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Potentialities of a New Class of Anticlotting and Antihemorrhagic Polymers

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SUMMARY

Major blood anticlotting agents may be grouped into two classes: 1) heparin and heparin analogs, 2) coumarin and coumarin analogs.

The first class of compounds interferes with the formation of thrombin and thromboplastin whereas the second inhibits the formation of prothrombin. Both classes have their own antagonists; namely, protamine sulfate types and vitamin K types exhibiting antihemorrhagic properties.

On a molecular level the heparinlike structures and their antagonists may be regarded as polyanions and polycations, respectively, whereas the coumarinlike structures and their antagonists as electron donors and acceptors, respectively, which may form charge transfer complexes.

Heparin belongs to the class of mucopolysaccharides consisting of repeating units of glucosamine and glucuronic acid. It can either be grafted on a plastic surface or it can be incorporated into functional cellulose derivatives. This polyanion combines in effective group ratios with other cationic compounds such as basic dyes (Azure A) or other dications; i.e., compounds such as nitro blue tetrazolium chloride. It also forms complexes with polycations, e.g., ionenes, which are known antihemorrhagic reagents. Structural analogs of heparin such as dextran sulfate or chitin sulfate behave similarly.

A polymer consisting of repeating units of bishydroxycoumarin units was prepared. The antagonist, a polymer with repeating units of menadione, was also prepared. The properties and potential uses of such systems are discussed.

INTRODUCTION

Substances which prolong the coagulation time of blood are referred to as anticlotting reagents. This term is commonly synonymous with anticoagulants. Anticlotting and antihemorrhagic are antonyms which express converse relations; thus the latter reagents are included in the class of compounds called procoagulants or simply coagulants.

The mechanism of blood clotting is still not established. Its normal course involves a series of complex reactions that, once initiated, result in the formation of a blood clot. However, by the use of appropriate techniques, these reactions can be stopped at certain definite stages. A three-stage mechanism is generally recognized as the classical scheme for the clotting reactions.

$$\text{Platelet and other factors} + \text{surface} \rightarrow \text{thromboplastin}$$

$$\text{Thromboplastin} + \text{prothrombin} \rightarrow \text{thrombin}$$

$$\text{Thrombin} + \text{fibrinogen} \rightarrow \text{fibrin}$$

According to the above equations, the first stage can be considered as the formation of thromboplastin, while the second and the third stages are the conversion of prothrombin to thrombin and fibrinogen to fibrin, respectively.

An anticoagulant may act directly by inhibiting one of the factors needed for the normal courses of coagulation or by the chemical

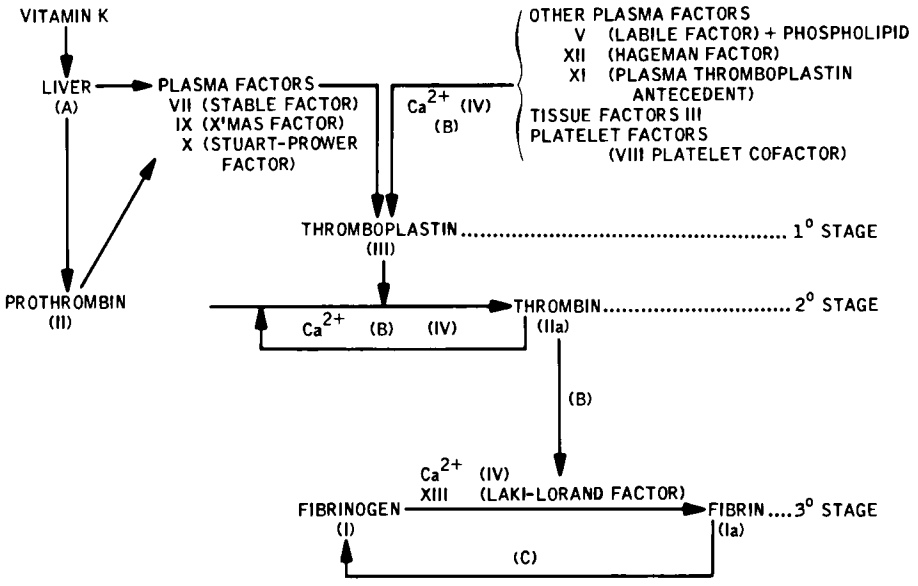


Fig. 1. Schematic three-stage mechanism of blood coagulation. (Roman numerals indicate blood clotting factors set by international committee. Capital letters represent site of actions of three major anticlotting agents.)

combination with any or several of the components in the above stages. It may also act indirectly through formation of anticoagulating factors such as heparin or antithrombin liberators. A procoagulant acts in the opposite sense. A detailed description of the 3-stage mechanism is summarized in Fig. 1.

In Fig. 1, the interrelation of the blood clotting factor with thromboplastin, prothrombin, thrombin, fibrinogen, and fibrin can be located. All Roman numerals designate systems based on standards according to the International Committee on Blood Clotting Factors. Major anticlotting agents may be grouped into 3 classes:

(A) Oral anticoagulants
(B) Heparins
(C) Fibrinolysin and related enzymes

The known mechanisms for each of these classes of compounds that interfere with the normal blood coagulation scheme are indicated at the appropriate places in the chart of Fig. 1.

