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The Anticoagulant Action of Some Derivatives of 3,3′-(Arylidene)-bis-4-hydroxycoumarin

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In a previous publication, we discussed the effect on prothrombin levels of halogen and nitro derivatives of 2-arylindandiones-1,3.[1] Our investigations of the relationship between chemical structure and biological activity of anticoagulant compounds have now been extended to include a number of derivatives of 4-hydroxycoumarin. With the purpose of studying the effect of electrophilic substituents and their position on the antivitamin-K activity of this group of compounds, we have studied derivatives of the following types:

(1) 3,3′-(Benzylidene)-bis-4-hydroxycoumarin containing the chloro[2] or nitro[3] groups in the phenyl residue in the *o*-, *m*- or *p*-position (Table I, compounds I–VI).

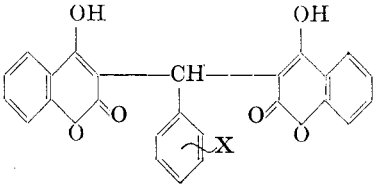
(2) 3,3′-(Furfurylidene)-bis-4-hydroxycoumarin and its derivatives containing the bromo, iodo[2] or nitro[3] group in the furan residue in position 5″ (Table I, compounds VII–X).

Our investigations of the derivatives of indandione-1,3,[4] which have been confirmed by other investigators,[5–9] have shown that the isomer with the α-naphthyl substituent, i.e. 2-α-naphthylindandione-1,3, is more active as an anticoagulant, whereas the corresponding β- compound has little effect on the prothrombin level. Similarly, among the 3-α- and 3-β-naphthyl derivatives of 4-hydroxycoumarin, only the α- isomer possessed biological activity.[5] According to the hypothesis of Mentzer and coworkers,[10] antivitamin-K activity depends on general molecular symmetry. The greater symmetry of 3-α-naphthyl-4-hydroxycoumarin as compared with 3-phenyl-4-hydroxycoumarin is considered to result in the greater biological activity of the former.

The absence of activity in β- isomers of naphthyl derivatives of both indandione-1,3[4, 6] and 4-hydroxycoumarin,[5] which possess equal symmetry of structure, as well as the greater activity of the α-naphthyl isomers, conflicts with this hypothesis. In our opinion, the biological activity of α-naphthyl derivatives is specifically linked with the α-naphthyl group.

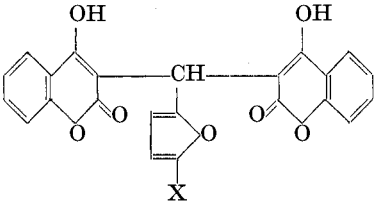
To provide further information on the roles of the α- and β-naphthyl radicals in antivitamin-K compounds, 3,3′-(α- and β-naphthylidene)-bis-4-hydroxycoumarins were obtained[11] (Table I, compounds XI–XII) and their effect on prothrombin levels studied.

Table I. Formulae of derivatives of bis-4-hydroxycoumarin investigated for anticoagulant activity



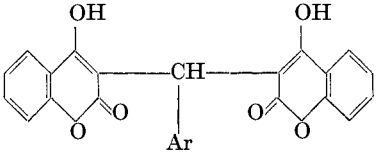
(I–VI)

(I); X = *o*-Cl
(II); X = *m*-Cl
(III); X = *p*-Cl
(IV); X = *o*-NO_2
(V); X = *m*-NO_2
(VI); X = *p*-NO_2

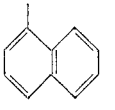


(VII–X)

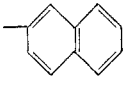
(VII); X = H
(VIII); X = Br
(IX); X = I
(X); X = NO_2



(XI); Ar =



(XII); Ar =



Methods

Methods described in a previous publication[2, 4] were employed. Dicoumarol in a standard dose of 10 mg/kg was used as the reference compound. Determinations of each anticoagulant substance at each dosage level were carried out on three rabbits. The results were calculated as prothrombin levels according to Quick's standard curve (Figs. 1–4). In addition, with the purpose of comparing the anticoagulant activity of the studied compounds with the analogous action of dicoumarol as reference, the relative anticoagulative index was calculated. For dicoumarol, the anticoagulative index, I_a, calculated per millimole at a dosage of 10 mg was assumed to be equal to 100:

$$I_a = t \times \frac{336 \text{ mg}}{10 \text{ mg}} \times 0{\cdot}223 = 100$$

where t = mean prolongation of prothrombin time in seconds, calculated from deviations from normal of prothrombin times after 24, 48 and 72 h; 336 mg = millimoles of dicoumarol; 10 mg = standard dose of dicoumarol; and 0·223 = factor for converting the dicoumarol index to 100.

