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The Crystal and Molecular Structure and Absolute Configuration of (−)-(*S*)-Warfarin

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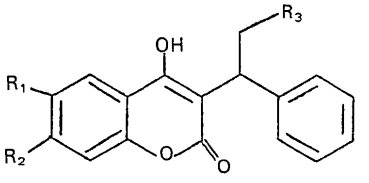
The crystal and molecular structure and the absolute configuration of (−)-(*S*)-warfarin, $C_{19}H_{16}O_4$, have been determined by X-ray crystallographic techniques. Crystals of (−)-(*S*)-warfarin are orthorhombic, space group $P2_12_12_1$, with $a=10{\cdot}883$ (3), $b=9{\cdot}562$ (3), and $c=14{\cdot}902$ (5) Å. Solution of the structure was by direct methods, and refinement by least-squares calculations led to a conventional R of 0·053 (Mo $K\alpha$ data). The molecule crystallizes as the intramolecular hemiketal and thus may be described as (2*S*,4*S*)-2,3*H*-2-methyl-4-phenyl-5-oxobenzopyrano[3,4-*e*]dihydropyran-2-ol. The absolute configuration was confirmed by recollecting with Cu $K\alpha$ radiation a group of reflections predicted to have the greatest observable Bijvoet differences based on the anomalous scattering of oxygen and the parameters from the refinement with Mo $K\alpha$ data. A group of 51 Friedel pairs, 86% of which indicate the *S* enantiomer, gave a 17% decrease in the residual over the *R* enantiomer. Refinement of the imaginary part of the anomalous dispersion of oxygen gave a value of 0·037 for $\Delta f''$.

Introduction

Warfarin [3-(α-acetonylbenzyl)-4-hydroxycoumarin, I*a*] is an extensively used rodenticide and clinically effective oral anticoagulant.



(I*a*)	$R_1=-H$;	$R_2=-H$;	$R_3=-COCH_3$
(I*b*)	$R_1=-H$;	$R_2=-OH$;	$R_3=-COCH_3$
(I*c*)	$R_1=-H$;	$R_2=-H$;	$R_3=-CHOHCH_3$
(I*d*)	$R_1=-OH$;	$R_2=-H$;	$R_3=-COCH_3$

Considerable concern has developed in recent years over the appearance of a resistant strain of rats in Scotland and their subsequent spread to Northern Europe (Jackson, 1969*a*, *b*; Bentley, 1969; Drummond, 1970) and the United States (Jackson, Spear & Wright, 1971). At the clinical level, problems have arisen from use of the drug because of the variability in biologic response sometimes produced by the concomitant administration of other medications (Koch-Weser & Sellers, 1971).

The drug exists in two enantiomeric forms and it is known that the (*S*) isomer is approximately five to six times as potent as the (*R*) isomer in both the rat (Eble, West & Link, 1966; Hewick, 1972; Breckenridge & Orme, 1972) and man (Hewick & McEwen, 1973), although the clinically available form of the drug is the racemate. Recent evidence has demonstrated that in man, the two enantiomeric forms are metabolized differently (Chan, Lewis & Trager, 1972; Lewis, Trager, Chan, Breckenridge, Orme, Roland & Shung, 1974). The (*S*) isomer is stereoselectively oxidized to the inactive 7-hydroxywarfarin, (I*b*), while the (*R*) isomer is stereospecifically reduced to the slightly active (*R*,*S*) alcohol, (I*c*). Both isomers are oxidized to the inactive 6-hydroxywarfarin, (I*d*). It has further been demonstrated that prior administration of other drugs, *e.g.* phenylbutazone, will quantitatively affect these metabolic pathways to different degrees (Lewis *et al.*, 1973) and as a consequence offer in part an explanation at the molecular level of the effects that are observed at the clinical level. Thus we felt that it was of some importance to establish the assignment of the absolute configuration of the warfarin isomers by an independent means.

Originally the assignment of (−)-(*S*)-warfarin was derived by relating it to (−)-(*R*)-β-phenylcaproic acid through a series of reactions not involving the asymmetric center (West, Preis, Schroeder & Link, 1961). We report here confirmation of the above assignment by the anomalous scattering of oxygen.

Experimental

Crystals of (−)-warfarin, grown from acetone by slow evaporation of the solvent, are colorless elongated prisms, melting at 172·5–173 °C, and having $[\alpha]_D^{25}=$

Table 1. *Crystal data*

Crystal system	Orthorhombic
Space group	$P2_12_12_1$
Cell dimensions (based on Mo $K\alpha=0{\cdot}71069$ Å)	$a=10{\cdot}883$ (3) Å $b=9{\cdot}562$ (3) $c=14{\cdot}902$ (5)
Unit-cell volume	1550·8 Å^3
Z	4
D_{obs} (KI density gradient)	1·34 (1) g cm^{-3}
D_{calc}	1·33 g cm^{-3}

$-127{\cdot}4$ (2)°, 1·2 g/100 ml, 0·5 *N* NaOH. Crystal data are listed in Table 1.

Intensity data were collected on a computer-controlled four-circle diffractometer from a crystal measuring $0{\cdot}5\times0{\cdot}6\times0{\cdot}5$ mm mounted along **c**. The $\omega/2\theta$ scan technique was used at a scan speed of 2° min^{-1} in 2θ. 20 s background counts were collected at each limit of the scan range which was varied to account for the α_1–α_2 separation. Five monitor reflections were collected at intervals of 200 reflections, and a decrease in intensity of 1·2% was observed in collecting the 2564 reflections to $\sin\theta/\lambda=0{\cdot}70$ Å^{-1} (excluding those systematically absent).

