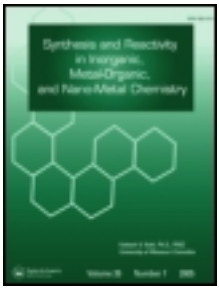

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Synthesis, Characterization and Anticoagulant Action of Lanthanide Complexes of Warfarin

Tian Quan Jiao [a] , Ji Gui Wu [a] , Fu Li Zeng [b] , Yun Long Fun [a] & Ru Wen Deng [a]

[a] Department of Chemistry, Lanzhou University, Lanzhou, 730000, China

[b] Department of Biology, Lanzhou University, Lanzhou, 730000, China

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SYNTHESIS, CHARACTERIZATION AND ANTICOAGULANT ACTION OF LANTHANIDE COMPLEXES OF WARFARIN

Tian Quan Jiao[a], Ji Gui Wu[a], Fu Li Zeng[b]*, Yun Long Fu[a] and Ru Wen Deng[a]

[a]Department of Chemistry, Lanzhou University, Lanzhou, 730000, China
[b]Department of Biology, Lanzhou University, Lanzhou, 730000, China

ABSTRACT

Thirteen lanthanide complexes of warfarin, $LnL_3 \cdot nH_2O$ [n = 6 (Ln = La-Yb) or n = 4 (Ln = Y); L = $(C_{19}H_{15}O_4)^-$] have been synthesized and characterized by elemental analyses, IR, ^1H NMR, thermoanalysis and molar conductance. The lanthanide ions combine with warfarin through the oxygen anion of the phenolic group by losing the proton and the oxygen atoms of two carbonyl groups. The warfarin lanthanide complexes show good anticoagulant action, especially the warfarin neodymium complex, which was less toxic than the sodium salt of warfarin.

INTRODUCTION

The first oral anticoagulant, dicumarol, was isolated from spoiled sweet clover, and then numerous analogues were synthesized and tested for anticoagulant activity. Fifty percent of hospitalized patients that die show evidence of ante mortem thromboembolism[1-3]. Warfarin, [3-(α-acetonylbenzyl)-4-hydroxycoumarin, $C_6H_4C_3O(OH)(O)CH(CH_2COCH_3)C_6H_5$, (HL)], Fig. 1, is one of the most important oral anticoagulants for the prevention and treatment of a variety of venoms and, to a lesser extent, arterial thromboembolic disorders. In recent years, many studies showed that the lanthanide elements have been used clinically as antithrombotic agents. Some complexes such as those with 3-sulfoisonicotinic acid and β-acetylpropionic acid fairly recently were still in clinical use and, therefore, in this study we tried to prepare lanthanide complexes of warfarin to examine their properties and anticoagulant actions[4,5].

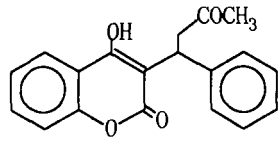

Fig. 1. Structure of Warfarin

RESULTS AND DISCUSSION

As seen from Table I, $LnL_3 \cdot nH_2O$ type complexes has been prepared [Ln = trivalent lanthanide cations, L = univalent anion of warfarin, $(C_{19}H_{15}O_4)^-$; n = 6 (Ln = La -Yb) or n = 4 (Ln = Y)]. Except for the yellow complex of europium, the others all have the corresponding colour of their hydrous cations. Under ultraviolet light the gadolinium and terbium complexes emit white or yellow-green fluorescence, respectively. These complexes are soluble in methanol, ethanol, acetone, dimethyl sulfoxide and dimethylformamide, but insoluble in chloroform, ethyl ether and benzene. Their molar conductances in dimethylformamide (nearly 10^{-3} mol/L) show that they exist as non-electrolytes.

IR Spectra

The infrared spectra of warfarin, the sodium salt of warfarin (NaL) and some warfarin lanthanide complexes are listed in Table II. The IR spectra of these lanthanide complexes are fundamentally similar which suggests that they have the same structure. Their bands at about 3390 cm^{-1} are ascribable to water which is in keeping with the elemental analyses. Warfarin has three interconverting tautomeric structures[6-8] in solutions of Me_2SO-d_6, $CDCl_3$ or mixtures of both solvents: (a) diastereomeric hemiketal; (b) open-chain form; (c) eight-membered ring with hydrogen bond. The diastereomeric hemiketal (a) is the main form in the crystalline state[9] (Fig. 2).

