Notes

Absolute Configuration of Acenocoumarin

Conrad R. Wheeler and William F. Trager*

Department of Pharmaceutical Sciences, University of Washington, Seattle, Washington 98195. *Received February 20, 1979*

The absolute configuration of acenocoumarin is assigned by chemical conversion of (-)-acenocoumarin to (S)-(-)-warfarin through a series of reactions not involving the asymmetric center.

Warfarin, phenprocoumon, and acenocoumarin are related coumarin anticoagulants possessing a single asymmetric center. All three compounds lead to anticoagulation by competitive inhibition of the vitamin K dependent synthesis of specific clotting factors. A number of studies have shown that for warfarin and phenprocoumon one enantiomer is more active than the other in both man[1-6] and rat.[7-13] For these two compounds, the topographically related *S* enantiomers are two to five times more potent than the corresponding *R* enantiomers. It might therefore be expected that the *S* enantiomer of acenocoumarin, which is also topographically related to the *S* enantiomers of warfarin and phenprocoumon, would also be the most potent. It has been recently reported, however, that the exact opposite is true.[14] The *R* enantiomer of acenocoumarin is several times more potent than the *S* enantiomer in both man and rat. However, this conclusion is based on absolute configurational assignments for acenocoumarin, which to our knowledge have not appeared in the literature.

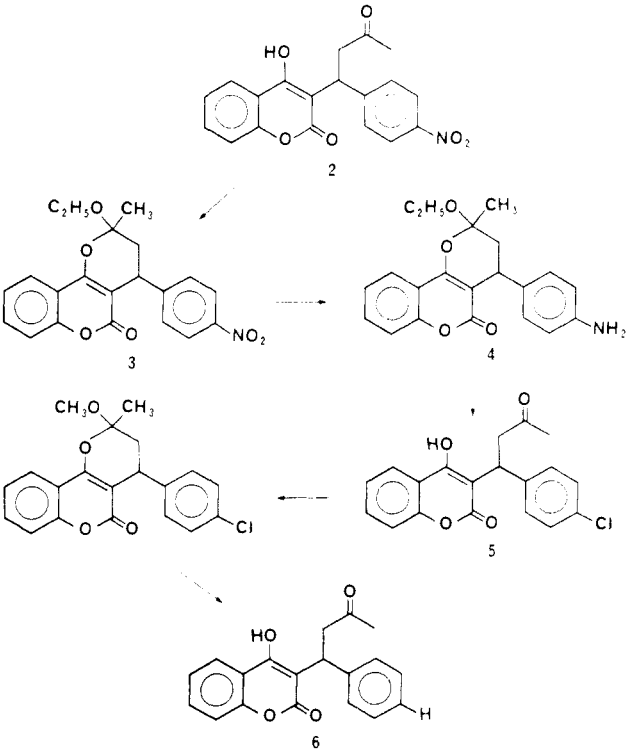
A number of reasons can be postulated to account for this apparent reversal in stereoselectivity: (1) The *R* configuration has been incorrectly assigned to the dextrorotatory enantimomer of acenocoumarin. (2) The pharmacokinetic properties of acenocoumarin (metabolism, distribution, protein binding, etc.) may be different from those of warfarin and phenprocoumon and of such a nature as to give rise to an apparent reversal in stereoselectivity.[15] (3) There is an actual reversal in stereoselectivity exhibited by the receptor for acenocoumarin at the level of vitamin K inhibition.

The absolute configuration of warfarin is well established through the correlation of (*S*)-warfarin to (*S*)-(-)-β-phenylcaproic acid, a compound of known absolute configuration, by a series of reactions not involving the asymmetric center.[13] In addition, the absolute configuration has been further substantiated by the direct determination of absolute configuration via X-ray crystallography.[16] Therefore, the chemical conversion of one enantiomer of acenocoumarin to warfarin by a series of reactions not involving the asymmetric center would provide unambiguous evidence for its assignment.

Results and Discussion

Acenocoumarin was synthesized and the (-) enantiomer resolved to optical purity via crystallization of the quinidine salt. After protection of the side-chain carbonyl by ethyl ketal formation, the aromatic nitro group was reduced with $NaBH_4$ in the presence of Pd on C. The 4'-aminowarfarin ethyl ketal thus obtained was diazotized and converted to 4'-chlorowarfarin with CuCl. Reductive dehalogenation of the methyl ketal of chlorowarfarin with $NaBH_4$ and Pd on C in refluxing ethyl acetate yielded the methyl ketal of warfarin. Hydrolysis of the ketal yielded warfarin of the same absolute configuration as the acenocoumarin from which it was derived.

