Analogs of 3-(1-Phenyl-3-oxobutyl)-4-hydroxycoumarin (Warfarin) Prepared from Substituted Salicylic Acids

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Some derivatives of salicylic acid containing substituents *meta* to the carboxyl group were used to prepare analogs of the anticoagulant drug warfarin, 3-(1-phenyl-3-oxobutyl)-4-hydroxycoumarin, containing substituents in either the 6- or 8-position of the courmarin ring. When the substituent was the hydroxyl group, the resulting products are previously identified metabolites of warfarin. The substituted salicylic acid is first acetylated with acetic anhydride, then either converted to the acid chloride and condensed with diethyl malonate in the presence of sodium hydroxide or converted to the mixed anhydride with formic acid and condensed with ethoxymagnesium diethyl malonate to yield, in either case, the corresponding 3-carbethoxy-4-hydroxycoumarin substituted in the 6- or 8-position of the coumarin ring. These compounds readily condense with benzalacetone to form the corresponding substituted warfarin in the presence of 5 mole % tertiary amine catalyst. This method offers an improved route for the synthesis of 8-hydroxywarfarin.

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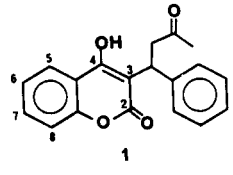
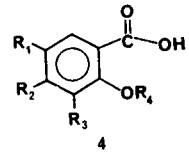
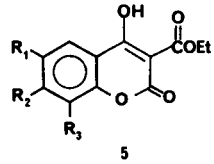
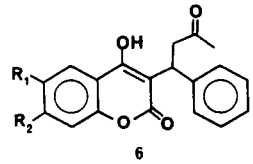
Warfarin (**1**, 3-(1-phenyl-3-oxobutyl)-4-hydroxycoumarin) has recently shown promise as a model substrate for characterizing cytochrome P-450 mixed-function oxidase isoenzymes [1-5]. 8-Hydroxywarfarin (**2**) (Scheme I) is a very minor metabolite of warfarin when liver microsomal mixed-function oxidase enzymes from uninduced animals are employed. However, pretreatment of the animals with polycyclic aromatic hydrocarbon inducing agents, such as 3-methylcholanthrene, β-naphthoflavone, and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, causes a 3-18-fold increase in the rate of formation of 8-hydroxywarfarin from warfarin [3-5]. Because this particular metabolite is characteristically formed only by those cytochrome P-450 isoenzymes that are inducible with polycyclic aromatic hydrocarbons, we required reliable methods to synthesize this metabolite for use as an analytical standard in biochemical studies.

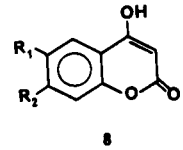
The previously reported synthesis of this metabolite [6] requires eight steps, with an overall yield of less than 1% (Scheme I). Most of the losses occur in the tedious synthesis of 8-benzyloxy-4-hydroxycoumarin (**3**). Warfarin itself has been prepared from salicylic acid (**4**, $R_1 = R_2 = R_3 = R_4 = H$) *via* the intermediate 3-carbethoxy-4-hydroxycoumarin (**5**, $R_1 = R_2 = R_3 = H$) [7,8]. We have adapted this procedure for the synthesis of the 8-hydroxy metabolite of warfarin (Schemes II and III), with a yield of 24%, based on the starting substituted salicylic acid. The same procedure was followed to produce 6-hydroxywarfarin (**6**, $R_1 = OH$, $R_2 = H$) and 6-chlorowarfarin (**6**, $R_1 = Cl$, $R_2 = H$) but failed to yield 7-hydroxywarfarin (**6**, $R_1 = H$, $R_2 = OH$).