Both intravascular hemorrhage and thrombosis constitute a serious threat

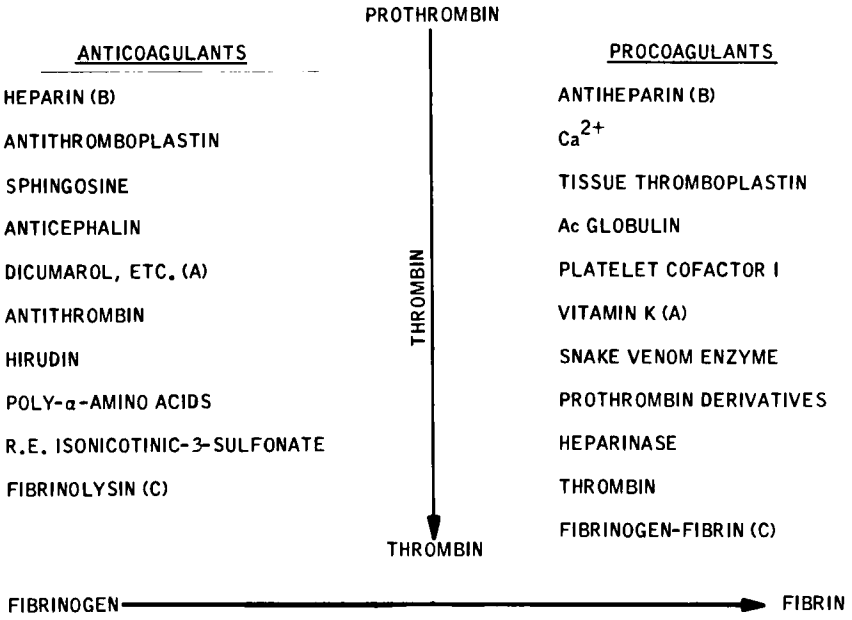


Fig. 2. General classes of anticlotting and antihemorrhagic substances. Major three types are designated by A, B, and C following the subject.

to human life. For the normal, healthy individual, the dynamic nature of circulating blood within the vascular system requires a proper balance of all normal factors. It is to be noted that this delicate balance is upset when the body responds to local injuries, blood is shed, and clotting occurs. The proper coagulation response to blood shed is as important to the well-being of the individual as is the maintenance of vascular fluidity.

In a broad sense all anticlotting and antihemorrhagic substances can be grouped as anticoagulants and procoagulants as indicated in Fig. 2. Among some of the known and common classes of anticoagulants and procoagulants are the heparins versus the antiheparins and the dicumarols versus vitamin K, and these deserve special attention. (A) is the designation for the latter class and (B) for the former class.

In the pesent discussion there is a difference between an antihemorrhagic agent and a hemostatic agent. The latter involves materials such as gelatin sponge and oxidized cellulose, and it is active during the last stage of coagulation. The former is similar to hemostatic agents, but excludes absorbents, and is active in all three stages of coagulation with emphasis in the first and

second stages. The initial stage may control the anticlotting activities for all the succeeding stages. The first stage of the clotting of blood is of primary importance in the understanding of anticlotting or antihemorrhagic properties. In this paper, discussion will be limited to the anticlotting activities of class (A) and class (B) substances.

Classes (B) and (C) are composed of high molecular weight polymeric materials. Heparin is a mucopolysaccharide with MW of about 20,000. Fibrinolysin is an enzyme, therefore a polypeptide. However, in class (A), the anticoagulant is monomeric. Yet its antagonist, vitamin K, and the analog of the latter, are known to associate into a polymeric precursor form.

Research concerning type (A) anticoagulants, namely the 4-hydroxycoumarins and indandiones, and particularly their application to the antithrombogenic surfaces of biomaterials, is sparse despite the fact that there is a great amount of work published in the field of antithrombogenic properties of materials, although most of them deal with class (B) (heparin salts and their analogs). Further study of class (B) is a challenge to an understanding of the blood clotting mechanism as well as to the development of a group of new polymeric substances that could have potential anticlotting and antihemorrhagic properties.

Known oral anticoagulants (A) and vitamin K homologs (A) are monomeric or dimeric. There are a number of advantages for the anticlotting or antihemorrhagic agents to be in a polymeric form. The major reasons can be summarized as follows:

1. Clotting factors and enzymes are polymeric; polymer-polymer interaction is unique.
2. Prolonged "repository" effect may be achieved; absorption for the monomer is rapid.
3. Oligomer activity can be additive as well as cooperative; configuration may be important.
4. Convenience for incorporation of other functional groups; for altering properties, e.g., solubility.
5. Fabrication for implant uses; can be grafted or copolymerized for time delay use.
6. Vitamin K precursors stored in liver are macromolecules; ubiquinone has a 50-C side chain.

In the following, the mechanism of heparin and antiheparin of class (B) and that of dicumarol and vitamin K of class (A) are discussed. Attention is focused on the mode of action of the competitive nature of the

Table 1. Common Sulfated Mucopolysaccharides Which are Heparinoids

Saccharides	Components	Linkages
Heparin	D-glucuronic acid (50% 2-Sulfo) D-glucosamine (2,6-Sulfo)	Major α (1→4)
Dextran sulfate	D-glucose	α(1→6)
Nigeran sulfate	D-glucose	α(1→3) (1→4)
Cellulose sulfate	D-glucose	β(1→4)
Sulfated hyaluronic acid	D-glucuronic acid D-glucosamine	β(1→3) (1→4)
Chitin sulfate	D-glucosamine	β(1→4)
Chondroitin sulfate A	D-glucuronic acid D-galactosamine (4-Sulfo)	β(1→4) (1→3)
Chondroitin sulfate C	D-glucuronic acid D-galactosamine (6-Sulfo)	β(1→4) (1→3)
β-Heparin	L-iduronic acid D-galactosamine (4-Sulfo)	α(1→4) β(1→3)

anticlotting and clotting (antihemorrhagic) properties. Of course, the polymeric nature will be emphasized. Finally, the synthesis of multimers and oligomers of bishydroxycoumarin (dicumarol) and menadione (vitamin K) will be discussed.

