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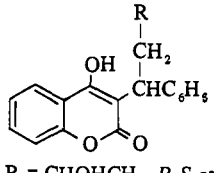
Absolute Configurations of the Four Warfarin Alcohols†

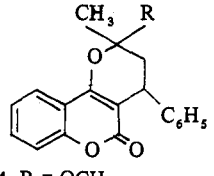
Kenneth K. Chan, Richard J. Lewis, and William F. Trager*

Department of Pharmaceutical Chemistry, School of Pharmacy, University of California, San Francisco, California 94122, and Research Service, Veterans Administration Hospital, San Francisco, California 94121. *Received May 8, 1972*

Chemical synthesis, specific stable isotope labeling, mass spectrometry, and nmr spectroscopy are utilized to establish the absolute configurations of the four stereoisomers of 3-[α-(2-hydroxypropyl)-benzyl]-4-hydroxycoumarin. The absolute configurations of the four stereoisomers of 2,3-dihydro-2-methyl-4-phenyl-4*H*-pyrano[3,2-*c*]benzopyran-5-one are also reported. Preliminary biological data indicate that in man the *"in vivo"* reduction is stereoselective and that the resultant metabolites are biologically active although not as active as warfarin itself.

In an earlier report we demonstrated that the diastereomeric alcohols (1), resulting from reduction of the carbonyl group in the side chain of warfarin (2), occur as metabolites in normal man.[2] Since phenprocoumon (3) and cyclocoumarol (4) are active as anticoagulants,[3] it appeared that minor modification of the side chain would not drastically affect this property. It seemed reasonable, therefore, to anticipate that the various isomers of 1 might have significant biological activity in their own right. In addition, the *S* forms of 2[4] and 3[5] are more potent than the corresponding *R* forms. For these reasons we initiated a study to establish the absolute configuration of the four possible isomers to determine their relative biological potencies as anticoagulants, and to determine the stereochemistry of the *"in vivo"* reduction of warfarin.





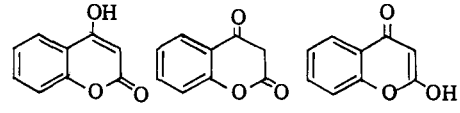
1a, R = $CHOHCH_3$, *R,S* or *S,R*
1b, R = $CHOHCH_3$, *R,R* or *S,S*
2, R = $COCH_3$
3, R = CH_3

4, R = OCH_3
5a, R = H, *R,S* or *S,R*
5b, R = H, *R,R* or *S,S*

Diastereomers 1a and 1b can readily be obtained by reducing sodium warfarin with $NaBH_4$ followed by separation utilizing a combination of elution chromatography and fractional recrystallization. These isomers can also be separated utilizing preparative tlc. The faster moving isomer (1a) was characterized as described below; the slower moving material was designated 1b.

In the initial development of tlc systems for the separation of the diastereomeric alchols it was noticed that with time a third spot appeared with higher R_f which seemed to originate from the breakdown of 1b. This compound was isolated and characterized by mass spectroscopy and nmr as 5. Subsequently, it was found that it could also be obtained by thermal or Lewis acid catalyzed cyclic dehydration of 1b. Under similar conditions 1a afforded a different isomer of 5. Formation of cyclic analogs 5a and 5b are of particular significance, since the conformational possibilities are now limited and assignment of stereochemistry based on nmr spectroscopy is less ambiguous than similar analysis of the respective precursors 1a and 1b. If the relative stereochemistry of the isomers of 5 can be determined and the mechanism of their formation from the isomers of 1 elucidated, then it follows that the stereochemistry of the flexible isomers of 1 will also be known.

It had been pointed out by earlier investigators[6] that 4-hydroxycoumarin (6) can exist in two other tautomeric forms, the diketo structure (7) and the chromone structure (8). The contribution of 7 is considered minimal because of the lack of stabilization by conjugation. The existence of the tautomeric forms 6 and 8 has been demonstrated by Arndt[6a] who obtained a mixture of 4-methoxycoumarin and 3-methoxychromone on treatment of 4-hydroxycoumarin with diazomethane.



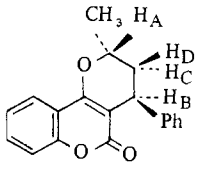
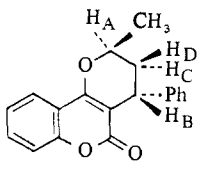
6 7 8

The determination of chromone or coumarin structures for 5a and 5b is important to this work, since two structurally different isomers could result upon dehydration of the alcohols. Neither nmr nor mass spectroscopy can *a*

†A preliminary report of this work was presented at the 162nd National Meeting of the American Chemical Society.[1] This investigation was supported in part by the University of California Academic Senate Grant 10, San Francisco Division, in part by a National Institutes of Health Grant GM-16496, and in part by the American Heart Association (R. J. L.). The authors are grateful to Endo Laboratories for supplying samples of resolved (*R*)- and (*S*)-warfarin.

*To whom correspondence should be addressed at the School of Pharmacy, University of Washington, Seattle, Wash. 98195.