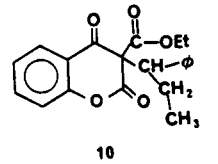






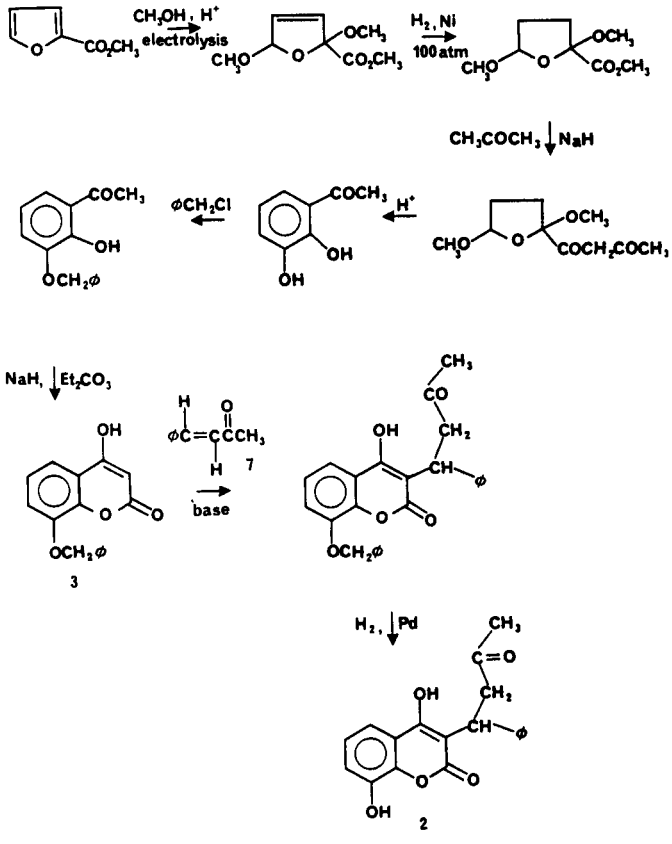






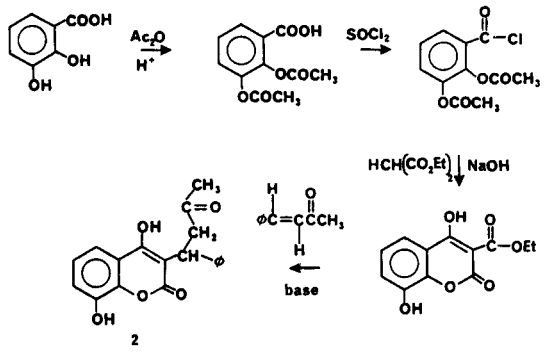
Two routes were explored for the formation of the variously substituted 3-carbethoxy-4-hydroxycoumarin (**5**) intermediates. Using the method employed by Hultquist [8] for the preparation of unsubstituted 3-carbethoxy-4-hydroxycoumarin (**5**, $R_1 = R_2 = R_3 = H$), the appropriately substituted salicylic acid (**4**, $R_4 = H$) was first acetylated,

Scheme I



then converted to its acid chloride, and then condensed with diethylmalonate in aqueous alkali (Scheme II). Alternately, using the method described by Tarbell and Prince [10] for the synthesis of the parent 3-carbethoxy-4-hydroxycoumarin (**5**, $R_1 = R_2 = R_3 = H$), the appropriately substituted salicylic acid was again first acetylated, then converted to its mixed anhydride with formic acid, and then condensed with ethoxymagnesium diethyl malonate (Scheme III). The latter method generally gave better yields.

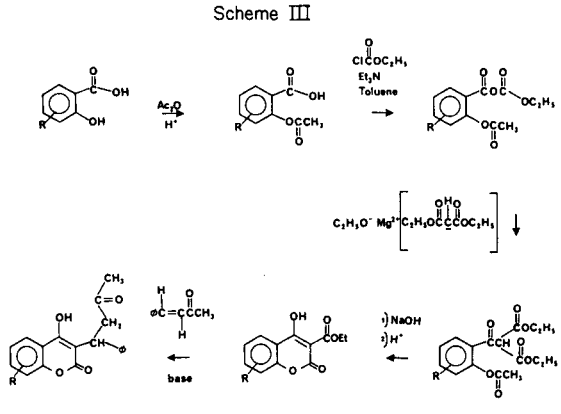
Scheme II



Once the required substituted 3-carbethoxy-4-hydroxycoumarin was obtained, it was condensed with *trans*-1-phenyl-2-buten-3-one (**7**). The solvent used in this reaction was found to have a major influence on the rate of the reaction. The reaction was very slow in pure water, pure dioxane, or pure ethanol. A mixture of 50% dioxane in water gave the best results for the synthesis of 8-hydroxywarfarin, although the reaction proceeded moderately in 50% ethanol in water, 25% dioxane in water or 75% dioxane in water. In each solvent studied, the reaction was catalyzed by the inclusion of 5 mole % triethylamine.

