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ANTICOAGULANT ACTIVITY OF 3,3'-ALKYLTHIOALKYLIDENE- AND 3,3'-(5-ALKYLTHIOFURFURYLIDENE)-*BIS*-4-HYDROXYCOUMARINS AND THEIR OXIDATION PRODUCTS

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Abstract—The anticoagulant activities of 3,3'-alkylthioethylidene- (series E), 3,3'-alkylthiopropylidene- (series P) and 3,3'-(5-alkylthiofurfurylidene)-*bis*-4-hydroxycoumarins (series F) and of their sulphoxy- and sulphonyl derivatives were studied by the one-step method of Quick. All the investigated substances were active anticoagulants. Derivatives of the P series displayed greatest activity, and derivatives of the F series were the least active. In each series the sulphoxides caused maximal increase of activity. Compounds containing $-SO_2$-groups were less active and displayed shorter action. These studies indicate an important role of sulphur containing functional groups in compounds with antivitamin K_1 activity. All the investigated compounds acted more quickly than dicoumarol. Derivatives with an ethyl chain at the sulphur atom were more active than the methyl homologs. The most interesting preparation, PE_{SO}, at a dose of 25 mg/kg exerted a similar biological effect as dicoumarol at a dose of 10 mg/kg. At higher doses when even the potentiation of biological effect was observed, PE_{SO} was devoid of side effects such as mortal haemorrhages. The activating influence of sulphur containing functional groups is dependent on the length of both the alkylidene chain and of the *S*-bound alkyl group as well as on the degree of sulphur oxidation.

In the course of studies on compounds with antivitamin K activity it was found that the introduction of sulphur containing functional groups such as alkyl-S-, alkyl-SO- or alkyl-SO_2- into the para position of the benzylidene residue of biologically inactive 3,3'-benzylidene-*bis*-4-hydroxycoumarin gave compounds with strong anticoagulant activity [1]. The activating influence of these groups depended on the number of C-atoms in the alkyl group bound with a sulphur atom, as well as on the degree of sulphur oxidation.

The most active preparation in this group was 3,3'-(4-ethylsulphoxybenzylidene)-*bis*-4-hydroxycoumarin, which in oral doses of 20–25 mg/kg lowered the prothrombin level in rabbits similarly to dicoumarol at a dose of 10 mg/kg [1].

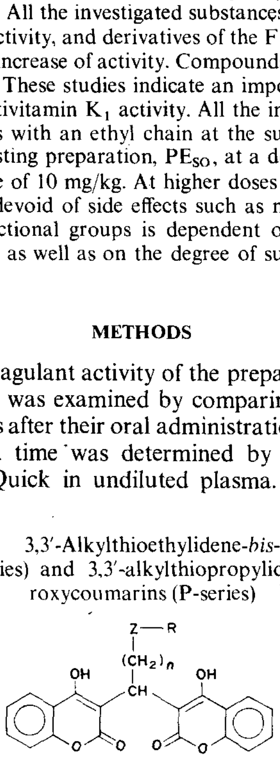
The present study was concerned with the relationship between the anticoagulant activity and the structure of the functional groups containing sulphur in three series of compounds: 3,3'-alkylthioethylidene (series E), 3,3'-alkylthiopropylidene (series P) and 3,3'-(5-alkylthiofurfurylidene)-*bis*-4-hydroxycoumarins (series F) and products of sulphur oxidation in these compounds, i.e. sulphoxide and sulphonyl derivatives (Table 1).

The selected groups of compounds provides a better insight into the relationship between structure and anticoagulant activity of 4-hydroxycoumarin derivatives, and particularly into the role of alkyl-S-, alkyl-SO- and alkyl-SO_2- groups in compounds with antivitamin K_1 activity.

METHODS

The anticoagulant activity of the preparations mentioned above was examined by comparing the prothrombin levels after their oral administration to rabbits. Prothrombin time was determined by the one-step method of Quick in undiluted plasma. In order to

Table 1. (a) 3,3'-Alkylthioethylidene-*bis*-4-hydroxycoumarins (E-series) and 3,3'-alkylthiopropylidene-*bis*-4-hydroxycoumarins (P-series)



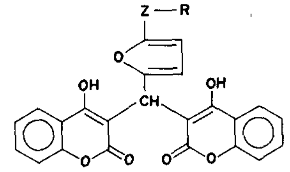
			R = CH_3	R = C_2H_5
Series	n	Z	Symbol	Symbol
E	1	-S-	EM_S	EE_S*
	1	-SO-	EM_{SO}	EE_{SO}
	1	-SO_2-	EM_{SO_2}	EE_{SO_2}
P	2	-S-	PM_S†	PE_S
	2	-SO-	PM_{SO}	PE_{SO}
	2	-SO_2-	PM_{SO_2}	PE_{SO_2}

* Thiotan® [5, 6].
† Thiocouma®, Thioporan® [3, 4].