The relative anticoagulative index (I_{aD}) is given by:

$$I_{aD} = \frac{t_x \, M_x/\text{dose of substance } x}{t \times 336/10 \text{ mg}}$$

$$= \frac{t_x/M_x}{\text{dose of substance } x} \times 0{\cdot}223$$

where t_x = mean prolongation of prothrombin time in seconds after administration of substance x; and M_x = molecular weight of the investigated substance.

The relative anticoagulative indices are presented collectively in Table II.

In addition, a study was made of the degree of accumulation of 3,3′-(α-naphthylidene)-bis-4-hydroxycoumarin after repeated administration of various doses. The initial dose was 10 mg/kg,

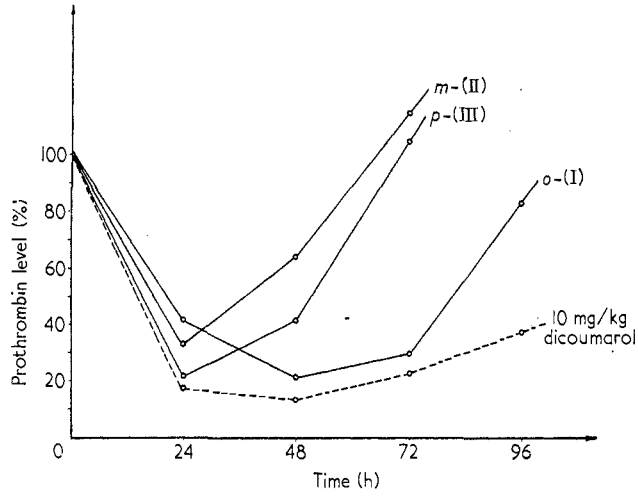


Fig. 1. Comparison of the prothrombin contents after administration of 3,3′-(*o*-, *m*- or *p*-chlorobenzylidene)-bis-4-hydroxycoumarins (I–III) and dicoumarol in doses of 10 mg/kg.

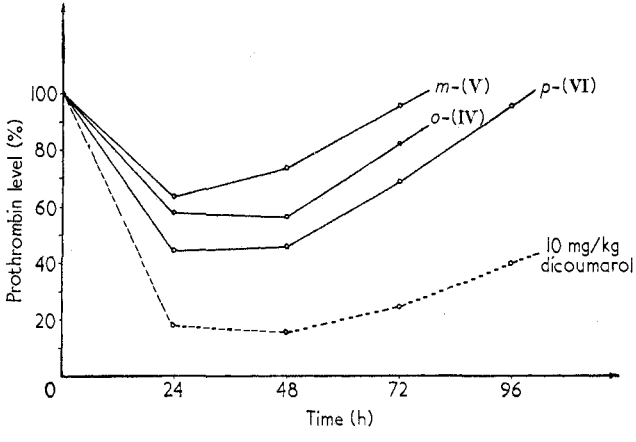


Fig. 2. Comparison of the prothrombin contents after administration of 3,3′-(*o*-, *m*- or *p*-nitrobenzylidene)-bis-4-hydroxycoumarin (IV–VI) in doses of 100 mg/kg and dicoumarol in a dose of 10 mg/kg.