The absorption spectrum of warfarin exhibits a band at 3283 cm^{-1}, ascribable to OH. The above band is not present in the sodium salt of warfarin and the warfarin lanthanide complexes which shows that the univalent anion of warfarin, losing the OH proton, combines with sodium or lanthanide cations. The absorption band at 1680 cm^{-1} can be assigned to the carbonyl group of the internal ester of the hemiketal form of warfarin, but the warfarin sodium salt and warfarin lanthanide complexes have two absorption bands for carbonyl groups. The bands for NaL were observed at 1720 and 1666 cm^{-1}, the former is assigned to the stretching vibration of

TABLE I Analytical Data and Molar Conductances ($cm^2 \cdot ohm^{-1} \cdot mol^{-1}$) of the Complexes

Complexes	Emp. formula	F. wt.	Ln%	C%	H%	Yield %	Mol. cond.	Decomp. temp., ℃
$LaL_3 \cdot 6H_2O$	$C_{57}H_{57}O_{18}La$	1168.9	11.67 (11.88)	58.70 (58.52)	4.72 (4.92)	95	40.5	253
$CeL_3 \cdot 6H_2O$	$C_{57}H_{57}O_{18}Ce$	1170.1	11.83 (11.97)	58.58 (58.46)	4.72 (4.91)	94	39.0	253
$PrL_3 \cdot 6H_2O$	$C_{57}H_{57}O_{18}Pr$	1170.9	12.01 (12.05)	58.51 (58.42)	4.70 (4.91)	92	38.2	254
$NdL_3 \cdot 6H_2O$	$C_{57}H_{57}O_{18}Nd$	1174.2	12.25 (12.28)	58.40 (58.30)	4.69 (4.89)	95	40.2	254
$SmL_3 \cdot 6H_2O$	$C_{57}H_{57}O_{18}Sm$	1180.4	12.52 (12.74)	58.17 (58.00)	4.68 (4.87)	92	32.6	255
$EuL_3 \cdot 6H_2O$	$C_{57}H_{57}O_{18}Eu$	1182.0	12.65 (12.86)	58.12 (57.92)	4.65 (4.86)	94	32.2	255
$GdL_3 \cdot 6H_2O$	$C_{57}H_{57}O_{18}Gd$	1187.3	13.03 (13.25)	57.86 (57.66)	4.66 (4.84)	93	32.2	255
$TbL_3 \cdot 6H_2O$	$C_{57}H_{57}O_{18}Tb$	1188.9	13.32 (13.37)	57.63 (57.54)	4.68 (4.83)	94	28.0	257
$DyL_3 \cdot 6H_2O$	$C_{57}H_{57}O_{18}Dy$	1192.5	13.52 (13.63)	57.37 (57.41)	4.67 (4.82)	92	23.5	260
$ErL_3 \cdot 6H_2O$	$C_{57}H_{57}O_{18}Er$	1197.3	13.84 (13.97)	57.34 (57.18)	4.61 (4.80)	94	26.0	262
$TmL_3 \cdot 6H_2O$	$C_{57}H_{57}O_{18}Tm$	1198.9	14.05 (14.09)	57.42 (57.10)	4.78 (4.79)	93	30.2	265
$YbL_3 \cdot 6H_2O$	$C_{57}H_{57}O_{18}Yb$	1203.0	14.35 (14.38)	56.93 (56.91)	4.66 (4.78)	93	30.0	266
$YL_3 \cdot 4H_2O$	$C_{57}H_{53}O_{16}Y$	1082.9	8.20 (8.21)	63.35 (63.17)	4.73 (4.93)	92	28.2	261

$L = (C_{19}H_{15}O_4)^-$; calculated values in parentheses

TABLE II
Main IR Bands (cm^{-1}) and Assignments for the Ligand, Warfarin Sodium Salt and Complexes

Absorp. peak	HL	NaL	$LaL_3 \cdot 6H_2O$	$NdL_3 \cdot 6H_2O$	$GdL_3 \cdot 6H_2O$	$YL_3 \cdot 4H_2O$
$\nu(H_2O)$	—	—	~3400	~3395	~3390	~3390
ν(OH)	3283	—	—	—	—	—
ν(C=O)	1680	1720	1692	1692	1692	1692
		1666	1653	1652	1652	1652
ν(Ph-C)	1384	1413	1406	1406	1405	1405

