

Warfarin metabolism in man: identification of metabolites in urine

Richard J. Lewis, William F. Trager

J Clin Invest. 1970;49(5):907-913. <https://doi.org/10.1172/JCI106310>.

Research Article

After administration of the coumarin anticoagulant racemic warfarin to normal humans, seven fluorescent compounds were chromatographically separated from extracts of their urine. Four of these were identified using mass spectrometry, thin-layer chromatography, and ultraviolet absorption spectroscopy. One metabolic pathway, reduction of the acetyl side chain of warfarin, resulted in the formation of a second asymmetric carbon atom, and two diastereoisomer alcohols were identified. These warfarin alcohols are structurally similar to pharmacologically active coumarin derivatives. They have not been reported in animal studies. In addition, 6- and 7-hydroxywarfarin were identified. These are the first studies to document the metabolic fate of warfarin in the normal human.

Find the latest version:

<https://jci.me/106310/pdf>



Warfarin Metabolism in Man: Identification of Metabolites in Urine

RICHARD J. LEWIS and WILLIAM F. TRAGER

From the Research Service, Veterans Administration Hospital, San Francisco, California 94121, and the Department of Medicine, School of Medicine and the Department of Pharmaceutical Chemistry, School of Pharmacy, University of California, San Francisco, California 94122

A B S T R A C T After administration of the coumarin anticoagulant racemic warfarin to normal humans, seven fluorescent compounds were chromatographically separated from extracts of their urine. Four of these were identified using mass spectrometry, thin-layer chromatography, and ultraviolet absorption spectroscopy. One metabolic pathway, reduction of the acetonyl side chain of warfarin, resulted in the formation of a second asymmetric carbon atom, and two diastereoisomer alcohols were identified. These warfarin alcohols are structurally similar to pharmacologically active coumarin derivatives. They have not been reported in animal studies. In addition, 6- and 7-hydroxywarfarin were identified. These are the first studies to document the metabolic fate of warfarin in the normal human.

INTRODUCTION

In the 18 yr that have elapsed since its introduction for clinical use, warfarin, *rac*-(3-(α -acetonylbenzyl)-4-hydroxycoumarin), has become the oral anticoagulant most frequently employed in this country. Despite this extensive experience, major difficulties are still encountered in attempts to maintain stable hypoprothrombinemia. The mechanism by which a hemorrhagic diathesis results from the same drug dose which may also produce acceptable anticoagulation remains, in many cases, unexplained. In addition, resistance to warfarin has been observed, and also remains unexplained.

Although warfarin has been the subject of pharmacogenetic investigations and studies of enzyme induction phenomena, these have been limited by the absence of information concerning the metabolism of warfarin in man. Studies of warfarin "metabolism" have been re-

stricted to analyses of data obtained on plasma levels of unmetabolized drug.

For these reasons, therefore, it was felt that a detailed investigation of the metabolic fate of warfarin would be worthwhile. Efforts to elucidate the problem employed the following three approaches: thin-layer chromatography, absorption spectroscopy, and mass spectrometry. The data were compared with those obtained with synthetic warfarin derivatives.

METHODS

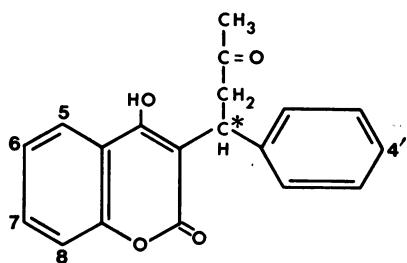
Sodium warfarin (Coumadin, Endo Laboratories, Inc., Garden City, N. Y.) was administered to informed normal volunteers in a single oral dose of 1.5 mg/kg body weight. Single pooled collections of urine were made over the subsequent 3 days. Urine was also collected from patients receiving 5–15 mg of warfarin daily. The samples, without preservative, were frozen for up to 3 months until processed.

