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High-Yield Synthesis of Warfarin and Its Phenolic Metabolites: New Compounds

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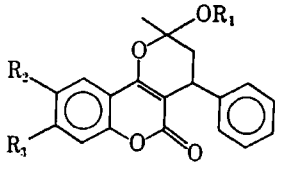
Abstract □ A novel synthesis of warfarin and phenolic warfarin metabolites is presented which results in higher yields than previous methods.

Keyphrases □ Warfarin—phenolic metabolites, new high-yield synthetic method □ Synthesis—warfarin and its phenolic metabolites, new high-yield method

The oral anticoagulant warfarin [4-hydroxy-3-(1-phenyl-3-oxobutyl)-2*H*-1-benzopyran-2-one] (I) has found extensive clinical use in the treatment of such pathological conditions as thrombophlebitis, pulmonary emboli, and myocardial infarction (1). It is also used widely as a rodenticide to help control rat populations (2) and more recently has been used as a probe to investigate the multiplicity and catalytic activity of microsomal and purified cytochrome P-450 preparations (3–7). Because of its clinical and pharmacological importance, considerable effort has been expended to develop analytical methods to quantitate both warfarin and its metabolites from biological matrices (8–11). However, one of the impediments to progress in this area has been the lack of a high-yield synthetic procedure for these materials. Since mechanistic work in this laboratory on cytochrome P-450 required the synthesis and optical resolution of specifically deuterated warfarin analogues as substrates and multideuterated metabolites as GC–MS assay internal standards, the need for a reproducible, high-yield synthesis for these compounds was evident.

Although successful synthetic routes to these materials are documented in the literature, the reported yields are poor to moderate at best (12). Warfarin has been synthesized by the Michael addition of 4-hydroxycoumarin to benzalacetone under a number of acid- or base-catalyzed conditions (13). Traditionally, the reaction has most often been run in water containing a catalytic amount of triethylamine (~5 mole %). Hermodson *et al.* (12), using essentially the same conditions, extended this synthesis to the phenolic metabolites of warfarin by condensing benzalacetone with the appropriately substituted 4-hydroxycoumarin. Fasco *et al.* (14) followed a similar route, but to obtain a homogeneous system substituted dioxane as the solvent and piperidine as the catalyst. A further refinement was reported by Cook *et al.* (15) who, in their synthesis of 3′-bromowarfarin, heated a solution of *m*-bromobenzalacetone and 4-hydroxycoumarin in pyridine at reflux. However, rarely was the overall yield of warfarin or hydroxywarfarin >65%. In the case of 7-hydroxywarfarin, it was invariably much lower.



		R_1	R_2	R_3
I:	Warfarin	H	H	H
II:	Warfarin Ethyl Ether	C_2H_5	H	H
III:	Cyclocoumarol	CH_3	H	H
IV:	6-Hydroxywarfarin	H	OH	H
V:	7-Hydroxywarfarin	H	H	OH
VI:	6-Hydroxycyclocoumarol	CH_3	OH	H
VII:	7-Hydroxycyclocoumarol	CH_3	H	OH

RESULTS AND DISCUSSION

Numerous attempts in this laboratory to improve previous yields by alternate reaction pathways failed. A new approach was conceived when it was found that warfarin was quantitatively converted to its ethyl ether (II) by refluxing in absolute ethanol. This observation suggested that in absolute ethanol, the acidity of 4-hydroxycoumarin might be sufficient to catalyze its condensation with benzalacetone to generate warfarin. Once formed, the reaction would be driven to completion by the subsequent and essentially irreversible formation of the ethyl ether under these conditions. Initial experiments investigating this possibility proved successful. Complete removal of the ethanol, however, proved to be difficult; therefore, methanol was substituted as the solvent, with equal success. This extended the necessary reaction time, presumably because of the lower reaction temperature; however, the lower temperature also allowed the relatively unstable starting material (4,7-dihydroxycoumarin) to be converted to the warfarin methyl ether (VII) in high yield.

The reaction involved stirring an appropriate coumarin analogue with benzalacetone in refluxing methanol. After 4–24 hr, as determined by TLC, the corresponding methyl ether was obtained in high yield. The ether can be quantitatively converted back to the warfarin analogue by acid hydrolysis. Typically, overall yields are >70%, and often yields as high as 95% are obtained. This dramatic increase in reaction yields should significantly aid in the development of warfarin as a tool to probe metabolic pathways.

EXPERIMENTAL[1]

Warfarin [4-Hydroxy-3-(1-phenyl-3-oxobutyl)-2*H*-1-benzopyran-2-one] (I)—4-Hydroxycoumarin (1.0 g, 0.0069 mole) was stirred

[1] Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. NMR spectra were recorded on a Varian EM-360A spectrometer using tetramethylsilane as internal standard. 4-Hydroxycoumarin was purchased from the Aldrich Chemical Co., and benzalacetone was purchased from MCB Reagents. 4,6- and 4,7-Dihydroxycoumarin were gifts from Dr. Lawrence Low, University of Washington. TLC was performed on EM Reagents analytical silica gel chromatography plates with flourescent indicator (no. 5539). All other solvents and reagents were of reagent purity.

