The Synthesis and Structure of Some 4-Hydroxycoumarins and Their Pharmacological Activity

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Coumarin is a structural fragment of different natural and synthetic compounds which demonstrate a wide range of pharmacological activities. Its derivatives are of interest because of their properties as oral anticoagulants or rodenticides, photosensitizers, anti-HIV agents and antibiotics. There has been continuous interest in the synthesis of these compounds.

This book describes various experiments for modifying the structure of clinically used anticoagulants, and establishes the structure of the synthesized products through X-ray analysis. It also identifies unexpected structures in this regard, and highlights the usefulness of all these structural modifications through different biological, toxicological and pharmacological experiments.

The most widely used antithrombotic drug in the European countries is the racemic Acenocoumarol (Syntrom, Niffcoumar). Chemical modifications of the Acenocoumarol structure seem to be a promising route to obtain compounds with a good biological activity, lower toxicity and fewer side effects.

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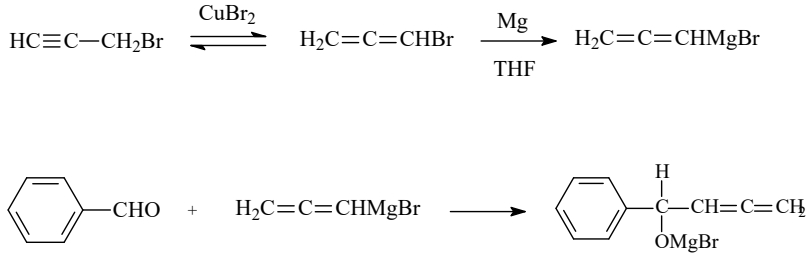
A METHOD FOR THE SYNTHESIS OF ANTICOAGULANTS - 4-HYDROXYCOUMARIN DERIVATIVES WITH CLINICAL APPLICATIONS

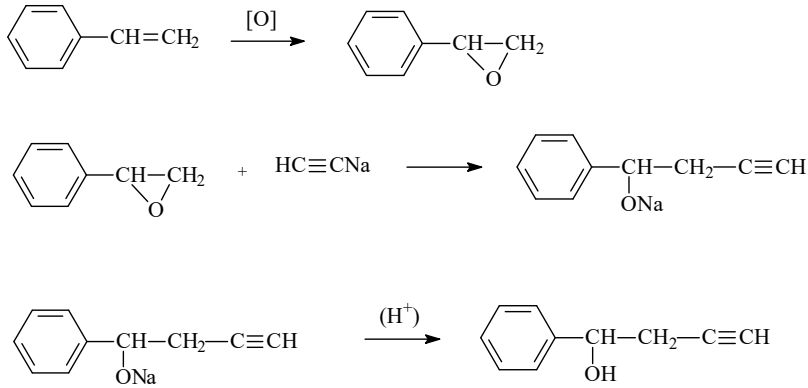
The first investigational steps in the area of 4-hydroxycoumarin derivatives were connected with experiments aiming to develop a patent pure technology for the synthesis of 4-hydroxy-3-[1-(4-nitrophenyl)-3-oxobutyl]-2H-chromen-2-one. This substance is known in the clinical practice under the names Acenocoumarol, Syntrom, Syncoumar, and later the Bulgarian name Niffcoumar.

Different catalysts have been used till now in the preparation of the anticoagulants warfarin and its 4'-substituted derivative acenocoumarol by Michael addition of 4-hydroxycoumarin to the corresponding 4-aryl-3-buten-2-ones. Among them are: ammonia [Starr and Haber, US Pat. 2 666 064, Jan. 12, 1954; CA, 49, 380h, 1955; Krey, DDR Pat. 15 092, 1958; CA, 3458de, 1960], aliphatic or aromatic amines [Krey, DDR Pat. 15 092, July 25, 1958 (Cl. 12q); CA, 3458de, 1960; Joshi and Bose, 1972, 461-462; Rizzo and Davis, 1989, 183-189], pyridine [Joshi and Bose, 1972, 461-462; Ikawa, Stahmann and Link. 1944, 902-906, US. Pat. 2 427 578, 1947; CA, 42, P603h, 1948; Stoll and Litvan, US Pat. 2 648 682, 1953, Geigy AG, Basel; CA, 49, P 2522fg, 1955], quinolone [Joshi and Bose, 1972, 461-462; Lacobescu and Gostea. Fr. Pat. 1 271 976; CA, 56, 15488g, 1962], alkali phosphates [Joshi and Bose, 1972, 461-462; Spiess and Spiess, Brit. Pat. 734 142, 1955; CA, 50, 7143a, 1956; Knoevenagel. D.O.S. 948 507, 1956, Spiess & Sohn and Norddeutsche Affinerie; CA, 53, 2258g, 1959], sodium acetate or citrate [Joshi and Bose, 1972, 461-462], borax [Joshi and Bose, 1972, 461-462] and alkali alkoxides [Stoll and Litvan, US Pat. 2 648 682, 1953, Geigy AG, Basel; CA, 49, P 2522fg, 1955].