Extraction. Since warfarin and similar compounds are organic acids, it was anticipated that, in a mixture of aqueous and organic solvents, they would be soluble in an alkaline aqueous phase, and soluble in the organic phase upon acidification of the aqueous phase. For the initial extraction, the urine (usually about 1000 ml) was transferred to polypropylene containers. The urine was acidified to pH < 2 with concentrated hydrochloric acid and an equal volume of organic solvent (*n*-butanol: ethylenedichloride, 1:1) was added. After the mixture was agitated for 30 min on a horizontal shaker, the phases were separated by centrifugation. The organic solvent was removed with a transfer pipet, and the extraction was repeated an additional two times. The pooled solvent was evaporated to dryness on a rotary vacuum evaporator. The residue was taken up in a small volume of 5 N NaOH, which was then transferred to a 60 ml glass stoppered jar. This solution was adjusted to pH > 10, if necessary. The NaOH solution was washed two times with a $\frac{1}{2}$ volume of *n*-butanol: ethylenedichloride, 1:1, by agitating the mixture for 10 min on a horizontal shaker. After each wash, the organic phase was removed and discarded. The aqueous phase was acidified to pH < 2 with concentrated hydrochloric acid. This was extracted three times with the *n*-butanol: ethylenedichloride as before. The

An abstract of portions of this work appeared in *Clin. Res.* 18: 119, 1970.

Received for publication 24 October 1969.

WARFARIN



WARFARIN ALCOHOL

FIGURE 1 The structural formula of warfarin, and warfarin alcohol. On the structural formula of warfarin (left), the numerals indicate the position of the hydroxy substitutions to which reference is made in the text. The single asymmetric carbon atom is indicated with an asterisk. On the right, in the structural formula of warfarin alcohol, the two asymmetric carbon atoms are indicated by asterisks.

solvent was evaporated *in vacuo* to dryness. The evaporating flask was rinsed with a small volume of acetone. The insoluble material was discarded; the acetone-soluble compounds were then separated by thin-layer chromatography.

Chromatographic purification. 1–2 ml of the acetone were applied to each of several preparative (2000 μ) silica gel chromatography plates (Analtech, Inc., Wilmington, Del.). The plates were initially developed in a solvent system of ethylenedichloride: acetone, 9:1 or 7:3. Fluorescent bands were located by short wave ultraviolet illumination (Ultra-Violet Products, Inc., San Gabriel, Calif.) and were transferred to a 60 ml glass stoppered jar. Loci with corresponding R_f values were pooled. To elute the compound,

n-butanol and 3 N HCl (4 parts to 1) were added in a volume about double that occupied by the silica gel. This mixture was agitated for 10 min and the solvent was recovered. The elution was repeated twice and the pooled solvent was then evaporated *in vacuo*. The residue was redissolved in acetone, and this was applied to another preparative chromatography plate. This was developed with either cyclohexane: ethyl formate: formic acid, 100:200:1, or t-butanol: benzene: concentrated NH₄OH: water, 90:40:18:6. The compound of interest was located with short wave ultraviolet light, transferred to a polypropylene container, and eluted as described. This solvent was evaporated. The residue was taken up in acetone and a portion of this was applied across an analytical (100 μ) silica gel thin-layer chromatography sheet (No. 6061, Eastman Kodak Co., Rochester, N. Y.). The sheet was then chromatographed with the third solvent system.

Characterization of compounds. After the third chromatographic purification, the fluorescent band was cut out, moistened with several drops of 0.25 N NaOH, and scraped into a polyethylene tube with a razor blade. Additional NaOH was added to give a total volume of about 1.5 ml. The compound was eluted by shaking the mixture for 10 min on the horizontal shaker. The silica particles suspended in the NaOH were removed by centrifugation at 10,000 g for 10 min. The ultraviolet absorption spectrum of the supernate was determined with a Zeiss PMQ II spectrophotometer (Carl Zeiss, Inc., New York); the spectrum was repeated after the addition of two drops of 3 N HCl to the 1 ml cuvette. This acidified sample was then reextracted into n-butanol, as described, and set aside for further chromatographic analysis and determination of its mass spectrum.