Table I. Chemical Shifts and Observed Coupling Constants for 5a and 5b, 100 MHz

	Proton(s)	Chemical shift, ppm	Coupling constants, Hz
 5a	H_A, H_B	4.25	
	H_C, H_D	2.1	
	Me	1.45	
	Ar (9)	7.3	
 5b	H_A	4.4	$J_{AD} = 11.0$ $J_{AC} = 2.1$ $J_{AMe} = 6.2$
	H_B	4.1	$J_{BD} = 10.7$ $J_{BC} = 7.3$
	H_C	2.45	$J_{gem} = 14.2$ $J_{CB} = 7.3$ $J_{CA} = 2.1$
	H_D	1.85	$J_{gem} = 14.2$ $J_{DA} = 11.0$ $J_{DB} = 10.7$
	Me	1.52	$J_{MeA} = 6.2$
	Ar (9)	7.3	

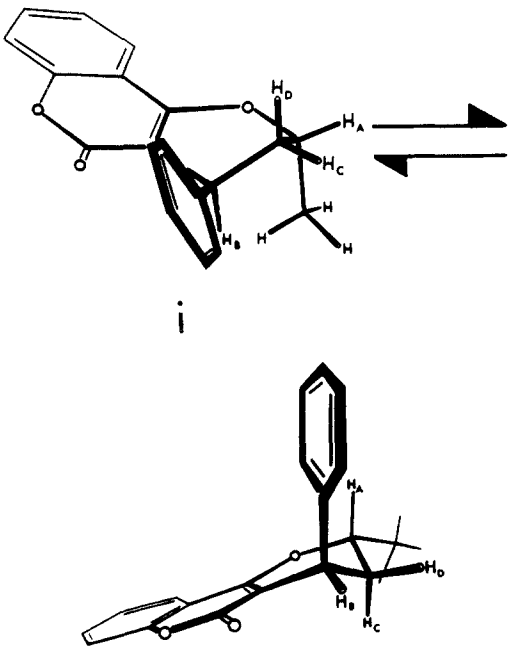
priori unambiguously distinguish between the chromone and coumarin structures. However, both the ir and uv spectra of **5a** and **5b** strongly support the coumarin structure for these materials, as the spectra are consistent with those given by 4-methoxycoumarin and not 2-methoxychromone.

Nmr Analysis. The nmr spectral paramaters‡ of **5a** and **5b** are tabulated in Table I. Examination of the data shows that the spectra are dramatically different. Isomer **5a** obtained from the thermal dehydration of **1a** shows a rather simple spectrum, while **5b** obtained from 1b shows a rather complex spectrum.

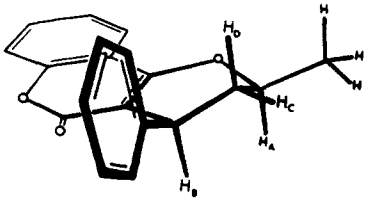
These data suggest that **5a** exists in a rather mobile ring system such that the observed nmr spectrum is a time average of at least two extreme conformations resulting in a relatively simplified spectrum. On the other hand, the nmr spectrum of **5b** is suggestive of a rather rigid ring system or at least one displaying a pronounced preferance for a single conformation resulting in a more complex spectrum due to the nonequivalance of the various protons in that system.

An examination of Dreiding models shows that in the structure having either the *R,S* or *S,R* configuration, significant contributions by at least two conformations are expected. In one conformation (i) the methyl group is axial, causing a 1,3 methyl-H_B interaction; while in the other conformation (ii) the phenyl group is pseudo-axial, with a resultant 1,3 phenyl-H_A interaction. The expected rapid equilibrium between these two conformations should result in a simplified nmr spectrum, because the chemical shift differences implicit in the two conformations would average out. Analysis of the nmr spectrum is most consistent with **5a** having the relative configuration indicated.

Dreiding models indicate that the isomer having either the *S,S* or *R,R* configuration would exist in a rather rigid ring system in which the dihydropyran portion of the molecule is in a half-chair conformation (iii). In this conformation both the methyl and phenyl groups occupy either an equatorial position or a pseudo-equatorial position. Hence the contribution of other conformations would be expected to be minimal. This should result in a relatively more complex nmr spectrum. These data are most consistent with **5b** having this relative configuration.



i ii



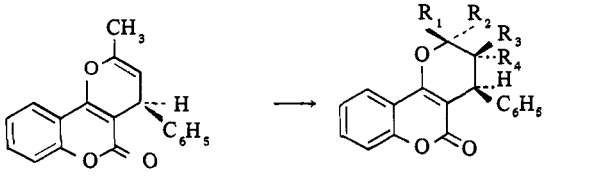
iii

Chemical Evidence for the Assigned Configurations. Although the spectroscopic evidence was indicative of the structural assignments for **5a** and **5b**, we desired a definitive chemical proof. One approach that seemed attractive involved cyclic dehydration of warfarin (**2**)[7] to **9**, followed by catalytic reduction to **5b**. Since catalytic reductions generally proceed stereoselectively *via* cis addition[8] the major (or single) product should be the one in which the methyl and phenyl groups bear a "cis" relationship to each other.

Cyclic dehydration of both the racemic and (*S*)-warfarin proceeded smoothly in the presence of P_2O_5 in benzene; catalytic hydrogenation over PtO_2 afforded the saturated analog. Glc, nmr, ir, mp, etc., showed that the sole product was identical with the one obtained in the thermal dehydration of 1b. These results are consistent with the assigned stereochemistry.

To confirm our assumption that cis addition occurred and proceeded from the least sterically hindered side, the reduction was carried out under D_2. Four products (**10**, **11**, **12** and **13**) are theoretically possible and they are shown below. For compounds **10** and **11** one would expect a coupling constant of ~11 Hz between the benzylic hydrogen and the adjacent methylene hydrogen. Conversely, products **12** and **13** should show a smaller coupling between the analogous hydrogens, since the spectral relationship they

‡The spectra will appear following these pages in the microfilm edition of this volume of the journal. Single copies may be obtained from the Business Operations Office. Books and Journals Division, American Chemical Society, 1155 Sixteenth St., N.W., Washington, D. C. 20036. Remit check or money order for $3.00 for photocopy of $2.00 for microfiche, referring to code number JMED-72-1265.

