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SYNTHESIS OF FOUR TRITIUM-LABELLED AMINOWARFARIN METHYL KETAL REGIOISOMERS

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SUMMARY

6-Bromo-4'-nitro-, 4'-bromo-6-nitro-, 4'-bromo-7-nitro- and 4'-bromo-8-nitrowarfarin methyl ketals were prepared and together subjected to catalytic tritiation over palladium on charcoal to produce tritium-labelled aminowarfarin methyl ketals. The four regioisomers were separated by thin-layer chromatography to yield pure compounds of high (24.9-29.8 Ci/mmol) specific activity.

Key Words: Warfarin, Cyclocoumarol, Catalytic Tritiation

INTRODUCTION

In addition to its pharmacological utility, the anticoagulant drug warfarin [4-hydroxy-3-(3-oxo-1-phenylbutyl)-2H-1-benzopyran-2-one], has proven to be a useful tool for the study of the cytochrome P450 (P450) superfamily of enzymes (1). Since several different P450 enzymes metabolize the two enantiomers of warfarin at different sites (2,3), this compound is an important probe for defining the nature of the substrate-binding sites of these enzymes. To this end, we have prepared four azido-substituted, regioisomeric analogues of warfarin for use as photoaffinity probes of P450 substrate-binding sites (4). All four compounds (4'-, 6-, 7-, and 8-azidowarfarin) inactivate P4501A1, when photoactivated in its presence (4).

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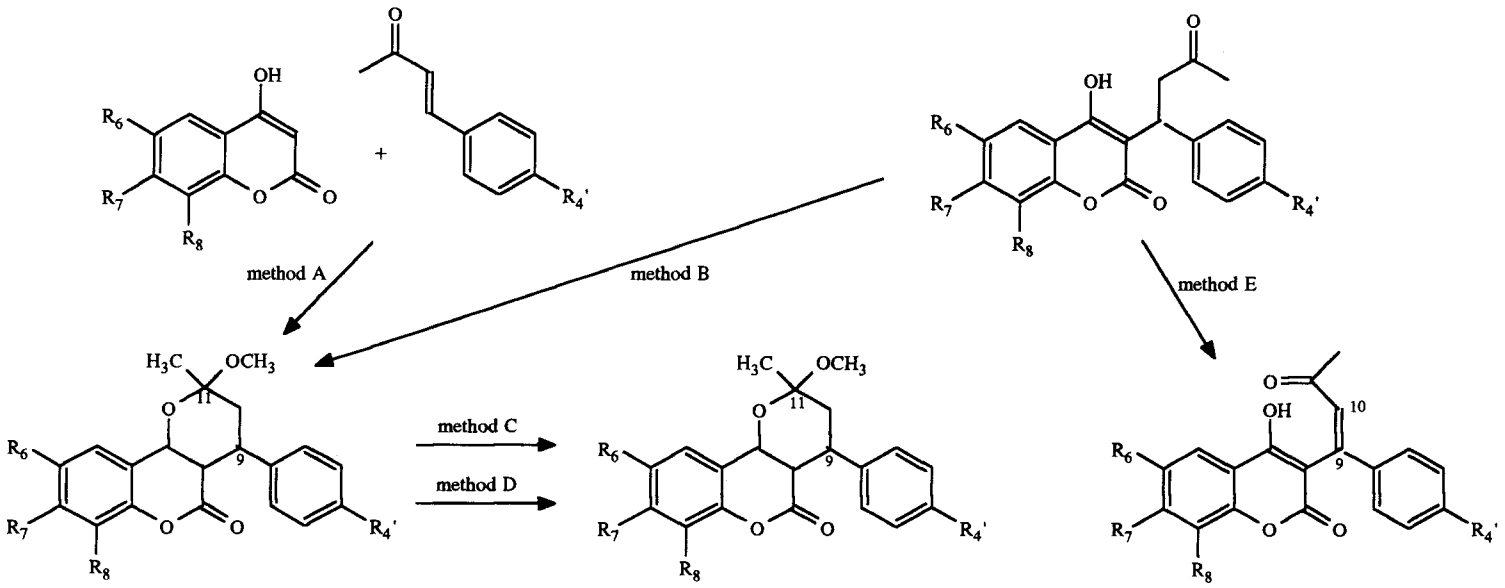
To determine the identities of the P450 substrate-binding-site amino acids that undergo covalent modification by photoactivated azidowarfarins it is necessary to obtain radiolabelled photoaffinity probes. We report here the preparations of 26 new nitro-, amino-, and halo-substituted analogues of warfarin and warfarin methyl ketal (cyclocoumarol; 3,4-dihydro-2-methoxy-2-methyl-4-phenyl-2H,5H-pyrano[3,2-c][1]benzopyran-5-one). These compounds are subsequently used in the successful and unsuccessful preparations of tritium-labelled aminowarfarin methyl ketal regioisomers to be used in the production of tritium-labelled azidowarfarins.