The mass spectra of the materials thus isolated were determined on an AEI MS 902 high resolution mass spectrometer, at approximately 200°C, via the direct insertion inlet system. The empirical formulas of the structurally significant ions were established by exact mass measurement.

Synthetic samples of 5-hydroxywarfarin and the aliphatic side chain alcohols of warfarin were prepared in this laboratory. The authors are grateful to Dr. Mark Hermodson and Professor Karl Paul Link of the University of Wisconsin for samples of synthetic 6-, 7-, and 8-hydroxywarfarin. Synthetic materials were dissolved in acetone and chromatographed once before the determination of their ultraviolet absorption spectra.

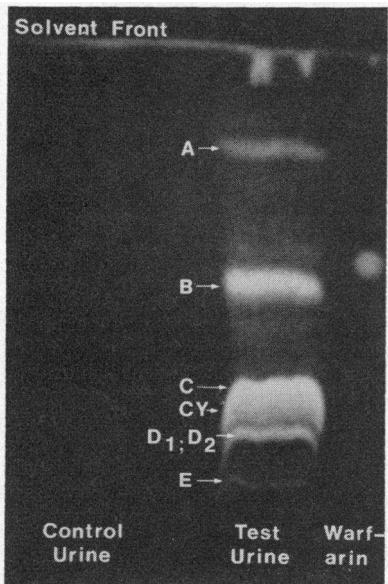


FIGURE 2 Chromatography plate photographed in short wave ultraviolet light. Several areas of fluorescence were resolved from extracts of urine obtained after warfarin administration (center). The control urine is on the left, warfarin on the right. The developing system was ethylenedichloride: acetone, 9:1.

RESULTS

The structural formula of warfarin and the formula of warfarin alcohol are presented in Fig. 1. The numerals on the coumarin nucleus refer to the position of the hydroxy substitutions.

Thin-layer chromatography. Chromatography of the urine extracts with a 9:1 ethylenedichloride:acetone solvent system separated blue fluorescent loci at R_f 0.75, 0.50, 0.23, 0.10, and 0.00 (Fig. 2). These were designated compounds A through E respectively. The compound with yellow fluorescence migrating with an R_f of 0.19 was designated CY. A small amount of a compound with the R_f of warfarin, 0.54, was also noted. In the cyclohexane:ethyl formate:formic acid (100:200:1) solvent system, compound D was separated into two components, designated D_1 and D_2 . No differences were apparent whether samples were obtained from patients on long-term warfarin maintenance or from volunteers receiving a single dose. Similar results were obtained when urine samples were processed immediately, without storage. These fluorescent loci were readily distinguished from the minor components in control samples.

In all solvent systems tested, identical chromatographic results were noted for synthetic 7-hydroxywarfarin and compound C (Table I). Synthetic 6-hydroxywarfarin and CY were also chromatographically identical to each other. Compounds D_1 and D_2 were chromatographically indistinguishable from the synthetic aliphatic side chain alcohol derivatives of warfarin. Both 7-hydroxywarfarin

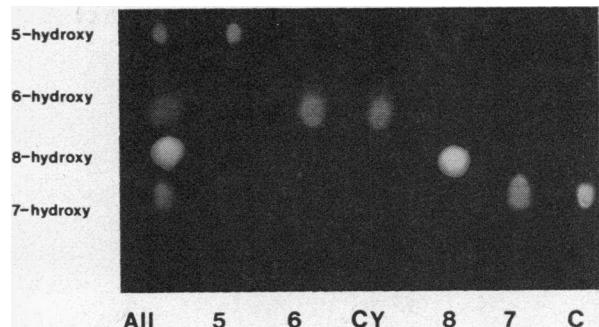


FIGURE 3 Chromatography of the four coumarin ring hydroxy derivatives of warfarin. At the origin, were applied from left to right: a mixture of all six compounds; 5-hydroxywarfarin; 6-hydroxywarfarin; compound CY; 8-hydroxywarfarin; 7-hydroxywarfarin; and compound C. These were separated in a solvent system of *t*-butanol:benzene:NH₄OH:water. Compound CY from the urine was identified as 6-hydroxywarfarin. Compound C was identified as 7-hydroxywarfarin.