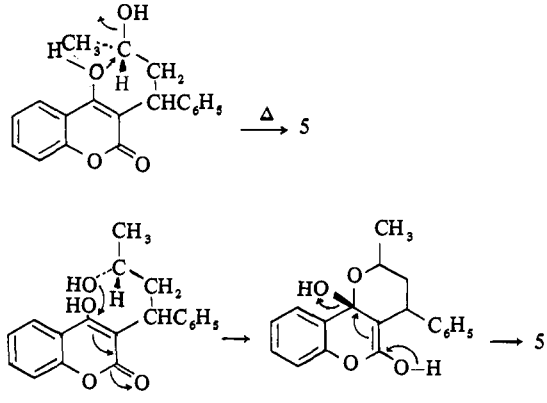


9

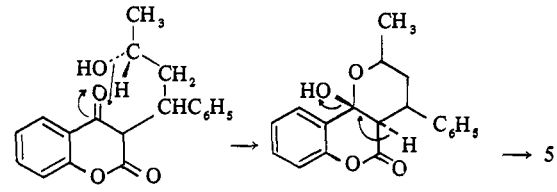
10, $R_1 = CH_3$; $R_2 = D$; $R_3 = H$; $R_4 = D$
11, $R_1 = D$; $R_2 = CH_3$; $R_3 = H$; $R_4 = D$
12, R_1 D; R_2 CH_3; $R_3 = D$; $R_4 = H$
13, $R_1 = CH_3$; $R_2 = D$; $R_3 = D$; $R_4 = H$

bear to each other is approximately axial equatorial. A single product was obtained from reduction of **9** under D_2. The nmr spectrum was determined and showed a doublet for H_B with a coupling constant of 10.7 Hz. Similarly H_D appeared as a doublet with a coupling constant of 10.7 Hz. Structures **12** and **13** can be eliminated on the basis of the observed coupling constant. Structure **11** is a trans product which presumably could arise mechanistically in two ways: (i) a Horiuti-Polanyi type mechanism[9] which involves stepwise isomerization of the methyl and/or benzylic carbons, (ii) a stereospecific stepwise trans addition of deuterium across the double bond. The former mechanism can be eliminated on the basis of the stereochemical purity of the product. No deuterium scrambling was found in the system as determined from the nmr and a parent ion at *m/e* 294 in its mass spectrum. In addition, the optical activity of the H_2 reduced product from **6** (obtained from (*S*)-warfarin) is identical with that shown by **5b** obtained from (*S*)-warfarin *via* thermal dehydration of **1b**. Further, no deuterium incorporation was found in the H_2 reduced product after exposing it to the conditions of deuterium reduction. These results indicate that no racemization is occurring during the H_2 and D_2 reductions. Mechanism ii is also unlikely since the "trans" addition mode would have to be stereospecific. This is, of course, an exceedingly unlikely possibility. Structure **10** resulting from "cis" addition from the least sterically hindered side is most consistent with the data presented.

In order to relate the established relative stereochemistry of **5** to the stereochemistry of **1**, it was necessary to determine the stereochemical course of cyclic dehydration. At least three mechanisms seem possible: (1) an SN2 inversion such that the stereochemistry at the methyl bearing carbon would be opposite that of the starting alcohol at this center of asymmetry, as shown below. (2) A retention of configuration at the methyl-bearing carbon can be envisioned



as proceeding first through a Michael type of addition reaction followed by the elimination of water. (3) The third mechanism also involves retention of configuration and can be depicted as proceeding *via* the keto form of the coumarin.



This mechanism involves an attack of the side chain hydroxyl group on the ring carbonyl generating the hemiketal followed by a facile trans diaxial elimination of water. To distinguish between the possibility of a mechanism involving inversion as opposed to one involving retention, warfarin was labeled with ^{18}O specifically in the side-chain carbonyl (see Experimental Section). The ^{18}O-(40%) containing warfarin was reduced with $NaBH_4$. The resultant diasteriomeric alcohols were separated and each was thermally dehydrated. If mechanism 1 were operative then the ^{18}O should be lost in the conversion of **1** to **5**. However, if either 2 or 3 were operative then ^{18}O should be retained in the products. The mass spectra of all these materials was determined to ensure that no ^{18}O was lost *via* exchange in the reaction sequence and to determine the ^{18}O content in **5**. The results of the experiments indicate that ^{18}O was retained in the conversion of **1** to **5**. Hence either 2 or 3 or a similar mechanism is operative and the reaction proceeds with retention of configuration. Thus isomer **1a** has the same relative configuration as **5a**, which is either trans *R,S* or *S,R*. Isomer **1b** possesses the same relative stereochemistry as **5b**, having cis geometry and a *R,R* or *S,S* configuration.

Having established the relative configurations of **1** and **5**, it now remained to determine the absolute configurations of the four possible stereoisomers in each case. Link, *et al.*, established the absolute configuration of the enantiomers of warfarin by relating the (−)-*S* isomer to (−)-(*R*)-β-phenylcaproic acid through a series of reactions which did not involve the asymmetric centers.[10] Knowing the absolute configuration at a given center of asymmetry, the absolute configuration at a second center follows once the relative configuration between the two centers is established.