HEPARINS AND ANTIHEPARINS

Heparin is considered to be a mucopolysaccharide. Generically, a mucopolysaccharide can be defined as a carbohydrate that contains heteroatoms. Thus, a mucopolysaccharide may be a polysaccharide (protein complex, a glycoprotein, or an amino sugar) containing polysaccharide [1]. Structurally, heparin consists of equal portions of partially sulfonated units of D-glucuronic acid and D-glucosamine joined by an $\alpha(1\rightarrow4)$ linkage. It is believed that within the tetrasaccharide units there are 7 anions consisting of 2 carboxylic groups from the uronic moiety, 2 N-sulfamino groups from the hexoamine moiety, and 3 O-sulfo groups on the hydroxyls of both sugar moieties (Fig. 3). Heparins usually include heparin derivatives such as 4-heparin (N-desulfated heparin), heparinoids such as chondroitin sulfate A and B, and heparin analogs such as the sulfate of polyvinyl alcohol (elvanol). A list of the common heparinoids with their structural information can be found in Table 1.

Two properties commonly shared by the heparins are (a) they contain sulfate groups and (b) they are negatively charged polyanions. Evidently it is not essential that heparins contain nitrogen since, for example, polyvinyl alcohol sulfate is a heparin analog that exhibits antiheparin activities but is without nitrogen atoms. On a molecular basis the activity is not proportional to the sulfur content of the polysaccharide but is instead a function of the distribution of the anionic groups [2]. Thus, it is possible to state that activity depends upon the spacial arrangement of the negative anions attached to the polysaccharide. These polyanions, which by virtue of the α-glycosidic linkage have helical networks, may be largely responsible for the unique activity of heparin.

As a result, the heparins are all capable of forming complexes. For example, collagen is a complex of protein (gelatin) and chondroitin sulfate A which may be dissociated by $CaCl_2$ solutions and recombined to give fibers. Heparin can form insoluble complexes with many proteins characterized by electrophoretic patterns and also with organic bases, such as cetyltrimethyl ammonium bromide and benzidine which are known to form insoluble complexes with heparins. In general, a cation, a dication, or a polycation will form a more or less neutral complex with polyanions. Or

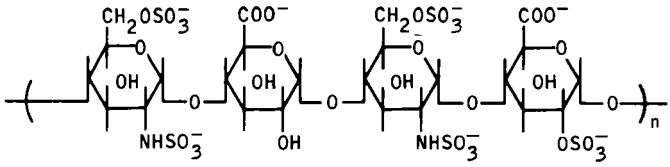


Fig. 3. Tentative structure of heparin. (Based on Jaques.)

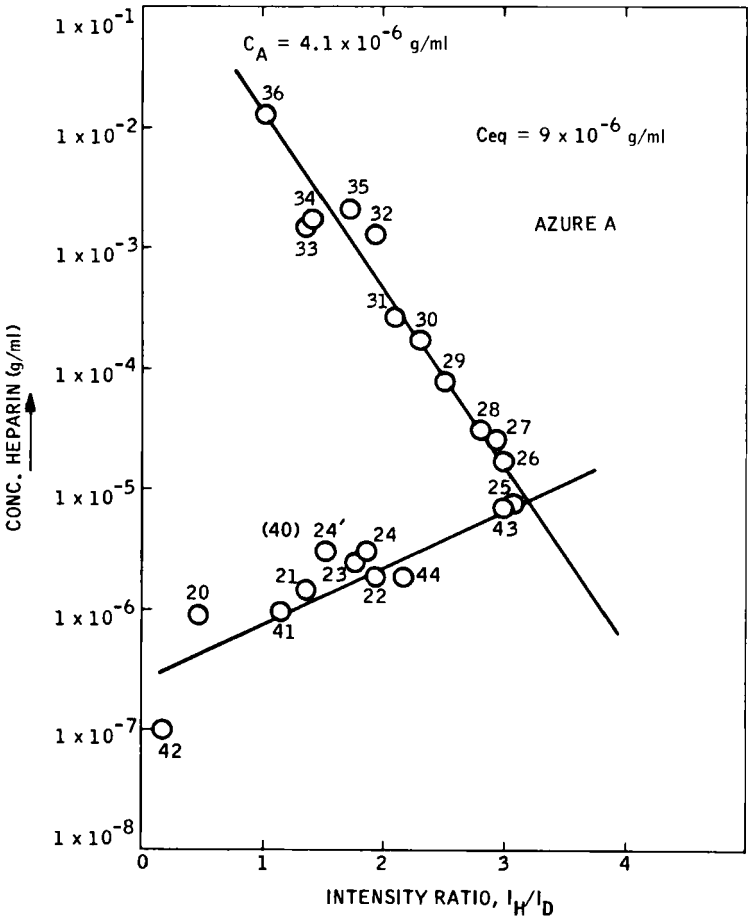


Fig. 4. Relation of heparin concentrations and intensity ratio of complex and Azure A absorptions. (The Azure A concentration is kept at 4.1 X 10^{-6} g/ml.)

$$A:^{-}Na^{+} + C^{+}Cl^{-} \rightarrow A:^{-}C^{+} + NaCl$$

$$A:^{-}C^{+} + A:^{-}Na^{+} \rightarrow CA_2^{-}Na^{+}$$

where A is a polyanion and C is a polycation. This neutralization requires a 2-electron transfer process.