(and the corresponding compound C from the urine) and 6-hydroxywarfarin (and the corresponding compound CY) could be separated from the other coumarin ring hydroxy derivatives of warfarin (Fig. 3).

Absorption spectroscopy. The ultraviolet absorption spectra obtained for synthetic 7-hydroxy warfarin and compound C were indistinguishable. Both exhibited extinction maxima at 329 and 256 m μ in NaOH, and a maximum at 312 after acidification. This hypsochromic shift in acid was characteristic of all the warfarin derivatives studied (Fig. 4). The absorption maxima obtained for synthetic 6-hydroxy warfarin and compound CY were identical: 302 m μ in NaOH with a shift to 279 m μ in acid. Compounds D_1 and D_2 had absorption spectra that were not only identical to each other, but also to the two synthetic warfarin alcohols: maxima at 309 m μ in NaOH, 285 and 275 m μ in acid were observed for all four. For synthetic 5-hydroxywarfarin, maxima in NaOH at 312 and 300 m μ , and a maximum in acid at 299 m μ were observed.

Mass spectrometry. The mass spectrum of warfarin was determined and its fragmentation pattern was elucidated by a combination of exact mass measurements, metastable scanning, and a comparison of this spectrum with that of a specifically deuterated warfarin. Warfarin has an empirical formula of C₂₀H₁₆O₄, and a molecular weight (expressed as mass to charge ratio, m/e) of 308. In addition to the molecular ion (M⁺) at m/e 308, a base peak ion at m/e 265 (M⁺ less 43) resulted from cleavage of the acyl group of the aliphatic side chain. This was followed by loss of the phenyl side chain to an ion at m/e 187 (m/e 265 less 78), which represented the coumarin nucleus. The empirical formulas of these ions were determined by exact mass measurements. The

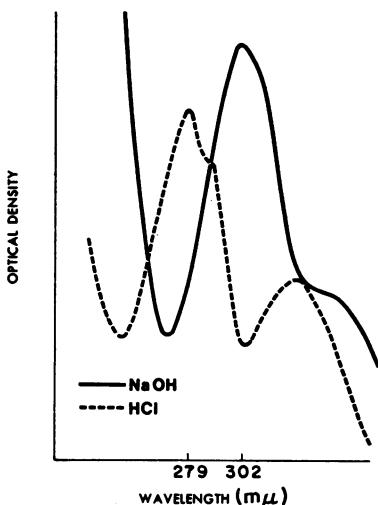
TABLE I
Thin-Layer Chromatography of Warfarin Derivatives*

Compound	Solvent system†		
	E:A	C:EF:FA	T:B:A:W
7-Hydroxywarfarin	0.56	0.47	0.22
C (urine)	0.56	0.47	0.22
6-Hydroxywarfarin	0.52	0.45	0.32
CY (urine)	0.52	0.45	0.32
Warfarin alcohol ₁	0.16	0.74	0.45
D ₁ (urine)	0.16	0.74	0.45
Warfarin alcohol ₂	0.12	0.66	0.40
D ₂ (urine)	0.12	0.66	0.40
5-Hydroxywarfarin	0.10	0.49	0.43
8-Hydroxywarfarin	0.58	0.67	0.27
Warfarin	0.83	0.76	0.55

* Values are expressed as R_f = distance traveled by compound per distance (10 cm) traveled by solvent front.

† E:A = ethylenedichloride:acetone, 7:3. C:EF:FA = cyclohexane:ethyl formate:formic acid, 100:200:1. T:B:A:W = *t*-butanol:benzene:concentrated ammonium hydroxide:water, 90:40:18:6.

