# Anomalous Chiroptical Properties of Warfarin and Phenprocoumon

Edward J. Valente and William F. Trager*

*Departments of Chemistry and Pharmaceutical Sciences, University of Washington, Seattle, Washington 98195.
Received May 17, 1977*

The configurationally similar enantiomers of warfarin and phenprocoumon are found to exhibit circular dichroism curves which are nearly mirror related in the range of 240–340 nm. This effect is interpreted as being due to spatial similarities of the preferred conformations of opposite configurations of the two drugs in solution. An inherently dissymmetric chromophore ($\theta \sim 1.2 \times 10^5$) is observed at approximately 220 nm for warfarin, the cyclic hemiketal tautomeric forms, and the cyclic methyl ketals.

The anticoagulant warfarin (1) exists in solution in a
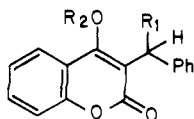
1, $R_1 = -CH_2COCH_3$; $R_2 = -H$
2, $R_1 = -CH_2CH_3$; $R_2 = -H$
3, $R_1 = -CH_2COCH_3$; $R_2 = -CH_3$
4, $R_1 = -CH_2CH_2CH_3$; $R_2 = -H$
5, $R_1,R_2 = -CH_2C(OCH_3)-$
               |
               CH_3

tautomeric equilibrium between two cyclic diastereomeric hemiketals and an open side-chain form.[1] Since the S-(−) enantiomer is considerably more potent than the R-(+) and since the difference is not entirely due to pharmacokinetic differences such as half-life,[2,3] it would appear that the configuration of the drug is important to its interaction with the receptor site leading to biologic activity. Phenprocoumon (2), an oral anticoagulant which is even more potent than warfarin,[4] can only exist in an open-chain form and its most potent enantiomer, (S)-(−)-2, is configurationally related to (S)-(−)-warfarin. This observation led to the conclusion that the open-chain tautomer of warfarin is the active inhibitory form of the drug at the vitamin K dependent site.[5] Recent metabolic work has shown that microsomal enzymes obtained from 3-methylcholanthrene induced rat liver, which catalyze hydroxylation of the coumarin ring in both phenprocoumon and warfarin, are highly stereoselective.[6] However, in the course of hydroxylation of these two compounds, opposite enantiomers are selected, e.g., (R)-(+)-1 and (S)-(−)-2. This result suggests that either the absolute stereochemical assignment of one of these drugs is incorrect or that corresponding configurations of the two drugs are interacting differently with the metabolic enzymes. The latter possibility has been observed by several groups of workers.[7–9] For example, Hein et al.[7] demonstrated that D-1-keto-3-carbomethoxy-1,2,3,4-tetrahydroisoquinoline and acylated L-phenylalanine methyl esters under similar conditions are hydrolyzed at comparable rates in the presence of α-chymotrypsin while L-1-keto-3-carbomethoxy-1,2,3,4-tetrahydroisoquinoline and acylated D-phenylalanine methyl esters are hydrolyzed at slower rates and function as competitive inhibitors. Wilson and Erlanger[8] explained the data of Hein et al. by demonstrating that the usual acylated L-phenylalanine methyl ester substrates of α-chymotrypsin have a conformation in common with D-1-keto-3-carbomethoxy-1,2,3,4-tetrahydroisoquinoline but one which is not accessible to the L isomer.

The assignment of absolute configuration to warfarin and phenprocoumon appears solid[10–12] and since 1 is capable of existing in several distinct tautomeric forms, the latter possibility, although somewhat surprising given the structural similarity of the drugs, may account for the data. To explore both the configurational and conformational aspects of the optically active forms of warfarin and phenprocoumon with respect to the above problem, a study was undertaken to measure, compare, and correlate their circular dichroism (CD) spectra. Since the strength of this approach relies on observations made on groups of similar compounds, several open-chain, analogous to 2, and cyclic, analogous to 1, model compounds were also studied.