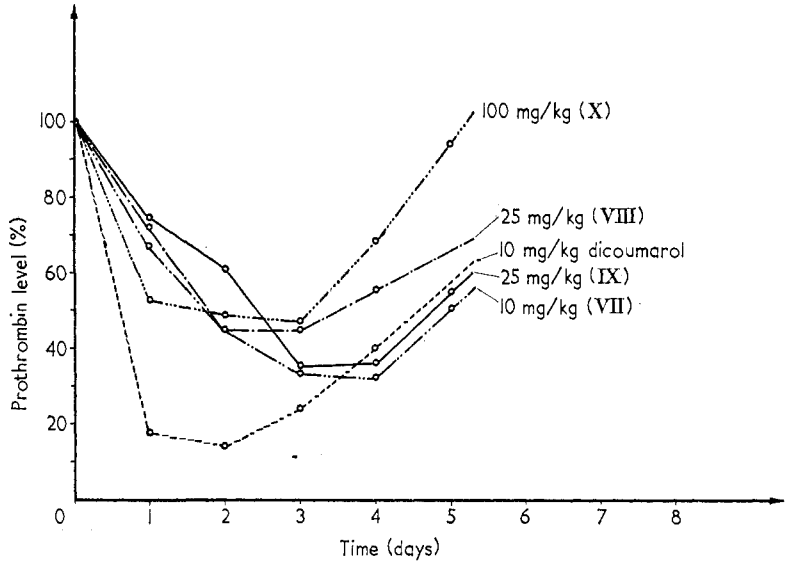


Fig. 3. Prothrombin content after administration of 3,3′-(furfurylidene-2″)-bis-4-hydroxycoumarin (VII) in a dose of 10 mg/kg; 3,3′-(5″-bromo, iodo- or nitro-furfurylidene-2″)-bis-4-hydroxycoumarin in doses of 25 mg/kg or 100 mg/kg; and dicoumarol in a dose of 10 mg/kg (VIII–X).

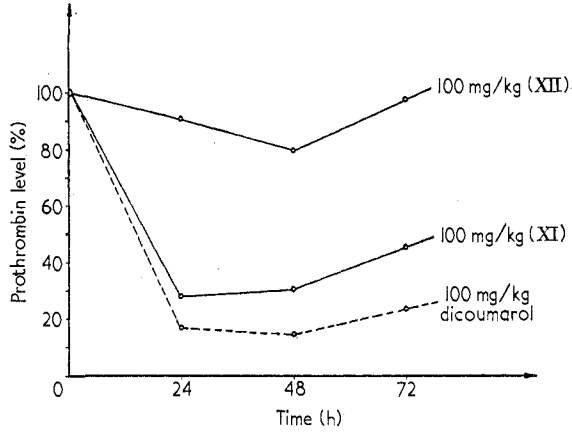


Fig. 4. Prothrombin content after administration of 3,3′-(α-naphthylidene)-bis-4-hydroxycoumarin (XI) in a dose of 10 mg/kg; 3,3′-(β-naphthylidene)-bis-4-hydroxycoumarin (XII) in a dose of 100 mg/kg; and dicoumarol in a dose of 10 mg/kg.

Table II. Relative anticoagulative indices of the compounds studied

No.	Name of anticoagulant compound	Relative anticoagulative index, I_{aD}	Dose for which index was calculated, mg/kg
	Dicoumarol	100	10
I	3,3′-(*o*-Chlorobenzylidene)-bis-4-hydroxycoumarin	70·6	10
II	3,3′-(*m*-Chlorobenzylidene)-bis-4-hydroxycoumarin	19·0	10
III	3,3′-(*p*-Chlorobenzylidene)-bis-4-hydroxycoumarin	42·5	10
IV	3,3′-(*o*-Nitrobenzylidene)-bis-4-hydroxycoumarin	1·5	100
V	3,3′-(*m*-Nitrobenzylidene)-bis-4-hydroxycoumarin	0·9	100
VI	3,3′-(*p*-Nitrobenzylidene)-bis-4-hydroxycoumarin	2·5	100
VII	3,3′-(Furfurylidene)-bis-4-hydroxycoumarin	37·4	10
VIII	3,3′-(5″-Bromofurfurylidene)-bis-4-hydroxycoumarin	12·6	25
IX	3,3′-(5″-Iodofurfurylidene)-bis-4-hydroxycoumarin	16·8	25
X	3,3′-(5″-Nitrofurfurylidene)-bis-4-hydroxycoumarin	3·2	100
XI	3,3′-(α-Naphthylidene)-bis-4-hydroxycoumarin	45·4	10
XII	3,3′-(β-Naphthylidene)-bis-4-hydroxycoumarin	0·3	100

followed by $\frac{1}{3}$ to $\frac{1}{5}$ of the initial dose every 24 hours for 8 days (Fig. 5).