Scheme I. Conversion of (-)-Acenocoumarin to (*S*)-(-)-Warfarin



The reaction sequence proceeded well, except for a minor problem encountered in the dehalogenation of the methyl ketal of chlorowarfarin. Initially, attempts at reductive dehalogenation were carried out in a mixture of MeOH and EtOH employing $NaBH_4$ as reductant and Pd on C as catalyst. No detectable reduction occurred at room temperature; therefore, refluxing conditions were tried. Under these conditions, partial reduction was obtained, but large amounts of $NaBH_4$ were necessary. For example, the addition of $NaBH_4$ (1 g, 26.4 mmol) in 100-mg portions for a period of 4 h gave only 50% reduction. Since it appeared that the problem was due to a more rapid reaction of the borohydride with the solvent than the reactant, change to a nonprotic solvent was indicated. Dehalogenation in refluxing EtOAc proceeded cleanly and smoothly to give a product that was 95% warfarin and 5% chlorowarfarin.

The CD spectra of (*S*)-(-)-warfarin and the warfarin produced from (-)-acenocoumarin are identical. Therefore, the *S* configuration can be unambiguously assigned to

0022-2623/79/1822-1122$01.00/0

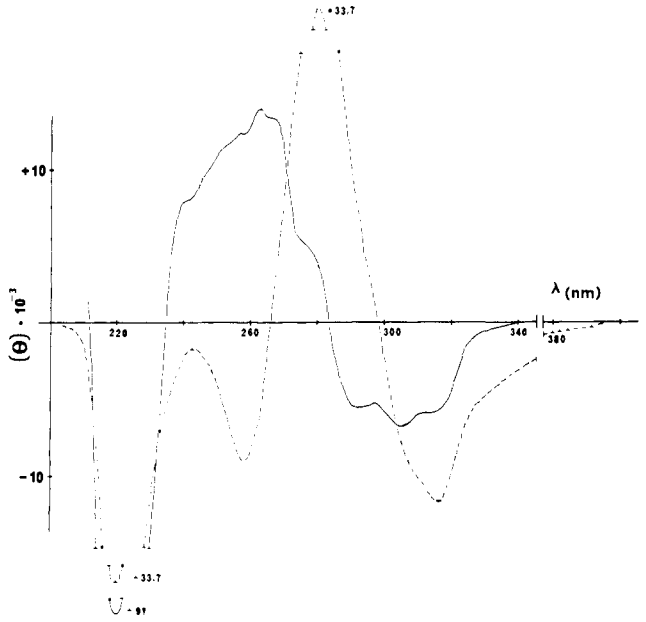


Figure 1. Circular dichroism spectra of (*S*)-(−)-acenocoumarin (- - -) and (*S*)-(−)-warfarin (—) in methanol.

(−)-acenocoumarin. This result is in agreement with the stated assignment of Minertz et al.[14] and implies that the reversed stereoselectivity with respect to potency observed by these authors is not due to a misassignment of absolute configuration.

Experimental Section

Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. NMR spectra were recorded on a Varian EM 360A spectrometer using tetramethylsilane as an internal standard. IR spectra were recorded on a Perkin-Elmer 727B infrared spectrophotometer, optical rotations on a Jasco DIP-4 digital polarimeter using 1-dm cells, and CD spectra on a Jobin Yvon dichrographe 111. For recording CD spectra, cell path lengths ranged from 0.1 to 1 cm, while the concentration remained close to 4 μg/mL. The scan rate was 12 nm/min and the time constant 10. EIMS were determined on an AEI MS-9 high-resolution mass spectrometer at 70 eV. Exact mass measurements were determined within 15 ppm utilizing an on-line PDP-12 computer and a high-resolution program. Methane CI mass spectra were determined on a Biospec Quadrapole mass spectrometer. All mass spectra were obtained by direct-insertion probe at a source temperature of 200 °C. TLC was performed on EM Reagents analytical silica gel chromatography plates with fluorescent indicator (no. 5539).

***p*-Nitrobenzalacetone** (1). This compound was prepared according to the method of Nishimura[17] in a 37% yield after two recrystallizations from EtOH: mp 104–105 °C; IR (KBr) 3400 (br), 1695, 1670, 1615, 1595, 1515, 1345, 1110, 980, 865, 830, 750 cm^{-1}; NMR (60 MHz, $CDCl_3$) δ 2.4 (s, 1 H), 6.3 (d, J = 16 Hz, 1 H), 7.5 (d, J = 16 Hz, 1 H), 7.62 (d, J = 8 Hz, 2 H), 8.19 (d, J = 8 Hz, 2 H).