A correction for coincidence loss was applied to the intense reflections, increasing the count of the most intense reflection (013) by 14·7%. Although absorption is small, an empirical correction based on the variation in the intensity of the 002 as a function of φ at $\chi=90°$ (range, 5·5% in *I*) was applied.

Atom scattering factors for carbon and oxygen were from *International Tables for X-ray Crystallography* (1962) and the bonded form factors of Stewart, Davidson & Simpson (1965) for hydrogen were used.

Determination of the structure and refinement

The 200 *E*-values greater than 1·8 were used in the program *MULTAN* (Main, Woolfson & Germain, 1971) to generate a starting set of phases ($R_{Karle}=21{\cdot}42$, absolute figure of merit = 1·01). An *E* map based on these phases showed what appeared to be the coumarin ring fragment. Further examination of the *E* map revealed the presence of yet another ring, fused to the coumarin at C(3) and C(4) (see Fig. 1), and a region of electron density at a position significantly out of the plane roughly described by the coumarin rings. A plausible model for part of the molecule could be inferred, however, and three cycles of structure factor–Fourier synthesis revealed all the remaining heavy atoms. Three cycles of least-squares calculations with data limited to $\sin\theta/\lambda=0{\cdot}40$ Å^{-1} converged to an $R(=\sum||F_o|-|F_c||/\sum|F_o|)$ of 0·28, but bond lengths were unreasonable, and it was apparent that the model was incorrect.

Using the coumarin ring atoms in the above trial, we performed multiple Patterson superpositions, but were unable to deduce a satisfactory model. Accordingly, a second set of phases ($R_{Karle}=21{\cdot}96$, absolute figure of merit = 1·00) was tried, giving an *E* map with what appeared to be the coumarin ring fragment in nearly the same position as the first set. A third starting set ($R_{Karle}=22{\cdot}02$, absolute figure of merit = 0·95) gave an *E* map again revealing the coumarin ring and hemiketal portions of warfarin as before, but shifted by $y=-0{\cdot}44$. A single F_o synthesis revealed 18 of the 23 non-hydrogen atoms, and a subsequent difference map showed the remaining five. *R* at this point was 0·31.

With data to $\sin\theta/\lambda=0{\cdot}55$ Å^{-1} (1265 reflections), two cycles of least-squares refinement on all non-hydrogen atoms with isotropic temperature factors reduced *R* to 0·105. The data set was now expanded to include all the reflections to $\sin\theta/\lambda=0{\cdot}70$ Å^{-1}, those with net negative counts being set to zero. Anisotropic temperature factors were included in the next least-squares refinement, and *R* decreased to 0·092. A difference map was computed and 15 of the 16 hydrogen atoms were located. One cycle of block-diagonal least-squares refinement adjusting only the hydrogen atoms with isotropic thermal parameters (initially set to the value of the adjacent heavy atom) was carried out and a new difference map revealed the remaining hydrogen atom, H(31). At this point we applied a 2σ threshold in *F* [equivalent to a threshold of $\sigma(I)$] and introduced weights of $1/\sigma^2(F)$, unit weights having been used up to this point. Four final least-squares cycles (blocks of approximately 140 parameters in two passes for each cycle) led to convergence. The final *R* was 0·053 for 2264 reflections to $\sin\theta/\lambda=0{\cdot}70$ Å^{-1} with *F* greater than $2\sigma(F)$ and $R_w[=\{(\sum w||F_o|-|F_c||^2/\sum w|F_o|^2)\}$ was 0·024 for the same data set. The average shift/error during the last cycle was 0·31 σ for 272 parameters.* Final parameters are listed in Tables 2 and 3.

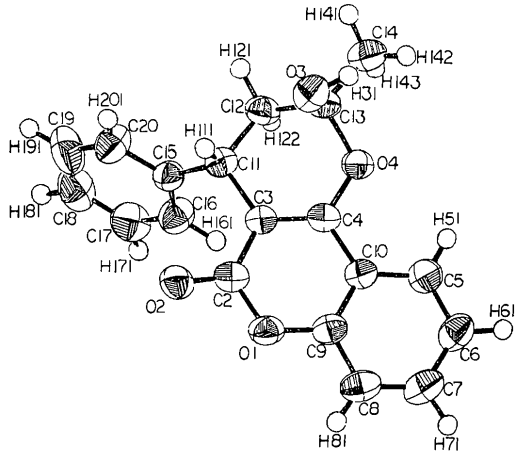


Fig. 1. Thermal-ellipsoid plot of the structure of (−)-(*S*)-warfarin and the numbering of the atoms. View in *z* direction.

Results and discussion

The primary feature of the structure of (−)-warfarin (Fig. 1) is the hemiketal ring formed by cyclization of the side-chain keto function and the phenolic hydroxyl in the 4 position of the coumarin ring system. The crystal structure of racemic warfarin (Bravic, Gaultier

* A list of structure factors has been deposited with the British Library Lending Division as Supplementary Publication No. SUP 30769 (16 pp., 1 microfiche). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 13 White Friars, Chester CH1 1NZ, England.

