Synthesis and Relative Urinary Excretion Rates of the Enantiomers of Warfarin-4-^{14}C and Phenprocoumon-2-^{14}C[1]

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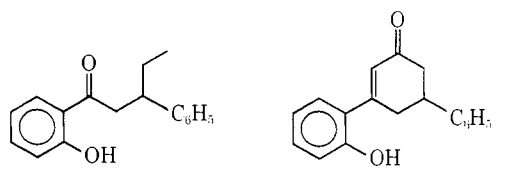
The enantiomers of warfarin-4-^{14}C (3-α-acetonylbenzyl-4-hydroxycoumarin) and phenprocoumon-2-^{14}C (3-α-ethylbenzyl-4-hydroxycoumarin) have been prepared. The urinary excretion rates of the separate isomers in the rat were not sufficiently different to explain their differences in anticoagulant potencies. No activity was detected in expired CO_2 from phenprocoumon-2-^{14}C.

This study was undertaken to determine if the observed differences between the anticoagulant potencies of the enantiomers of warfarin (3-α-acetonylbenzyl-4-hydroxycoumarin) and phenprocoumon (3-α-ethylbenzyl-4-hydroxycoumarin) could be explained by correspondingly different rates of excretion. (−)(*S*)-Warfarin has been shown[2] to be five to eight times more potent in the rat than the (+)(*R*) isomer, the actual ratio varying with the type of assay used. (−)(*S*)-Phenprocoumon has been shown similarly to be three to five times more potent than the (+)(*R*) isomer.[3]

The preparation of the ^{14}C-labeled phenprocoumon enantiomers is given in the Experimental Section.

Warfarin enantiomers could not be labeled in the same way because hydrolytic decarboxylation is accompanied in this case by cyclization to 1-*o*-hydroxy-



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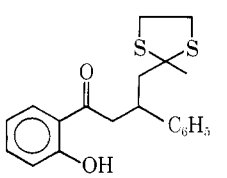

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phenyl-5-phenylcyclohexen-3-one (**1**). Hydrolysis and decarboxylation of the ethylene thioketal of warfarin[4] yielded the expected uncyclized *o*-hydroxy ketone (**2**) but many variations of the recarboxylation procedure using isotopic ethyl carbonate failed. Warfarin was instead prepared in the 4-^{14}C-labeled racemic form by the method of Underwood[5] and resolved by a modification of the earlier procedure.[4]

Results and Discussion

Rates of excretion of the ^{14}C label from the warfarin enantiomers (Figure 1) and the phenprocoumon enantiomers (Figure 2) differed slightly, the less potent isomer appearing more rapidly in each case. The data agree well with Berg's[6] who included feces in his estimate of total excretion of racemic warfarin. We estimate from a separate summation of ^{14}C activity in the feces that in 150 hr, a total of 9% of the dose of either enantiomer of warfarin is excreted there. Measurement of ^{14}C activity in $BaCO_3$ recovered from air scrubbers attached to a sealed cage showed that none of doses of either enantiomer of phenprocoumon was excreted as CO_2 (limit of detectibility, 1% of dose). This finding agrees with a similar one by Berg for warfarin-4-^{14}C. It is interesting that the lactonic carbonyl carbon, the most labile in the molecule does not appear to a measureable extent as CO_2.

We feel that the *in vivo* potency ratio of the drug enantiomers we have studied is not explained by the moderate difference in gross excretion data we have presented. We conclude that the basis for the difference between potencies must be sought at some point closer to the anticoagulant event.

Experimental Section

Diethyl Carbonate-Carbonyl-^{14}C.—This procedure is a modification of the method of Eisenhauer, *et al.*[7] $BaCO_3$ (60 mg) and 8.5 mg of $Ba^{14}CO_3$ (sp act., 25 mCi/mmole) were treated in a sealed, evacuated 10-ml distillation apparatus with 3 ml of 20% $HClO_4$. The $^{14}CO_2$ was allowed to diffuse for 24 hr into 1.4 ml of 0.51 *M* NaOH in the receiver flask. Excess $AgNO_3$ solution was added to the receiver and 85 mg of $Ag_2{}^{14}CO_3$ was collected, dried, and refluxed with 1.0 ml of EtI and 2.0 ml of Et_3N for 4 hr. All volatile substances were removed by vacuum distillation into a receiver in Dry Ice–acetone. The distillate contains unreacted EtI and Et_3N (it is important that Et_3N be in excess) which continue to react during storage. The Et_3N–$Et_2{}^{14}CO_3$ mixture was filtered immediately before use with a syringe pressure filter to remove precipitated quaternary salt. The presence of Et_3N in the product caused no ill effect.