Results and Discussion

The CD spectra of (S)-(−)-warfarin and (S)-(−)-phenprocoumon are shown in Figure 1. Except for a large difference in the intensity of the band at 220 nm, the curves for these configurationally identical molecules are virtually mirror related. This surprising result suggests that either the original assignments of configuration for warfarin and phenprocoumon were wrong or that these molecules have decidedly different solution conformations. Warfarin has been shown to exist predominantly in the cyclic hemiketal tautomeric forms.[1] If it is assumed that such forms could account for the opposite CD Cotton effects of warfarin relative to phenprocoumon, the CD spectra of derivatives of (S)-(−)-1 which are entirely in the open-chain form should be similar to the spectrum of (S)-(−)-2. The opposite result would indicate a stereochemical misassignment.

To test this, two different open-chain derivatives were prepared from (S)-(−)-1, the open-chain (S)-warfarin 4-methyl ether, (S)-(−)-3, and the open-chain aliphatic derivative, (S)-(−)-4, obtained from reduction of the side-chain carbonyl group of warfarin in two steps. In Figures 2 and 3, the CD spectra of (S)-(−)-3 and (S)-(−)-4 are compared with those of (S)-(−)-1 and (S)-(−)-2. Since the spectra of (S)-(−)-3 and (S)-(−)-4 derived from (S)-(−)-1 are now similar in sign and magnitude to that of (S)-(−)-2, the previous configurational assignments are correct. Thus the differences in the CD spectra of the same configurational isomers of warfarin and phenoprocoumon must result from differences in their solution conformations.

To test the possibility that the cyclic hemiketal forms of warfarin are responsible for the conformational differences, (S)-(−)-1 was converted to a mixture of the diastereomeric cyclic methyl ketals, (SS)-(+)- and (SR)-(−)-5, which were subsequently separated and studied individually. In Figure 4, the CD spectrum for (SS)-(+)-5 is compared with those of (S)-(−)-1 and (S)-(−)-2. It is nearly superimposable with that of the former. Except for the less intense band at 220 nm, the spectrum of (SR)-(−)-5 (not shown) is similar to that of the SS isomer. Thus the cyclic structure of warfarin appears to be the cause of the strikingly opposite CD spectra of (S)-(−)-warfarin and (S)-(−)-phenprocoumon.
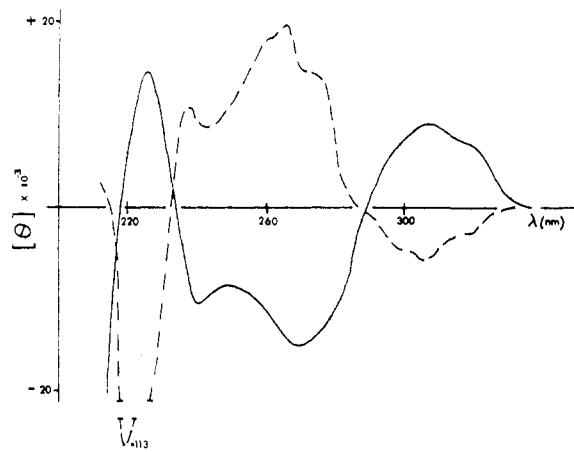
Phenprocoumon can only exist in an open-chain form,

Figure 1. Circular dichroism spectra of (S)-phenprocoumon (2, —) and (S)-warfarin (1, ---) in methanol.
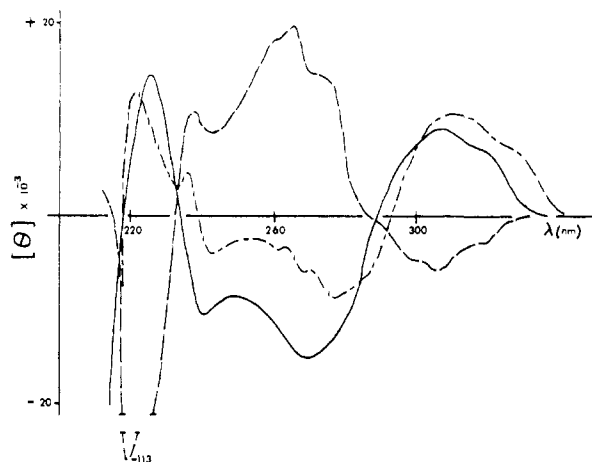