6-OH WARFARIN AND COMPOUND CY



7-OH WARFARIN AND COMPOUND C

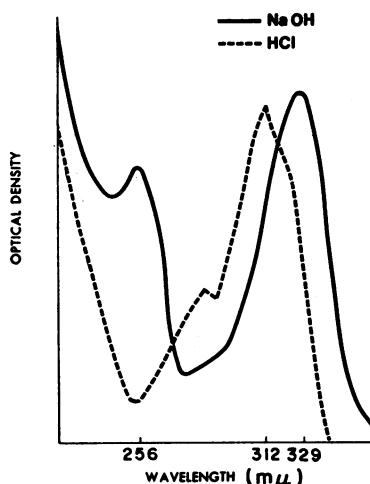
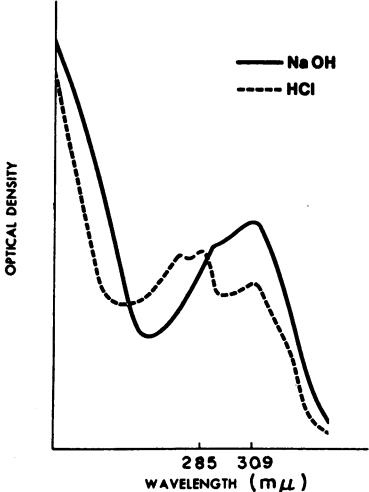
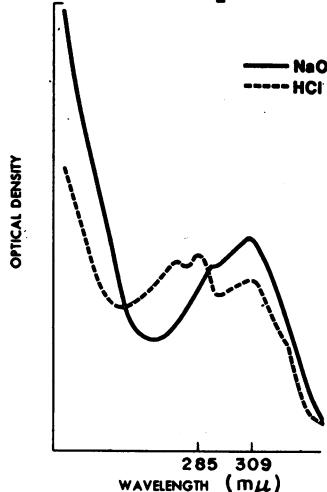
WARFARIN ALCOHOL₁ AND COMPOUND D₁WARFARIN ALCOHOL₂ AND COMPOUND D₂

FIGURE 4 Ultraviolet absorption spectra of urine metabolites of warfarin and the corresponding synthetic derivatives. These were determined in NaOH (solid lines) and after acidification (dashed lines). Upper left: 6-hydroxywarfarin and compound CY from the urine. Upper right: 7-hydroxywarfarin and compound C from the urine. Lower left: warfarin alcohol₁ and urine compound D₁. Lower right: warfarin alcohol₂ and urine compound D₂.

fragmentation sequence could then be further described as $M^+ = m/e 308 (C_{19}H_{16}O_4) \rightarrow m/e 265 (C_{17}H_{13}O_3) \rightarrow m/e 187 (C_{11}H_7O_3)$.

The fragmentation pattern of synthetic 6- and 7-hydroxywarfarin was similar to that observed for warfarin. From the molecular ion, cleavage of the acyl group gave rise to a base peak ion. This was followed by loss of the phenyl group, leaving the ion representing

the coumarin nucleus. The exact mass measurements of these ions differed from the corresponding ions of warfarin only by the increase of 16 mass units due to the addition of an oxygen atom: $M^+ = m/e 324 (C_{19}H_{16}O_5) \rightarrow m/e 281 (C_{17}H_{13}O_4) \rightarrow m/e 203 (C_{11}H_7O_4)$. Compound C and compound CY gave rise to ions at the same exact mass as did synthetic 7- and 6-hydroxywarfarin.

The mass spectra of compounds D₁ and D₂ were dif-

ferent from those of warfarin and the hydroxylated derivatives of warfarin. Compounds D₁ and D₂ both gave a molecular ion at m/e 310. By exact mass measurement this was shown to be C₁₉H₁₈O₄, which differed from the empirical formula of warfarin by the addition of two atoms of hydrogen. A prominent ion at m/e 292 (M⁺ less 18) was observed, and exact mass measurements of this ion indicated an empirical formula of C₁₉H₁₆O₃. Thus the elements of water were lost from the molecular ion. The data obtained with the synthetic aliphatic side chain alcohols of warfarin were identical to those obtained with compounds D₁ and D₂.