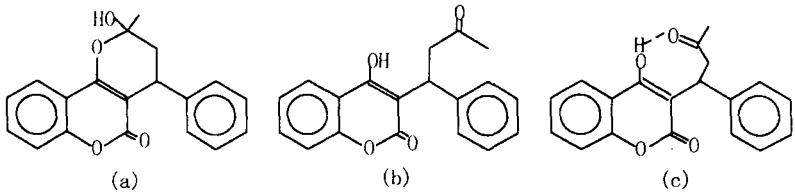

Fig. 2. Tautomeric Forms of Warfarin

the carbonyl group of the side chain ketone, and the latter may be assigned to the carbonyl group of the internal ester, which reveals that warfarin combines with the sodium ion to form the salt of the open-chain form. The carbonyl group of the internal ester shifts to lower wave number owing to loss of the OH proton, as the negative charge of the enol oxygen moves to the ring, so the π bond of C=O of the internal ester weakens. The two vibrational peaks of warfarin lanthanide complexes occur at 1692 and 1652-1653 cm^{-1} due to the carbonyl group, which exhibited a red-shift in comparison with NaL. These changes indicate that, after deprotonation, the two oxygen atoms of the carbonyl groups of warfarin take part in coordination to lanthanide ions.

The absorption peak ν(Ph-C) of benzopyrone ring for warfarin occurs at 1384 cm^{-1}, but for warfarin sodium salt this peak is shifted to higher frequency (1413 cm^{-1}) as a result of the increase of the bond order upon losing the OH proton. The ν(Ph-C) bands of benzopyrone ring for warfarin lanthanide complexes exist at 1406 or 1405 cm^{-1}. We conclude that in the lanthanide complexes the warfarin anion exists in the open chain form, binding to the lanthanide cations through the phenol oxygen after loss of the OH proton, and the carbonyl groups of the side chain and internal ester.

^{1}H NMR Spectra

The proton magnetic resonance chemical shifts (ppm) for warfarin were recorded in dimethyl sulfoxide solution (s = singlet, t = triplet, m = multiplet): (δ) 1.63 (3H, s, $-CH_3$), 1.89-2.4 (2H, s, $>CH_2$), 4.0 (1H, t, $\geq CH$), 7.19-7.88 (9H, m, Ar-H). These experimental data are in keeping with reference 9, which showed that warfarin exists mainly as cyclic interconverting tautomeric structures. However, the metal compounds in dimethylsulfoxide solution exhibit different chemical shifts: warfarin sodium salt, (δ) 2.03 (3H, s, $-CH_3$), 3.23-3.32 (2H, s, $>CH_2$), 4.87, (1H, t, $\geq CH$), 7.03-7.75 (9H, m, Ar-H); warfarin lanthanum complex, (δ) 2.05 (3H, s, $-CH_3$), 3.24-3.34 (2H, s, $>CH_2$), 4.92 (1H, t, $\geq CH$), 7.02-7.98 (9H, m, Ar-H); warfarin yttrium complex, (δ) 2.04 (3H, s, $-CH_3$), 3.36-3.49 (2H, s, $>CH_2$), 5.00 (1H, t, $\geq CH$), 7.06-8.06 (9H, m, Ar-H). The characteristic peaks of methylene and methyne for open chain compounds occur at about 3.3 and 5 ppm, respectively, which shows that the metal salt or complexes of warfarin have the open chain form consistent with IR data. The chemical shifts of warfarin sodium salt and lanthanum or yttrium complexes undergo a shift to lower field in the above sequence. It is possible that the ionic potentials and the attraction forces of the electron for Na^+, La^{3+} and Y^{3+} are enhanced in this order, so that the deshielding effects for these metal ions on related warfarin compounds increase in the same sequence.

Thermal Analyses

The thermal analytical data of some warfarin complexes are presented in Table III. The DTA and TG thermographs of the complexes are similar for analogous structures, but obviously differ with the ligand. About 58° C all complexes begin to lose weight and up to about 255° C two endothermic peaks ascribable to dehydration processes were recorded. The weight loss percentages are fundamentally consistent with the calculated values which revealed that the dehydrating occurs step by step, and the water of the complexes exists in two different forms. Upon further heating the dehydrated complexes decompose at once, and some exothermic peaks occur owing to combustion of the organic components under the oxidizing atmosphere. At about 600° C the weight loss and DTA curves become constant, and the residue weight corresponds to the theoretical value calculated for Ln_2O_3 (Ln = trivalent lanthanide cations). Comparing the decomposition temperatures, the combinative forces of warfarin anion with trivalent lanthanide cations strengthen with an increase of the atomic number (except yttrium cation). From the above results, the structure in Fig. 3 is proposed for these complexes.