Scheme 1. The synthesis of 1-phenyl-3-butin-1-ol by using 3-bromopropin



Scheme 2. The synthesis of 1-phenyl-3-butin-1-ol by using styrene



We found out that by accomplishing this reaction in an aqueous medium either alkali fluorides (KF, NaF) or quaternary ammonium salts (phase-transfer catalysts) could be applied as highly efficient catalysts (Table 1). For comparison of the reaction times, the addition process was carried out without any catalyst (entries 1 and 12) or in the presence of trimethylamine or trisodium phosphate (entries 10 and 11) which were the most recommended catalysts according to the literature. When accomplished in pyridine or in glacial acetic acid in the presence of sodium acetate, the

reaction gave dark-coloured by-products and the purification of the products was complicated. Diluted sulphuric acid inhibited or stopped this reaction.

The following general conclusions can be drawn from the experimental data presented in Table 1.

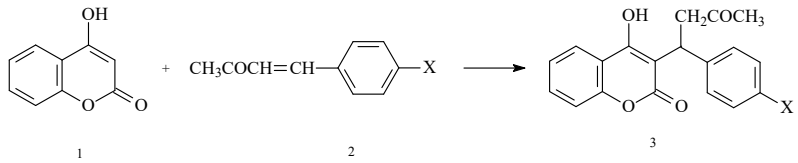
First, for the preparation of warfarin (**3a**) the phase-transfer catalysts in an optimum concentration of 5 mol per cent were very suitable. The yield of **3a** was not essentially influenced by the nature of the quaternary salt. The use of these catalysts reduces the reaction time to about 5 % while the use of KF reduced it only to about 50 % (entry 9). Even more, the catalysed processes gave markedly higher yields of **3a**.

Second, the alkali fluorides in a concentration of 5 mol per cent were more useful for the preparation of acenocoumarol (**3b**). There was practically no difference in the effects of the salts used, while triethylbenzylammonium chloride (entry 17) shortened it to about 50 %. The simultaneous application of both catalysts (entry 18) did not enhance the catalytic effect, and the product in these trials was of low quality.

High yields were achieved when using the catalysts described above – nearly 80 % for triethylbenzylammonium chloride in **3a** (entry 3) and for NaF in **3b** (entry 16). The reaction mixture remained pale-coloured till the end of the process and the corresponding product could be isolated easily in a rather pure state.

The Michael addition occurred by refluxing in a heterogeneous mixture, since 4- hydroxycoumarin (**1**) was soluble in hot water and the unsaturated ketone **2** was a melt. Obviously, fluoride ions acted as a weak basic catalyst with good solubility in the organic phase [Curci, Fiorentino, Troisi, Edwards et al. 1980, 4758-4760]. However, additional studies should be performed in order to get a reasonable explanation of the effect of the quaternary ammonium salts in the absence of any base (in contrast to the numerous examples in the literature) [Dehmlow and Dehmlow. Phase Transfer Catalysis, 2nd Ed., Verlag Chemie, Weinheim, 1983].

Scheme 3. The interaction of 4-hydroxycoumarin and unsaturated ketones



1 2 3

2 , 3	a	b
x	H	NO_2

Table 1. The influence of some catalysts on the synthesis of Warfarin (3 a) and Acenocoumarol (3 b)

Entry No	Product	Catalyst (amount)	Duration, h	Yield, %,[a)]	M.p. [o] C, [b)]
1	**3a**	Without a catalyst	42	46	160-161
2		Triethylbenzylammonium chloride (2.5 mol %)	2	66	155-158
3		Triethylbenzylammo-nium chloride (5 mol %)	2	83	155-158
4		Triethylbenzylammo-nium chloride (10 mol %)	2	84	155-158
5		Tetrabutylammonium chloride (5 mol %)	3	77	150-154
6		Benzyltributylammonium chloride (5 mol %)	2	83	156-159
7		Tetraoctylammonium bromide (5 mol %)	3	75	155-157
8		Aliquat 336 (5 mol %)	2	68	158-160
9		KF (5 mol %)	20	71	158-162
10		Triethylamine (7.7 mol %)	3	67	159-161
11		Na_3PO_4 (6 mol %)	10	57	159-161
12	**3b**	Without a catalyst	6	78	c)
13		KF (5 mol %)	2	77	c)