& Hauw, 1973) also displays the hemiketal ring feature. Moreover, an acetone-d_6 n.m.r. spectrum of the resolved (−)-warfarin (Porter, Trager & Valente, 1973) indicates that the hemiketal also exists in solution. An examination of the bond lengths within the hemiketal ring reveals that the atoms have retained some of the character expected for the open side chain (keto) compound. The C(4)–O(4) bond length of 1·351 (2) Å is close to a phenolic C–O bond. Also, the bond lengths between C(12) and C(13) and between C(13) and C(14) are 1·504 (3) and 1·507 (3) Å respectively, close to expected values for C–C bonds adjacent to carbonyl groups.

A striking feature is the unusually long C–O bond between C(13) and O(4) of 1·483 (2) Å. Examination of representative hemiketals such as are found in sorbose (Kim & Rosenstein, 1967), tagatose (Takagi & Rosenstein, 1969), and coriose (Taka, Osaki & Okuda, 1970) show that bond lengths in the range 1·42–1·45 Å are expected (see Table 4). The conformation of the hemiketal is affected by the near planarity of the coumarin ring system which maintains C(11) and O(4) in approximate coincidence with that plane. C(12) alone is predominantly out of that plane, imparting the half-chair conformation to the hemiketal ring. Atoms C(2)–C(11), O(1), O(2) and O(4) have a standard deviation of 0·086 Å from the least-squares plane through them. O(2) and C(11) are the farthest from the plane, deviating by +0·16 and −0·19 Å respectively (Table 5). Data for the phenyl ring are also included there.

Within the coumarin system, the length of the double bond C(3)–C(4), 1·351 (3) Å, is reasonable for a C=C conjugated to a carbonyl, and the adjacent C(2)–C(3) bond of 1·439 Å is shortened because of resonance. We have compared the bond lengths and angles of

Table 3. *Parameters for hydrogen atoms with estimated standard deviations in parentheses*

Positional parameters × 10^4; thermal parameters × 10^3; temperature-factor expression exp $[-8\pi^2U^2(\sin^2\theta/\lambda)]^2$.

	x/a	*y/b*	*z/c*	*U*
H(31)	−1519 (25)	2421 (25)	−2562 (15)	111 (11)
H(51)	1596 (20)	4729 (22)	−3644 (14)	69 (7)
H(61)	3352 (22)	6050 (24)	−3503 (15)	98 (9)
H(71)	5231 (20)	4850 (21)	−3192 (15)	79 (7)
H(81)	5209 (20)	2330 (22)	−3027 (16)	75 (8)
H(111)	− 61 (16)	−773 (17)	−3187 (12)	48 (5)
H(121)	−1617 (19)	−14 (19)	−4206 (12)	52 (6)
H(122)	−612 (18)	692 (19)	−4798 (14)	58 (6)
H(141)	−1682 (21)	3012 (22)	−4737 (16)	81 (8)
H(142)	−1925 (20)	3715 (24)	−3738 (14)	75 (8)
H(143)	−2697 (23)	2391 (23)	−3973 (16)	91 (9)
H(161)	1731 (21)	−482 (21)	−5185 (14)	71 (7)
H(171)	1955 (27)	−2229 (28)	−6334 (18)	123 (10)
H(181)	904 (23)	−4443 (26)	−6151 (17)	96 (9)
H(191)	−366 (22)	−4738 (25)	−5145 (16)	83 (8)
H(201)	−585 (24)	−2972 (26)	−3840 (17)	87 (9)

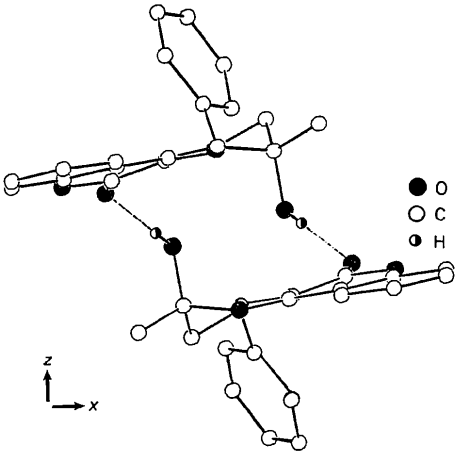


Fig. 2. Projection illustrating hydrogen bonding between pairs of molecules related by the twofold screw axis parallel to *y*.

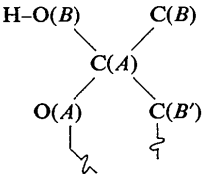
Table 2. *Parameters for non-hydrogen atoms with estimated standard deviations in parentheses*

Positional parameters × 10^5; thermal parameters × 10^4; temperature-factor expression exp $[-2\pi^2(U_{11}h^2a^{*2}+\ldots+2U_{23}klb^*c^*)]$.