Acenocoumarin [4-Hydroxy-3-[1-(4-nitrophenyl)-3-oxobutyl]-2*H*-1-benzopyran-2-one (2)]. Acenocoumarin was prepared by the condensation of 1 with 4-hydroxycoumarin in a procedure similar to that utilized for the synthesis of warfarin.[18] 4-Hydroxycoumarin (13.74 g, 85 mmol), 1 (13.5 g, 70 mmol), Et_3N (1.3 mL), and H_2O (400 mL) were refluxed in a 1000 mL round-bottom flask with magnetic stirring for 10 h. To the cooled reaction mixture, NaOH (1 N, 200 mL) was added. Stirring was continued until all solid material had dissolved. Unreacted 1 was removed by several extractions with Et_2O (3 × 200 mL), and the aqueous layer was filtered and acidified (HCl) to pH 2. The resulting precipitate was collected by suction filtration and after one recrystallization from Me_2CO–H_2O yielded **2** (16.31 g, 75% theor): mp 193–195 °C, lit.[19] 196–199 °C; IR (KBr) 3400 (br), 2910, 1695, 1625, 1575, 1515, 1390, 1350, 1175, 1110, 1090, 1000, 905, 850, 760, 700 cm^{-1}; NMR (60 MHz, Me_2CO-d_6) δ 1.7 (s, 0.66 H), 1.73 (s, 2.34 H), 2.43 (m, 2 H), 4.38 (m, 1 H), 7.7 (m, 8 H). The two noninteger proton resonances are a consequence of the fact that the compound exists as two diastereomeric hemiketals in solution.

Resolution of Acenocoumarin. Quinidine (13.77 g, 42.4 mmol) and (±)-**2** (15 g, 42.4 mmol) were dissolved in a warmed mixture of Me_2CO (125 mL) and $CHCl_3$ (85 mL).[13] The resulting solution was allowed to stand overnight at room temperature. The following day, the mixture was kept at 4 °C for 12 h prior to collecting a first crop (15.7 g): $[\alpha]^{25}_D$ +39.0 ± 0.2 (*c* 1, 95% EtOH). This material was dissolved in a boiling mixture of Me_2CO (400 mL) and EtOH (300 mL). Crystallization proceeded at 4 °C for 15 h, to yield a second crop (9.2 g) of the salt: $[\alpha]^{25}_D$ +26.7 ± 0.2 (*c* 1, 95% EtOH). A final recrystallization from Me_2CO (300 mL) and EtOH (300 mL) yielded 2.3 g of the salt: $[\alpha]^{25}_D$ +26.0 ± 0.5 (*c* 1, 95% EtOH). Acenocoumarin was recovered by partitioning the salt from the last recrystallization between $CHCl_3$ (50 mL) and NaOH (1 N, 100 mL) and acidifying (HCl) the separated aqueous layer. The precipitate was recovered by filtration and recrystallized from EtOH–H_2O to yield 0.8 g of white material. Recrystallization from EtOH–H_2O led to problems with spontaneous ethyl ketal formation. To remove the ethyl ketal, the entire 0.8 g was dissolved in 1 N base and the insoluble ketal removed by filtration. The basic solution was acidified (HCl) to give a white precipitate (0.65 g): $[\alpha]^{25}_D$ −224.2 ± 0.4 [*c* 0.62, 0.5 N NaOH, lit.[14] −225 (1% in 0.5 N NaOH)]; mp 191–192 °C; CD (×10^{-3}; 42.6 μg/mL of MeOH) $[\theta]_{410}$ 0, $[\theta]_{315}$ −12.1, $[\theta]_{290}$ 0, $[\theta]_{281}$ +33.7, $[\theta]_{267}$ −8.6, $[\theta]_{244}$ −16, $[\theta]_{220}$ −33.7, $[\theta]_{200}$ 0.

(−)-Acenocoumarin Ethyl Ketal [2,3-Dihydro-2-methyl-2-ethoxy-4-(4-nitrophenyl)-5-oxo-3*H*-benzopyrano[3,4-*e*]-pyran (3)]. (-)-Acenocoumarin (0.6 g, 1.7 mmol) was added to EtOH (50 mL) in a 100-mL flask. HCl gas was briefly bubbled through the EtOH and the flask stoppered. The mixture was stirred for 24 h and then filtered to remove the white precipitate: yield 0.59 g (91% theor); mp 237–239 °C; IR (KBr) 3420 (br), 2980, 2940, 2740, 1715, 1630, 1515, 1385, 1350, 1180, 1110, 1085, 1055, 995, 915, 860, 760 cm^{-1}; NMR (60 MHz, Me_2CO-d_6) δ 1.07 (t, J = 6 Hz, 3 H), 1.77 (s, 3 H).