14	KF (10 mol %)	2	83	c)
15	NaF (15 mol %)	2	75	c)
16	NaF (5 mol %)	2	79	c)
17	Triethylbenzylammonium chloride (5 mol %)	3	88	c)
18	KF (5 mol %) + triethyl-benzylammonium chloride (5 mol %)	2	92	157-180 coloured

Notes:
a) The yield of the isolated product without recrystallization, TLC homogeneous;
b) The m.p. corresponds to the product obtained with the given yield;
c) The typical m.p. of the crude acenocoumarol is in the range 192-196 °C [Ivanov, Manolov and Alexandrova. 1990, 521-522] (lit. m.p. 196-199 °C) [The Merck Index, 10th Ed.,p. 29, Rahway, USA, 1983].

The synthesis of warfarin sodium (**1**) [US Pharmacopeia XXI, p. 1121, 1985] was realized using an original technology protected by an authorship certificate [Manolov and Ivanov, BG Pat. 48021, 14.04.1989], in view of the commercial production of the drug form. This required an appropriate method for its analysis.

THE ANALYTICAL INVESTIGATIONS

In the past the quantitative determination of **1** was carried out spectrophotometrically. However, this technique had some drawbacks, its relatively low accuracy being the major one. The performance of the determination was quite complex, as several parallel analyses (of the sample, standard and blank) were required.

Warfarin sodium is a weak base of the charge type A (negative), so its quantitative determination can be performed by acid-base titration. The performance of the latter in an aqueous medium is, however, strongly hampered since the conjugated acid (warfarin) is water-insoluble and a two-phase system is obtained in the course of the titration. Due to its acidic properties, the precipitate formed hampers the exact visual determination of the equivalence volume since the colour transition of the indicator is vague. The presence of a two-phase system renders difficult the potentiometric performance of the analysis as well, since the electrode potential is vacillating and does not reach an equilibrium state.

Due to these reasons, as a first step, an appropriate medium readily dissolving both forms of the substance was supposed to be found. It was experimentally established that methanol was quite appropriate as a solvent.

It should be noted that the titration of **1** as a base of the charge type $\overline{A}$ in methanol offered considerable advantages from a theoretical point of view, since an elongation of the equivalence part of the titration curve was achieved. In order to reduce the waste of methanol, a methanol/water mixture was used. It was established that the optimum titration medium was methanol/water (70:30 v/v). When the water content was higher than 30 %, a precipitate was formed.

For the visual establishment of the equivalence point we had to choose an appropriate indicator. For that purpose an experimental titration curve of **1** in methanol/water mixture was drawn by potentiometric titration with small additions of a standard solution. The titration curve is shown in Fig. 1. It could be seen that its equivalence part was relatively short, i.e. the error of the indicator determination would not be more than 2-3 %. The equivalence part of the titration curve was from 6 to 3 pH units. In this pH range suitable indicators were methyl orange and 2,6-dinitrophenol. Five determinations of the chromatographically pure substance **1** were performed

with both indicators. The preliminary chromatographic studies of the substance were carried out by TLC on Kieselgel GF 254 in cyclohexane/chloroform/acetic acid (10:10:4). Under these conditions the substance proved to be free of any intermediate products of the synthesis. The results of the indicator determination of **1** showed that both indicators gave higher results with great systematic error, which was + 3.8 % for methyl orange and + 2.9 % for 2,6-dinitrophenol. Furthermore, the exact determination of the equivalence volume was strongly hampered due to the lack of a clear colour change. Only a slight decrease of the colour intensity was observed in the equivalence point while a relatively clear colour change was achieved by the addition of an excess (10-15 %) of the titrant.