Table 1 *continued on next page*

Table 1—*continued*

(b) 3,3′-5-Alkylthiofurfurylidene-*bis*-4-hydroxycoumarins (F series)



Series	Z	R=CH_3	R=C_2H_5
		Symbol	Symbol
F	-S-	FM_S	FE_S
	-SO-	FM_{SO}	FE_{SO}
	-SO_2-	FM_{SO_2}	FE_{SO_2}

All compounds were synthesized in the Department of the Chemical Technology of Drugs, Medical Academy, Cracow, Poland.

compare activities, relative anticoagulant indices were calculated in relation to dicoumarol (I_{aD}), which was used as a standard reference preparation at a dose of 10 mg/kg. The details of the method were described in previous papers [1, 2].

RESULTS

Compounds of the ethylidene series (*E*) (*Fig.* 1, *Table* 2). All the compounds of this group were biologically less active than dicoumarol. Lowering of the prothrombin level similar to that observed after dicoumarol required administration of 100 mg/kg. Of the methyl derivatives of the ethylidene series (EM), the sulphide (EM_S) and sulphoxide (EM_{SO}) exhibited similar indices at doses of 25 and 50 mg/kg. At higher doses (100 mg/kg), EM_{SO} enhanced the biological effect and was the most active compound of this group. The sulphone (EM_{SO_2}) was the least active preparation.

Among the ethyl derivatives of the ethylidene series (EE), the sulphide (EE_S) and sulphoxide (EE_{SO}) in equivalent doses showed higher activity than their methyl homologs. Considering the intensity of action and its duration, the sulphoxide (EE_{SO}) was the most active compound of the E series. Both sulphones (EM_{SO_2} and EE_{SO_2}) were least active; their effect, which was most distinct after doses of 50 mg/kg, was of briefest duration, especially that of EE_{SO_2}. In this series, compounds containing a S-ethyl group were invariably more active than the analogous compounds with the S-methyl group.

Compounds of the propylidene series (*P*) (*Fig.* 2, *Table* 2). Compounds of this series were about twice as active as those of the E series. The most active preparations were PM_{SO} and PE_{SO}. At doses of 25 mg/kg, the former was about one-third, and the latter half as active as dicoumarol at a dose of 10 mg/kg. Their maximal effect occurred 24 hr after administration. As in the case of EM_{SO}, increasing doses enhanced the antiprothrombin effect. It should be emphasized that PE_{SO}, even in high doses with prolonged prothrombin time, caused no mortal haemorrhages in the animals, such as occur during oral administration of other anticoagulants even in small doses.

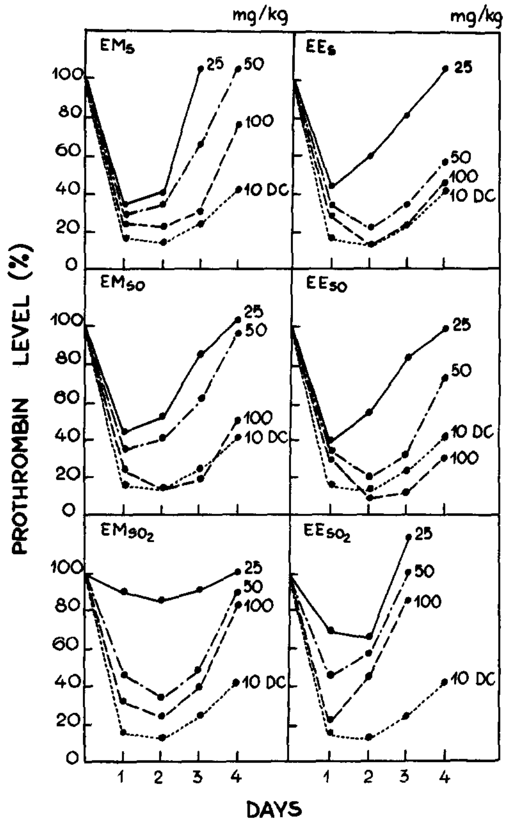


Fig. 1. Prothrombin levels after administration of derivatives of 3,3′-alkylthioethylidene-*bis*-4-hydroxycoumarin (series E) at a dose of 25 mg/kg——, 50 mg/kg —·—· and 100 mg/kg ----, and of dicoumarol at a dose of 10 mg/kg ....

The sulphides, PM_S and PE_S, were about 2–3 times as active as analogous compounds of the ethylidene series, i.e. EM_S and EE_S, and 3·5–5 times less active than dicoumarol. As in the E series, distinct differences in antivitamin K activity were noted between the methyl and ethyl groups. Compounds of the P series containing a S-ethyl group (PE) were more active than their analogues with a S-methyl group (PM). In the P series, the sulphones PM_{SO_2} and PE_{SO_2} were also least active.