DISCUSSION

For each of the three criteria of identity which were employed in this study, ultraviolet absorption spectroscopy, thin-layer chromatography, and mass spectrometry, the data obtained with the metabolite isolated from the urine and the synthetic compound with the structure proposed for that metabolite were indistinguishable.

Bishydroxycoumarin, ethyl biscoumacetate, phenprocoumon, and warfarin are 3-substituted-4-hydroxycoumarins having ultraviolet absorption maxima in NaOH near 310 m μ , indicating that the substitutions in these compounds have little effect on the absorption spectrum of the molecule (1). The maxima at 309 m μ observed for compounds D₁ and D₂ and the synthetic aliphatic side chain alcohols of warfarin are consistent with this observation. The 6- or 8-hydroxy substitutions on the coumarin ring have only a limited effect on the spectrum. Extinction maxima near 302 m μ in NaOH have been observed for both of these compounds (2). The exception to this general pattern, the bathochromic shift to 329 m μ in the absorption spectrum of compound C and 7-hydroxywarfarin, is presumably due to the extension of conjugation resulting from resonance structures such as depicted in Fig. 5. Although similar resonance structures are at least possible for 5-hydroxywarfarin, their contributions would be minimized by the formation of an intramolecular hydrogen bond between the 4- and 5-hydroxy groups. Such resonance structures are not possible for warfarin, 6-, or 8-hydroxywarfarin.

Mass spectrometry was of great aid in the efforts to identify these compounds. From the viewpoint of the fragmentation pattern, the warfarin molecule could be considered to consist of the following three parts: the acyl group, the phenyl group, and the coumarin nucleus. Structural modifications in any of these parts of the molecule could be detected by changes in the fragmentation pattern and appropriate alterations in the exact mass measurements of the ions.

The fragmentation pattern and exact mass measurement of the ions given compounds C and CY confirmed that these substances were hydroxylated derivatives of

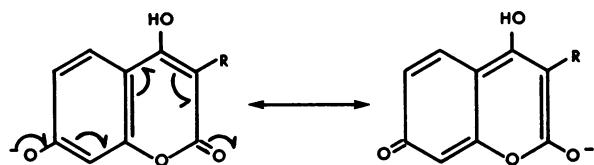


FIGURE 5 Resonance structures for hydroxywarfarin substituted in the seven position. In NaOH, this type of resonance would extend conjugation and is presumed to account for the distinctive bathochromic shift in the absorption spectrum of 7-hydroxywarfarin.

warfarin. Since the ion at m/e 187 in the mass spectrum of warfarin represented the coumarin nucleus, the occurrence of the ion at m/e 203 (i.e., m/e 187 + 16) in the mass spectrum of compounds C and CY indicated that the oxygen addition was on the coumarin nucleus. Compounds C and CY therefore represented either 5-, 6-, 7-, or 8-hydroxywarfarin. On the basis of mass spectrometry, a distinction between the four coumarin ring hydroxy derivatives was not possible. Definitive identification was then accomplished by comparative thin-layer chromatography with the demonstration that these four could be separated (Fig. 3). Therefore, compound C was identified as 7-hydroxywarfarin and compound CY was identified as 6-hydroxywarfarin.

The exact mass measurements of the ions given by compounds D₁ and D₂ confirmed that these were reduced derivatives of warfarin. Loss of the elements of water which was observed in the fragmentation pattern of these compounds suggested further that these were alcohols of warfarin (3). Indistinguishable chromatographic and mass spectral data were obtained with the synthetic alcohols, and therefore compounds D₁ and D₂ were identified as aliphatic side chain alcohols of warfarin.

The warfarin which was available for these studies was the racemic mixture of R and S isomers (4). Acetonyl reduction produced a second asymmetric carbon atom (Fig. 1). For the warfarin alcohols, four configurations (enantiomers RR, RS, SR, and SS) are thus possible. These consist of two pairs of mirror images (RR-SS and RS-SR), and two pairs of diastereoisomers (RR-RS and SS-SR). Although mirror image compounds have identical chromatographic behavior, diastereoisomers can be resolved (5). The warfarin alcohols, compounds D₁ and D₂, therefore each represented either RR and SS or RS and SR in unknown proportions.