In order to avoid a systematic error related to the indicator determination of the equivalence point, a potentiometric version of the same determination was developed, using a glass pH-electrode. In view of the slight potential change, due to the weak basic properties of the substance studied, the determination of the equivalence point was performed by the method of the linearized titration curves, proposed by Gran [Gran, Analyst, 1952, 661-671]. The enlarged equation [Pehrsson, Ingman and Johansson, 1976, 769-780] for the determination of the equivalence volume V_e adapted for the titration of weak bases was used:

$$V_e = V + K_a \left[\frac{V}{H} + \frac{V_o + V}{C} \left(\frac{K_s}{H^2} - 1 \right) \right] + \frac{V_o + V}{C} \left(\frac{K_s}{H} - H \right)$$

where

V_o = the initial volume of the sample;

V = the volume of titrant added;

C = the concentration of the titrant;

K_s = the autoprotolysis constant of the solvent;

K_a – the protolysis constant of warfarin;

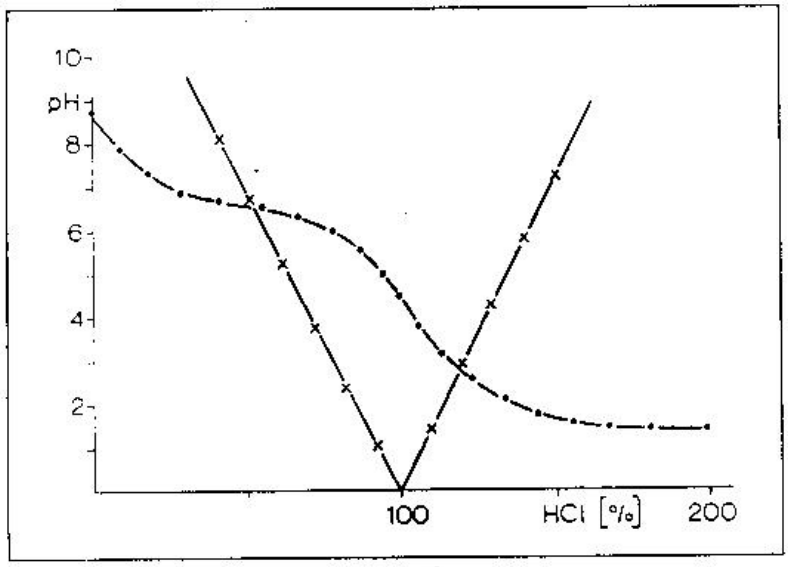
H = the concentration of hydrogen ions [mol/L].

The equation involved the concentration of hydrogen ions so the results of the experimental data had to be presented in pH units. This imposed the preliminary calibration of the electrode system. However, since there were

no corresponding buffers and the pH-function in this medium differed from that of water, the calibration might introduce a systematic error in the results. In order to overcome this problem, we employed the method of the electrode system calibration in the course of the titration proposed by Tencheva et al. [Tencheva, Velinov and Budevsky, 1976, 65-74]. Thus, the titration was performed by reading the millivolt data with their subsequent recalculation into pH units.

The Gran-plot of the determination of **1** according to the proposed method is shown in Fig. 1. It is seen that in this case the determination of the equivalence point is quite reliable and with high accuracy.

Figure 1. The titration of warfarin sodium with 1 mol/L HCl in methanol/water (70:30, v/v).



The experimental titration curve, x: The Gran-plot of the same analysis

Five parallel analyses of **1** were carried out using the developed potentiometric method and the method of USP XXI. The results obtained are presented in Table 2. The coincidence of the mean values obtained by the two methods can be seen and both results are well in agreement with the pharmacopeia requirements for the percentage of **1** (97 to 102 %). Concerning the accuracy and precision of the two methods, the advantage of the proposed potentiometric method over the spectrophotometric one is

obvious. Furthermore, the developed method offers one more advantage: it presents the value of the protolysis constant pK_a of warfarin (shown in Table 2), which is an indication of identity.

In order to enhance the accuracy of the results by eliminating the human factor, the method was automated. For this purpose it was adapted to the possibilities of the automatic titration system. It was established that the automatic performance of the analysis not only enhanced its precision but also shortened the time for a single determination to 1-2 min [Panushev, Velinov and Manolov, 1992, 302-303].

Table 2. The results of the parallel analyses of warfarin sodium according to the proposed potentiometric method compared with the spectrophotometric method of USP XXI

№	Potentiometric method			Spectrophotometric method	
sample (mg)	results (mg)	content (%)	pK_a	content (%)	
1.	294.0	290.8	98.9	5.93	97.6
2.	296.2	292.3	98.7	5.90	98.2
3.	295.9	292.0	98.7	5.88	99.5
4.	295.3	291.9	98.8	5.84	99.9
5.	299.6	295.7	98.7	5.82	98.4
Mean value:			98.76		98.72
Relative standard deviation:			0.09		0.96

The derivatives of 4-hydroxycoumarin are of interest because of their physiological, photodynamic, anticoagulant, spasmolytic, bacteriostatic, and rodenticidal activities. Some coumarin derivatives are known for their antibiotic and antifungal activities. They are also used as analytical reagents.