Both (*R*)- and (*S*)-warfarin were reduced and the resultant diastereoisomers separated as described previously for racemic warfarin. Thus isomer **1a** obtained from the reduction of (*R*)-warfarin must have the *R,S* absolute configuration, while isomer **1b** must have the *R,R* configuration. Likewise, **1a** obtained from reduction of (*S*)-warfarin must have the *S,R* absolute configuration, while **1b** has the *S,S* configuration.

Biological Results. Having established the absolute stereochemistry of the four possible warfarin alcohols and having standards available, it was now possible to determine their relative biological potencies and the stereochemistry of the *"in vivo"* reduction.

After separate oral administration, 1.5 or 3.0 mg/kg, of each of the four warfarin alcohol stereomers to two human volunteers, citrated plasma samples and quantitative urine collections were obtained at time intervals. Analysis showed that prothrombin times were increased, while the plasma levels of each of the vitamin K dependent clotting factors II, VII, IX, and X were significantly decreased.§ Essentially similar coagulation factor responses were elicited by each of the isomers, although a more sustained depression of coagulation activity was noted after administration of the

§R. J. Lewis, W. F. Trager, A. J. Robinson, and K. K. Chan, manuscript in preparation.

R,S alcohol. This later observation is presumably due to the much longer plasma half-life of the *R,S* alcohol relative to the other three stereomers. The plasma half-lives of the *S,S*, *S,R*, and *R,R* isomers are approximately 13 hr while the *R,S* alcohol has a plasma half-life of approximately 34 hr.

Similar experiments were conducted in two rhesus monkeys and one-stage prothrombin determinations were taken as a measure of anticoagulant activity. As in man, the alcohols were found to be active as anticoagulants. Dose levels, iv, of 4.5, 8.0, and 18.0 mg/kg were studied in one monkey and from these studies it appears that the *R,S* enantiomer is the most active, while the *R,R* is the least active.

Although these data are preliminary in nature and lack statistical significance, two observations seem firm. First, the alcohols are active as anticoagulants but have only about 0.1–0.05 the activity of racemic warfarin and second, the rate of elimination of one of the isomers, *R,S*, is much slower than the other three.

Two normal male volunteers were administered (oral) 0.75 mg/kg of (*S*)- and (*R*)-warfarin on separate occasions. Periodic blood and urine samples were collected over 10 days, stored, and frozen. The blood samples were processed and analyzed in a manner similar to that described for unchanged warfarin.[11,#] In one individual after administration of (*R*)-warfarin the *R,S* alcohol was found to be the major metabolite that could be detected in the plasma having a peak level of approximately 860 ng/ml at 60 hr. In addition, small amounts of 7-hydroxywarfarin, peak level 38 ng/ml at 29 hr, and *R,R* alcohol, peak level 25 ng/ml at 30 hr, were found. After administration of (*S*)-warfarin, however, the major plasma metabolite was found to be 7-hydroxywarfarin, peak level 1000 ng/ml, a smaller amount of *S,S* alcohol, peak level 130 ng/ml at 32 hr, and the *S,R* alcohol, peak level 37 ng/ml at 30 hr. The results obtained from the second individual were qualitatively the same as those obtained from the first. These data imply that the reductase is stereoselective and preferentially generates the *S* configuration at the hydroxyl-bearing carbon. In addition, the smaller amounts of alcohol produced from (*S*)-warfarin imply that the stereochemistry at the benzyl group significantly modulates both the ease and course of reduction. Two explanations for the small amounts of the *S,R* and *R,R* alcohol found in the two studies seem possible: (1) the reductase is not completely stereospecific or (2) the reductase is totally stereospecific and the observed *R,R* and *S,R* alcohol obtained from the reduction in fact have the *S,S* and *R,S* configurations, respectively, and arise from a minor contamination of the (*R*)- and (*S*)-warfarin with their respective enantiomers. For example, a contamination in (*S*)-warfarin of less than 5% (*R*)-warfarin could produce the level observed.

Initial pharmacokinetic calculations based on the results of these experiments show that the metabolic pattern is not equivalent to the sum of the patterns obtained from administration of the individual isomers. These data imply that there is shunting of metabolic pathways *via* competitive inhibition between the enantiomers. This in turn may have significant meaning at the clinical level. A more complete and detailed analysis along with the urine and pharmacokinetic data will be reported at a later date.

#The assay has been refined to include the metabolites. The method involves extraction, separation by tlc, and quantification by spectrophotofluorometry. The details of the method will be published separately.