Compounds of the furfurylidene series (*F*) (*Fig.* 3, *Table* 2). All the compounds of this series were the least active and had the shortest duration of all the compounds investigated. In comparison with the E series, their activity was about 2 times, and in comparison with P series 3–5 times weaker. Nevertheless, similar though less pronounced relationships were observed in

Table 2. The relative anticoagulative indices (IaD)

Dose of compound (mg/kg body wt)	Series					
	Ethylidene (E)		Propylidene (P)		Furfurylidene (F)	
	EM_S	EE_S	PM_S	PE_S	FM_S	FE_S
10	—	—	18·3	23·2	—	—
25	11·7	7·6	25·7	23·8	6·2	7·0
50	8·1	13·8	28·4	26·5	5·6	4·9
100	7·4	8·7	23·0	28·4	3·5	4·3
	EM_{SO}	EE_{SO}	PM_{SO}	PE_{SO}	FM_{SO}	FE_{SO}
10	—	—	24·7	18·6	—	—
25	9·2	9·5	31·3	51·0	13·3	18·8
50	7·4	14·6	23·4	165·0	8·5	25·7
100	11·9	14·3	—	—	—	—
	EM_{SO_2}	EE_{SO_2}	PM_{SO_2}	PE_{SO_2}	FM_{SO_2}	FE_{SO_2}
10	—	—	14·8	23·2	—	—
25	1·9	3·2	13·8	16·4	7·3	8·4
50	7·7	3·4	9·5	20·4	6·0	7·1
100	5·7	4·9	—	—	5·1	5·2

For dicoumarol the anticoagulative index (I_a) calculated per millimole in a dose of 10 mg/kg was assumed to be equal to 100.

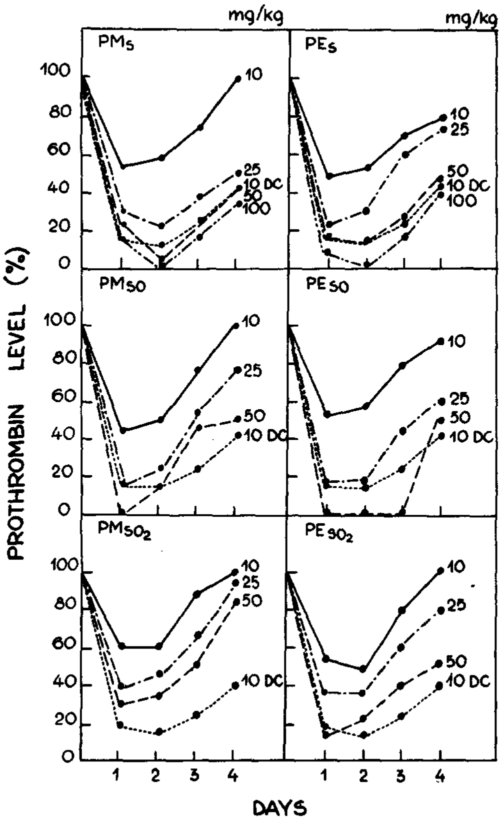


Fig. 2. Prothrombin levels after administration of derivatives of 3,3′-alkylthiopropylidene-*bis*-4-hydroxycoumarin (series P) at a dose of 10 mg/kg ——, 25 mg/kg -·-·- and 50 mg/kg ----, and of dicoumarol at a dose of 10 mg/kg ····.

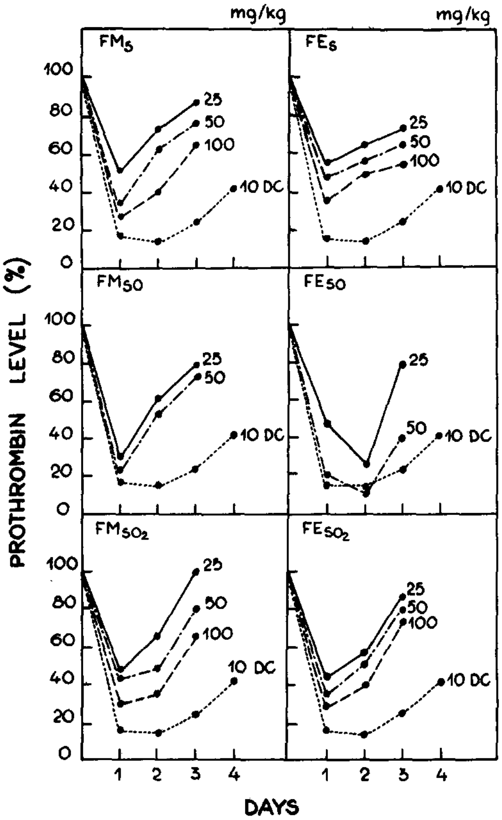


Fig. 3. Prothrombin levels after administration of derivatives of 3,3′-(5-alkylthiofurfurylidene)-*bis*-4-hydroxycoumarin (series F) at a dose of 25 mg/kg ——, 50 mg/kg -·-·-, and 100 mg/kg ----, and of dicoumarol at a dose of 10 mg/kg ······.

the F series as in the preceding ones (E and P). Sulphoxides were the most active derivatives, especially FE_{SO}, which was only slightly less active than PM_{SO}; sulphones and sulphides were the least active. An activating influence of the S-ethyl group was distinct only in sulphoxides.