Figure 2. Circular dichroism spectra of (S)-phenprocoumon (2, —), (S)-warfarin (1, ---), and (S)-warfarin 4-methyl ether (3, —·—·-) in methanol.
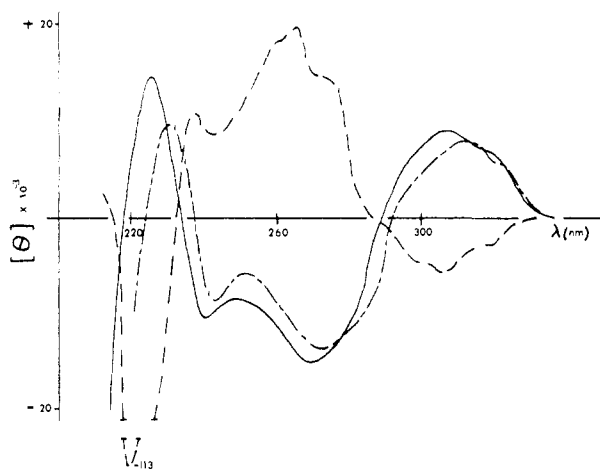

Figure 3. Circular dichroism spectra of (S)-phenprocoumon (2, —), (S)-warfarin (1, ---), and (S)-4 (—·—·-) in methanol.

and the S-(−) isomer is selectively hydroxylated by the 3-methylcholanthrene induced microsomal enzymes.[5] One might expect that warfarin would be similarly selectively hydroxylated to the extent that it exists in the open-chain form or to the extent that the tautomeric rate of interconversion is sufficiently rapid. However, this does not appear to be the case since (R)-(+)- rather than (S)-(−)-warfarin is selectively hydroxylated[13-15] and thus there must be a spatial relationship between the opposite isomers
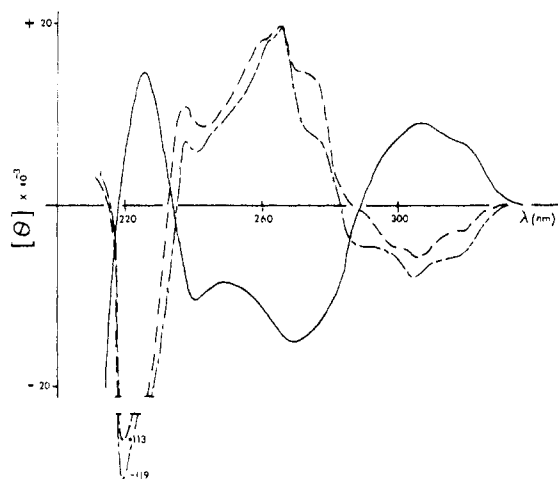
Figure 4. Circular dichroism spectra of (S)-phenprocoumon (2, —), (S)-warfarin (1, ---), and (S,S)-cyclocoumarol (5, —·—·-) in methanol.
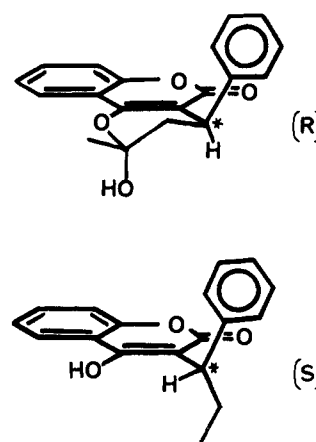

Figure 5. Comparison of the preferred conformations of (R)-warfarin and (S)-phenprocoumon.

of warfarin and phenprocoumon for the purposes of metabolism. If one assumes that for (R)-(+)-warfarin a cyclic tautomer is the preferred spatial form of the molecule relative to its interaction with the microsomal hydroxylase, then (S)-phenprocoumon can adopt a closely related form in which the phenyl and coumarin rings are in spatially related orientations to the fixed cyclic warfarin system (Figure 5). If this interpretation is correct, then important information concerning the topology of the active site of the 3-methylchloanthrene induced enzyme(s) has been gained. Thus, further work to more exactly define the structural parameters of the active site should lead to the development of a model which could be utilized predictively in the study of similar substrates.