EXPERIMENTAL

Materials. The following synthetic precursors were synthesized by published methods: 4'-nitrowarfarin (5), 6-, 7-, and 8-nitrowarfarin (4), *p*-nitrobenzalacetone (6), *p*-bromo- and *p*-chlorobenzalacetone (7), 6-bromo- and 6-chloro-4-hydroxycoumarin (8), 6-nitro-4-hydroxycoumarin (9), 7-nitro-4-hydroxycoumarin (10), and 8-nitro-4-hydroxycoumarin (11). CuCl was prepared as described (12). Warfarin methyl ketal was prepared and separated into *cis* and *trans* diastereomers as described (13). Commercial sources of the following reagents were: benzalacetone (Fluka Chemical Co., Ronkonkoma, NY), 10% Pd/C, $Fe_3(CO)_{12}$, and butyl nitrite (Aldrich Chemical Co., Milwaukee, WI), and silica TLC sheets, "Baker-flex" (J.T. Baker Chemical Co., Phillipsburg, NJ).

Instrumentation. HPLC analyses were performed using a Waters 990 photodiode array detection system. Ultraviolet spectra were determined using a Beckman DU-70 uv/vis spectrophotometer. The HPLC/MS system included a Waters HPLC system with a Vestec Model 101 thermospray interface. C,H,N analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN. Melting points were determined on a Thomas Hoover apparatus and are uncorrected.

Method A, Figure 1: Nitrowarfarin Methyl Ketals (**2**-**6**, **8**-**11**). The nitrowarfarin methyl ketals **2**-**6**, **8**-**11** were prepared from the corresponding substituted 4-hydroxycoumarin (5.1 mmol) and benzalacetone (3.1 mmol), which were refluxed in methanol (50 mL) under a drying



Compounds 1 - 11
Nitrowarfarin Methyl Ketals

	$R_{4'}$	R_6	R_7	R_8
1	NO_2	H	H	H
2	NO_2	Cl	H	H
3	NO_2	Br	H	H
4	H	NO_2	H	H
5	Cl	NO_2	H	H
6	Br	NO_2	H	H
7	H	H	NO_2	H
8	Cl	H	NO_2	H
9	Br	H	NO_2	H
10	H	H	H	NO_2
11	Br	H	H	NO_2

Compounds 12 - 22
Aminowarfarin Methyl Ketals

	$R_{4'}$	R_6	R_7	R_8
12	NH_2	H	H	H
13	NH_2	Cl	H	H
14	NH_2	Br	H	H
15	H	NH_2	H	H
16	Cl	NH_2	H	H
17	Br	NH_2	H	H
18	H	H	NH_2	H
19	Cl	H	NH_2	H
20	Br	H	NH_2	H
21	H	H	H	NH_2
22	Br	H	H	NH_2

Compounds 23 - 26
Nitrodehydrowarfarins

	$R_{4'}$	R_6	R_7	R_8
23	NO_2	H	H	H
24	H	NO_2	H	H
25	H	H	NO_2	H
26	H	H	H	NO_2

Figure 1. Reaction scheme for the preparation of Compounds 1 - 26. Method A: CH_3OH, Δ; Method B: CH_3OH/HCl, Δ; Method C: 10% Pd/C, H_2, EtAc; Method D: $Fe_3(CO)_{12}$, CH_3OH, C_6H_6, Δ; Method E: CuCl, pyridine, 37°C.

tube. After 18 hr to 3 days, the reactions were complete (no 4-hydroxycoumarin remained, as determined by HPLC) and the mixture was allowed to cool. The reaction mixture was filtered and the crystalline product washed with cold methanol. All nitrowarfarin methyl ketals (compounds 1-11) were recrystallized from ethyl acetate.