	x/a	*y/b*	*z/c*	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
O(1)	34532 (12)	7017 (15)	−31554 (10)	401 (7)	652 (9)	611 (9)	−26 (7)	100 (7)	−55 (8)
O(2)	25520 (12)	−13441 (14)	−30565 (9)	530 (8)	598 (8)	640 (9)	36 (8)	−8 (8)	−136 (8)
O(3)	−11704 (13)	15937 (16)	−27673 (10)	647 (9)	659 (10)	504 (8)	90 (9)	−149 (7)	−96 (8)
O(4)	1932 (11)	26738 (13)	−37447 (9)	399 (7)	534 (8)	559 (8)	1 (7)	−7 (6)	−38 (7)
C(2)	24196 (18)	−1146 (22)	−32432 (13)	448 (11)	636 (13)	394 (11)	−6 (10)	−16 (10)	−14 (10)
C(3)	12959 (16)	5307 (20)	−35398 (12)	392 (10)	510 (11)	369 (10)	−20 (10)	7 (8)	13 (9)
C(4)	12311 (16)	19406 (20)	−35816 (12)	391 (10)	568 (12)	352 (10)	2 (10)	−9 (9)	−8 (9)
C(5)	23296 (22)	42671 (25)	−35321 (16)	550 (14)	588 (15)	565 (14)	−36 (13)	−51 (12)	87 (12)
C(6)	34204 (25)	49960 (27)	−34337 (16)	668 (16)	646 (15)	616 (15)	−174 (14)	−77 (13)	136 (13)
C(7)	44944 (23)	42825 (30)	−32523 (17)	575 (15)	820 (19)	576 (15)	−230 (15)	−32 (13)	105 (14)
C(8)	44997 (21)	28544 (30)	−31603 (16)	466 (14)	851 (19)	520 (14)	−105 (14)	54 (12)	−12 (14)
C(9)	34098 (19)	21302 (23)	−32714 (14)	434 (12)	610 (14)	402 (11)	−65 (11)	63 (10)	35 (11)
C(10)	23126 (17)	28103 (22)	−34544 (13)	431 (11)	548 (13)	365 (11)	−39 (10)	−30 (9)	60 (10)
C(11)	2059 (18)	−3990 (22)	−37456 (15)	416 (11)	540 (13)	457 (12)	−52 (11)	−6 (10)	−40 (11)
C(12)	−8128 (20)	5106 (24)	−41553 (17)	389 (12)	614 (14)	573 (14)	−37 (12)	58 (11)	45 (12)
C(13)	−9705 (17)	18753 (21)	−36673 (14)	370 (11)	612 (13)	486 (11)	−20 (10)	−23 (10)	−67 (10)
C(14)	−19183 (24)	28317 (32)	−40767 (20)	448 (14)	772 (19)	678 (18)	90 (14)	14 (13)	−77 (16)
C(15)	4879 (18)	−15763 (22)	−44013 (14)	431 (11)	500 (13)	526 (12)	−24 (11)	64 (10)	−25 (10)
C(16)	12586 (21)	−13841 (28)	−51310 (15)	651 (15)	639 (15)	599 (14)	98 (14)	−63 (12)	−92 (14)
C(17)	14023 (25)	−24248 (31)	−57735 (18)	731 (17)	827 (19)	645 (16)	−48 (16)	23 (15)	−179 (16)
C(18)	7823 (28)	−36525 (33)	−56981 (21)	920 (20)	709 (19)	766 (19)	−126 (19)	259 (17)	−222 (18)
C(19)	417 (30)	−38667 (30)	−49791 (22)	1105 (25)	597 (18)	904 (22)	−204 (19)	213 (20)	72 (18)
C(20)	−1017 (26)	−28467 (28)	−43272 (20)	702 (17)	651 (16)	794 (18)	−180 (14)	85 (15)	−17 (15)

Table 4. *Comparison of bond lengths in the hemiketal ring*

Estimated standard deviations in parentheses where given.



Compound*	C(A)–O(A)	C(A)–O(B)	C(A)–C(B)	C(A)–C(B')
Coriose	1·452 (11) Å	1·403 (11) Å	1·560 (13) Å	1·554 (12) Å
α-D-Tagatose	1·43	1·42	1·52	1·53
α-L-Sorbose	1·420 (5)	1·415 (5)	1·515 (6)	
(−)-Warfarin	1·483 (2)	1·385 (2)	1·504 (3)	1·507 (3)

* See text for references.

Table 5. *Least-squares planes for coumarin and phenyl rings and deviations of atoms from planes*

Coumarin plane: $2{\cdot}3806x-0{\cdot}9458y-14{\cdot}4662z=5{\cdot}3137$

O(1)	0·007 Å	C(7)	0·056 Å
O(2)	−0·157	C(8)	0·059
C(2)	0·035	C(9)	0·029
C(3)	−0·065	C(10)	−0·032
C(4)	−0·023	O(4)	−0·103
C(5)	−0·053	C(11)	0·192
C(6)	−0·005	C(12)*	0·456
		C(13)*	−0·417

Phenyl plane: $8{\cdot}3708x-3{\cdot}6201y+7{\cdot}6722z=-2{\cdot}3853$

C(15)	−0·012 Å	C(18)	−0·009 Å
C(16)	0·003	C(19)	0·000
C(17)	0·007	C(20)	0·010
		C(11)*	−0·172

* These atoms given zero weight.

(−)-(*S*)-warfarin and the racemate (Bravic *et al.*, 1973) and found them to be in close agreement, the largest differences arising in regions involving the hydrogen-bonding scheme (see Fig. 2). Comparison has also been made with a number of other coumarin-containing structures (Table 6: the first four entries are of comparatively low precision).

The phenolic proton has been transferred to O(3) which is hydrogen bonded to O(2′) of the symmetry-related molecule at $-x,y+\frac{1}{2},-\frac{1}{2}-z$. The O(3)–O(2′) distance is 2·767 (5) Å and the O–H–O angle is 166 (2)°, the proton being not quite directed at O(2′). There are no other intermolecular hydrogen bonds. Along *z*, pairs of molecules related by twofold screw axes pack with the coumarin rings nearly parallel.

