mL of absolute EtOH) was added dropwise to a stirred solution of **18** (2.1 g, 10 mmol) in dry dioxane (20 mL). With each drop of ethoxide, a yellow color formed and dissipated. After the solution was stirred for 10 min, the solvent was removed by rotary evaporation, 1 M HCl (10.5 mL) was added to the yellow oil residue, and the product was extracted with 3 × 20 mL of ether. The combined ether extracts were dried ($MgSO_4$) and evaporated to 1.92 g (87%) of an almost colorless oil. After distillation, a colorless oil was obtained: bp 84 °C (0.15 mm); NMR ($CDCl_3$) δ 1.21 (t, 3 H), 1.71 (s, 3 H), 4.20 (q, 2 H), 6.95–7.25 (m, 2 H), 7.45–7.8 (m, 2 H); IR (film) 2990 (m), 1750 (s), 1720 (s), 1605 (s), 1470 (m), 1460 (m), 1260 (s), 1125 (m), 1090 (m) cm^{-1}. Anal. Calcd for $C_{12}H_{12}O_4$; C, H.

Reaction of 18 with Ethanethiol and Triethylamine (26b). Ethanethiol (0.67 mL, 9 mmol) was added dropwise to a stirred solution of **18** (1.9 g, 9 mmol) in dry dioxane (25 mL) under Ar at 10–15 °C. Then triethylamine (1.26 mL, 9 mmol) was added, whereupon a white precipitate (Et_3N·HCl) formed immediately. The mixture was stirred at 10–15 °C for 15 min and then at room temperature for 30 min. The solvent was removed by rotary evaporation and the semisolid residue was triturated with anhydrous ether (3 × 30 mL) and filtered. The combined filtrates were evaporated to give 1.73 g (82%) of a pale-yellow oil, which crystallized upon standing for 1 h. The product was Kugelrohr distilled (150 °C, 0.07 mm) to give a colorless oil which crystallized: mp 49–50 °C; NMR ($CDCl_3$) δ 1.13 (t, 3 H), 1.79 (s, 3 H), 2.60 (q, 2 H), 6.95–8.0

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(m, 4 H); IR (KBr) 1770 (s), 1760 (s), 1690 (s), 1685 (sh), 1608 (s), 1458 (s) cm^{-1}. Anal. Calcd for $C_{12}H_{12}O_3S$; C, H, S.

Reaction of 18 with Water (20). A solution of **18** (431 mg, 2 mmol) in 50% aqueous dioxane (20 mL) was allowed to stand at room temperature for 12 h and then concentrated to about one-fourth volume and extracted with ether (3 × 10 mL). The combined extracts were dried ($MgSO_4$), and the solvent was evaporated to give 281 mg (76%) of a yellow oil. Distillation yielded a pale-yellow oil: bp 74 °C (0.4 mm) (lit.[31] bp 126–128 °C (10 mm)); NMR ($CDCl_3$) δ 1.72 (d, 3 H), 5.27 (q, 1 H), 6.7–7.9 (m, 4 H), 11.8 (s, 1 H); IR (film) 3100 (b), 1640 (s), 1610 (m), 1575 (m), 1480 (m), 1450 (m) cm^{-1}.

3-Allyl-3-methyl-2,4-chromandione (21). Triethylamine (3.5 mL, 25 mmol) was syringed into a solution of **1c** (4.4 g, 25 mmol) in dry THF (100 mL)) under N_2. Allyl bromide (3.5 mL, 40 mmol) was added and the reaction solution was heated at 70 °C for 45 h. The reaction mixture was evaporated to an off-white solid which was triturated with anhydrous ether (4 × 100 mL) and filtered. The combined filtrates were evaporated to 4.0 g (74%) of a white solid, which was recrystallized from *n*-hexane to give the product as white needles: mp 65–65.5 °C; NMR ($CDCl_3$) δ 1.55 (s, 3 H), 2.70 (dd, 2 H), 4.85–5.9 (m, 3 H), 7.05–8.05 (m, 4 H); IR (KBr) 1787 (s), 1771 (s), 1690 (s), 1602 (s), 1455 (s), 1328 (s), 1095 (s) cm^{-1}. Anal. Calcd for $C_{13}H_{12}O_3$; C, H.

The ether-insoluble solid was Soxhlet extracted with ether for 25 h and the ether extract was evaporated to give 0.93 g (21%) of starting material.