The metachromasia of basic dyes when they react with heparins can also be interpreted as a 2-electron transfer process [3]. In this instance the basic dye is the positive cation. For example, in the reaction of heparins with Azure A (Fig. 4), the heparin concentration is plotted versus the ratio of the intensity of the shortwave length band (I_H) to the intensity of the long wavelength band (I_D) and this ratio I_H/I_D increases to a maximum indicating the complete conversion of the dye cation to the complex $A:^{-}C^{+}$. At still higher concentrations, the $CA_2^{-}Na^{+}$ is formed. Most basic dyes behave similarily, and usually the heparin is found to react with them according to the ratio of 2 dye molecules per each tetrasaccharide unit as shown in Table 2.

Many cations possess a sufficiently high affinity to block the activity of heparins and heparinoids. Examples can be found in trypaflavin, toluidine blue, tetracyclines (aureomycin and tetramycin) stibamidine isothionate, DPP (1,5-dimethyl decamethylene polymethyl bromide) histone and globin, protamine, and ionenes [9]. Basic dyes which can be treated as monocations along with some dications, such as lucigenine, and polycations, such as ionenes, can all form stable complexes with the polyanionic heparin. Consequently, these organic bases form the basis for antiheparin activity. The antiheparin activity can be treated as the process of the affinity for a specific polyanion. The order of activity of heparin with antiheparins is:

β-lipoprotein < thrombin clotting system < Christmas factor IX < platelet protein < protamine sulfate

In general, when the basic groups of various compounds react with the acidic groups of the heparins, the anticoagulant properties of the latter class of compounds are nullified. This is the reason for using protamin or basic dyes as heparin antidotes. Heparin binds with organic bases (benzidine, etc.), with alkaloids (quinine, brucine), and with basic amino acid-containing polypeptides (polylysine). The specific nature of the binding topology of this 2-electron transfer reaction has not been elucidated.

Table 2. Equivalent Ratio of Basic Dyes with Heparin

Dye	MW	Equivalent concentration (μg/ml)	Dye concentration (μg/ml)	Equiv. wt.	Equiv. ratio[a]
Azure A	291.8	9.0	4.1	642	0.52
Methylene Blue	373.9	7.0	3.6	725	0.59
Basic Fuchsin	337	8.0	3.3	808	0.66
Brilliant Cresyl Blue	332.5	25	9.7	856	0.69

[a]Theoretical value of interaction of two dye molecules with one tetrasaccharide unit of heparin (1228) is 0.50.

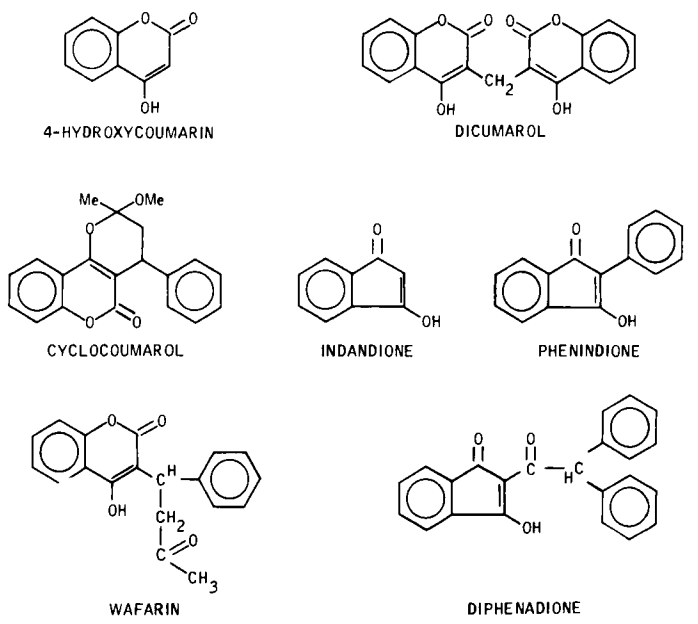


Fig. 5. Structures of common oral anticoagulants.

ORAL ANTICOAGULANTS AND VITAMIN K SERIES

The coumarin and indandione derivatives have been known anticoagulants since the discovery of the cause of the sweet clove disease. Structures of some common oral anticoagulants are summarized in Fig. 5. Despite the simplicity of the structure, it is quite difficult to establish the essential chemical characteristic that produces the anticoagulant activity. In general, each has a 4-hydroxycoumarin structure with 3 positions which are either substituted by carbon residue or hydrogen atoms.

The vitamin K series are known antagonists of coumarin. The skeleton structure of this series is the menadione (2-methyl-1,4-naphthoquinone) structure. The common vitamin K series is listed in Fig. 6. The minimum structural requirement here is the menadione structure with the 3-position either substituted with carbon or hydrogen atoms.

Neither the oral anticoagulants nor the vitamin K series show pharmacological effects in vitro. They exert activities in vivo only after a latent period of a half to a whole day. They both interfere and inhibit the normal