Experimental Section**

3-[α-(2-Hydroxypropyl)benzyl]-4-hydroxycoumarin (1a and 1b). A mixture of the title compds was obtained by $NaBH_4$ reduction as described previously.[2a] To 140 g of silica gel (Brinkman Instruments Inc., extrapure, 70–325 mesh) suspended in *n*-hexane in a 4-ft column was added 2.5 g (0.0081 mole) of a mixture of 1a and 1b dissolved in a minimal amount of Me_2CO. The column was fitted to an automatic fraction collector, then eluted with the following solvents: *n*-hexane, 200 ml; 30% *n*-hexane–70% C_6H_6, 100 ml; C_6H_6, 200 ml; 70% C_6H_6–30% EtOAc, 200 ml; 50% C_6H_6–50% EtOAc, 300 ml; 30% C_6H_6–70% EtOAc, 100 ml; EtOAc, 300 ml; 90% EtOAc–10% MeOH, 100 ml; 80% EtOAc–20% MeOH, 100 ml; 50% EtOAc–50% MeOH, 200 ml; 30% EtOAc–70% MeOH, 100 ml; MeOH, 200 ml. In this fashion 250 8-ml fractions were collected, and the solvent was evapd in a H_2O bath under a stream of $CaCl_2$–dried N_2. Two major fractions of material were obtained. Fractions 88–97 were pooled and recrystd to yield 0.5 g (0.0016 mole) of 1a: mp 173–175°, Me_2CO; ir 3400, 2900, 1690, 1650 (split), 1580, 765, 705 cm^{-1} (KBr). In similar fashion fractions 103–117 were pooled and recrystd from Me_2CO to yield 0.5 g (0.0016 mole) of 1b: mp 162–168°; ir 3300, 3050, 1690 (singlet), 1530, 765, 710 cm^{-1} (KBr). The purity of these materials was checked by tlc on silica gel (chromagram sheet, Eastman 6060 with fluorescent indicator) and a solvent system of toluene–ethyl formate–formic acid, 10:5:1, R_f 1a, 0.46; 1b, 0.21.

Subsequently increased quantities of pure 1a and 1b were obtained by fractional recrystn of the mixture in isopropyl alcohol using the pure materials obtained above as seed crystals. The mixture was dissolved in a small amount of isopropyl alcohol, 1 g/10 ml, and seeded with a crystal of 1a. In 3 days the first crop of crystals rich in 1a was collected. The mother liquor from the first crystn was seeded with pure 1b, and, after about 2 days, the crystals rich in 1b were collected. Recrystn in the same fashion as for 1a yielded pure 1b. It should be noted that good analyses for these materials were never obtained. The analysis indicated 1.5 moles of water of hydration in the case of 1a and 0.5 mole in the case of 1b. Attempts to remove the water thermally resulted in cyclic dehydration. The spectral evidence and analyses of the cyclic products, however, leave no doubt as to the identity of these materials.

1a (*S,R*) and 1b (*S,S*). (*S*)-Warfarin (5.0 g, 0.016 mole) was reduced ($NaBH_4$) as described previously. The resultant mixture of diastereoisomers were sepd as described above: 1a (*S,R*), mp 192.5–193.5°, $[\alpha]^{24}D$ −128.2° (*c* 1.3, 95% EtOH), $[\alpha]^{25}D$ −127.5° (*c* 1.05, 0.5 *N* NaOH); 1b (*S,S*), mp 171–172.5° $[\alpha]^{25}D$ −85.7° (*c* 0.72, 95% EtOH), $[\alpha]^{25}D$ −126.8° (*c* 0.98, 0.5 *N* NaOH).

1a (*R,S*) and 1b (*R,R*). (*R*)-Warfarin (5.0 g, 0.016 mole) was reduced with $NaBH_4$ as previously described, The resultant mixture of diastereoisomers was sepd as described above: 1a (*R,S*), mp 191–192.5°, $[\alpha]^{25}D$ +129.7° (*c* 1.4, 95% EtOH), $[\alpha]^{25}D$ +127.4° (*c* 1.26, 0.5 *N* NaOH); 1b (*R,R*), mp 172–173.5°, $[\alpha]^{25}D$ +82° (*c* 0.68, 95% EtOH), $[\alpha]^{25}D$ +129.4 (*c* 0.99, 0.5 *N* NaOH).

cis-2,3-Dihydro-2(*S*)-methyl-4(*S*)-phenyl-4*H*-pyrano[3,2-*c*]benzopyran-5-one, 5b (*S,S*). Pure 1b (*S,S*) (0.1 g, 0.00032 mole) was placed in a sealed glass tube and immersed in an oil bath at 170–180° for 2 hr. The tube was cooled and opened, and the crude product analyzed by glc and tlc. Glc (4 ft, 5% Versamid on Chromosorb W, 60–80 mesh, 290°) showed essentially one peak. Tlc on silica gel (usual system) indicated the reaction was complete. The R_f of 1b (*S,S*) is 0.21, while the R_f of the product is 0.58. Recrystn (Me_2CO), followed by Et_2O yielded colorless needles of 5b: mp 222–224°; $[\alpha]^{25}D$ −73.6° (*c* 0.88, dioxane); mass spectrum, molecular ion *m/e* 292 ($C_{19}H_{16}O_3$); uv λ(nm)(log ε), 277 (4.05), 283 (4.10), 306 (401), 3.19 (3.85) shoulder, 95% EtOH; ir 1717 cm^{-1}, carbonyl (KBr). *Anal.* ($C_{19}H_{16}O_3$) C, H.

cis-2,3-Dihydro-2(*R*)-methyl-4(*R*)-phenyl-4*H*-pyrano[3,2-*c*]-benzopyran-5-one, 5b (*R,R*) (a). The title compd was obtained

**Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Mass spectra were determined on an AE1 Model MS-902 mass spectrometer *via* a direct insertion probe and were vaporized at temperatures between 200 and 250°. Exact mass measurements were determined by the electrical peak-matching technique using perfluorotributylamine as standard. Uv spectra were recorded on a Cary Model 14 and/or 15 spectrophotometer. Ir spectra were recorded on a Perkin-Elmer Model 457 grating infrared spectrophotometer. Nmr spectra were recorded on a Varian Model A-60-A or a Jeolco Model 100 M spectrometer in $CDCl_3$ ($(CH_3)_4Si$). Optical rotations were determined in a Bendix automatic polarimeter Series 1100 in a 5.002-mm quartz cell. Combustion values were within ±0.4%.