These data differ somewhat from those obtained in animal studies, but such species differences are well known. Barker (6, 7) reported 6-, 7-, and 8-hydroxy-warfarin, in the rat, and Hermodson (8, 9) described 4'-hydroxywarfarin, a pyranocoumarin and either a sulphate (8) or glucuronide (9) conjugate of 7-hydroxy-warfarin. The warfarin alcohols have not been previously

noted. 6- and 7-Hydroxywarfarin were reported (8) in the urine of an unusual patient who required 145 mg of warfarin daily (10), but the pertinence of this observation to the normal human is uncertain.

Species differences have been observed in the metabolism of compounds structurally related to warfarin. Ethylbiscoumacetate metabolism involves hydroxylation in man, but deesterification in the rabbit (11). The metabolism of coumarin is to 7-hydroxycoumarin in man (12); but all six monohydroxy coumarins in the rabbit (13); and hydroxy derivatives and carbon dioxide in the rat (14). In all these species, cleavage of the heterocyclic ring of coumarin has been demonstrated, a metabolic pathway which is also present in the gastrointestinal microorganisms of these species (15).

The 6-, 7-, and 8-hydroxy substitutions are without significant pharmacologic activity (in the rat) (8). Whether this apparent lack of potency of coumarin ring substitutions of warfarin is due to species specificity, is due to decreased protein binding with presumed rapid renal excretion of polar compounds (2), or rather implies that an intact 4-hydroxycoumarin nucleus is necessary for activity (16) is unclear. On the other hand, compounds D₁ and D₂, the warfarin alcohols, may well be mediators of an anticoagulant effect.

A variety of compounds which are structurally similar to warfarin are pharmacologically effective, whether the substitutions are on the phenyl (e.g., acenocoumarol) or aliphatic (e.g., phenprocoumon) side chains. The warfarin alcohols differ from phenprocoumon only by a hydroxyethyl instead of a methyl group. Phenprocoumon is even more potent, on a molar basis, than warfarin. Although the relative potency of the four warfarin alcohol enantiomers has not yet been established, the S forms of both warfarin (17) and phenprocoumon (18) have been shown to be about five times more potent than the R isomers. It seems reasonable to anticipate that the stereochemical configuration of the warfarin alcohols may be of similar significance in this regard.

The determinants of the relative production of these metabolites are unknown, although genetic influences have been implicated in dicumarol metabolism (19, 20). Resistance to warfarin has been described in man (10, 21) and animals (22, 23) and has been shown to be an inherited phenotype (10, 22). The evidence that, after administration of racemic warfarin, only one warfarin enantiomer is converted to a particular metabolite (8) not only emphasizes the stereospecific nature of these reactions but also leads to the speculation that an alteration in a stereospecific enzyme may be responsible for states of unusual warfarin sensitivity or resistance by alterations in the production of a pharmacologically active metabolite.

ACKNOWLEDGMENTS

This work could not have been completed without the support of Paul M. Aggeler and Ralph R. Cavalieri, the sponsors of one of us (R. J. L.) as a V. A. Research Associate. Discussions with Samuel M. Gordon provided additional impetus. Technical assistance was rendered by Mr. Leon P. Illnicki and Miss Maritta Carlstrom.

This work was carried out in part under a Grant-in-Aid of the American Heart Association supported by the Alameda, San Francisco, San Mateo, and Santa Clara Heart Associations, and in part by a University of California Academic Senate Grant.