Method B, Figure 1: Nitrowarfarin Methyl Ketals (1,7). The nitrowarfarin methyl ketals 1 and 7 were prepared from the corresponding substituted nitrowarfarins (0.89 mmol), which were refluxed in methanolic HCl (25 mL) under a drying tube. After 17-22 hr, the mixture was maintained at 4° C to crystallize the product. Suction filtration and washing with cold methanol yielded crystalline products.

Method C, Figure 1: Aminowarfarin Methyl Ketals (12, 15-22). The aminowarfarin methyl ketals 12, 15-22 were prepared from the corresponding substituted nitrowarfarin methyl ketals 1, 4-11 (1.0 mmol), which were dissolved in ethyl acetate (35 mL) and placed in a sealed one-liter flask with 10% Pd/C (0.1 g). The flask was evacuated and washed twice with N_2, then evacuated and filled with H_2. The reaction was rapidly stirred for 1-19 hr, after which the catalyst was removed by filtration through Whatman #1/celite and the solvent removed *in vacuo* to yield solid products. Compounds 12 and 15-22 were recrystallized from heptane/ethyl acetate.

Method D, Figure 1: Aminowarfarin Methyl Ketals (13, 14). 6-Halo-4'-nitrowarfarin methyl ketals 2, 3 (0.9 mmol) and $Fe_3(CO)_{12}$ (0.5 g) were refluxed in benzene (9 mL) and methanol (0.24 mL) for 45-90 min. The hot reaction mixture was filtered through Whatman #1 paper and the filtrate cooled to 4° C to yield crystalline products.

Method E, Figure 1: Nitrodehydrowarfarins (23-26). The nitrodehydrowarfarins 23-26, were prepared from the correspondingly substituted nitrowarfarins (2.6 g, 7.4 mmol), which were shaken at 37° C in an open 50-mL flask with freshly prepared CuCl (1.2 g) in pyridine (9 mL). After 17-21 hr, water (100 mL) and conc. HCl (12 mL) were added and the products were extracted into CH_2Cl_2 (3 x 100 mL). The organic layer was back-extracted with 1M NaOH (3 x 100 mL), acidified with conc. HCl, and the product extracted into ether (3 x 50 mL). The ether was dried (Na_2SO_4), filtered, and the solvent removed *in vacuo* to yield solid

products. All nitrodehydrowarfarins (compounds **23**-**26**) were crystallized from acetone/water.

Method F, Figure 2: Tritiated Aminowarfarin Methyl Ketals. A mixture containing equal quantities of 6-bromo-4'-nitro-, 4'-bromo-6-nitro-, 4'-bromo-7-nitro-, and 4'-bromo-8-nitrowarfarin methyl ketals **3**, **6**, **9**, and **11** (5.0 mg) in dioxane (1.0 mL) and triethylamine (0.112 mL) was catalytically tritiated with 4.5 mg 10% Pd/C and 10 Ci tritium gas. The reaction was vigorously stirred for 90 min, after which the uptake of gas had ceased. The catalyst was removed by filtration, the filter washed with 20 mL methanol, and the solvent removed *in vacuo*. Labile tritium was removed with 3 x 2 mL methanol to yield product containing 200 mCi tritium. (Catalytic tritiation and labile tritium removal was performed by the Amersham Corporation, Arlington Heights, IL.)

The four regioisomers were separated on silica TLC (Baker-flex plates) with ether/heptane/ethyl acetate (3:1:1) (R_f 4'-aminowarfarin methyl ketal **12**: 0.27-0.32; 6-aminowarfarin methyl ketal **15**: 0.35-0.38; 7-aminowarfarin methyl ketal diastereomers **18**: 0.51-0.56; 8-aminowarfarin methyl ketal **21**: 0.63-0.66). The bands were scraped and eluted with methanol (2 x 1.0 mL), and purity was assessed by co-elution with unlabeled standards on HPLC. Yields were: 4'-aminowarfarin methyl ketal-6-^{3}H **12**: 58 mCi (70% chemical yield, 69% radiochemical yield); 6-aminowarfarin methyl ketal-4'-^{3}H **15**: 38 mCi (46% chemical yield, 46% radiochemical yield); 7-aminowarfarin methyl ketal-4'-^{3}H **18**: 38 mCi (50% chemical yield, 45% radiochemical yield); and 8-aminowarfarin methyl ketal-4'-^{3}H **21**: 32 mCi (46% chemical yield, 38% radiochemical yield). Specific activity of each compound was determined by HPLC/UV analysis: 4'-aminowarfarin methyl ketal-6-^{3}H **12**: 29.4 Ci/mmol; 6-aminowarfarin methyl ketal-4'-^{3}H **15**: 29.8 Ci/mmol; 7-aminowarfarin methyl ketal-4'-^{3}H **18**: 27.2 Ci/mmol; 8-aminowarfarin methyl ketal-4'-^{3}H **21**: 24.9 Ci/mmol.