The most widely used antithrombotic drug in the USA and Canada is the racemic sodium Warfarin which is a substance with few side effects.

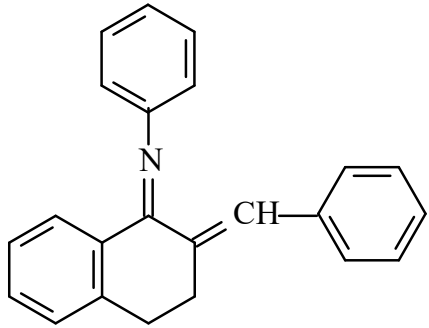
THE ALDEHYDE CONDENSATION PRODUCTS OF 4-HYDROXYCOUMARIN AND SCHIFF BASES

Nearly forty years ago Dike and Merchant investigated the reaction of 4-hydroxycoumarin with Schiff bases in an acidic medium. The experiments involved a condensation of one mole 4-hydroxycoumarin with two moles benzylideneaniline in glacial acetic acid at 30 °C for 1-3 hrs. The product was benzopyranooxazine with a molecular mass of 431 ($C_{29}H_{21}NO_3$) and a melting point 169 °C.

Our attempts to repeat these experiments and results failed. The reaction described above was carefully carried out with 4-hydroxycoumarin and benzylideneaniline, p-methoxybenzylideneaniline and p-chlorobenzylideneaniline at the same molar ratio in glacial acetic acid at 30 °C for 1, 3, 10 hrs and longer but there was no interaction. Only the starting substances were in the reaction mixture. In addition, there was no condensation process as well even when the reaction was carried out in methanol as a solvent.

The same reaction mixture in glacial acetic acid as a solvent was refluxed for 13 hrs and after cooling a crystalline product was separated. After recrystallization, the m.p. of the pure product was 299-302 °C. The elemental analysis revealed a molecular formula $C_{22}H_{15}NO_2$ confirmed by its MS (M+ 325) taken at 70 eV (Jeol 300 D – Japan). ^1H NMR ([D_6]-DMSO) showed a singlet for a proton at δ 5.3 ppm and a multiplet at δ 6.8 – 7.7 ppm corresponding to 14 aromatic protons. The IR (nujol) spectrum showed a band at 1660-1620 cm^{-1} for the conjugated double bonds and a band at 750 cm^{-1} for the monosubstituted aromatic nucleus.

On the basis of the structure of the new product we supposed that the Schiff base was probably hydrolysed in an acetic acid medium. After the Schiff base had been hydrolysed, the aromatic aldehyde attacked No 3 carbon atom and aniline attacked No 4 carbon atom in 4-hydroxycoumarin. The final product was 3-benzylidene-4-phenyliminocoumarin after a subsequent (or a simultaneous) detachment of two water molecules.



3-benzylidene-4-phenyliminocoumarin

In order to confirm this type of interaction, a condensation process between 4-hydroxycoumarin and aniline at a molar ratio 1:17 (w.p.) was carried out. The reaction mixture was refluxed for 45 min. and 4-phenyliminocoumarin was obtained (m.p. 273-274 ^{o}C, yield 44 %, TLC pure product). Then 4-phenyliminocoumarin was refluxed with benzaldehyde in glacial acetic acid for 17 hrs. The product was 3-benzylidene-4-phenyliminocoumarin, the same as the one obtained by the condensation process between 4-hydroxycoumarin and benzylideneaniline in glacial acetic acid.

The reaction followed a different pathway with new end products when the starting substances were 4-hydroxycoumarin and 4-methoxybenzylideneaniline or 4-chlorobenzylideneaniline. 3,3'-(Arylmethylidene)-bis-4hydroxycoumarins were obtained instead of 3-arylmethylidene-4-phenylimino-coumarins. The structures of these compounds were confirmed by elemental analyses, IR, ^{1}H NMR and MS spectra.

This unexpected way of the condensation process could be explained by the presence of substituents with electronodonors' properties at para-position of the aromatic nucleus in the Schiff base between arylmethylidenechromanocoumarin and phenylammonium acetate. After alkalization, aniline was found in the filtrate.

These products were synthesized by the interaction of 4-hydroxycoumarin with aromatic aldehydes (4-methoxybenzaldehyde and 4-chlorobenzaldehyde) in boiling alcohol or in glacial acetic acid medium at reflux. The structures of the end products were identical to the ones of the substances above, which fact was proven in an unequivocal way [Manolov, 1998, 3041-3042].