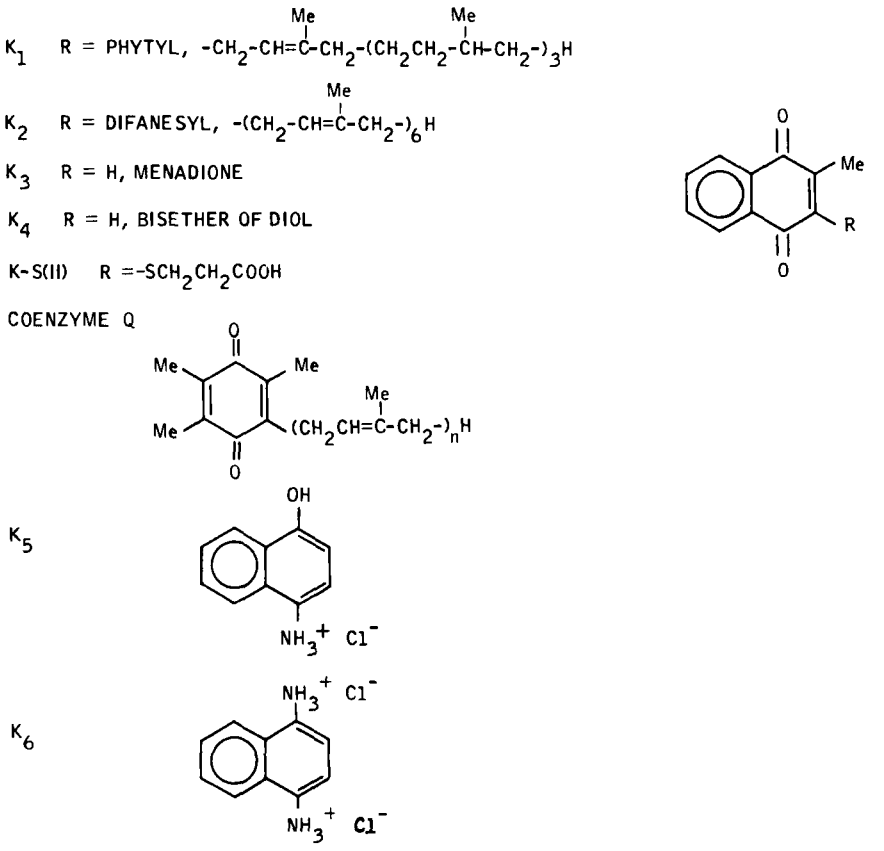


Fig. 6. Structures of vitamin K series. (Structure of coenzyme Q is also included.)

synthesis of prothrombin and other factors such as VII, IX, and X in the liver as indicated by (A) in Fig. 1.

Up to now there has been no satisfactory mechanism proposed for the vitamin K and antivitamin K activity. Chmielewska and Cieslak [4] have investigated the structure-activity relation of the vitamin K series and found, as shown in Fig. 7, that the group $CH_2{=}C(R){-}C{=}C{-}O^-$ is responsible for the

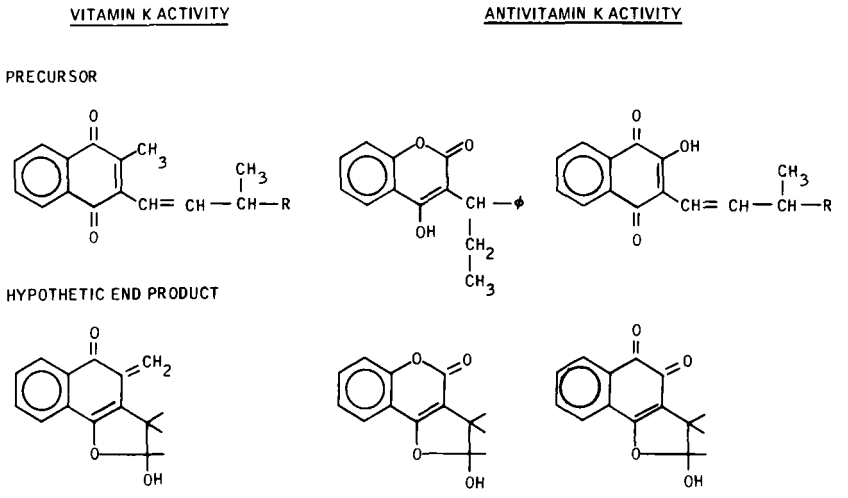
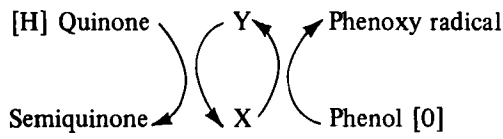


Fig. 7. Anticoagulation mechanism based on Chmielewska and Cieslak.

vitamin K activity whereas the group O=C–C=C–O– is responsible for
|
R

the antivitamin K activity. R is a cyclic carbonyl group for both classes, but also can be a cyclic ether group for the latter. Although this theory can explain a number of observed facts, its greatest drawback is that it cannot account for the strong anticoagulant properties of the indandiones.

A generalized mechanism for both antivitamin and vitamin K properties [5] may be that the antivitamin K property is associated with the nature of an oxidation inhibitor whereas the vitamin K property is associated with an oxidation accelerator. The ease with which the 1,3-dione system forms stable radicals [6] that interfere with oxidation sequences is the reason that indandiones and coumarins are anticoagulants. Based on this mechanism, the 2-hydroxy-substituted menadione can now be identified as an anticlotting agent and not as a procoagulant of vitamin K homologs. Furthermore, the clotting activity of ellagic acid can also be explained on the basis that ortho-quinone and the para-isomer behave similarly. The vitamin K and antivitamin K activity can be treated as a one-electron transfer of the donor-acceptor reaction scheme



The X and Y evolve in an in vivo system.

MENADIONE POLYMER AND COUMARIN POLYMER

Oligomers and polymers with multiple units of menadione and coumarin will be discussed in this section. A recent survey of the literature revealed that there has been virtually no work done in this area. It is known that the dimer form of courmarin has enhanced activity over that of the monomeric form [7]. A good comparison may be made with compound 48/80 which is a polymer of p-methoxyphenylmethylamine and is the most active compound with respect to histamine release.

Simple arene polymers are prepared from arenes such as benzene, naphthalene, anthracene, and tetracene with formaldehyde [8]. The modified procedure is one in which glacial acetic acid is used to give the arene polymer with methylene bridges whereas sulfuric acid will give $-CH_2O-$ linkages (more than 50%) in addition to $-CH_2-$ linkages.