***o*-[2-(*N*-Propylcarboxamide)-2-allylpropionyl]phenol (22a).** *n*-Propylamine (125 μL, 1.5 mmol) was added dropwise to a stirred solution of **21** (324 mg, 1.5 mmol) in dry dioxane (2 mL) under Ar. After 2 min, the reaction solution was rotary evaporated to give 388 mg (94%) of an off-white solid, which was recrystallized from *n*-hexane to give **22a** as shiny white needles: mp 121.5–122 °C; NMR ($CDCl_3$) δ 0.96 (t, 3 H), 1.4 (m, 2 H), 1.49 (s, 3 H), 2.74 (d, 2 H), 3.17 (m, 2 H), 4.8–5.95 (m, 3 H), 5.15 (s, 1 H), 6.6–7.95 (m, 4 H), 12.1 (bs, 1 H); IR (KBr) 3300 (s), 3200 (b), 1650 (s), 1622 (s), 1600 (s), 1540 (s), 1486 (s), 1437 (s) cm^{-1}. Anal. Calcd for $C_{16}H_{21}NO_3$; C, H, N.

2-Chloro-2-phenyl-1,3-indandione (23). Triethylamine (4.5 mL, 32.3 mmol) was added dropwise to a stirred solution of **2a** (6.67 g, 30 mmol) in dry THF (200 mL) under Ar. The wine-red solution was cooled in an ice bath and then dry Cl_2 was bubbled slowly into it for 30 min. The red solution turned to a thick off-white solid in a yellow solution. Excess Cl_2 was removed by bubbling Ar into the reaction mixture for 15 min in an ice bath and then for 15 min in a room-temperature water bath. The solvent was removed by rotary evaporation (a drying tube was placed in the line from the aspirator). The pale-yellow semisolid residue was triturated with anhydrous ether (3 × 100 mL) and filtered. The combined ether extracts were evaporated to an off-white solid, which upon standing in a closed container overnight turned brown. The color was removed by applying a CH_2Cl_2 solution of the product to a column of silica gel (3.7 × 52 cm; 350 g), eluting with CH_2Cl_2. The UV-fluorescent material was collected and the solvent was evaporated to give 6.82 g (89%) of **23** as a white solid, mp 114.5–116 °C. After recrystallization from *n*-hexane, shiny colorless needles were obtained: mp 115–116 °C, NMR ($CDCl_3$) δ 7.15–7.7 (m, 5 H), 7.75–8.2 (m, 4 H); IR (KBr) 1755 (m), 1720 (s), 1585 (m), 1255 (s) cm^{-1}. Anal. Calcd for $C_{15}H_9ClO_2$; C, H, Cl.

Reaction of 23 with *n*-Propylamine (24a). *n*-Propylamine (83 μL, 1.0 mmol) was added dropwise to a stirred solution of **23** (257 mg, 1.0 mmol) in dry dioxane (2 mL) under Ar. The reaction solution turned yellow within a minute of addition of the amine. After 15 min, the solvent was removed by rotary evaporation to give a semisolid, which was triturated with *n*-hexane. The resulting pale-yellow solid product (270 mg, 86%) was recrystallized from $CHCl_3$–hexane, yielding **24a** as shiny white needles: mp 155.5–156.5 °C; NMR ($CDCl_3$) δ 0.85 (m, 3 H), 1.67 (m, 2 H), 3.33 (m, 2 H), 5.37 (s, 1 H), 6.4–8.0 (m, 10 H); IR (KBr) 3230 (b), 1690 (s), 1675 (s), 1615 (m), 1470 (m) cm^{-1}. Anal. Calcd for $C_{18}H_{18}ClNO_2$; C, H, Cl, N.