Thermal motion of the atoms in the phenyl ring is quite large, causing appreciable bond shortening, particularly bonds C(17)–C(18) and C(18)–C(19). The average aromatic C–C bond is 1·375 Å. Table 7 lists the bond lengths and angles in (−)-warfarin.

Absolute configuration

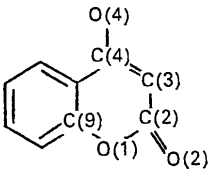
Although there are no atoms heavier than O, several attempts to determine the absolute configuration of such compounds have been successful (Engel, 1972; Hope & De la Camp, 1972). We felt that such an attempt could be successful in the case of warfarin in view of the good level of refinement attained.

Attempts to prepare crystalline sodium, potassium, and cesium salts of (−)-warfarin all yielded glasses. The synthesis of a selectively brominated derivative was deemed impractical in view of the time that would be required to separate and identify the various products. The most direct approach is to examine the free drug itself.

No enantiomer-sensitive reflections could be found in the data used to solve the structure because the anomalous scattering of oxygen is vanishingly small with Mo $K\alpha$ radiation. With Cu $K\alpha$ radiation, however, the relatively small anomalous scattering of four oxygens out of 23 non-hydrogen atoms may be detectable. Rather than recollecting a full set of Cu data, the best reflections for enantiomer indication were chosen from the calculated structure factors including anomalous scattering ($\Delta f'_C=0{\cdot}019$, $\Delta f''_C=0{\cdot}010$, $\Delta f'_O=0{\cdot}049$, $\Delta f''_O=0{\cdot}032$). The list of reflections was scanned for those with the largest differences between the Bijvoet pairs ΔF_{Bij}, and a group of 34 reflections was chosen, which had $\Delta F_{Bij}/F_{Cu}$ greater than 0·006. Since these reflections are all relatively weak, it was necessary to improve precision by increasing the scan time. An approximate calculation suggested count times of 320 to 650 s for the largest reflection (taking into account an estimated count increase of Cu $K\alpha$ over Mo $K\alpha$ radiation because of its greater efficiency of scattering).

The scan speed was reduced to 0·125° min^{-1} and the $\omega/2\theta$ scan technique used over a scan range in 2θ of 1·2° plus an amount accounting for the α_1–α_2 separation. Collection times varied from 610 to 800 s. Background counts of 200 s were collected at each limit of the scan range. The eight most intense reflections of the 34 chosen were measured in each octant yielding four sets of Friedel pairs, while the remaining 26 were observed only for their *hkl* and $\bar{h}\bar{k}\bar{l}$. Data were measured with the same diffractometer and on the same crystal as used for the Mo $K\alpha$ data set. The source was a fine-focus Cu-target X-ray tube operating at 30 mA

Table 6. *Bond-length comparisons of coumarins*



Compound	C(9)–O(1)	O(1)–C(2)	C(2)–O(2)	C(2)–C(3)	C(3)–C(4)	C(4)–O(4)
Dibromodicoumarol[a]	1·36 Å	1·36 Å	1·22 Å	1·42 Å	1·38 Å	1·34 Å
Dicoumarol[b]	1·40	1·30	1·22	1·51	1·30	1·32
	1·41	1·33	1·21	1·46	1·31	1·33
4-Hydroxycoumarin[c]	1·37	1·37	1·20	1·44	1·35	1·35
3-Bromo-4-hydroxycoumarin[d]	1·43	1·31	1·26	1·43	1·41	1·34
3-(1-Naphthyl)-4-hydroxycoumarin[e]	1·38	1·36	1·23	1·43	1·37	1·33
Phenprocoumon[f]	1·38	1·37	1·23	1·42	1·37	1·34
Racemic warfarin[g]	1·38	1·38	1·21	1·42	1·36	1·35
(−)-(*S*)-Warfarin[h]	1·38	1·38	1·22	1·44	1·35	1·35
Coumarin[i]	1·38	1·37	1·20	1·45	1·34	–
Coumarin[j]	1·38	1·37	1·22	1·44	1·34	–

(*a*) Alcock & Hough (1972); (*b*) Bravic, Gaultier & Hauw (1968); (*c*) Gaultier & Hauw (1966); (*d*) Gaultier & Hauw (1965); (*e*) Bravic, Gaultier, Geoffre & Hauw (1974); (*f*) Bravic, Gaultier & Hauw (1971); (*g*) Bravic, Gaultier & Hauw (1973); (*h*) this work; (*i*) Miasnikova, Davydova & Simonov (1973); (*j*) Gavuzzo, Mazza & Giglio (1974).