In glacial HOAc:

$$HCH{=}O + H^+ \rightleftharpoons HCH^+{-}CH \tag{1}$$

$$ArH + HCH^+ - OH \rightleftharpoons ArCH_2^+ + H_2O \tag{2}$$

$$ArCH_2^+ + ArH \rightleftharpoons ArCH_2Ar + H^+ \tag{3}$$

$$ArCH_2Ar + HCH^+{-}OH \rightleftharpoons ArCH_2ArCH_2^+ + H_2O \tag{4}$$

$$ArCH_2ArCH_2CH_2^+ + ArH \rightleftharpoons ArCH_2ArCH_2Ar + H^+ \tag{5}$$

In H_2SO_4:

$$ArCH_2^+ + HCH{=}O \rightleftharpoons ArCH_2OCH_2^+ \tag{3a}$$

$$ArCH_2OCH_2^+ + ArH \rightleftharpoons ArCH_2OCH_2Ar + H^+ \tag{3b}$$

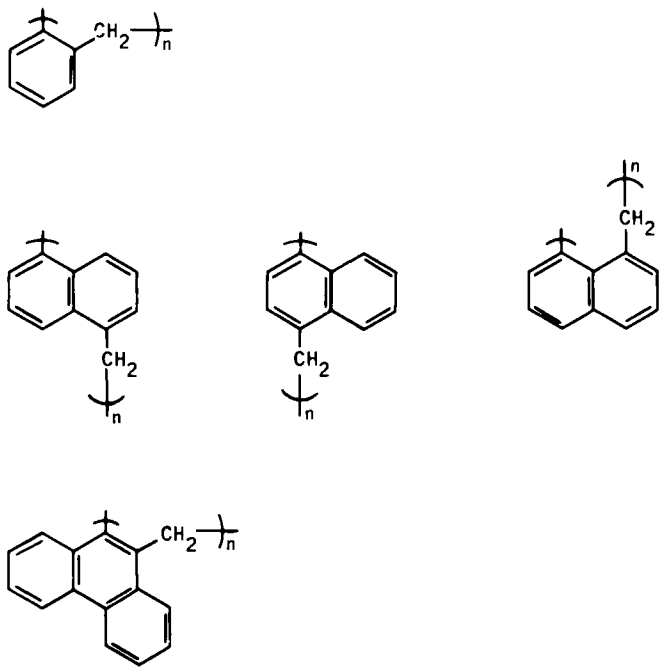


Fig. 8. Structure of simple arene polymers. (From upper to lower: benzene, naphthalene, and phenanthrene, respectively.)

The simple arene polymers made can be represented by the structures shown in Fig. 8. The naphthalene-formaldehyde system resulted in polymers containing about equal proportions of 1,4; 1,5; and 1,8-structures due to the high reactive index of the α-position of naphthalene. This fact is well-supported by NMR and ir spectra.

The naphthalene-formaldehyde condensation polymers thus made have the structure shown in Fig. 9. After chromic acid anhydride oxidation, the quinone yield is 33% based on the structure. Repeated runs always have this as a limiting value. However, when the initial arene is 1,4-dihydroxynaphthalene, the condensation polymerization converts to 100% quinone after oxidation (Fig. 10). The ir spectra gave a strong 1730 cm^{-1} absorption band resembling that of the spectrum of menadione which absorbs strongly at 1670 cm^{-1}. Color tests for quinone are positive as indicated by aqueous KOH

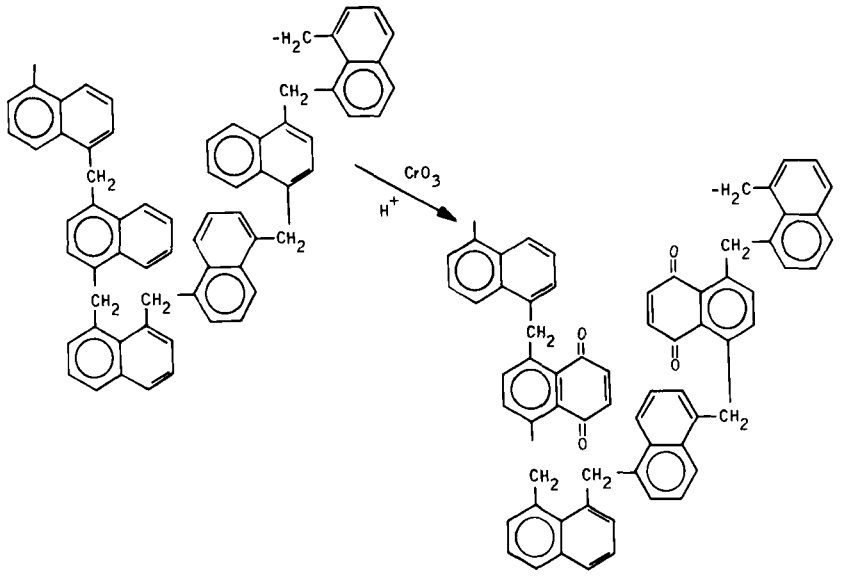
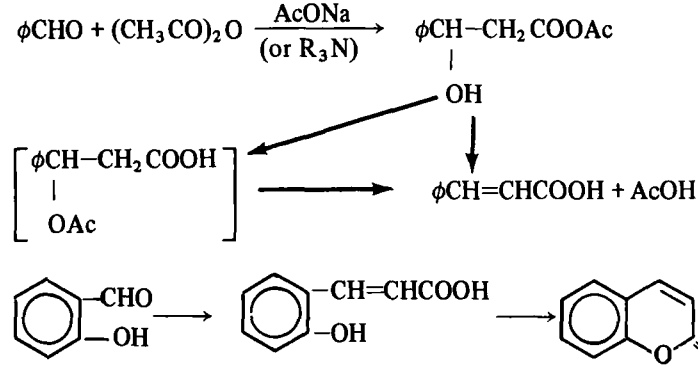


Fig. 9. Structure of naphthoquinone polymer. (Only 33% of the polymer contains quinone groups.)

and 2,4-dinitrophenylhydrazine. This scheme for the preparation of repeating menadione units is quite general, for the 3-substituted repeating units of phytyl and difanesyl units can also be prepared.