from pure 1b (*R,R*) (0.1 g, 0.00032 mole) by thermal dehydration as described above. Recrystn as above yielded colorless needles of 5b: mp 222.5–223.5°; $[\alpha]^{25}D$ +75.8° (*c* 1.15, dioxane). (b). 1b (*R,R*) (0.025 g, 0.00008 mole) was placed in a small vial containing 1.5 ml of $CH_3OH \cdot BF_3$ (Applied Science Lab, Inc., State College, Pa.). The mixture was gently boiled for about 15 min on a hot plate. Then 25 ml of distilled H_2O was added to the solution. The ppt was collected and recrystallized in Et_2O. The ir, tlc, and glc behavior of the crystalline material (0.02 g, 87%) was identical with 5b (*R,R*) obtained from thermal treatment, mp 222–223°.

trans-2,3-Dihydro-2(*R*)-methyl-4(*S*)-phenyl-4*H*-pyrano[3,2-*c*]-benzopyran-5-one, 5a (*R,S*) (a). The title compound was obtained from pure 1a (*R,S*) (0.2 g, 0.00064 mole) by thermal dehydration as described, except a temp of 190–200° was used for a period of 4–6 hr. Tlc analysis on silica gel (usual system) indicated that the reaction was essentially complete while glc analysis (usual system) indicated a mixture of diastereomeric dehydration products. The relative peak heights of these two isomers indicated that the mixture contained 20–30% of 5b and 70–80% of the title compd. Recrystn (Me_2CO) yielded two distinct types of crystals. One appeared to be white and powdery while the other was transparent and crystalline. Physical separation of these two crystalline forms followed by glc analysis indicated that the powdery material was a mixture (approx 50/50) of the two diastereomers, while the crystn material was essentially pure. Further recrystn (Me_2CO) followed by Et_2O gave while needles: mp 194–194.8°; $[\alpha]^{25}D$ −128° (*c* 0.56, dioxane); mass spectrum, molecular ion *m/e* 292; uv λ(nm)(log ε), 2.69 (4.06), 280 (4.11), 304 (4.01), 317 (3.83), 95% EtOH; ir 1717 cm^{-1}, carbonyl (KBr). *Anal.* ($C_{19}H_{16}O_3$) C, H. (b) 1a (*R,S*) (0.25 g, 0.0008 mole) was boiled in $CH_3OH \cdot BF_3$ as before. The ppt isolated after recrystallization was identical by ir, tlc, and glc with 5a (*R,S*) obtained from thermal treatment, mp 193–194°.

trans-2,3-Dihydro-2(*S*)-methyl-4(*R*)-phenyl-4*H*-pyrano[3,2-*c*]-benzopyran-5-one. The title compd was obtained from pure 1a (*S,R*) (0.2 g, 0.00064 mole) by therm dehydration as described above for 1a (*R,S*). After physical sepn of the two forms of crystals, recrystn (Me_2CO) followed by Et_2O yielded white needles: mp 194–195°; $[\alpha]^{25}D$ +137° (*c* 0.69, dioxane).

4-Methoxycoumarin. To 6, 0.1 g, in a 10-ml erlenmeyer flask was added 3 ml of BF_3–MeOH and the mixture was boiled gently for 5 min. Addition of approx 50 ml of H_2O yielded a colorless ppt which was collected by filtration. Tlc analysis on silica gel (usual system) indicated this material was a mixture of two components, one of which corresponded to starting material. The mixture was dissolved in Et_2O and was extracted 4 times with a satd NaCl–$NaHCO_3$ soln. The Et_2O was dried (Na_2SO_4), decanted and evapd. The residue was a crystn material, mp 124–125° (lit.[6a] mp 125°). The ir of this material (KBr pellet) is also identical with the title compound obtained by the following method: uv λ(nm)(log ε), 264 (4.07), 275 (4.06), 303 (3.85), 317 shoulder, 95% EtOH; ir 1720 cm^{-1}, carbonyl (KBr).

2-Methoxychromone. A mixture of the title compd and 4-methoxycoumarin was prepared by treatment of 6 with CH_2N_2 according to the method of Arndt.[6a] The title compd was sepd as described[6a] and recrystd: mp 105–106° (petr ether) (lit.[6a] mp 108° (C_6H_6)); uv λ (nm)(log ε), 244 (3.8), 266 (3.99), 286 (3.81), 295 shoulder, 95% EtOH; ir 1655 cm^{-1}, carbonyl (KBr).

2-Methyl-4-phenyl-4*H*-pyrano[3,2-*c*]benzopyran-5-one (9). A soln of racemic 2 (2.0 g, 0.0065 mole) in 100 ml of anhyd C_6H_6 in a one-neck, round-bottom flask equipped with a condenser, $CaCl_2$ drying tube, and a distillation head was heated to 80° and a small quantity of C_6H_6 distilled over. To this soln was added 2.0 g of P_2O_5 and 3 drops of AcOH and the mixture was refluxed overnight. The C_6H_6 soln was decanted into a separatory funnel. The residue was dissolved in H_2O and extracted with 50 ml of 5% NaOH 3 times followed by extraction with 50 ml of H_2O 3 times. The C_6H_6 soln was dried ($MgSO_4$) and evapd. The residue, 1.7 g (90%), was recrystd (Et_2O): mp 145–147°; ir 1720, 1645, 1630, 1200, 1035 (split), 767, 712 cm^{-1} (KBr pellet).