The efficacy of various tertiary amine catalysts in promoting the condensation of 3-carbethoxy-4,6-dihydroxycoumarin (**5**, $R_1 = OH$, $R_2 = R_3 = H$) with *trans*-1-phenyl-2-buten-3-one (**7**) was studied in 25%, 50% and 75% aqueous dioxane. The rate of formation of 6-hydroxywarfarin (**6**, $R_1 = OH$, $R_2 = H$) was greatest in 50% or 75% aqueous dioxane when either simple tertiary aliphatic amines (triethylamine, tripentylamine, trihexylamine, or trioctylamine) or substituted tertiary amines [triethanolamine, tris(hydroxylmethyl)aminomethane, 1,4-piperazinebis(ethane-sulfonic acid), 4-dimethylaminopyridine, or *N*-methylimidazole] were used at 5 mole % concentration.

Scheme III



When *N*-methylimidazole was used either as the reaction medium or at 5 mole % concentration in pure water, no 6-hydroxywarfarin was formed. Instead, another highly fluorescent product was obtained.

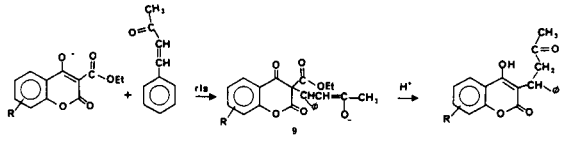
The condensation reaction to form the substituted warfarin analogs was very slow when the substituent desired was the hydroxyl group. The reaction to form 6- or 8-hydroxywarfarin required nearly two weeks to go to completion in boiling 50% aqueous dioxane. However, when the synthesis of 7-hydroxywarfarin was attempted, a large number of products, none of them identifiable as the desired product, were formed instead. Recovery of unreacted starting material was possible when the reaction between any of the other 3-carbethoxy-4-hydroxycoumarins (**5**) and *trans*-1-phenyl-2-buten-3-one (**7**) was halted at a time before the reactants were exhausted.

We have previously observed that the condensation of

either 4,6-dihydroxycoumarin (**8**, R_1 = OH, R_2 = H) or 4,7-dihydroxycoumarin (**8**, R_1 = H, R_2 = OH) with *trans*-1-phenyl-2-buten-3-one (**7**) in 50% aqueous dioxane in the presence of 5 mole % triethylamine gives good yields of 6-hydroxywarfarin (**6**, R_1 = OH, R_2 = H) or 7-hydroxywarfarin (**6**, R_1 = H, R_2 = OH) within 24 hours [10]. This suggests that the rate-limiting step in the condensation with the 3-carbethoxy derivatives (**5**) either involves the hydrolysis and decarboxylation of the side-chain ester group or the direct addition of the anion of the carbethoxy compound (**5**) to the α,β-unsaturated ketone (**7**) (Scheme IV). The latter mechanism would give rise to a tetrahedral intermediate (**9**) similar in structure to the structural analog (**10**), which has been found to be readily hydrolyzed by hot alkali and decarboxylated by neutralization with acid to *p*H 3 (11). Since 3-carbethoxy-4-hydroxycoumarin itself (**5**, R_1 = R_2 = R_3 = H) is only partly converted to 4-hydroxycoumarin in boiling water in the presence of 5 mole % triethylamine within 8 hours but reacts completely with *trans*-1-phenyl-2-buten-3-one (**7**) when treated under identical conditions for the same time [7], the pathway illustrated in Scheme IV appears to be more likely than a mechanism requiring decarboxylation to 4-hydroxycoumarin as a first step.

The reaction sequence described herein is a useful route for the preparation of certain 4-hydroxycoumarin derivatives substituted in both the 6- (or 8-) position and the 3-position of the coumarin ring.