REFERENCES

- French, W. N., and A. Wehrli. 1965. Identification and assay of some coumarin anticoagulants. *Can. Pharm. J.* **98**: 174.
- O'Reilly, R. A. 1969. Interaction of the anticoagulant drug warfarin and its metabolites with human plasma albumin. *J. Clin. Invest.* **48**: 193.
- Biemann, K. 1962. Mass Spectrometry: Organic Chemical Applications. McGraw-Hill Book Company, New York. 95.
- West, B. D., S. Preis, C. H. Schroeder, and K. P. Link. 1961. Studies on the 4-hydroxy coumarins. XVII. The resolution and absolute configuration of warfarin. *J. Amer. Chem. Soc.* **83**: 2676.
- Lederer, E., and M. Lederer. 1957. Chromatography. Elsevier, Amsterdam. 2nd edition. 420.
- Barker, W. M. 1965. The Metabolism of 4-C¹⁴ Warfarin Sodium in the Rat. Doctorate Thesis. University of Wisconsin, Madison.
- Link, K. P., D. Berg, and W. M. Barker. 1965. Partial fate of warfarin in the rat. *Science (Washington)*. **150**: 378.
- Hermodson, M. A. 1968. Biochemical Studies on Warfarin. Doctorate Thesis. University of Wisconsin, Madison.
- Barker, W. M., M. A. Hermodson, and K. P. Link. 1970. The metabolism of 4-C¹⁴-warfarin sodium by the rat. *J. Pharmacol. Exp. Ther.* **171**: 307.
- O'Reilly, R. A., P. M. Aggeler, M. S. Hoag, L. S. Leong, and M. L. Kropatkin. 1964. Hereditary transmission of exceptional resistance to coumarin anticoagulant drugs. *N. Engl. J. Med.* **271**: 809.
- Burns, J. J., M. Weiner, G. Simson, and B. B. Brodie. 1953. The biotransformation of ethylbiscoumacetate (Tromexan) in man, rabbit, and dog. *J. Pharmacol. Exp. Ther.* **108**: 33.
- Shilling, W. H., R. F. Crampton, and R. C. Longland. 1969. Metabolism of coumarin in man. *Nature (London)*. **221**: 664.
- Kaighen, M., and R. T. Williams. 1961. The metabolism of (3-C¹⁴) coumarin. *J. Med. Pharm. Chem.* **3**: 25.
- VanSumere, C. F., and H. Teuchy. 1968. The metabolism of coumarin-2-C¹⁴ and 7-hydroxycoumarin-2-C¹⁴ (umbelliferone-2-C¹⁴) in the rat. *Arch. Int. Physiol. Biochem.* **76**: 968.
- Scheline, R. R. 1968. Studies on the role of the intestinal microflora in the metabolism of coumarin in rats. *Acta Pharmacol. Toxicol.* **26**: 325.
- Overman, R. S., M. A. Stahmann, C. F. Huebner, W. R. Sullivan, L. Spero, D. G. Doherty, M. Ikawa, L. Graf, S. Roseman, and K. P. Link. 1944. Studies on the hemorrhagic sweet clover disease. XIII. Antico-

- agulant activity and structure in the 4-hydroxycoumarin group. *J. Biol. Chem.* **153**: 5.
17. Eble, J. N., B. D. West, and K. P. Link. 1966. A comparison of the isomers of warfarin. *Biochem. Pharmacol.* **15**: 1003.
18. West, B. D., J. N. Eble, W. M. Barker, and K. P. Link. Unpublished data cited by West, B. D., and K. P. Link. 1965. The resolution and absolute configuration of "Marcumar" (I). *J. Heterocyclic Chem.* **2**: 93.
19. Vesell, E. S., and J. G. Page. 1968. Genetic control of dicumarol levels in man. *J. Clin. Invest.* **47**: 2657.
20. Motulsky, A. G. 1964. Pharmacogenetics. In *Progress in Medical Genetics*. A. G. Steinberg and A. G. Bearn, editors. Grune & Stratton Inc., New York. **3**: 49.
21. Lewis, R. J., M. Spivack, and T. H. Spaet. 1967. Warfarin resistance. *Amer. J. Med.* **42**: 620.
22. Pool, J. G., R. A. O'Reilly, L. J. Schneiderman, and M. Alexander. 1968. Studies on warfarin resistance in the rat. *Amer. J. Physiol.* **215**: 627.
23. Rowe, F. P., and R. Redfern. 1965. Toxicity tests on suspected warfarin resistant house mice (*Mus musculus* L.). *J. Hyg.* **63**: 417.