2-Methyl-4(*S*)-phenyl-4*H*-pyrano[3,2-*c*]benzopyran-5-one (9) (*S*). 2 (*S*) (3.5 g, 0.0113 mole) was dehydrated as above. The title compd displays polymorphism yielding both needles, mp 139–140°, and prisms, mp 139–141°. The ir, KBr pellet, of these materials are slightly different in the finger print region and are both different from the racemic compound. The solution spectra, $CHCl_3$, of all these compounds, however, are identical.

cis-2,3-Dihydro-2-methyl-4-phenyl-4*H*-pyrano[3,2-*c*]benzopyran-5-one. To a soln of 9 (0.02 g, 0.00065 mole) in 75 ml of abs EtOH in a Paar pressure bottle was added 0.05 g of PtO_2. The reaction vessel was fitted to a Paar low-pressure hydrogenator for 6 hr. The reaction mixture was filtered and the volume of EtOH reduced. Recrystn from EtOH yielded 0.18 g (94%) of crystn material, mp 188–189°, which had identical glc, tlc, mmp, and ir to that obtained from the thermal dehydration of racemic 1b (lit.[7] mp 188–189°).

Table II. Mass Spectral Data of ^{18}O Incorporation into Warfarin and Its Derivatives

Compound	*m/e* 312 / *m/e* 310	*m/e* 310 / *m/e* 308	*m/e* 294 / *m/e* 292	*m/e* 267 / *m/e* 265
Warfarin		0.41[a]		0.035
1a	0.42			0.053
1b	0.417			0.054
5a from 1a			0.39	
5b			0.36	

[a] Theoretical 0.424.

cis-2,3-*cis*-Dideuterio-2(*S*)-methyl-4(*S*)-phenyl-4*H*-pyrano-[3,2-*c*]benzopyran-5-one (10). To a soln (0.3 g, 0.00098 mole), in 75 ml of purified EtOAc,†† of 9 (*S*) in a Paar pressure bottle was added 0.072 g of PtO_2. The reaction vessel was fitted to a Paar low-pressure hydrogenator and was flushed several times with deuterium gas (Bio-Rad, 99.65 g-atom %). The reduction was allowed to proceed at room temperature for 24 hr at 30 lb above atm pressure. The reaction mixture was filtered and the filtrate concentrated and placed in the refrigerator overnight. The crystn material, 0.25 g (85%), so obtained was recrystallized (Et_2O) to yield 0.22 g (76%) of a cryst compound: mp 221–222°; nmr $CDCl_3$Ar, 9 H's, 7.3 ppm; Bz, H, db, J = 10.7 Hz, 4.0 ppm; methylene H, db, J = 10.7 Hz, 1.8 ppm; Me, S, 1.47 ppm.

cis-2,3-Dihydro-2(*S*)-methyl-4(*S*)-phenyl-4*H*-pyrano[3,2-*c*]-benzopyran-5-one. Compound 9(*S*), 0.3 g (0.00098 mole), was hydrogenated as previously described for 9. The product after recrystallization, EtOH the Et_2O, gave 0.22 g (76%) of white crystalline material: mp 222–223°; $[\alpha]^{25}D$ −75.2° (*c* 88, dioxane).

3-[α-(2-Oxopropyl-^{18}O)benzyl]-4-hydroxycoumarin (14). Warfarin sodium (0.050 g, 0.00016 mole) was equilibrated with 205 μl of 40% $H_2{}^{18}O$ (normalized, Miles Lab) in a capped tube which was submerged in a water bath (40–60°). After 90 hr 120 μl was removed and placed in a small vial containing 5 drops of 2 *N* HCl and approximately 0.5 ml of pure $CHCl_3$. The warfarin was extracted into the $CHCl_3$ layer which was then removed and dried briefly over $MgSO_4$. The dried $CHCl_3$ soln was evaporated and the residue analyzed by mass spectrometry. Taking into account the isotopic abundance of ^{13}C the peak height of the ion at *m/e* 308 indicated a 15% incorporation of ^{18}O. The base peak ion of unlabeled warfarin occurs at *m/e* 265 and is due to the loss of an acyl radical from the side chain.[2a] The lack of any significant ion at *m/e* 267 indicates that essentially all of the ^{18}O has been incorporated into the side-chain carbonyl. At the end of 7 days the rest of the warfarin was isolated as before. Mass spectral analysis showed 40% incorporation of ^{18}O.

3-[α-(2-Hydroxypropyl-^{18}O)benzyl]-4-hydroxycoumarin (15). Compound 14 (40% ^{18}O) (0.02 g, 0.00008 mole) was dissolved in 2 ml of dry MeOH and was treated with $NaBH_4$ (0.15 g, 0.004 mole) for 3 hr at room temperature. The reaction solution was added to 5 ml of 5% HCl, and the precipitated alcohols were filtered and analyzed by tlc (25% EtOH in C_6H_6) for starting material. The tlc indicated that the reaction had proceeded to completion. The diastereomeric alcohols were separated by tlc (Mallinckrodt, Chromaplate, Silica-Ar, tlc-4G, 250 μ without fluorescent indicator) using the usual solvent system. The resulting bands were scraped off and extracted in a Soxhlet extractor with spectrograde Me_2CO. After evaporation, the residue was taken up in a small volume of 1 *N* NaOH and acidified after filtration. Each of the products was dried in a vacuum dessicator for mass spectral analysis.