With regard to the CD properties of these drugs, work is in progress in our laboratory to develop suitable sector rules for relating the preferred solution conformations to the sign of the Cotton effects, to investigate the inherently dissymmetric chromophore at 220 nm observed in the cyclic compounds, and finally to study changes in the tautomeric equilbria of warfarin produced by changes in pH.

Experimental Section

Optical rotations were measured on a Rudolph polarimeter in 2- and 4-dm tubes. Circular dichroism spectra were taken on a Cary Model 60 spectrophotometer at 22 °C, the cell compartment was purged with a continuous flow of N_2, and concentrations were typically 1–4 mg/mL in MeOH with precautions taken to ensure

that small differential absorptions in regions of high extinctions were real. Typically, spectra were recorded at a scan rate of 3 nm/min and cells of 0.1- and 0.5-mm path lengths were utilized as required.

Warfarin [3-(1-Phenyl-3-oxobutyl)-4-hydroxycoumarin (1)]. Sodium warfarin, U.S.P., was converted to warfarin by acidification with HCl and resolved by the method of West et al.[10] (S)-(-)-1: $[\alpha]^{25}_D$ -149.5 (1.0)° (c 1.1, 0.5 N NaOH); CD (×10⁻³) (0.98 mg/mL of MeOH) $[\theta]_{325}$ 0, $[\theta]_{300}$ -4.4, $[\theta]_{287}$ 0, $[\theta]_{268}$ +21.4, $[\theta]_{236}$ +8.8, $[\theta]_{234}$ 0, $[\theta]_{219}$ -113, $[\theta]_{216}$ 0. (R)-(+)-1: $[\alpha]^{25}_D$ +145.9 (1.0)° (c 1.2, 0.5 N NaOH); CD (×10⁻³) (1.24 mg/mL of MeOH) $[\theta]_{325}$ 0, $[\theta]_{304}$ +6.4, $[\theta]_{287}$ 0, $[\theta]_{263}$ -23.3, $[\theta]_{245,237}$ -9.4, $[\theta]_{235}$ 0, $[\theta]_{219}$ +129, $[\theta]_{212}$ 0.

Phenprocoumon [3-(1-Phenylpropyl)-4-hydroxycoumarin (2)]. Marcumar, U.S.P., was resolved by the method of West et al.[12] (S)-(-)-2: $[\alpha]^{25}_D$ -120.0 (1.0)° (c 1.5, 95% EtOH); CD (×10⁻³) (1.32 mg/mL of MeOH) $[\theta]_{335}$ 0, $[\theta]_{308}$ +9.8, $[\theta]_{290}$ 0, $[\theta]_{270}$ -14.8, $[\theta]_{255}$ -8.5, $[\theta]_{240}$ -10.6, $[\theta]_{236}$ 0, $[\theta]_{227}$ +15.9, $[\theta]_{219}$ 0. (R)-(+)-2: $[\alpha]^{25}_D$ +122.1 (0.6)° (c 1.5, 95% EtOH); CD (×10⁻³) (1.18 mg/mL of MeOH) $[\theta]_{335}$ 0, $[\theta]_{308}$ -9.0, $[\theta]_{288}$ 0, $[\theta]_{267}$ +12.4, $[\theta]_{248}$ +4.8, $[\theta]_{240}$ +5.7, $[\theta]_{236}$ 0, $[\theta]_{226}$ -15.2, $[\theta]_{220}$ 0.