The coumarin type of compound usually can be prepared either by the Perkin synthesis or by the Von Pechmann reaction.

Perkin synthesis:



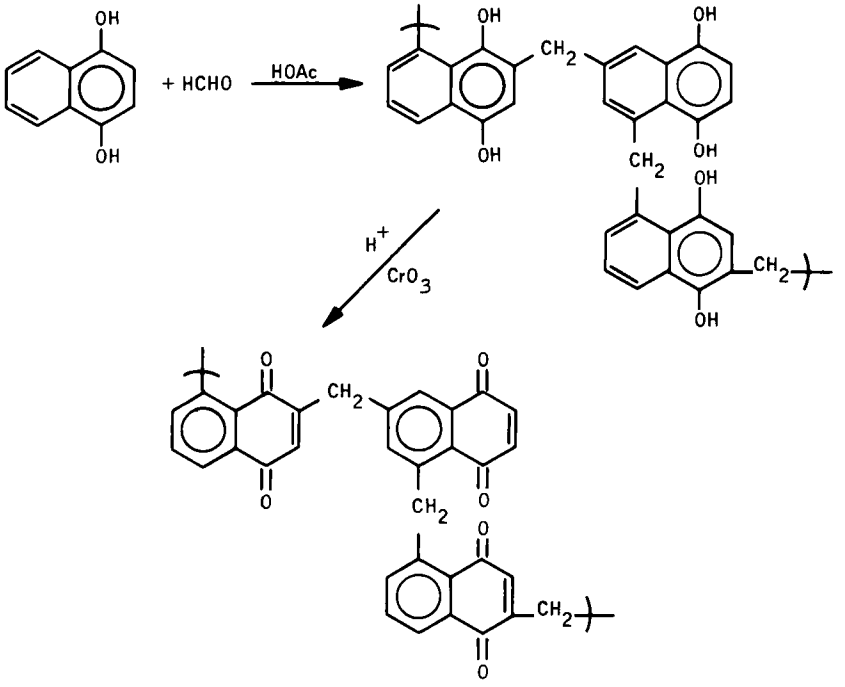
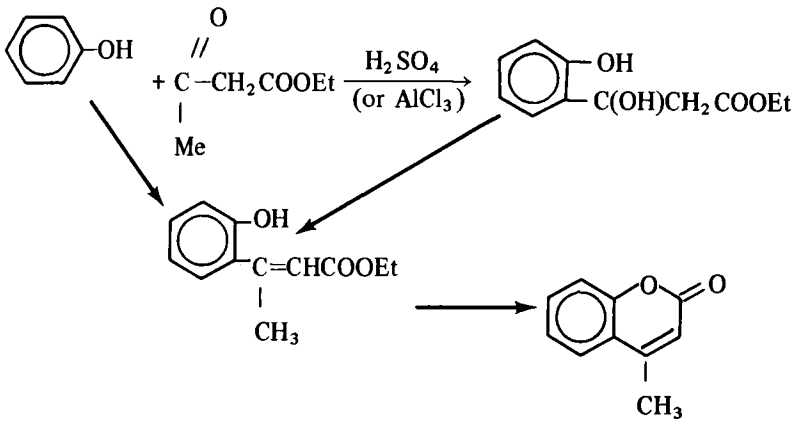


Fig. 10. Menadione polymer. (Notice the position of propagation is on 2, 5, 7, and 8 of naphthalene.)

Von Pechmann reaction:



The salicylic acid-formaldehyde polymerized condensate in sulfuric acid was prepared under the Perkin condition to yield a coumarin polymer as shown in Fig. 11. The condensate has a softening temperature at ca. 297°C and is soluble in THF and DMAc whereas the coumarin polymer has a m.p. of 360°C and is soluble only in DMAc and pyridine. These acetate groups can be hydrolyzed to give the free hydroxy group and thus result in a "coumarol" (4-hydroxycoumarin) repeating sequence.

A coumarin dimer can easily be obtained when 1,5-dihydroxynaphthene is subjected to the Von Pechmann conditions as shown on the following page. This yellow product is soluble in pyridine, o-dichlorobenzene, and nitrobenzene.

Coumarin oligomers or polymers often have high melting points and are insoluble, but these drawbacks can be remedied by the modification of the structure by adding solubilizing groups, such as sulfonate or acetate, in coumarin polymers which can be administered orally or intravenously either in solution or emulsion form.