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at reflux for 20 hr with benzalacetone (1.0 g, 0.0069 mole) in 50 ml of methanol. At this time, TLC (cyclohexane–ether, 1:1) indicated that all of the starting material had been converted to cyclocoumarol (III). The solvent was removed under reduced pressure and the residue was dissolved in 50 ml of acetone. An equal volume of 5 *N* HCl was added, and the solution was agitated in a 37° water bath for 4 hr or until TLC indicated complete hydrolysis to warfarin. Saturated aqueous NaCl (10 ml) was added and the mixture was extracted three times with 50 ml of ether. The combined ether extracts were back-extracted three times with 20 ml of 10% NaOH; the aqueous layer was filtered and reacidified to pH 2 with 5 *N* HCl. The resulting precipitate was collected by filtration, triturated with ether, and dried. Recrystallization from acetone–water (13) gave 1.98 g (93% yield) of I, mp 159–161° [lit. (12) 161°]. The NMR (dimethyl sulfoxide-d_6) was identical to that of authentic warfarin.

6-Hydroxywarfarin [4,6-Dihydroxy-3-(1-phenyl-3-oxobutyl)-2*H*-1-benzopyran-2-one] (VI)—6-Hydroxywarfarin was synthesized following the aforementioned procedure using 4,6-dihydroxycoumarin (0.5 g, 0.0028 mole) and benzalacetone (0.5 g, 0.0034 mole) in 30 ml of methanol. The product was recovered as described above and recrystallized from acetone–chloroform (12) to give 0.83 g (91% yield) of VI, mp 217–220° [lit. (12) 219–220°].

7-Hydroxywarfarin [4,7-Dihydroxy-3-(1-phenyl-3-oxobutyl)-2*H*-1-benzopyran-2-one] (VII)—7-Hydroxywarfarin was synthesized following the aforementioned procedure using 4,7-dihydroxycoumarin (0.2 g, 0.0011 mole) and benzalacetone (0.2 g, 0.0014 mole) in 20 ml of methanol. The product was recovered as described above and recrystallized from acetone–chloroform (12) to give 0.27 g (77% yield) of VII, mp 206–209° [lit. (12) 208–210°].

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Synthesis of 4-Substituted *N*-[(Dimethylamino)methyl]benzamides: New Compounds

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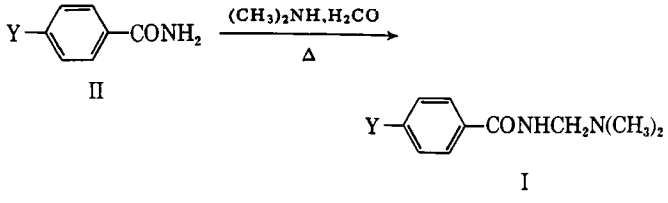
Abstract □ Nine 4-substituted-*N*-[(dimethylamino)methyl]benzamides were obtained in high yield from the corresponding 4-substituted benzoic acids *via* the corresponding benzamides.

Keyphrases □ *N*-[(Dimethylamino)methyl]benzamides—4-substituted synthesis *via* Mannich–Einhorn reaction □ Amidomethylation reaction—synthesis of 4-substituted *N*-[(dimethylamino)methyl]benzamides

To investigate the possible relationship between electronic structure and local anesthetic properties, simple model compounds were required. The 4-substituted *N*-[(dimethylamino)methyl]benzamides[1] (I) were chosen as models since they are closely related to the procainamides, which are widely used as local anesthetics (1). These compounds have the advantage of being readily available from the 4-substituted benzamides (II) in a single step, using the Mannich–Einhorn reaction (2, 3). The 4-substituted benzamides (II) are either commercially available (4), or are readily prepared by conventional methods (5–9).



Y = H,F,Cl,Br,I,OCH$_3$,CH$_3$,N(CH$_3$)$_2$.

Scheme I

This study reports the application of the Mannich–Einhorn reaction (Scheme I) to the synthesis of I, and discusses the preparation of the starting materials (II), which were not commercially available.

EXPERIMENTAL[2]

4-Substituted *N*-[(dimethylamino)methyl]benzamide (I) was prepared by refluxing a mixture of a 4-substituted benzamide (II) (0.071 mole), 40% aqueous dimethylamine (17.4 ml; 0.142 mole), 35% aqueous formaldehyde (12.2 ml; 0.142 mole), and water (15 ml) with stirring for 4 hr.

[1] The key to the labeling of the 4-substituted amides (I) is in Table I.

[2] Melting points were determined in a Kofler apparatus and are uncorrected. IR spectra were determined in a Perkin-Elmer 457A spectrophotometer. NMR spectra were obtained with a Varian T-60 spectrometer using tetramethylsilane as the internal standard. Elemental analyses were performed at the Microanalysis Laboratories of the Instituto de Química—USP, São Paulo.

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