DISCUSSION

The results of this study confirm our previous observation that in anticoagulant compounds derived from 4-hydroxycoumarin, the presence of sulphur-containing functional groups, has a significant influence on their activity [1, 3–6]. Alkylsulphide groups induced substantial antivitamin K_1 activity in inactive compound such as 3,3′-benzylidene-*bis*-4-hydroxycoumarin [1]. Oxidation of sulphur to the sulphoxide group in these compounds caused maximal increase of activity [1].

In the present study, compounds were examined which on the methylene bridge of 3,3′-methylene-*bis*-derivatives of 4-hydroxycoumarin contain thioether groups (series E and P), besides structurally similar compounds of the furfurylidene series (series F). Of the compounds investigated only EE_S (Thiotan®) was previously screened by Trčka [5, 6] and PM_S (Properton, Thiocoumar®, Thioporan®) by Gori [3, 4]. According to Trčka [5] EE_S is more active than EM_S as an anticoagulant in rabbits. He did not observe any differences in the activity of EE_S and the corresponding oxygen containing analogue 3,3′-metoxyethylidene-*bis*-4-hydroxycoumarin. Veldstra *et al.* [7] also did not observe any significant differences in the anticoagulant activity of metoxy- and etoxy-derivatives in the group of 3,3′-alkoxyethylidene-*bis*-4-hydroxycoumarins. In our experiments, in all three series, E, P and F (Table 1), compounds with a sulphoxide group were the most active. As a rule, the presence of an S-ethyl group caused a stronger increase of anticoagulant activity. All the compounds containing a SO_2 group were undoubtly less active.

Our observations on three large groups of 4-hydroxycoumarin derivatives, namely 3,3′-alkylthioarylidene-*bis*-4-hydroxycoumarins [1], 3,3′-alkylthioalkylidene and 3,3′-(5-alkylthiofurfurylidene)-*bis*-4-hydroxycoumarins and on the products of sulphur oxidation in these compounds indicate an important role of sulphur-containing functional groups in compounds with antivitamin K_1 activity. Compounds of this type usually act quicker than dicoumarol and stronger than the respective 3,3′-ethylidene and propylidene-*bis*-4-hydroxycoumarins [4, 5]. This may be connected with the interaction of –S-alkyl or –SO-alkyl groups with sulphur-containing proteins involved in blood coagulation. According to Henschen and Blombäck [8, 9] human fibrinogen is a protein with an exceptionally high sulphur content. The observations of Jeener [10] and Lyons [11] indicated the role of –SH groups in blood coagulation. Potentiation of the anticoagulant indices by increasing doses of sulphoxides (PE_{SO}, EE_{SO} and FE_{SO}) observed by us seems to support this opinion. Besides the basic antivitamin K_1 effect of the *bis*-4-hydroxycoumarin moiety, the sulphoxide group exerts probably an additional direct effect on the proteins of the blood coagulation system. This type of mechanism could also explain the relatively rapid and brief action of the sulphur containing derivatives of 4-hydroxycoumarin studied by us.

The higher activity of –S-ethyl compared with –S-methyl compounds is noteworthy. The former are probably less easily dealkylated so that their effect is more intensive and of longer duration than the effect of –S-methyl compounds. The observation of Witte *et al.* [12] that ethionine, in contrast to methionine, inhibits blood coagulation is of interest in connection with our observations.

Introduction of –S-ethyl, or –SO-ethyl groups into structures with antivitamin K_1 activity has a favourable effect on their biological action because it shortens the time of their activities to 24 hr and increases it slightly, too. The propylidene (P) series is generally more active than the ethylidene (E) series. It could be probably a consequence of a longer chain in which the –S-alkyl group is better accessible to chemical interaction.

Of the preparations investigated, PE_{SO} is most interesting. A dose of 25 mg/kg of this compound lowers the prothrombin level in rabbits to nearly the same degree as 10 mg/kg of dicoumarol. Moreover, at this and higher doses, PE_{SO} is devoid of side effects such as internal haemorrhages leading to death of the animal, as was often observed after other anticoagulant drugs.

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