2,3-Dihydro-3-methyl-4-phenyl-4*H*-pyrano-^{18}O-[3,2-*c*]-benzopyran-5-one. About 5 mg of each of the diastereomers of 15 was placed into a sealed vial and was thermally cyclized as before. The products purified by glc (usual versamid) afforded the pure diastereomers of the title compound.

Mass Spectral Analysis of the Warfarin-^{18}O, Warfarin-^{18}O Alcohol Diastereomers, and the Cyclized ^{18}O Diastereomers. The % ^{18}O incorporation was calculated by the following equation: % ^{18}O = $[(M+2) + (M+3)]100/[M + (M+1) + (M+2) + (M+3)]$, where the $(M+3)$ term in the numerator arises from $^{13}C + {}^{18}O$.

The accuracy of the measurement is arbitrarily assigned to be ±5% which is probably well above the noise level. The exact mass

†† Unless meticulous care is taken to dry and purify the EtOAc, hydrogen is incorporated into the reduced product.

measurement was determined on 15 and was found to be consistent with the formula $C_{19}H_{16}{}^{16}O_2{}^{18}O$ within 4 ppm. The location of the ^{18}O in warfarin was checked by the ratio of *m/e* 267/*m/e* 265 fragment ions (Table II).[2a]

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Naphthylalkyl Lactamimides as Inhibitors of Blood Platelet Aggregation

Edward M. Roberts, J. Martin Grisar,* Robert D. MacKenzie, George P. Claxton, and Thomas R. Blohm

Merrell-National Laboratories, Division of Richardson-Merrell Inc., Cincinnati, Ohio 45215.

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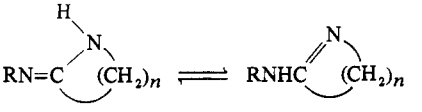
Hexahydro-2-[1-(1-naphthyl)ethylimino]azepine·HCl (2) (RMI 7822) was found to inhibit human blood platelet aggregation induced by ADP and other agents with minimal release of procoagulant platelet factor 3. The compound was selected after careful modification of structural parameters, such as α-substitution, lactam ring size, aromatic substitution, as well as evaluation of its optical isomers.

Arterial thrombosis, especially in arteries supplying heart muscle and brain, is a leading cause of death and disability.[1] The blood platelets play a dominant role in such thromboses, both in the initial event and at the occlusive stage.[2] The so-called white thrombus that develops before fibrin clot formation is composed primarily of aggregated platelets. A number of tissue constituents can initiate platelet aggregation.[3] Among these substances adenosine diphosphate (ADP) seems to be especially important, since it not only can initiate aggregation, but appears to mediate aggregation due to other agents as well.[4] Physiologic functions of platelets include hemostasis and possible repair of vascular endothelium.[5] In performing these functions platelets must undergo controlled adhesion and aggregation. Agents that would normalize abnormal platelet functions in the thrombosis-prone individual would have great therapeutic value. For these reasons we have adopted and developed techniques to evaluate large numbers of compounds available to us from earlier synthetic programs in our laboratories. Using human platelet-rich plasma (PRP) we measured *in vitro* inhibition of platelet aggregation induced by ADP, collagen, and several other agents.[6] Compounds that showed activity were then checked for release of platelet factor 3 (PF3) or PF3-like activity by measuring Stypven time. PF3 is a phospholipoprotein that acts as a cofactor in the coagulation process. Since we had established earlier that a normal breakfast causes PF3-like activity of from 0.1 to 0.3%, we adopted this as our limit of acceptability.[7] Selected compounds were then evaluated further. We reported earlier our findings on certain piperidineethanols of benzyl- and benzylidenefluorene,[8,9] and on a member of a series of anilines,[10] obtained from Zellner.[11] We now wish to report our findings on certain lactamimides.

An exploratory series of lactamimides was prepared by one of us (E.M.R.) as potential antihypertensive agents. Later one of these, compound 2† in Table I, was found to inhibit ADP-induced platelet aggregation. We then explored systematically the effect of structural modifications on this activity by preparing and evaluating the compounds listed in Table I. This study revealed that activity is distributed broadly throughout the series. The structural parameter that most affects inhibition of platelet aggregation in this series was found to be the substituent R on the carbon atom adjacent to the lactamimide function (compounds **1-6**). The unsubstituted congener **1** was found to be less active than several of the alkyl-substituted congeners, and of these the methyl-substituted compound **2** showed least effect on PF3 release. The sterically hindered *tert*-butyl congener **5** and the phenyl-substituted congeners **16-19** were less active. Exploration of the influence of the lactam ring size (compounds **2, 7-10**) showed that the 5- and 6-membered lactam ring congeners are less active (*cf.* also **14** *vs.* **13** and **16** *vs.* **17**), while the larger 8- and 13-membered ring congeners showed enhanced PF3 release. Several examples of aromatic substitution (compounds **11-14, 16, 17**) revealed no trends, nor did the structural changes represented by compounds **15** and **20**. No significant differences were found between the *d* and *l* isomers of **2**.

Compound 2† also inhibited aggregation induced by thrombin, epinephrine, and serotonin. It did not inhibit clot retraction and this property may be advantageous. More detail on these and additional evaluations will be reported elsewhere.

Lactamimides, also named cyclic or semicyclic amidines,[19] exist in two tautomeric forms A and **B**. This tautomerism



A B

has been studied by Kwok and Pranc.[20] It is not known, however, which tautomer prevails in the crystalline monohydrochloride salts, not to mention solutions under physio-

†Also named RMI 7822.