Reaction of 23 with Ethanol and Triethylamine (24b). A solution of **23** (1.028 g, 4 mmol) in absolute ethanol (20 mL) containing triethylamine (2.4 mL, 4.1 mmol) under Ar was heated to reflux for 1 h. The red solution was cooled to room temperature and the solvent rotary evaporated. The resulting red oil with some fine needles in it was triturated with ether, and the insoluble off-white needles ($Et_3N{\cdot}HCl$, 38 mg) were removed by filtration. The filtrate was evaporated to a clear red–orange oil (1.186 g), which was taken up in CH_2Cl_2 and applied to a column of silica gel (2 × 39 cm, 75 g) and eluted with CH_2Cl_2. Fractions (8 mL) were collected and two products were obtained in the UV-fluorescent fractions. The fractions containing only material with R_f 0.74 (silica gel, CH_2Cl_2) were combined and evaporated to 63 mg of a white solid. The fractions containing only material with R_f 0.63 were combined and evaporated to 866 mg (72%) of a pale-yellow viscous oil, which was identified as **24b**. The intermediate fractions containing mostly **24b**, but some of the compound with R_f 0.74, were evaporated to give 199 mg (16%) of a pale-yellow oil. The product was Kugelrohr distilled (190 °C, 0.2 mm) to give a yellow oil; NMR ($CDCl_3$) δ 1.36 (t, 3 H), 4.36 (q, 2 H), 5.84 (s, 1 H), 6.7–6.95, 7.1–7.7, 7.8–8.1 (m, 9 H); IR (film) 2980 (m), 1772 (m), 1690–1730 (s), 1592 (m), 1570 (m), 1250–1310 (s) cm^{-1}. Anal. Calcd for $C_{17}H_{15}ClO_3$; C, H.

Reaction of 23 with Ethanethiol and Triethylamine (27 and 28). Ethanethiol (75 μL, 1.0 mmol) was added dropwise to a stirred solution of **23** (257 mg, 1.0 mmol) in dry dioxane (2 mL) under Ar at 10 °C. There was no apparent reaction until triethylamine (140 μL, 1.0 mmol) was added, whereupon the solution turned reddish and a precipitate formed. After the solution was stirred at 10 °C for 15 min and at room temperature for 30 min, the solvent was removed by rotary evaporation to give a pink solid, which was triturated with anhydrous ether (2 × 10 mL) and filtered. The ether-insoluble solid, identified by NMR an IR as $Et_3N{\cdot}HCl$, was obtained in quantitative yield. The filtrate was evaporated to 236 mg (84%) of **27** as an orange solid. The product was chromatographed on silica gel (1 × 22 cm) with CH_2Cl_2 as eluant and the first yellow band (R_f 0.61) was collected. The solvent was evaporated and the resulting viscous yellow oil crystallized overnight (201 mg). After recrystallization from *n*-hexane, the product was obtained as fine off-white needles: mp 103.5–104 °C; NMR ($CDCl_3$) δ 1.12 (t, 3 H), 2.67 (q, 2 H), 7.2–8.3 (m, 9 H); IR (KBr) 1730 (m), 1700 (s), 1680 (sh), 1580 (m), 1254 (s) cm^{-1}. Anal. Calcd for $C_{17}H_{14}O_2S$; C, H, S.

Ethanethiol (56 μL, 0.75 mmol) was added dropwise to a solution of **27** (211 mg, 0.75 mmol) in dry dioxane (1 mL) under Ar at 10 °C. There was no apparent reaction until triethylamine (105 μL, 0.75 mmol) was added, whereupon an immediate red color appeared. After the solution was stirred at 10 °C for 20 min and at room temperature for 4 h, the solvent was removed and the residue was triturated with ether. The insoluble red solid (221 mg, 91%) was identified as **28** by comparison with the NMR and IR of an authentic sample.

A heterogeneous mixture of **28** (209 mg) prepared above in ether (20 mL) was stirred while 0.1 M HCl (8 mL) was added. The orange ether solution was separated from the aqueous layer, which was extracted once with ether. The combined ether extracts were evaporated to an off-white solid (133 mg, 92%), identified by NMR and IR as **2a**.

Triethylammonium Salt of 2-Phenyl-1,3-indandione (28). Triethylamine (150 μL, 1.1 mmol) was added to a solution of **2a** (222 mg, 1.0 mmol) in dry dioxane (3 mL). An immediate red oil precipitated. The solvent was removed by rotary evaporation and the red oil solidified to give, after ether trituration, 290 mg (90%) of a red solid: NMR ($CDCl_3$) δ 1.00 (t, 9 H), 2.64 (q, 6 H), 6.9–7.4 and 7.8–8.1 (m, 9 H), 7.65 (s, 1 H); IR (KBr) 1635 (m), 1600 (m), 1588 (m), 1520 (s), 1505 (sh), 1495 (sh), 1480 (sh), 1436 (m), 1418 (m) cm^{-1}.

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