Scheme IV



EXPERIMENTAL

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer 599 B spectrophotometer using potassium bromide discs containing 1% sample. Ultraviolet spectra were recorded on a Perkin-Elmer 559 spectrophotometer. Proton magnetic resonance spectra were recorded on a Bruker HX 90E spectrometer. Mass spectra were determined by a Finnigan 4000 system using the direct insertion probe. Progress of the final condensation reaction was monitored by thin-layer (tlc) or high performance liquid chromatography (hplc). For tlc, Whatman LK6D Linear K silica gel plates with preadsorbant spotting zone were spotted with 25μl samples from the reaction mixtures. The plates were developed in 50% acetone/50% chloroform, dried, exposed to ammonia vapor, and observed under 365 nm uv light. For hplc, samples of the reaction mixtures were suitably diluted and injected (20 μl) onto an Altex Ultrasphere-IP ion-pair, reversed-phase column. The reaction products were eluted with a mobile phase containing 70% 44 m*M* phosphate buffer (*p*H 7.4) and 30% acetonitrile to which was added 1.2 g/l acetyltrimethylammonium bromide. Hydroxylated warfarins and 3-carbethoxy-4-hydroxycoumarins were detected fluorometrically using a Kratos FS 950 detector equipped with a Hg lamp and 313 nm excitation filter used with a 340 nm cutoff emission filter. Samples were diluted to contain products resulting from ~ 3 μg/ml of starting material. Acetylsalicylic acid (**4**, R_1 = R_2 = R_3 = H, R_4 = $COCH_3$) was obtained from Mallinckrodt (U.S.P. powder) and used directly.

2,3-Diacetoxybenzoic Acid (**4**, R_1 = R_2 = H, R_3 = $OCOCH_3$, R_4 = $COCH_3$).

2,3-Dihydroxybenzoic acid (**4**, R_1 = R_2 = R_4 = H, R_3 = OH) (38.5 g, 250 mmoles), acetic anhydride (145 ml) and sulfuric acid (0.7 ml) were warmed for 25 minutes on a steam bath and then poured onto 250 g of ice. The mixture was stirred. Precipitation of the product occurred as the ice melted. The precipitate was recovered by filtration and recrystallized from a mixture of benzene and acetic acid to give 44.2 g (74%) of the title compound, mp 160-163°; nmr (DMSO-d_6): δ 2.27 (s, 3H), 2.30 (s, 3H), 7.2-7.8 (m, 3H).

2,4-Diacetoxybenzoic Acid (**4**, R_1 = R_3 = H, R_2 = $OCOCH_3$, R_4 = $COCH_3$).

2,4-Dihydroxybenzoic acid (**4**, R_1 = R_3 = R_4 = H, R_3 = OH) (32.0 g, 208 mmoles), acetic anhydride (120 ml) and sulfuric acid (0.6 ml) were treated as described above for the 2,3-isomer to give 34.6 g (70%) of the title compound, mp 136-137° [lit [12] mp 136°]; nmr (DMSO-d_6): δ 2.27 (s, 6H), 7.1-8.1 (m, 3H).

2,5-Diacetoxybenzoic Acid (**4**, R_2 = R_3 = H, R_1 = $OCOCH_3$, R_4 = $COCH_3$).

2,5-Dihydroxybenzoic acid (**4**, R_2 = R_3 = R_4 = H, R_1 = OH) (48.0 g, 311 mmoles), acetic anhydride (180 ml) and sulfuric acid (0.9 ml) were treated as described above for the 2,3-isomer to give 54.8 g (74%) of the title compound, mp 125-126° [lit [13] mp 121°]; nmr (DMSO-d_6): δ 2.2 (s, 6H), 7.2-7.8 (m, 3H).

2-Acetoxy-5-chlorobenzoic Acid (**4**, R_2 = R_3 = H, R_1 = Cl, R_4 = $COCH_3$).

5-Chlorosalicylic acid (**4**, R_2 = R_3 = R_4 = H, R_1 = Cl) (97.5 g, 565 mmoles), acetic anhydride (350 ml) and sulfuric acid (1.8 ml) were treated as described above to give 88.1 g (73%) of the title compound, mp 153-154°; nmr (deuteriochloroform): δ 2.3 (s, 3H), 7.1-8.2 (m, 3H).