Method G, Figure 2: Attempt at Deuteration of Aminochlorowarfarin Methyl Ketals. A mixture containing equal quantities of **13**, **16**, and **19** (55.7 mg) was dissolved in ethyl acetate (3.5 mL) and mixed with 10% Pd/C (187.3 mg). The reaction was brought to reflux temperature and $NaBD_4$ (60.0 mg) suspended in ethyl acetate (5 mL) was added dropwise over

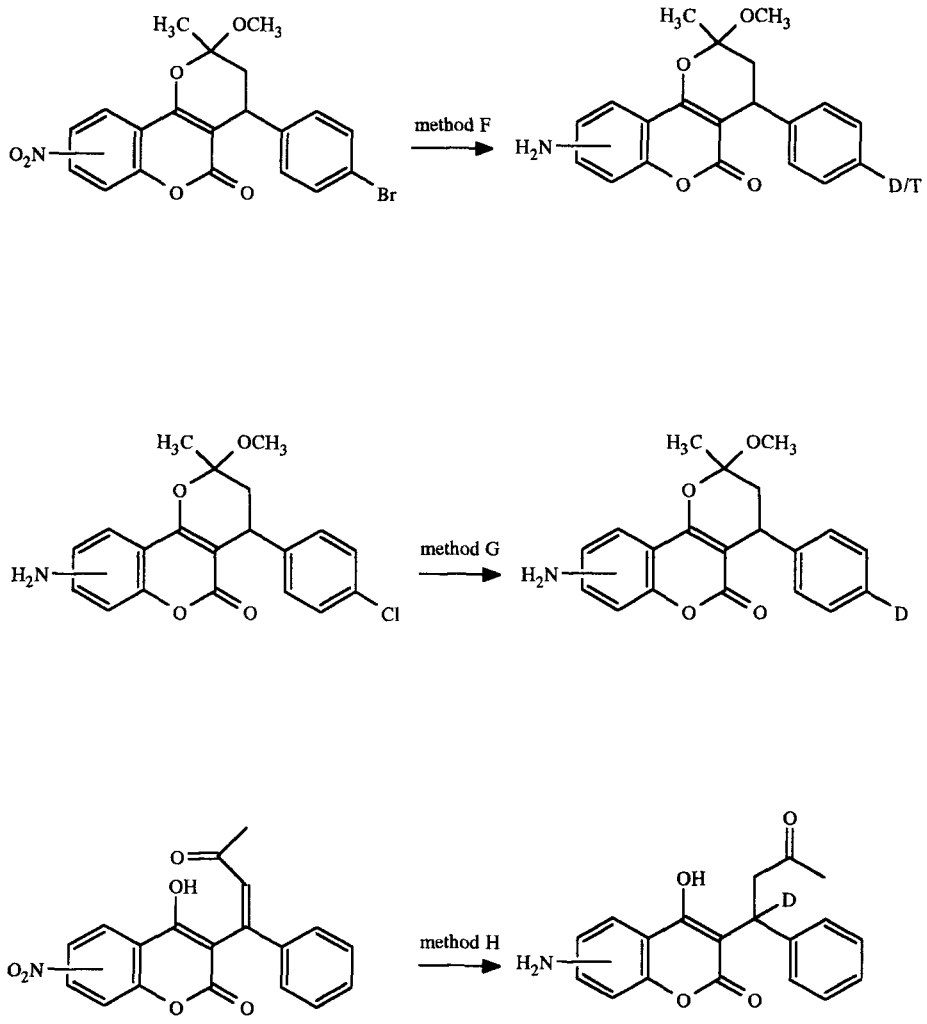


Figure 2. Reaction schemes attempted for the incorporation of tritium into aminowarfarin methyl ketals. Method F: 10% Pd/C, D_2 or T_2, dioxane, triethylamine; Method G: 10% Pd/C, $NaBD_4$, ethyl acetate; Method H: 10% Pd/C, D_2 or T_2, ethyl acetate.