Table 7. *Bond lengths* (Å) *and angles* (°)

O(1)—C(2)	1·375 (2)	C(2)—O(1)—C(9)	121·5 (2)
O(1)—C(9)	1·378 (3)	O(1)—C(2)—C(3)	118·7 (2)
O(2)—C(2)	1·217 (3)	O(1)—C(2)—O(2)	115·4 (2)
C(2)—C(3)	1·439 (3)	C(3)—C(2)—O(2)	125·8 (2)
C(3)—C(4)	1·351 (3)	C(2)—C(3)—C(4)	119·1 (2)
C(3)—C(11)	1·514 (3)	C(2)—C(3)—C(11)	118·4 (2)
C(4)—O(4)	1·351 (2)	C(4)—C(3)—C(11)	122·4 (2)
C(4)—C(10)	1·454 (3)	C(3)—C(4)—C(10)	121·5 (2)
C(9)—C(10)	1·387 (3)	O(4)—C(4)—C(10)	113·8 (2)
C(5)—C(10)	1·398 (3)	O(4)—C(4)—C(3)	124·7 (2)
C(5)—C(6)	1·384 (4)	C(4)—O(4)—C(13)	115·6 (2)
C(6)—C(7)	1·380 (4)	C(6)—C(5)—C(10)	120·3 (2)
C(7)—C(8)	1·372 (4)	C(5)—C(6)—C(7)	119·9 (2)
C(8)—C(9)	1·383 (3)	C(6)—C(7)—C(8)	121·0 (2)
C(11)–C(12)	1·536 (3)	C(7)—C(8)—C(9)	118·8 (2)
C(11)–C(15)	1·522 (3)	O(1)—C(9)—C(8)	116·9 (2)
C(12)–C(13)	1·504 (3)	O(1)—C(9)—C(10)	121·3 (2)
C(13)–C(14)	1·507 (3)	C(8)—C(9)—C(10)	121·8 (2)
C(13)–O(3)	1·385 (3)	C(4)—C(10)–C(5)	124·8 (2)
C(13)–O(4)	1·483 (2)	C(4)—C(10)–C(9)	117·0 (2)
C(15)–C(16)	1·378 (3)	C(5)—C(10)–C(9)	118·2 (2)
C(15)–C(20)	1·386 (3)	C(3)—C(11)–C(12)	108·3 (2)
C(16)–C(17)	1·385 (4)	C(3)—C(11)–C(15)	114·0 (2)
C(17)–C(18)	1·356 (5)	C(12)–C(11)–C(15)	108·0 (2)
C(18)–C(19)	1·359 (4)	C(11)–C(12)–C(13)	112·4 (2)
C(19)–C(20)	1·390 (4)	O(3)—C(13)–O(4)	108·0 (2)
O(3)—H(31)	0·93 (2)	O(3)—C(13)–C(12)	108·5 (2)
		O(3)—C(13)–C(14)	113·7 (2)
		O(4)—C(13)–C(12)	108·2 (2)
		O(4)—C(13)–C(14)	103·9 (2)
Average C–H lengths:		C(12)–C(13)–C(14)	114·1 (2)
Coumarin		C(11)–C(15)–C(20)	121·9 (2)
C——H	0·96	C(11)–C(15)–C(16)	120·4 (2)
		C(20)–C(15)–C(16)	117·5 (2)
Phenyl		C(15)–C(16)–C(17)	120·8 (3)
C——H	0·99	C(16)–C(17)–C(18)	121·0 (3)
		C(17)–C(18)–C(19)	119·4 (3)
Aliphatic		C(18)–C(19)–C(20)	120·4 (3)
C——H	0·99	C(19)–C(20)–C(15)	120·9 (2)

and 40 kV. The cell constants were redetermined with Cu $K\alpha$ radiation ($\lambda = 1{\cdot}5418$ Å), and are: $a = 10{\cdot}883$ (2), $b = 9{\cdot}563$ (2) and $c = 14{\cdot}904$ (3) Å. Reflections 1 through 8 in the four octants ($hkl, \bar{h}\bar{k}\bar{l}, \bar{h}k\bar{l}, h\bar{k}l$) were collected first. Then the Friedel pairs of reflections 9 through 34 were collected followed by reflections 1 through 8 in the remaining four octants ($hk\bar{l}, \bar{h}\bar{k}l, \bar{h}kl, h\bar{k}\bar{l}$). Friedel pairs were observed at $+2\theta$, χ and φ and then at $+2\theta$, $-\chi$ and $180° + \varphi$.

Data collection required 48 h of essentially continuous exposure to the beam. Some crystal deterioration was noted during Mo $K\alpha$ irradiation, and further deterioration was expected for the Cu $K\alpha$ data. The data were collected in the order noted above to aid in assessing the effects of deterioration and other possible systematic problems.

Standard deviations in the intensities were calculated by the formula

$$\sigma(I_{rel}) = [N_{pk} + (t/t_{bg})^2 N_{bg} + (0{\cdot}005\ N_{pk})^2]^{1/2}.$$

N_{pk} is the peak count, t is the peak scan time, t_{bg} is the background count time, and N_{bg} is the background count.

Since the model refined with Mo $K\alpha$ data was (R, R), both asymmetric centers had the same configuration* and agreement between inequalities of the Friedel pairs predicted and observed with Cu $K\alpha$ radiation would indicate the (R, R) configuration. Of the 34 unique Friedel pairs, 28 indicate the opposite of that predicted, *i.e.* 82% of the reflections indicate the (S, S) configuration. Including the symmetry-related reflections of 1 through 8 (all octants), we have 58 Friedel pairs, 45 indicating the (S, S) configuration, a 78% indication.

* Warfarin in the open-chain keto form has a single asymmetric center at C(11), but the formation of the hemiketal introduces a second center at C(13). The term (R, R) refers to the (2*R*, 4*R*) configuration and (S, S) refers to the (2*S*, 4*S*) configuration. Note that both centers are constrained to be the same. In the racemate (Bravic *et al.*, 1973) the configurations then become (2*R*, 4*R*) and (2*S*, 4*S*).