(+)(*R*)-Phenprocoumon.—(+)(*S*)-*o*-Hydroxy-3-phenylvalerophenone (**3**)[8] (0.20 ml) was dissolved in 5 ml of PhMe and this solution was dried by distilling out one-third of the solvent. Freshly cut Na (0.1 g) and 1 ml of absolute EtOH were added; the Na was allowed to react completely before excess EtOH was removed by distillation. To this NaOEt solution were added 0.40 ml of Et_2CO_3 and 1.2×10^8 dpm of the $Et_2{}^{14}CO_3$ solution prepared above and the mixture was refluxed with stirring until tlc on fluorescent silica gel plates showed absence of starting ketone (solvent system: toluene–HOAc, 3:1 v/v). H_2O was added to the mixture and the aqueous part was added to ice–HCl. The crude phenprocoumon (80 mg) was dissolved with quinine (108 mg) in 0.5 ml of EtOH and filtered, 1.0 ml of H_2O was added, and the solution was held at 5° for 24 hr. The resulting white salt was decomposed by partition between 5% NaOH and $CHCl_3$ and the (+)(*R*)-phenprocoumon (30 mg) was recovered by addition of the aqueous layer to ice–HCl. Purification of the product in this way was found to be superior to simple crystallization of the phenprocoumon alone or preparative tlc. The specific activity

(1) This work was supported by Public Health Service Grant No. RO1-HE 10527-03 from the National Heart Institute. We wish to thank the Department of Biology of this institution for the use of their liquid scintillation counter.

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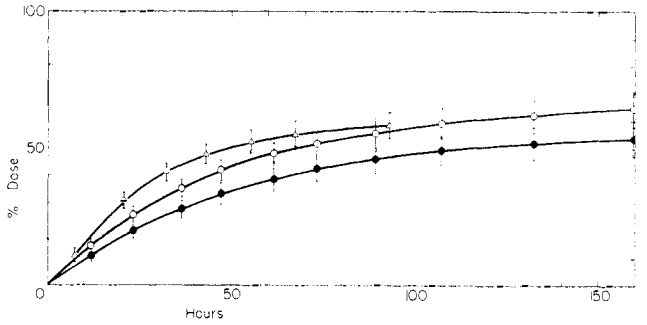

Figure 1.—Cumulative per cent of dose as ^{14}C in urine from groups of ten 350-g female rats given intraperitoneal injections of 1.0 (-Δ-) or 0.16 mg/rat (-○-) of (+)(*R*)-warfarin-4-^{14}C or 0.13 mg/rat of (−)(*S*)-warfarin-4-^{14}C (-●-). The 1.0-mg (*R*) and 0.13 mg (*S*) doses are approximately equipotent. Bars represent two standard counting errors.

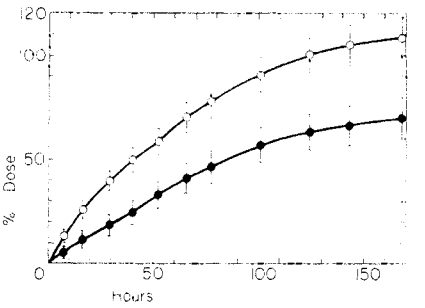

Figure 2.—Cumulative per cent of dose in urine as ^{14}C from groups of ten 350-g female rats given single intraperitoneal injections of 0.16 mg/rat, (+)(*R*)-phenprocoumon-2-^{14}C (-○-), or 0.14 mg/rat, (−)(*S*)-phenprocoumon-2-^{14}C (-●-). Bars represent two standard counting errors.

of the product was 102,000 dpm/mg. This procedure has been shown not to cause racemization.[8]

(−)(*S*)-Phenprocoumon.—(−)(*R*)-3[3] was used as above and quinidine was employed in place of quinine to facilitate purification. Crystallization solvent for the salt was $CHCl_3$-EtOH (3:1). The (−)(*S*)-phenprocoumon had a specific activity of 126,000 dpm/mg.