15 min. After 45 min the reaction was complete as judged by HPLC. The cooled mixture was filtered through Whatman #1 paper and the solvent removed *in vacuo* to yield a solid product. MS analysis demonstrated aminowarfarin methyl ketal products with undetectable deuterium incorporation.

Method H, Figure 2: Deuteration of Nitrodehydrowarfarins. A mixture containing equal quantities of **23**-**26** (49.0 mg) in ethyl acetate (12 mL) was stirred with 10% Pd/C (72.6 mg) under deuterium for 24 hr. HPLC analysis showed approximately 50-70% conversion to aminowarfarin products, with the remainder of the material being a more polar by-product. MS analysis demonstrated approximately 80% incorporation of deuterium.

RESULTS AND DISCUSSION

Compounds **1**-**11** (Figure 1, Table 1) were readily prepared from available precursors in yields ranging from 40-90%. The preparations of **1** and **7** involved cyclizing the nitrowarfarin starting materials in refluxing acidic methanol (Method B, Figure 1), while **2**-**6**, **8**-**11** were prepared by condensing the substituted hydroxycoumarin and benzalacetone and affecting cyclization *in situ* in the refluxing methanol reaction solvent (Method A, Figure 1). This method is based on a previously reported method for the preparation of warfarin methyl ketal and hydroxy-substituted analogues (14) and possesses two advantages: (i) By avoiding the use of aqueous reaction solvent, loss of nitrocoumarins due to lactone hydrolysis and decarboxylation (especially in the case of 8-nitro-4-hydroxycoumarin) is obviated. (ii) In all cases, the product crystallized out of the reaction mixture, making product work-up simple. Identities of compounds **1** - **11** were confirmed by LC/MS analysis. In each case protonated mass peaks were observed (*m/z* 368, 404/402, and 448/446 for unsubstituted, chloro-substituted, and bromo-substituted nitrowarfarin methyl ketals, respectively). Mass spectral peaks at *m/z* 385, 421/419, and 465/463 for some of these compounds arise from the formation of ammonium adducts from the ammonium acetate buffer in the solvent system used.

All but two nitrowarfarin methyl ketal compounds were readily converted to the corresponding amino compounds (Table 1) via catalytic hydrogenation using 10% Pd/C in ethyl acetate (Method C, Figure 1). However, the nitro group in compounds **2** and **3** could not be reduced to yield compounds **13** and **14** without concomitant hydrogenolysis of the halogen. For these compounds, reduction of the nitro group to the amine while leaving the halogen intact was affected through the use of $Fe_3(CO)_{12}/CH_3OH$ in refluxing benzene (Method D, Figure 1) (15).