3-Carbethoxy-4,6-dihydroxycoumarin (**5**, R_2 = R_3 = H, R_1 = OH). Method I (Scheme 2).

2,3-Diacetoxybenzoic acid (20 g, 84 mmoles) and thionyl chloride (20 ml) were mixed with diethyl ether (100 ml) and benzene (200 ml) and refluxed 16 hours. The solvent was removed under reduced pressure using a rotary evaporator and the residual yellow oil was dissolved in dimethoxyethane (50 ml). A 500 ml, 3-neck flask equipped with two 125 ml dropping funnels (one pressure-equalizing) and a mechanical stirrer was chilled in an ice-water bath to 0°. Diethyl malonate (redistilled, 12 g), ice (25 g) and water (25 ml) were mixed in the flask. A few drops of a saturated solution of alizarin yellow R in 5% (v/v) aqueous ethanol was added to the reactants and stirring was begun. The dimethoxyethane solution of the acid chloride was transferred to the pressure-equalizing dropping funnel; the other funnel was charged with 26 g of a 50% (w/w) aqueous solution of sodium hydroxide. Both the sodium hydroxide solution and the acid chloride solution were alternately added a few drops at a time while the contents of the flask were stirred rigorously. The rate of sodium hydroxide addition was adjusted to maintain a persistent red color in the reaction mixture. The reaction was allowed to continue for 25 minutes after the final addition of the acid chloride. Then water (200 ml) was added. Some insoluble material was removed by filtration, and the filtrate was acidified to *p*H 3 with concentrated hydrochloric acid and cooled to 4°. After standing 24 hours, the precipitate was recovered by suction filtration. The product was recrystallized from benzene and ethanol yielding 2.1 g of product (23%, based on 2,5-diacetoxybenzoic acid), mp decomposed > 250 λ max (DMSO): 290 nm; log ϵ max = 4.02; ir (1% w/w in potassium bromide): 1695, 1630, 1565 cm^{-1}; ms: (electron impact) m/z 250, 205, 204; nmr (DMSO-d_6): δ 1.32 (t, 3H), 4.36 (q, 2H), 7.2-7.8 (m, 3H).

Anal. Calcd. for $C_{12}H_{10}O_6$: C, 57.60; H, 4.00. Found: C, 57.39; H, 4.05.

Method II (Scheme 3).

Ethoxymagnesium diethyl malonate was prepared from Mg turnings (2.5 g, 0.1 mole) and diethyl malonate (16 g, 0.1 mole) dissolved in 15.5 ml of absolute ethanol to which 10 mg of iodine was added. The reaction mixture was heated, to reflux, protected by a calcium chloride drying tube. After two days, all of the Mg had reacted. After cooling, diethyl ether (30 ml) was added to decrease the viscosity of the gel-like product. The solvents were stripped under reduced pressure using a rotary evaporator. Benzene (35 ml) was added to the residue and the product was dissolved. The benzene was then removed under reduced pressure; this procedure aids in removing residual ethanol. The residue was then suspended in anhydrous diethyl ether (45 ml).

2,5-Diacetoxybenzoic acid (27.5 g, 116 mmoles), triethylamine (13.8 ml, 0.1 mole), and toluene (200 ml) were mixed in a 500 ml, four-neck flask equipped with a stirrer, dropping funnel, thermometer, and a condenser fitted with a calcium chloride drying tube. The flask was immersed in an ice-salt bath. After the reaction mixture was chilled to below 0°, ethyl chloroformate (9.5 ml, 0.1 mole) was added dropwise with stirring over 30 minutes. The reaction mixture was stirred an additional 45 minutes, then the suspension of ethoxymagnesium diethyl malonate was added dropwise. The reaction temperature was maintained below 0° for 60 minutes then the mixture was allowed to warm slowly to room temperature overnight with continued stirring. A solution of 5% (w/v) aqueous sulfuric acid (200 ml) was added cautiously; a gas was evolved. The organic phase was then separated, and the aqueous phase was washed with two portions of ethyl ether (50 ml each). The organic phases were combined and washed with 5% (w/v) aqueous sulfuric acid (50 ml), water (50 ml), and three portions of a saturated aqueous solution of sodium bicarbonate (50 ml each). The organic phase was percolated through about 20 g of anhydrous sodium sulfate to remove water and the dried organic phase was concentrated under reduced pressure. The oily residue was stirred for 2 hours with sodium hydroxide (12 g dissolved in 200 ml of water). The *p*H was then adjusted to 4.5 with acetic acid. A precipitate formed which was collected by suction filtration. The filtrate was acidified to *p*H 2 with phosphoric acid. After chilling to 4° overnight, a second crop was collected. Both precipitates had identical infrared spectra and were combined. The combined yield was 11.8 g (39%, based on 2,5-dihydroxybenzoic acid). The ms and ir were identical to those obtained for this product obtained *via* Method I.