Table 8 summarizes the results, and Table 9 shows the indications of the individual reflections, except for 1 through 8 which are averages.

Table 8. *Sign indications*

Group	Number of contrib. pairs	Number of (S,S)	% (S,S)
Unique Friedel pairs, 1–34*	34	28	82
All octant reflections, 1–8†	32	23	72
All Friedel pairs 1–34 and octant reflections of 1–8	58	45	78
Abridged set for R calculations‡	51	44	86

* hkl and $\bar{h}\bar{k}l$ only.
† hkl, $\bar{h}\bar{k}l$, $h\bar{k}l$, $hk\bar{l}$, $\bar{h}k\bar{l}$, $h\bar{k}\bar{l}$, $\bar{h}\bar{k}\bar{l}$.
‡ Omits reflections 223, 466, 126, and 5,2,11 (see text).

It is seen that an indication of absolute configuration is evident from the intensity inequalities alone, although with less certainty than for structures containing heavy atoms. The high proportion of the data indicating the (S,S) configuration over the other enantiomer is striking in view of the fact that oxygen makes up only 20% of the structure.

The data set was abridged to exclude reflections with uneven background counts and two with $\Delta\sigma(I)$ values much greater than average values. The structure factors of these reflections were calculated and compared with those calculated from the parameters obtained from the Mo $K\alpha$ structure but using the cell constants determined with Cu $K\alpha$ radiation, including both the real and imaginary terms of the anomalous scattering of oxygen and carbon. After refining the scale factor for the observed data, $R_+ = 0{\cdot}0214$* and R_-, obtained by inverting the sign of $\Delta f''$ was 0·0182, $R_+/R_- = 1{\cdot}18$ and S_+/S_-† $= 1{\cdot}17$. Thus, with fairly high confidence we can assign the (S,S) enantiomeric configuration to (−)-warfarin on the basis of intensity inequalities and R values.

Our only attempt to refine the anomalous scattering factors was to minimize R_- by adjusting the terms $\Delta f''_C$ and $\Delta f''_O$. The ratio of $\Delta f''_O/\Delta f''_C$ was held constant at 3·55 (Hope & De la Camp, 1972) and $\Delta f''_O$ was varied in the range 0·020 to 0·047 ($\Delta f''_C$ in the range

* '+' refers to the (R,R) configuration and '−' to the (S,S) configuration.
† $S_\pm = \sum((|F_o| - |F_c|^2/\sigma^2(F_o))$.

Table 9. *Enantiomer-determining reflections*

No.	h	k	l	$\Delta\sigma(I)^a$	D^b_{obs}	$100\Delta F_{Bij,o}$		$100\Delta F^c_{Bij,c}$ A	$100\Delta F^d_{Bij,c}$ B
1	4	2	3	2·8	1·0	8·0		14	22
2	2	2	3	7·9	*e*				
3	6	1	1	3·5	0·9	8·2		11	19
4	3	4	4	2·4	0·6	6·2	Average	11	17
5	2	2	4	8·2	1·5	11·0	of 4 observa-	13	22
6	6	1	2	3·1	0·6	−7·2	tions	−9	−16
7	5	1	7	1·7	0·5	4·8		9	15
8	1	1	9	3·1	0·3	−6·8		−8	−12
9	2	3	2	10·7	1·8	−17		−15	−21
10	3	5	2	6·5	2·1	−11		−17	−26
11	3	2	11	5·9	0·7	−11		−13	−20
12	1	2	6	28·8	*e*				
13	4	6	6	0·3	*e*				
14	5	2	11	24·0	*e*				
15	9	2	2	3·4	0·5	6		−10	−16
16	2	1	7	2·3	0·5	3		7	13
17	9	4	3	7·6	0·3	15		10	15
18	5	5	2	3·1	0·2	5		−7	−10
19	1	7	3	·8	0·4	−1		−9	−16
20	7	2	6	5·1	0·3	8		9	15
21	6	4	2	2·0	0·4	−3		−7	−12
22	6	3	3	7·9	0·5	13		9	15
23	5	2	13	4·0	0·2	7		9	12
24	7	7	8	11·0	0·1	19		9	12
25	7	4	2	7·4	0·3	13		9	15
26	8	3	6	7·6	0·3	14		7	13
27	1	5	9	3·4	0·2	−7		−10	−16
28	3	2	13	4·8	0·2	10		10	15
29	3	1	5	7·1	1·4	11		12	21
30	12	1	4	5·7	0·2	−10		−10	−14
31	4	2	6	10·7	0·4	15		10	14
32	4	2	11	4·5	0·3	−18		−10	−13
33	2	5	7	10·7	0·9	21		13	21
34	11	2	2	2·8	0·2	5		9	13

(*a*) $\Delta\sigma(I) = |I_{hkl} - I_{\bar{h}\bar{k}\bar{l}}|/2\sigma(\bar{I})$. (*b*) $D_{obs} = (\Delta F_{Bij})^2/[\sigma(F)]^2$. (*c*) $\Delta F_{Bij,c}$ based on $\Delta f''_C = 0{\cdot}011$, $\Delta f''_O = 0{\cdot}037$. (*d*) $\Delta F_{Bij,c}$ based on $\Delta f''_O = -0{\cdot}032$ only. (*e*) Reflections excluded from abridged data set.