Results

3,3′-(Chlorobenzylidene)-bis-4-hydroxycoumarins (see Fig. 1). All of the investigated compounds (I–III) were active at the 10 mg/kg dose level. Their activity and mode of action vary depending upon the position of chlorine in the benzylidene residue. The least active compound is 3,3′-(*m*-chlorobenzylidene)-bis-4-hydroxycoumarin (II). The greatest reduction in the prothrombin level

was observed after administration of the *p*- isomer (III). The effect after a single administration, similar to the effect of the *m*-isomer, is of short duration, achieving its maximum after 24 h. In spite of its somewhat weaker effect compared with that of the *p*- isomer, 3,3′-(*o*-chlorobenzylidene)-bis-4-hydroxycoumarin (I) has a more prolonged action. The greatest reduction in the pro-

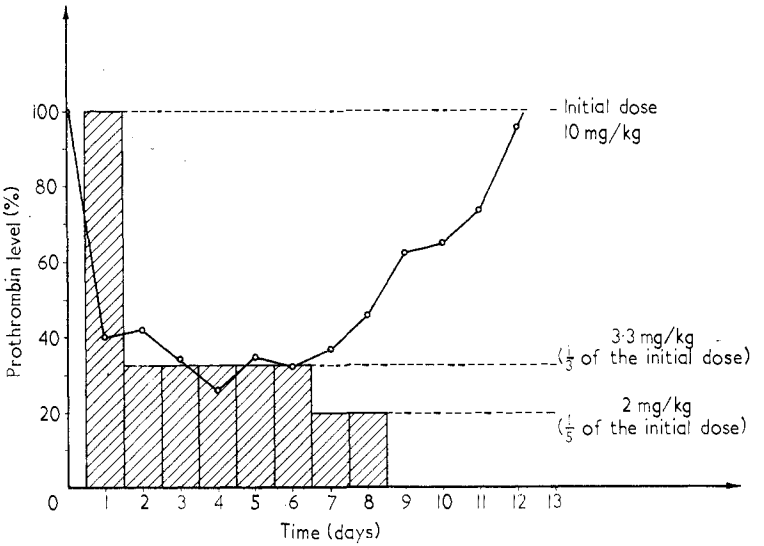


Fig. 5. Prothrombin content after daily administration of various doses of 3,3′-(α-naphthylidene)-bis-4-hydroxycoumarin (XI). The diagram illustrates the relative sizes of the doses. The curve in the background of the diagram represents the prothrombin level 24 h after the corresponding dose.

thrombin level occurs after 48 h, the prothrombin time becoming normal after 5 to 6 days.

3,3′-(Nitrobenzylidene)-bis-4-hydroxycoumarins (see Fig. 2). This group of compounds (IV–VI) displayed anticoagulant activity at doses of 100 mg/kg, and are much less active than the above discussed halogen derivatives. Nevertheless, the effect of the position of the nitro group is evident. 3,3′-(*p*-Nitrobenzylidene)-bis-4-hydroxycoumarin (VI) was the most active of these nitro-compounds.

3,3′-(Furfurylidene)-bis-4-hydroxycoumarin and its nitro-, bromo, and iodo- derivatives (see Fig. 3). 3,3′-(Furfurylidene)-bis-4-hydroxycoumarin (VII) at a dose level of 10 mg/kg displays medium activity, reducing the prothrombin level to about 35 per cent.

Of the investigated derivatives of this compound, the least active was 3,3′-(5″-nitrofurfurylidene)-bis-4-hydroxycoumarin, which only produces a distinct effect at a dose of 100 mg/kg. The prothrombin time returns to normal much more quickly than after administration of other furfurylidene derivatives of bis-4-hydroxycoumarin. 3,3′-(5″-Bromo- and 5″-iodo-furfurylidene)-bis-4-hydroxycoumarins are somewhat less active than the parent substance. A prolongation of the prothrombin time approximately equal to that produced by compound VII follows the administration of the 5″-iodo derivative, but this requires a 2·5 times larger dose. The activity of the bromo derivatives at the same dose level is somewhat weaker. On the whole, all of the investigated furfurylidene-bis-derivatives of 4-hydroxycoumarin (VII–X) displayed a more prolonged action than dicoumarol (Fig. 3). The maximum reduction in the prothrombin level occurs after 72–96 h and becomes normal after 6 to 7 days.