(+)- and (-)-3-(1-Phenyl-3-oxobutyl)-4-methoxycoumarin (3). To enantiomerically pure warfarin (1), 100 mg in 30 mL of anhydrous ether, was added an excess of ethereal CH_2N_2; the product on evaporation of the excess CH_2N_2 and ether was an oil. (-)-3 from (-)-1:[5,10] $[\alpha]^{25}_D$ -9.1 (2.0)° (c 1.8, MeOH); CD (×10⁻³) (3.76 mg/mL of MeOH) $[\theta]_{348}$ 0, $[\theta]_{315}$ +11.1, $[\theta]_{292}$ 0, $[\theta]_{277}$ -9.4, $[\theta]_{258}$ -2.1, $[\theta]_{243}$ -4.3, $[\theta]_{239}$ 0, $[\theta]_{221}$ +13.7, $[\theta]_{218}$ 0. (+)-3 from (+)-1: $[\alpha]^{25}_D$ +14.2 (2.0)° (c 1.5, MeOH); CD (×10⁻³) (1.01 mg/mL of MeOH) $[\theta]_{345}$ 0, $[\theta]_{315}$ -8.3, $[\theta]_{292}$ 0, $[\theta]_{276}$ +8.6, $[\theta]_{252}$ +3.0, $[\theta]_{243}$ +4.0, $[\theta]_{239}$ 0, $[\theta]_{222}$ -11.4, $[\theta]_{217}$ 0.

(-)-3-(1-Phenylbutyl)-4-hydroxycoumarin (4). Starting from (S)-(-)-1, this compound was prepared by conversion of 1 to the dithioketal and then reduced with Raney Nickel:[10] a white crystalline solid; mp 132.5-133.5 °C; $[\alpha]^{25}_D$ -105 (3)° (c 1.1, 95% EtOH); CD (×10⁻³) (0.98 mg/mL of MeOH) $[\theta]_{337}$ 0, $[\theta]_{312}$ +8.5, $[\theta]_{292}$ 0, $[\theta]_{275}$ -13.8, $[\theta]_{252}$ -5.9, $[\theta]_{244}$ -9.2, $[\theta]_{238}$ 0, $[\theta]_{230}$ +10.5, $[\theta]_{224}$ 0.

(SS)-(+)-, (SR)-(-)-, and (RR)-(-)-2(3H)-2-Methyl-2-methoxy-4-phenyl-5-oxobenzopyrano[3,4-b]dihydropyran (5). These compounds were prepared as described previously[16] beginning with enantiomerically pure 1 and were obtained as colorless crystalline solids. (SS)-(+)-5 from (-)-1: mp 178.6-180.0 °C; $[\alpha]^{25}_D$ +33.1 (0.6)° (c 1.9, CHCl₃); CD (×10⁻³) (1.1 mg/mL of MeOH) $[\theta]_{330}$ 0, $[\theta]_{306}$ -8.2, $[\theta]_{288}$ 0, $[\theta]_{268}$ +20.5, $[\theta]_{242}$ +6.7, $[\theta]_{238}$ +7.3, $[\theta]_{236}$ 0, $[\theta]_{218}$ -119, $[\theta]_{216}$ 0. (SR)-(-)-5 from (-)-1: $[\alpha]^{25}_D$

-30.0 (0.8)° (c 0.9, CHCl₃); CD (×10⁻³) (1.38 mg/mL of MeOH) $[\theta]_{325}$ 0, $[\theta]_{305}$ -5.6, $[\theta]_{284}$ 0, $[\theta]_{268}$ +14.5, $[\theta]_{245}$ +6.5, $[\theta]_{237}$ +8.4, $[\theta]_{233}$ 0, $[\theta]_{218}$ -84, $[\theta]_{211}$ 0. (RR)-(-)-5 from (+)-1: $[\alpha]^{25}_D$ -30.7 (1.2)° (c 2.1, CHCl₃); CD (×10⁻³) (10.6 mg/mL of MeOH) $[\theta]_{330}$ 0, $[\theta]_{306}$ +10.3, $[\theta]_{282}$ 0, $[\theta]_{268}$ -22.5, $[\theta]_{248}$ -8.6, $[\theta]_{238}$ -9.7, $[\theta]_{235}$ 0, $[\theta]_{219}$ +122, $[\theta]_{212}$ 0.