3-Carbethoxy-4,7-dihydroxycoumarin (**5**, $R_1 = R_3 = H$, $R_2 = OH$). Method I.

2,4-Diacetoxybenzoic acid (10 g, 42 mmoles) was treated as described above for the 2,5-isomer to yield 2.5 g (24%, based on 2,4-diacetoxybenzoic acid) of product, mp decomposed > 250°; λ max (DMSO): 294 nm log ε max 3.90; ir (1% w/w in potassium bromide): 1712, 1605, 1570 cm^{-1}; ms: (electron impact) m/z 250, 204, 167, 149; nmr (DMSO-d_6): δ 1.32 (t, 3H), 4.35 (q, 2H), 6.50-6.89 (m, 2H), 7.7 (d, 1H).

Anal. Calcd. for $C_{12}H_{10}O_6$: C, 57.60; H, 4.00. Found: C, 57.22; H, 4.12.

Method II.

2,4-Diacetoxybenzoic acid (47.6 g, 200 mmoles) was treated as described above for the 2,3-isomer to yield 32.5 g (62%, based on 2,4-diacetoxybenzoic acid) of product. The ms and ir spectra were identical to those obtained for this product obtained *via* Method I.

3-Carbethoxy-4,8-dihydroxycoumarin (**5**, $R_1 = R_2 = H$, $R_3 = OH$). Method I.

2,3-Diacetoxybenzoic acid (10 g, 42 mmoles) was treated as described above for the 2,5-isomer to yield 3.0 g (25%, based on 2,3-diacetoxybenzoic acid) of product, mp decomposed > 250°; λ max (DMSO): 299 nm log ε max = 4.05; ir (1% w/w in potassium bromide): 1700, 1620, 1610, 1565, 1510 cm^{-1}; ms: (electron impact) m/z 250, 205, 204; nmr (DMSO-d_6): δ 1.33 (t, 3H), 4.37 (q, 2H), 6.6-7.8 (m, 3H).

Anal. Calcd. for $C_{12}H_{10}O_6$: C, 57.60; H, 4.00. Found: C, 57.44; H, 3.88.

Method II.

2,3-Diacetoxybenzoic acid (23 g, 97 mmoles) was treated as described above for the 2,3-isomer to yield 10.7 g (44%, based on 2,3-diacetoxybenzoic acid) of product. The ms and ir spectra were identical to those obtained for the product obtained *via* Method I.

3-Carbethoxy-6-chloro-4-hydroxycoumarin (**5**, $R_1 = Cl$, $R_2 = R_3 = H$). Method I.

2-Acetoxy-5-chlorobenzoic acid (72 g, 336 mmoles) was treated as described above for 2,5-diacetoxybenzoic acid to yield 30 g (25 %, based on 2-acetoxy-5-chlorobenzoic acid) of product, mp 181-183°; λ max (DMSO); 297 log ε max = 3.82; ir (1% w/w in potassium bromide): 1665, 1580 cm^{-1}; ms: (electron impact) m/z 271, 270, 269, 268, 225, 224, 223, 222, 198, 196, 157, 156, 155, 154, 153, 152; nmr (deuteriochloroform): δ 1.4 (t, 3H), 4.5 (q, 2H), 7.2-8.0 (m, 3H).

Anal. Calcd. for $C_{12}H_9ClO_5$: C, 53.65; H, 3.35. Found: C, 53.69; H, 3.48.