(−)-4′-Aminowarfarin Ethyl Ketal [2,3-Dihydro-2-methyl-2-ethoxy-4-(4-aminophenyl)-5-oxo-3*H*-benzopyrano[3,4-*e*]dihydropyran (4)]. Compound **3** (0.4 g, 10.5 mmol) was placed in a 50-mL Erlenmeyer flask equipped with a magnetic stir bar, MeOH (25 mL) was added, and stirring begun. Palladium on carbon (10%, 25 mg) was added, and a stream of N_2 was directed into the flask. $NaBH_4$ (130 mg, 3.43 mmol) was added in several portions over a period of 5 min.[20] Ten minutes after completing the addition, the reaction was analyzed by TLC (solvent systems: Et_2O–THF–AcOH, 50:50:1). Since some **3** remained, additional $NaBH_4$ (30 mg, 0.79 mmol) was added. A TLC following this addition indicated the reduction was complete. The reaction mixture was filtered to remove the catalyst and HCl (10%, 1 mL) was added. The volume of the solution was then reduced to 0.2–0.3 mL under a stream of N_2 and the mixture stored in a freezer.

(−)-4-Chlorowarfarin [4-Hydroxy-3-[1-(4-chlorophenyl)-3-oxobutyl]-2*H*-1-benzopyran-2-one (5)]. The flask containing the aminowarfarin was removed from the freezer and to it was added HCl (6 N, 17 mL). The suspension was cooled to 0 °C in the ice–salt bath. Magnetic stirring was started, and $NaNO_2$ (140 mg, 2.03 mmol) in H_2O (1 mL) was added by Pasteur pipet. After 35 min, the diazotization was complete and the solution had a clear yellow appearance.

While the diazotization reaction was proceeding, freshly prepared CuCl[21] (0.5 g, 5.05 mmol) was added to HCl (6 N, 10 mL) in a 50-mL Erlenmeyer flask and the solution cooled in an ice bath to 0 °C. The diazotized warfarin was added to this solution over a period of 2 min with magnetic stirring. Immediately after the addition the ice bath was removed and the contents of the flask were allowed to come to room temperature. At the end of an hour, the reaction mixture was briefly heated to 45 °C to decompose any remaining copper complex. After cooling, a light brown precipitate was removed by filtration. The precipitate was dissolved in NaOH (1 N, 20 mL) and filtered. The filtrate was acidified (HCl), and upon standing the oil that initially formed crystallized. TLC analysis of the product indicated one

spot with a R_f different from aminowarfarin: yield 281 mg (78% theor); mp 103–106 °C; IR (KBr) 3380 (br), 2910, 1690, 1620, 1570, 1495, 1380, 1170, 1090, 1015, 900, 820, 760 cm^{-1}; NMR (60 MHz, Me_2CO-d_6) δ 1.66 (s, 0.6 H), 1.72 (s, 2.4 H), 2.48 (m, 2 H), 4.1 (q, J = 6 Hz, J = 11 Hz, 1 H), 7.4 (m, 8 H); CD ($\times 10^{-3}$; 40 μg/mL of MeOH) $[\theta]_{340}$ 0, $[\theta]_{315}$ −7.1, $[\theta]_{305}$ −8.5, $[\theta]_{290}$ −6.5, $[\theta]_{283}$ 0, $[\theta]_{266}$ +19.2, $[\theta]_{240}$ +10.2, $[\theta]_{235}$ 0, $[\theta]_{225}$ −56.5, $[\theta]_{210}$ 0; high-resolution mass measurement ($C_{19}H_{15}ClO_4$) calcd, 342.06595; found, 342.0660.