References and Notes

(1) E. J. Valente, E. C. Lingafelter, W. R. Porter, and W. F. Trager, *J. Med. Chem.*, **20**, 1489 (1977).
(2) A. Breckenridge and M. L'Orme, *Life Sci.*, **11**, 337 (1972).
(3) A. Yacobi and A. Levy, *J. Pharmacokinet. Biopharm.*, **2**, 239 (1974).
(4) R. G. Bell and P. Stark, *Biochem. Biophys. Res. Commun.*, **72**, 619 (1976).
(5) E. J. Valente, W. R. Porter, and W. F. Trager, *J. Med. Chem.*, submitted for publication.
(6) W. R. Porter, Ph.D. Thesis, University of Washington, 1976.
(7) G. E. Hein, R. B. McGriff, and C. Niemann, *J. Am. Chem. Soc.*, **82**, 1830 (1960).
(8) I. B. Wilson and B. F. Erlanger, *J. Am. Chem. Soc.*, **82**, 6422 (1960).
(9) E. S. Awad, H. Neurath, and B. S. Hartley, *J. Biol. Chem.*, **235**, 9 (1960).
(10) B. D. West, S. Preis, C. H. Schroeder, and K. P. Link, *J. Am. Chem. Soc.*, **83**, 2676 (1961).
(11) E. J. Valente, W. F. Trager, and L. H. Jensen, *Acta Crystallogr., Sect. B*, **31**, 954 (1975).
(12) B. D. West and K. P. Link, *J. Heterocycl. Chem.*, **2**, 93 (1965).
(13) L. R. Pohl, R. Bales, and W. F. Trager, *Res. Commun. Chem. Pathol. Pharmacol.*, **15**, 233 (1976).
(14) L. R. Pohl, S. D. Nelson, W. R. Porter, W. F. Trager, M. J. Fasco, F. D. Barker, and J. W. Fenton, II, *Biochem. Pharmacol.*, **25**, 2153 (1976).
(15) W. F. Trager in "Drug Metabolism Concepts, ACS Symposium Series, No. 44", D. M. Jerina, Ed., American Chemical Society, Washington, D.C., 1977, Chapter 5.
(16) M. Ikawa, M. Stahmann, and K. P. Link, *J. Am. Chem. Soc.*, **66**, 902 (1944).

Phosphorus–Nitrogen Compounds. 21. Murine Oncolytic and Antifertility Effect of Adamantylaziridine Compounds[1]

Lindley A. Cates,* Michael B. Cramer, and Louis Williams

College of Pharmacy, University of Houston, Houston, Texas 77004. Received March 17, 1977

P,P-Bis(1-aziridinyl)-N-adamantylphosphinic amide and N,N'-bis(ethylene)-P-(1-adamantyl)phosphonic diamide were synthesized as potential anticancer and male antifertility agents. Log P values (octanol–water) of the agents were determined and compared to calculated values. Both derivatives displayed intraperitoneal murine antileukemic activity and antifertility effects when given intraperitoneally and orally.

The high antileukemic activity of P,P-bis(1-aziridinyl)-N-adamantylphosphinic amide (1),[2,3] prompted the synthesis and bioevaluation of an analogue, N,N'-bis(ethylene)-P-(1-adamantyl)phosphonic diamide (2). These derivatives can be considered as belonging to the ethylenimine class of anticancer and male antifertility agents. Similar compounds previously investigated for the latter effect are ineffective antispermatogenics when given orally.

This paper describes the synthesis and antileukemic activity of 2 and the effect of 1 and 2, given intraperitoneally and orally, on male fertility in the mouse.

A comparison study of 1 and 2 was considered of interest since the carbon–phosphorus bond is stronger than an amide linkage and 2 might be resistant to in vivo loss of the adamantyl moiety. In addition, 2 has higher water solubility (0.94% vs. 0.13% for 1) and an experimentally