4,6-Dihydroxy-3-(1-phenyl-3-oxobutyl)coumarin (6-Hydroxywarfarin, **6**, $R_1 = OH$, $R_2 = H$).

3-Carbethoxy-4,6-dihydroxycoumarin (11.8 g, 47 mmoles), benzalacetone (7.4 g, 50 mmoles) and triethylamine (0.35 ml, 2.5 mmoles) were dissolved in 50% (v/v) aqueous dioxane (500 ml). The mixture was heated to reflux under a nitrogen atmosphere. Refluxing was continued for 14 days, at which time analysis of the reaction mixture by hplc showed the disappearance of the carbethoxycoumarin starting material. The solvent was removed under reduced pressure. The organic residue was dissolved in 5% (w/v) aqueous $NaHCO_3$ (400 ml), and the *p*H was adjusted to 9 with sodium hydroxide. The solution was extracted with 2 portions of diethyl ether (50 ml each). The aqueous phase was then cautiously acidified with concentrated hydrochloric acid to *p*H 2. After vigorous evolution of gas, a precipitate formed, which was collected by filtration and recrystallized from acetone/chloroform to yield 7.0 g (46%, based on 3-carbethoxy-4,6-dihydroxycoumarin) of product, mp 213-215° [lit [6] mp 219-220°]; λ max (DMSO): 279, 292, 330 nm; log ε max 3.87, 3.84, 3.86; ir (1% w/w in potassium bromide): 1668, 1630, 1578 cm^{-1}; MS (electron impact) m/z: 324, 282, 281, 267, 265, 203, 178. The ir and ms spectra were identical to an authentic sample prepared as described by Hermodson [6].

Anal. Calcd. for $C_{19}H_{16}O_5$: C, 70.37; H, 4.94. Found: C, 70.22; H, 4.96.

4,8-Dihydroxy-3-(1-phenyl-3-oxobutyl)coumarin (8-Hydroxywarfarin, **2**).

3-Carbethoxy-4,8-dihydroxycoumarin (2.38 g, 9.5 mmoles), benzalacetone (4.63 g, 31.2 mmoles) and 1,4-piperazinebis(ethane sulfonic acid) 1.5-Na salt (0.17 g, 0.6 mmoles) were refluxed in 50% (v/v) aqueous dioxane under nitrogen for 11 days, at which time no more starting material was detectable by hplc. The reaction mixture was worked up as described for the 6-hydroxy isomer to yield 1.35 g (57%, based on 3-carbethoxy-4,8-dihydroxycoumarin) of product, mp 195-196° [lit mp 189-191° on recrystallization from chloroform [6]]. λ max (DMSO): 290 nm, log ε max 4.08; ir (1% w/w in potassium bromide): 1700, 1628, 1585 cm^{-1}. MS (electron impact) m/z: 324, 306, 281, 229, 203. The ir and ms spectra were identical to an authentic sample prepared as described by Hermodson [6].

Anal. Calcd. for $C_{19}H_{16}O_5$: C, 70.37; H, 4.94. Found: C, 70.18; H, 4.94.

6-Chloro-4-hydroxy-3-(1-phenyl-3-oxobutyl)coumarin (6-Chlorowarfarin; **6**, $R_1 = Cl$, $R_2 = H$).

3-Carbethoxy-6-chloro-4-hydroxycoumarin (3.5 g, 13 mmoles), benzalacetone (7.0 g, 47.2 mmoles) and triethylamine (0.07 g, 0.69 mmoles) were refluxed in 50% (v/v) aqueous dioxane under nitrogen for 2 days, at which time no more starting material was detected by hplc. The reaction mixture was worked up as described for the 6-hydroxy isomer to yield 3.5 g (80%, based on 3-carbethoxy-6-chloro-4-hydroxycoumarin) after recrystallization from 95% (v/v) aqueous ethanol, mp 179-180°. ir (1% w/w in potassium bromide): 1685, 1615, 1567 cm^{-1}; ms: (electron impact) m/z 344, 343, 342, 301, 300, 299, 285, 282, 223, 221.

Anal. Calcd. for $C_{19}H_{15}ClO_4$: C, 66.59; H, 4.38. Found: C, 65.39; H, 4.41.

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REFERENCES AND NOTES

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