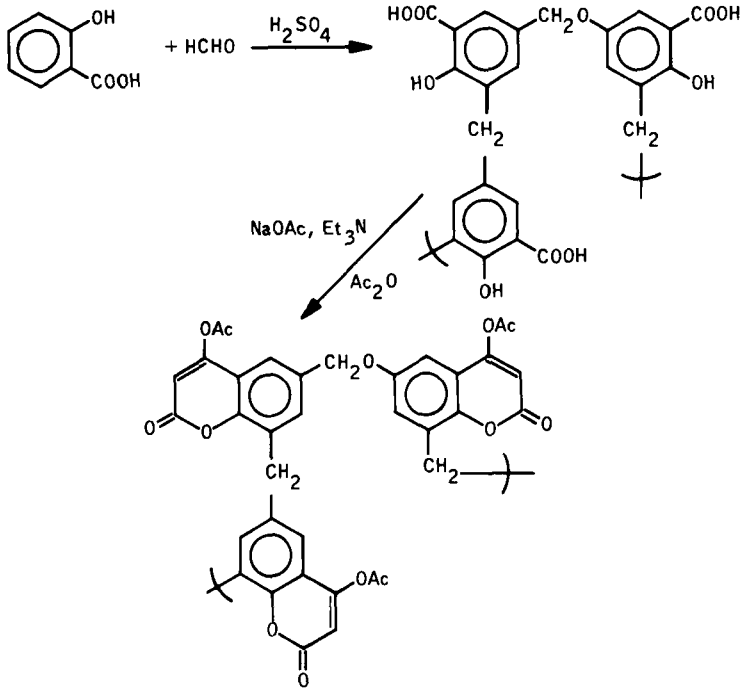
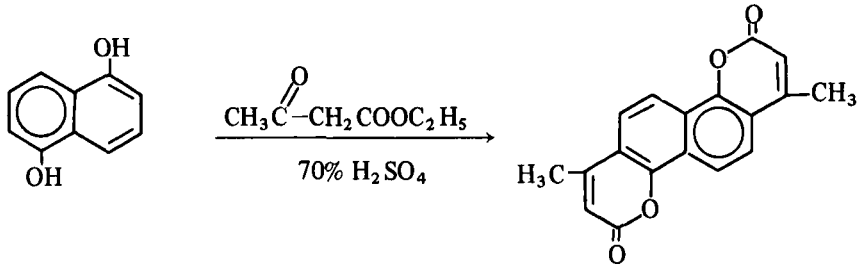


Fig. 11. Coumarin polymers prepared by Perkin reaction (the acetate on 4-hydroxyl position can be hydrolyzed.)



Ordinary Novolak (phenol-formaldehyde linear polymer) prepared by barium hydroxide catalysis can undergo Von Peckmann reaction, resulting in the red-colored coumarin-chromone polymer shown in Fig. 12. This polymer is soluble in a dilute alkaline aqueous medium.

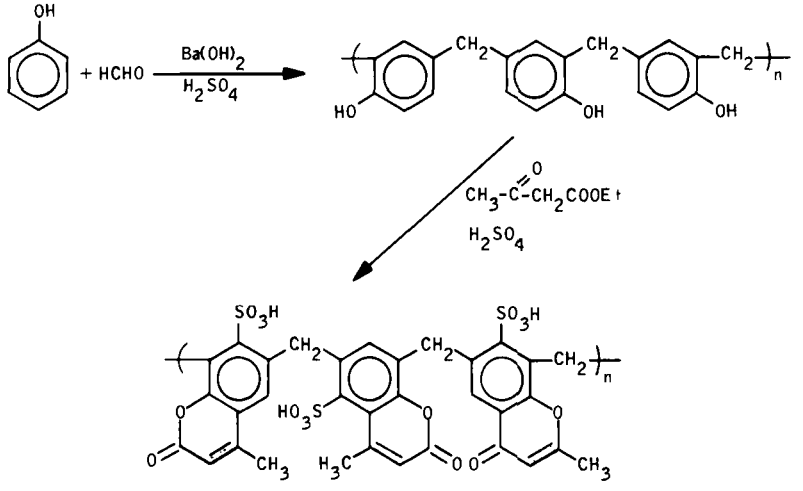


Fig. 12. Coumarin-chromone polymer prepared by Von Peckmann reaction. (The first 2 units of the final polymer are coumarin units, the last unit is chromone; the intermediate stage of the polymer is Novolak.)

Table 3. Average Doses and Speed of Action of Various Oral Anticoagulants

Compound	Initial dose (mg)	Maintenance dose (mg)	Peak prothrombin time effect (hr)	Duration (days)	Side effects
Dicumarol	300	100-200	36-48	5-6	Disturbance to Gastrointestine
Cyclocoumarol	1υ00	25-58	36-60	6-8	–
Wafarin	60	10	36-72	4-5	Dermatitis urticaria
Acenocoumarin	20-28	2-12	36-48	1½-2	Mouth ulceration
Phenindione	100-200	25-50	24-48	1-4	Rashes, leukopenia
Diphenadione	20-30	15	24-48	15-20	Nausea
Aninsindione	300	25-300	24-72	1½-3	Red-orange urine

CONCLUSIONS

The oral anticoagulants are known to inhibit the formation of prothrombin and other factors in the liver, especially factor VII. For various coumarins and indandiones, varying potency is reflected as varying dosages are taken, as shown in Table 3, under the comparable depressions in prothrombin concentration. In general there is a characteristic delay in the mode of action of these compounds. The same result is observed with the administration of vitamin K and its analogs. It is also known that the vitamin K series can reverse the coumarin-induced hypoprothrombinemia.

Metabolic transformation studies of oral anticoagulants reveal the fact that most active components are stored in the liver. Variations in liver storage may be due to individual variations in susceptibility. The difference in the use of polymeric or oligomeric anticoagulants is initial, and maintenance dosages (together with individual variations) may not be important because of the initial excess supply and their readiness to be consumed as the active forms by slow degradation, preferentially with a synergistic enzyme.

Clinical testing of these oligomeric anticlotting and antihemorrhagic compounds is scheduled and is expected to yield valuable information. Their uniqueness, which is summarized as follows, helps to support their potentiality as a useful class of chemotherapeutic reagents:

1. Low MW polymers could have more "residence time" for in vivo studies, especially for tagged molecules. Monomers are known to excrete from the body at a fast rate.
2. Both vitamin K and coumarin involve the first stage of clotting, and fundamental information on clotting mechanism can be obtained from them.
3. The results can help to further the understanding of factors VII, IX, and X and thromboplastin, as well as the biosynthesis of vitamins K and P.
4. Altering the physical properties of these polymers is easy, and introduction of functional groups for film forming is possible.

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