0·007 to 0·014). A minimum of $R_- = 0{\cdot}0181$ was found for $\Delta f''_O = 0{\cdot}037$ and $\Delta f''_C = 0{\cdot}011$. Although this is only 0·0001 lower than R_- found for $\Delta f''_O = 0{\cdot}032$ and $\Delta f''_C = 0{\cdot}010$ and $\Delta f''_O = 0{\cdot}049$ and $\Delta f'_C = 0{\cdot}019$, the enantiomer, $R_+ = 0{\cdot}0220$, increased by 0·0006, increasing the ratio R_+/R_- to 1·21 and S_+/S_- to 1·18, in agreement with previous work (Hope & De la Camp, 1972) in which $\Delta f''_O$ was found to be larger than that calculated by Hönl's (1933) formula of 0·032. Table 9 lists the data on the 34 reflections used in determining absolute configuration.

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References

ALCOCK, N. & HOUGH, E. (1972). *Acta Cryst.* B**28**, 1956–1960.

BENTLEY, E. (1969). *Schriftenreihe Ver. Wasserhyg.* **32**, 19–25.

BRAVIC, G., GAULTIER, J., GEOFFRE, S. & HAUW, C. (1974). *C. R. Acad. Sci. Paris, Sér. C*, **278**, 601–603.

BRAVIC, G., GAULTIER, J. & HAUW, C. (1968). *C. R. Acad. Sci. Paris, Sér. C*, **267**, 1790–1793.

BRAVIC, G., GAULTIER, J. & HAUW, C. (1971). *C. R. Acad. Sci. Paris, Sér. C*, **272**, 1113–1114.

BRAVIC, G., GAULTIER, J. & HAUW, C. (1973). *C. R. Acad. Sci. Paris, Sér. C*, **277**, 1215–1218.

BRECKENRIDGE, A. & ORME, M. (1972). *Life Sci.* **11**, 337–345.

CHAN, K., LEWIS, R. & TRAGER, W. (1972). *J. Med. Chem.* **15**, 1265–1270.

DRUMMOND, D. (1970). *Symp. Zool. Soc. Lond.* **26**, 351–367.

EBLE, N., WEST, B. & LINK, K. (1966). *Biochem. Pharmacol.* **15**, 1003–1006.

ENGEL, D. (1972). *Acta Cryst.* B**28**, 1496–1509.

GAULTIER, J. & HAUW, C. (1965). *Acta Cryst.* **19**, 927–933.

GAULTIER, J. & HAUW, C. (1966). *Acta Cryst.* **20**, 646–651.

GAVUZZO, E., MAZZA, F. & GIGLIO, E. (1974). *Acta Cryst.* B**30**, 1351–1357.

HEWICK, D. (1972). *J. Pharm. Pharmacol.* **24**, 661–662.

HEWICK, D. & MCEWEN, J. (1973). *J. Pharm. Pharmacol.* **25**, 458–465.

HÖNL, H. (1933). *Ann. Phys. Lpz.* **18**, 625.

HOPE, H. & DE LA CAMP, U. (1972). *Acta Cryst.* A**28**, 201–207.

International Tables for X-ray Crystallography (1962). Vol. III, pp. 202–203. Birmingham: Kynoch Press.

JACKSON, W. (1969*a*). *Pest Control*, **37** (3), 51–55.

JACKSON, W. (1969*b*). *Pest Control*, **37** (4), 40–43.

JACKSON, W., SPEAR, P. & WRIGHT, C. G. (1971). *Pest Control*, **39** (9), 13–14.

JOHNSON, C. K. (1965). *ORTEP*. Oak Ridge National Laboratory Report ORNL-3794.

KIM, S. H. & ROSENSTEIN, R. D. (1967). *Acta Cryst.* **22**, 648–656.

KOCH WESER, J. & SELLERS, E. M. (1971). *New Engl. J. Med.* **285** (10), 547–588.

LEWIS, R. J., TRAGER, W. F., CHAN, K. K., BRECKENRIDGE, A., ORME, M., ROLAND, M. & SHUNG, W. (1974). *J. Clin. Invest.* **53**, 1607–1617.

MAIN, P., WOOLFSON, M. M. & GERMAIN, G. (1971). *MULTAN, A Computer Program for the Automatic Solution of Crystal Structures*, Univ. of York Printing Unit, York.

MIASNIKOVA, R. M., DAVYDOVA, T. C. & SIMONOV, V. I. (1973). *Kristallografiya*, **18**, 720–724.

PORTER, W., TRAGER, W. F. & VALENTE, E. (1973). Unpublished results.

STEWART, J. M., KUNDELL, F. A. & BALDWIN, J. C. (1970). X-RAY System, Univ. of Maryland.

STEWART, R. F., DAVIDSON, E. R. & SIMPSON, W. T. (1965). *J. Chem. Phys.* **42**, 3175–3187.

TAKA, T., OSAKI, O. & OKUDA, T. (1970). *Acta Cryst.* B**26**, 991–997.

TAKAGI, S. & ROSENSTEIN, R. D. (1969). *Carbohydr. Res.* **11**, 156–158.

WEST, B. D., PREIS, S., SCHROEDER, C. H. & LINK, K. P. (1961). *J. Amer. Chem. Soc.* **83**, 2676–2679.