Anticoagulant Action

The experimental anticoagulation data of some lanthanide chlorides and lanthanide warfarin complexes are listed in Tables IV and V. The dose of warfarin

TABLE III
Thermal Analytical Data of Warfarin Lanthanide Complexes

Experiments	$LaL_3 \cdot 6H_2O$	$NdL_3 \cdot 6H_2O$	$GdL_3 \cdot 6H_2O$	$YbL_3 \cdot 6H_2O$	$YL_3 \cdot 4H_2O$
Temp. at beginning of wt. loss,° C	59	58	58	64	63
Endother. peak (dehydrated), ° C	108, 135	109, 136	110, 140	112, 155	112, 151
Wt. loss, %	8.93 (9.22)	8.65 (9.19)	8.60 (9.10)	8.65 (8.94)	6.15 (6.65)
Decompn. temp., ° C	253	254	255	266	261
Decompd. exother. peak, ° C	343, 456	360, 531	364, 523	389, 537	381, 527
Residue wt., %	13.05 (13.91)	14.00 (14.32)	16.17 (15.27)	17.19 (16.37)	11.21 (10.43)

Calculated values in parentheses.

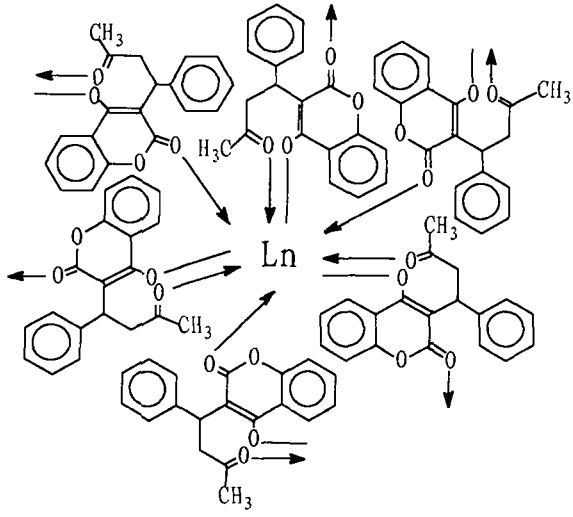

Fig. 3. The Proposed Structure for Lanthanide Complexes of Warfarin

TABLE IV
Data of Anticoagulant Action for Lanthanide Chlorides

Compounds	Sample vol. (mL)	Sample concn. (mol / L)	Dose (mg / kg)	Anticoag. time (min)
Control	0.25	0	0	4.0
$LaCl_3$	0.25	5.0×10^{-4}	1.53	7.1
$NdCl_3$	0.25	5.0×10^{-4}	1.57	9.0
$GdCl_3$	0.25	5.0×10^{-4}	1.65	5.9
$YbCl_3$	0.25	5.0×10^{-4}	1.75	4.0

$P < 0.05$ in the above experiments; P = probability of null hypothesis.

TABLE V
Data of Anticoagulant Action for Sodium Salt of Warfarin and Warfarin Lanthanide Complexes

Compounds	Sample vol. (mL)	Sample concn. (mol / L)	Dose (mg / kg)	Anticoag. time (min)
Control	0.25	0	0	4.0
NaL	0.25	3.0×10^{-3}	12.4	8.0
$LaL_3 \cdot 6H_2O$	0.25	2.5×10^{-4}	3.7	11.0
$NdL_3 \cdot 6H_2O$	0.25	2.5×10^{-4}	3.7	13.0
$GdL_3 \cdot 6H_2O$	0.25	2.5×10^{-4}	3.7	12.0
$YbL_3 \cdot 6H_2O$	0.25	2.5×10^{-4}	3.7	9.0

$P < 0.05$ in the above experiments; P = probability of null hypothesis.

sodium salt for humans is the basis point of the experimental operation. In order to arrange suitable experimental conditions, the dose of warfarin lanthanide complexes used was calculated to be one-fourth of the molar dose for warfarin sodium salt.