3,3′-(Naphthylidene)-bis-4-hydroxycoumarins (see Fig. 4). Of the two investigated 3,3′-(naphthylidene)-bis-4-hydroxycoumarins (XI–XII), only the α- isomer (XI) has significant activity and in a dose of 10 mg/kg reduces the prothrombin level to about 30 per cent. The shapes of the curves (Fig. 4) show that the mode of action of compound XI is of the dicoumarol type. The fairly high activity of this compound led us to study the behaviour of the prothrombin level after repeated administration. An initial dose of 10 mg/kg was administered, followed by doses sufficient to maintain the same prothrombin level, administered every 24 h for 8 days.

Fig. 5 shows that the maintaining dose is $\frac{1}{3}$ to $\frac{1}{5}$ of the initial dose. A reduction of prothrombin levels to therapeutic values (approx. 20 per cent) would require administration of a somewhat higher initial dose (15 mg/kg) and maintaining doses equal to $\frac{1}{2}$ to $\frac{1}{3}$ of the initial dose.

Discussion

In the course of investigations of the anticoagulant activity of derivatives of 4-hydroxycoumarin, comparatively little attention has been devoted to compounds of the type 3,3′-(arylidene)-bis-4-hydroxycoumarin. The slight interest taken in this group of compounds has probably been the result of the finding by Overman and co-workers[12] of the negligible activity of 3,3′-(benzylidene)-bis-4-hydroxycoumarin and its 4″-hydroxy, 4″-methoxy, 4″-dimethylamino, 3″-methoxy, and 3″- and 4″-methylenedioxy derivatives. These investigators explained the slight activity of these compounds (amounting to 1/100 to 1/500 of the activity of dicoumarol) by their facile transformation into poorly soluble and biologically inactive epoxy derivatives.

Overman and co-workers[12] expressed the activity of the compounds they investigated by means of a so-called relative anticoagulative index, which was calculated for doses giving maximal indices. For our compounds we have also calculated the relative anticoagulative indices (Table II); however, only an approximative comparison is possible, since the indices were calculated for doses producing about equal anticoagulant effects which does not always give a maximal index.

There is no mention in the literature of any observations dealing with the action of 3,3′-(arylidene)-bis-4-hydroxycoumarins containing electrophilic substituents.

The beneficial effect of electrophilic substituents, especially halogens, which was observed in the group of 3-substituted derivatives of 4-hydroxycoumarin,[13-19] and our own observations in the group of 2-phenyl- and 2-α-naphthylindandione-1,3,[1] induced us to study the influence of introducing halogens and the nitro group into the slightly active 3,3′-(benzylidene)-bis-4-hydroxycoumarin. The results indicate that electrophilic substituents, especially the halogens and even the nitro group to a lesser degree, enhance the antivitamin-K activity. The introduction of the chlorine atom into the *p*- and *o*- positions in the benzylidene residue is the most beneficial. The position of the chlorine atom in the benzylidene residue somewhat alters the nature of the effect, since *m*- and *p*-chloro- derivatives have a more rapid

action of shorter duration. The activity of the *o*- isomer approaches the dicoumarol type.

The nitro group in the benzylidene residue exerts a much weaker activating effect than chlorine. The character of the action of nitro derivatives is similar to that of dicoumarol. In this group too, the *p*- isomer has the strongest, and the *m*- isomer the weakest activity.

The results of our present investigations and those previously reported,[1] as well as the results of other investigators,[13-19] permit the generalization that the introduction of electrophilic substituents into the aryl radical situated in the 2- position in indandione-1,3, or into aryl radicals situated in the side-chain of 3-substituted derivatives of 4-hydroxycoumarin, and also in 3,3′-(arylidene)-bis-4-hydroxycoumarin, has a beneficial effect on anticoagulant activity. The activity of these derivatives may be represented as follows:

$$\underset{(\mathrm{Br})}{p\text{-Cl}} > \underset{(\mathrm{Br})}{o\text{-Cl}} > \underset{(\mathrm{Br})}{m\text{-Cl}} \gg p\text{-}NO_2 > o\text{-}NO_2 > m\text{-}NO_2$$