Warfarin [3-(α-Acetonylbenzyl)-4-hydroxycoumarin (6)]. The methyl ketal of **5** was synthesized by dissolving the reactant (98 mg, 0.301 mmol) in MeOH (4 mL) in a 50-mL round-bottom flask containing a magnetic stirring bar. After briefly bubbling HCl gas through the solution, the flask was capped and the contents were stirred for 3 h. The MeOH was then blown off with dry N_2 and replaced with EtOAc (7 mL) and Pd on C (10%, 35 mg). A condenser was added and the solution heated to reflux. Over a period of 30 min $NaBH_4$ (200 mg, 5.3 mmol) was added.[22] One hour after the addition was completed, a small amount was removed and analyzed by CI mass spectrometry. The chloro compound was about 5% of the total. Further addition of $NaBH_4$ (200 mg, 5.3 mmol) and refluxing for another 4 h did not diminish the amount of chlorowarfarin. The catalyst was filtered off and the filtrate added to a 50:50 mixture of THF and HCl (6 N, 15 mL). After stirring the mixture for 6 h, the THF was allowed to evaporate. The resulting precipitate was removed by filtration and partitioned between NaOH (10 mL) and Et_2O (3 × 20 mL). Acidification (HCl) of the aqueous solution yielded a white precipitate. Recrystallization from Me_2CO–H_2O gave 26 mg (28% theor): mp 167–168 °C, lit.[13] mp 170–171 °C; IR (KBr) 3350 (br), 2915, 1680, 1615, 1565, 1490, 1380, 1170, 1070, 755 cm^{-1}. Resolved (*S*)-(−)-warfarin,[13] $[\alpha]^{25}_D$ −150.83 ± 1.25 (*c* 1.2, 0.5 N NaOH), gave the following CD values ($\times 10^{-3}$; 48.6 μg/mL of MeOH): $[\theta]_{340}$ 0, $[\theta]_{315}$ −6.1, $[\theta]_{305}$ −6.7, $[\theta]_{292}$ −5.6, $[\theta]_{284}$ 0, $[\theta]_{263}$ +13.8, $[\theta]_{240}$ +8.2, $[\theta]_{235}$ 0, $[\theta]_{221}$ −91.0, $[\theta]_{213}$ 0. The shape and sign of the curve obtained from **6** and resolved (*S*)-warfarin were identical, while the absolute values of the maxima were within 10% of each other.

Acknowledgment. This investigation was supported in part by NIH Research Career Development Award 1K04GMO211, NIH Grant 1R01GM25136 (W.F.T.), and Training Grant 5T32GMO7348-01 (C.R.W.) from the Institute of General Medical Sciences.

References and Notes

(1) R. A. O'Reilly, *Mol. Pharmacol.*, **7**, 209 (1971).
(2) D. S. Hewick and J. McEwen, *J. Pharm. Pharmacol.*, **25**, 458 (1973).
(3) A. Breckenridge, M. Orme, H. Wesseling, R. J. Lewis, and R. Gibbons, *Clin. Pharmacol. Ther.*, **15**, 424 (1974).
(4) G. Levy, R. A. O'Reilly, and L. B. Wingard, Jr., *Res. Commun. Chem. Pathol. Pharmacol.*, **7**, 359 (1974).
(5) R. A. O'Reilly, *Clin. Pharmacol. Ther.*, **16**, 348 (1974).
(6) E. Jähnchen, T. Meinertz, H. J. Gilfrich, U. Groth, and A. Martini, *Clin. Pharmacol. Ther.*, **20**, 342 (1976).
(7) B. D. West and K. P. Link, *J. Heterocycl. Chem.*, **2**, 93 (1965).
(8) J. J. Ebele, B. D. West, and K. P. Link, *Biochem. Pharmacol.*, **15**, 1003 (1966).
(9) A. Breckenridge and M. Orme, *Life Sci.*, **11**, 337 (1972).
(10) D. S. Hewick, *J. Pharm. Pharmacol.*, **24**, 661 (1972).
(11) A. Yacobi and G. Levy, *J. Pharmacokin. Biopharm.*, **2**, 239 (1974).
(12) W. Schmidt and E. Jähnchen, *J. Pharm. Pharmacol.*, **29**, 266 (1977).
(13) B. D. West, S. Preis, C. H. Schrueder, and K. P. Link, *J. Am. Chem. Soc.*, **83**, 2676 (1961).
(14) T. Meinertz, W. Kasper, C. Kahl, and E. Jähnchen, *Br. J. Clin. Pharmacol.*, **5**, 187 (1978).
(15) W. Dieterle, J. W. Faigle, C. Montigel, M. Sulc, and W. Theobald, *Eur. J. Clin. Pharmacol.*, 11, 367 (1977).
(16) E. J. Valente, Ph.D. Thesis, University of Washington, Seattle, Wash., 1977.
(17) T. Nishimura, *Bull. Chem. Soc. Jpn.*, **26**, 253 (1953).
(18) M. Hermodson, W. Barker, and K. Link, *J. Med. Chem.*, 14, 167 (1971).
(19) U.S. patent 2 648 682.
(20) T. Neilson, H. Wood, and A. Wylie, *J. Chem. Soc.*, 371 (1962).
(21) C. S. Marvel and S. M. McElvain in "Organic Syntheses", Collect. Vol. II, H. Gilman and A. H. Blatt, Ed., Wiley, New York, 1941, p 170.
(22) T. R. Bosin, M. G. Raymond, and A. R. Buckpitt, *Tetrahedron Lett.*, **47**, 4699 (1973).