As seen from Tables IV and V, although the used quantities of warfarin or lanthanide in the warfarin lanthanide complexes is lower than that of the independent quantities of the warfarin sodium salt or lanthanide chloride, the anticoagulant action of warfarin lanthanide complexes is better, which showed that the warfarin anion and lanthanide cation have a synergistic anticoagulant effect. Among the compounds of these two series, the anticoagulant activities of neodymium compounds are the best, especially the warfarin neodymium complex. It was also discovered that the anticoagulant time of warfarin lanthanide complexes (4-96 h) is longer than that of warfarin sodium salt (4-72 h), although their anticoagulant times at the beginning are approximately equal. The anticoagulant mechanism of warfarin is different from that of the lanthanide ions, the former inhibits vitamin K and vitamin K 2,3-epoxide reductase activities[10], and the latter inhibit competitively the action of calcium ion in the blood coagulation process[4]. After the warfarin lanthanide complex was administered into the stomach, it is possible that the warfarin anion and lanthanide cation exhibit their anticoagulant actions according to their individual mechanism; later, the anticoagulant effect increases obviously due to their synergistic action. The warfarin anion and lanthanide cation attract each other, so that the release, decomposition, metabolism and excretion processes of the complexes slow down, and the effective anticoagulant times are prolonged.

Acute Toxicity

The experimental data of acute toxicity are listed in Table VI. The signs of death are fecal and urinary incontinences, staggering gait and general tremors. As seen from Table VI, the toxicity of the warfarin neodymium complex is lower than that of warfarin sodium salt. It is possible that the warfarin anion and lanthanide cation in the complex bind to each other, hence the free warfarin concentration of the warfarin neodymium complex is lower than that of the warfarin sodium salt.

EXPERIMENTAL

General

Lanthanide(III) chlorides were obtained by dissolving the corresponding oxide (99.95%) in a stoichiometric amount of 1:1 hydrochloric acid. The solutions obtained were put on a water bath to evaporate until a crystal film appeared. After

TABLE VI
Data of Acute Toxicity of the Sodium Salt of Warfarin and the Neodymium Warfarin Complex

Compd.	Obs. title	Dose (mL, 0.5 g / mL)				
		0.25	0.30	0.35	0.40	0.60
NaL	Death no.	0	0	2	3	4
	Survival no.	4	4	2	1	0
NdL_3	Death no.	0	0	0	0	4
	Survival no.	4	4	4	4	0

cooling to room temperature crystals separated out. The lanthanide content in all complexes was determined by heating the samples at 800 °C. The resulting residues were dissolved with 1:1 hydrochloric acid and the solution titrated with EDTA and xylenol orange as indicator. The contents of carbon and hydrogen were determined on a Carlo Erba 1106 elemental analyser. The sodium salt of warfarin as a drug was produced by the Shanghai Sixteenth Pharmaceutical Factory, China. IR spectra of the solid compounds were obtained in KBr pellets on a Nicolet 170SX infrared spectrometer. ^1H NMR spectra were recorded on a FT-80A NMR spectrometer in DMSO-d_6 solution, using TMS as internal reference. Thermal analyses were carried out with a Dupont 1090 thermal analyser in air.

Preparation of Warfarin (HL)

The sodium salt of warfarin was dissolved in water, adding 1:1 HCl solution until the product precipitated. After 0.5 h of stirring efficiently, the precipitate was separated by filtration, washed with water and dried in a desiccator to constant weight, m.p. : 160.5-161.0; lit.[7]: 159.5-160.5 °C.

Synthesis of the Complexes

A quantity of 2.0 g (6.1 mmol) sodium salt of warfarin was dissolved in 40 mL water. Then, with stirring, 10 mL of an aqueous solution of lanthanide chloride (2.1

mmol) was added dropwise. The product precipitated immediately. After 0.5 h of stirring, the precipitate was separated by filtration, washed with water five times, and dried in a vacuum desiccator with molecular sieves to constant weight. The yields of the thirteen warfarin lanthanide complexes are about 90-95%.

Investigation of the Anticoagulant Action

The calculated amounts of the different compounds were dissolved in the 1% Tween-80 aqueous emulsion, respectively. The mice weighing 20 ± 2 g and being in good health were chosen and divided stochastically, ten in each group. The test samples of 0.25 mL were perfused into their stomachs and a 1% Tween-80 aqueous emulsion was given to the control group. After 24 h the mice were anaesthetized with ether, 3 cm of the end of the tail was sheared off, so that blood flowed readily. The mouse blood was dipped gently with a filter paper one time per minute, until the wiredrawing appeared[5].

Investigation of the Acute Toxicity

Forty mice, weighing 20 ± 2 g and being in good health, were divided stochastically into 10 groups, four in each group. The different doses of the respective aqueous solutions were injected into the abdominal cavities of mice in each group; 4 h later, the survival and death numbers were observed.
Note: Ji Gui Wu did the actual writing.

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Referee I: P. K. Mascharak
Referee II: E. J. Valente