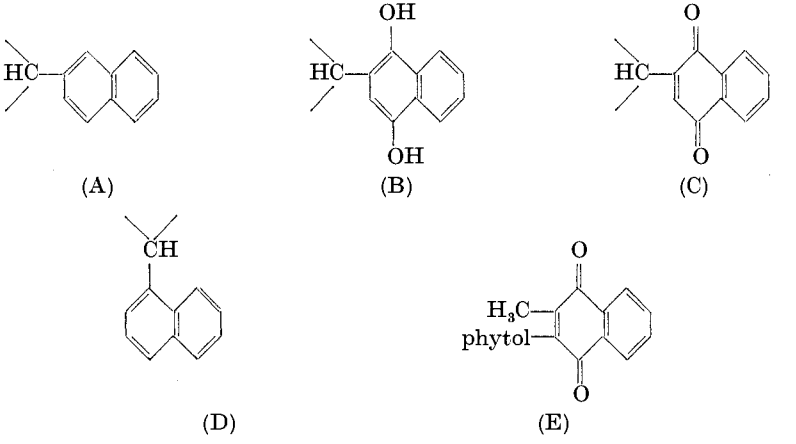
The investigated 3,3′-(furfurylidene)-bis-4-hydroxycoumarin derivatives are an example of compounds possessing a heterocyclic residue at the methylene bridge of the dicoumarol molecule. The furfurylidene derivative has only twice been mentioned in the literature[20, 21] as possessing activity; in general, these findings are in agreement with our own. All the investigated derivatives of 3,3′-(furfurylidene)-bis-4-hydroxycoumarin display prolonged action. This differs from the dicoumarol type in that the maximum reduction in the prothrombin level first occurs after 3 days; hence, their action is somewhat slower. The prothrombin time returns to normal within 5 to 6 days. Unexpectedly, in this group of compounds electrophilic substituents do not increase activity. The significant activity of 3,3′-(α-naphthylidene)-bis-4-hydroxycoumarin found in our investigations as compared with the β- isomer confirms our previously expressed opinion.[11]

Differences in activity of compounds XI and XII (also 2-α-naphthylindandione-1,3, 2-β-naphthylindandione-1,3, and 3-α- and 3-β-naphthyl-4-hydroxycoumarin, whose β- isomers do not possess antivitamin-K activity) contradict the hypothesis of

Mentzer that antivitamin-K activity is associated with general molecular symmetry.[10]

In our opinion, differences in the activity of α- and β- derivatives of naphthalene might be explained by possible oxidation of β-derivatives A (see Table III) in the course of metabolic transformations in the organism to 1,4-naphthoquinone (C) or 1,4-dihydronaphthoquinone (B) systems. Such oxidation products

Table III. Scheme of oxidation of β-naphthyl derivatives. For comparison, the α-naphthyl system and the formula of vitamin K are shown



(Table III), because of their great similarity to vitamin K (E), cannot possess antivitamin-K activity. Derivatives of the α-type (D), possessing a carbon atom in position 1, are not capable of forming analogous quinone or hydroquinone systems without breaking down the molecule; hence, they constitute antivitamins to vitamin K.

Summary. (1) The anticoagulant activity of derivatives of the following types was studied: (*a*) 3,3′-(benzylidene)-bis-4-hydroxycoumarins, containing chloro or nitro groups in the phenyl residue in the *o*-, *p*- or *m*-positions; (*b*) 3,3′-(furfurylidene)-bis-4-hydroxycoumarin and its derivatives containing bromo, iodo or nitro groups in the furan residue in position 5″; and (*c*) 3,3′-(α- and β-naphthylidene)-bis-4-hydroxycoumarins.

(2) The introduction of the chloro or even nitro groups as electrophilic substituents into the phenyl residue of 3,3′-(benzylidene)-bis-4-hydroxycoumarin produces an increase in the antivitamin-K activity. The

activity of the substituted derivatives of 3,3′-(benzylidene)-bis-4-hydroxycoumarin decreases in the following order:

$$p\text{-Cl} > o\text{-Cl} > m\text{-Cl} \gg p\text{-NO}_2 > o\text{-NO}_2 > m\text{-NO}_2$$

(3) 3,3′-(Furfurylidene)-bis-4-hydroxycoumarin displayed medium activity. This compound, as well as its derivatives containing bromo, iodo or nitro groups in the furan residue, has a somewhat slower action as compared with dicoumarol, the maximal effect occurring only after 3 days. Electrophilic substituents do not increase the antivitamin-K activity in this group of compounds.

(4) Among the 3,3′-(naphthylidene)-bis-4-hydroxycoumarins, differences in activity depend on the manner in which the naphthyl group is linked. The α- isomer is very active biologically, whereas the β- isomer is practically inactive; these differences in anticoagulant activity may be explained by different degrees of oxidation in the organism.

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