(−)(*S*)-Warfarin-4-^{14}C.—*rac*-Warfarin-4-^{14}C was prepared by the method of Underwood.[5] This product (38 mg, 1.8×10^7 dpm/mg) was mixed in 10 ml of warm Me_2CO with 410 mg of optically pure, nonradioactive (−)(*S*)-warfarin and 0.55 g of quinidine. The salt was collected after 12 hr storage at 5° and crystallized twice more in the same way from approximately 12 ml of Me_2CO/g. To test this preparation for optical purity, a small sample (estd 1 mg) was mixed with 1.25 g of optically pure (+)(*R*)-warfarin-quinine salt. The specific activity of the mixture was found to be 1030 dpm/mg. The test mixture was then recrystallized four times by dissolving the solid in the minimum volume of absolute EtOH, cooling to *ca.* 25°, adding the same volume of dry Et_2O and storing at −10°. The final head fraction had sp act. 8 dpm/mg which allows the calculation of a minimum optical purity of 99.2% for the (−)(*S*)-warfarin-4-^{14}C. The counting of initial and final specific activities were done at the same time in solutions of identical composition and the values were checked by combining aliquots identical with those counted separately to verify the identity of the individual efficiencies. Final yield was 40 mg of (−)(*S*)-warfarin-4-^{14}C.

(+)(*R*)-Warfarin-4-^{14}C.—The filtrate from the first crystallization of the (−)(*S*)-warfarin was concentrated to a glassy solid (0.78 g) which was stirred into a mixture of $CHCl_3$ and 5% NaOH. Acidification of the aqueous layer yielded 0.28 g of warfarin-4-^{14}C containing predominantly the (−)(*S*) isomer. This product was mixed with 0.34 g of quinine and 7.8 g of optically pure (+)(*R*)-warfarin quinine salt in 20 ml of absolute EtOH. Dry Et_2O (20 ml) was added to the solution and it was stored at −10° for 12 hr. The crystals were filtered quickly because they soften at room temperature and were recrystallized five times from 3 ml of EtOH/g to which was added 12 ml of Et_2O/g. To test the optical purity of the head fraction 4.2 mg of it and 1.59 g of (−)(*S*)-warfarin-quinidine salt were crystallized together (Me_2CO) four times. The initial specific activity of the mixed salts was 305 dpm/mg and the specific activity of the final head fraction was 3.7 dpm/mg which places a lower limit of 98.8% on the optical purity of the product. The yield was 75 mg.

Excretion-Rate Determinations.—Groups of ten female rats (av wt 350 g) were housed in large metabolism cages with fine screens for feces and metal funnels. Feces were removed frequently (9–24 hr) to prevent their absorbing urine and at each urine-collection interval the funnels were rinsed down to collect dried urine solids. Each rat was etherized lightly and dosed intraperitoneally with a $NaHCO_3$–isotonic saline solution of the drug. The rats had free access during the experiment to water and a paste made from powder form commercial rat food and water.

Radioactivity Measurements.—Urine and urine funnel washings were measured and 1-ml samples were pipetted into counting vials containing 3.75 ml of Triton X-100 (Beckman Instruments Co.) and 11.25 ml of a solution of 0.4% PPO and 0.01% POPOP in PhMe. The counting instrument (Beckman Model LS-100) corrected for efficiencies which were in the range 75–85%. The samples were counted to 3% standard error in the case of phenprocoumon and to 2% for warfarin.

Feces were dried and weighed and 1-g samples were burned in a wire basket suspended in a closed 4-l. suction flask. The flask was flushed (O_2) beforehand and the feces were ignited by sunlight using a 10-cm magnifying lens.

NaOH solution was added to the flask and allowed to react with the CO_2. $BaCl_2$ was added in excess and the resulting $BaCO_3$ was collected and dried at 50°. The $BaCO_3$ was weighed and small weighed samples (0.5 g) of it were powdered and suspended in counting vials with 15 ml of PhMe containing 5% thixotropic colloidal silica, 0.4% PPO and 0.01% POPOP.

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