Table 1. Summary of Properties of Compounds 1 - 26

	Compound	Method of Prep.	Time of Reaction	Yield	Product Stereochem.	m.p.	UV/VIS λ_{Max} (nm)	LC MS (*m/z*)	Analysis[a] C	H	N
1	4'-Nitrowarfarin Methyl Ketal	B	17 hr	90%	*trans* > cis (84:16)	–	221,284,306	385,368			
2	6-Chloro-4'-Nitrowarfarin Methyl Ketal	A	40 hr	71%	*cis*	255-6°	225,285,310	421,419,404,402,374,372			
3	6-Bromo-4'-Nitrowarfarin Methyl Ketal	A	40 hr	71%	*cis*	267-9°	225,284,310	448,446	53.66	3.98	2.83
4	6-Nitrowarfarin Methyl Ketal	A	3 d	47%	*trans*	226-8°	219,226,323(sh)	368			
5	4'-Chloro-6-Nitrowarfarin Methyl Ketal	A	22 hr	42%	*trans*	245-7°	222,266,320(sh)	404,402			
6	4'-Bromo-6-Nitrowarfarin Methyl Ketal	A	19 hr	48%	*trans*	246-8°	225,266,321(sh)	448,446,403,401	53.24	3.86	2.82
7	7-Nitrowarfarin Methyl Ketal	B	22 hr	73%	*trans*	214-6°	219,250,336	385,368,338			
8	4'-Chloro-7-Nitrowarfarin Methyl Ketal	A	18 hr	40%	*cis* ≅ *trans* (60:40)	–	224,250,334	404,402,374,372			
9	4'-Bromo-7-Nitrowarfarin Methyl Ketal	A	23 hr	62%	*cis* ≅ *trans* (58:42)	–	226,249,333	465,463,448,446,418,416	53.62	3.86	2.94
10	8-Nitrowarfarin Methyl Ketal	A	41 hr	68%	*cis* > *trans* (84:16)	–	221,279,321	385,368,338			
11	4'-Bromo-8-Nitrowarfarin Methyl Ketal	A	19 hr	40%	*cis*	224-5°	225,270,322	465,463,448,446,418,416	54.31	3.94	2.97
12	4'-Aminowarfarin Methyl Ketal	C	15 hr	67%	*trans* > *cis* (84:16)	–	228,269,280,304	338,306,266,213	70.92	5.83	3.96
13	4'-Amino-6-Chlorowarfarin Methyl Ketal	D	1.5 hr	16%	*cis*	188-90°(d)	229,270,280,313	391,389,374,372			
14	4'-Amino-6-Bromowarfarin Methyl Ketal	D	45 min	45%	*cis*	195-6°(d)	229,272,284,315	418,416,386,384,346,344,162			
15	6-Aminowarfarin Methyl Ketal	C	17 hr	84%	*trans*	215-6°	239,282,348	338,306,266,178	70.60	5.93	4.03
16	6-Amino-4'-Chlorowarfarin Methyl Ketal	C	18 hr	76%	*trans*	212-4°	238,271,348	374,372			
17	6-Amino-4'-Bromowarfarin Methyl Ketal	C	17 hr	78%	*trans*	215-7°	236,270,348	418,416,386,384,338,261,178			
18	7-Aminowarfarin Methyl Ketal	C	19 hr	73%	*trans*	212-4°	233,336	338,306,266,177	70.65	5.66	3.96
19	7-Amino-4'-Chlorowarfarin Methyl Ketal	C	14 hr	27%	*trans* > *cis* (71:29)	–	228,335	374,372			
20	7-Amino-4-'-Bromowarfarin Methyl Ketal	C	2 hr	30%	*trans* > *cis* (78:22)	–	230,335	418,416,386,384,346,344,146			
21	8-Aminowarfarin Methyl Ketal	C	3 hr	81%	*cis* > *trans* (79:21)	–	221,262,292	338,306,267,225,195,189	71.19	5.98	4.04
22	8-Amino-4'-Bromowarfarin Methyl Ketal	C	3 hr	64%	*cis*	208-9.5°	228,262,293	418,416			
23	4'-Nitrodehydrowarfarin	E	21 hr	45%	*cis*	248-55°(d)	225,306	352,351,334,333,303	65.06	3.66	4.04
24	6-Nitrodehydrowarfarin	E	17 hr	50%	*cis*	226-32°	225,287	352,351,334,333,303	64.96	3.79	3.90
25	7-Nitrodehydrowarfarin	E	21 hr	68%	*cis*	235-40°(d)	225,261,382	352,351,334,333,303	64.96	3.75	3.96
26	8-Nitrodehydrowarfarin	E	21 hr	64%	*cis*	224-6°(d)	227,291	369,352,351,334,304			

[a] Theoretical analysis values: Nitrobromowarfarin methyl ketals: C(53.83), H (3.61), N (3.14); Aminowarfarin methyl ketals: C (71.20), H (5.68), N (4.15); Nitrodehydrowarfarins: C(64.96), H (3.73), N (3.99)

Warfarin methyl ketals possess two chiral centers (at positions 9 and 11, Figure 1) thus generating the possibility of two diastereoisomeric pairs. In the case of warfarin methyl ketal itself, both *cis* and *trans* diastereomers are formed with a preponderance of the latter (13). Reacting compounds **12**, **15**, **18**, and **21** with butyl nitrite in dimethylformamide at 60°C results in replacement of the amino group by a hydrogen (without altering the stereochemistry of these compounds) (16). Assignment of *cis/trans* ratios of these compounds was then made by comparing the HPLC retention times of their warfarin methyl ketal products with known standards of *cis* and *trans* warfarin methyl ketal (13) (Table 1). The stereochemistry of the halo-substituted aminowarfarin methyl ketals was then inferred from that of the unsubstituted aminowarfarin methyl ketals on the basis of the fact that, under the analytical HPLC conditions used, all *cis* compounds investigated migrate faster than all *trans* compounds. Surprisingly, the ratio of diastereomers differed from that of the parent warfarin methyl ketal. In several cases, formation of one diastereomer predominates (>99%) during the reaction, and the isolated product is a pure diastereomer. In all cases, nitro-substitution at the 6-position (**4**, **5**, **6**) resulted in the formation of only *trans*-diastereomers. 7-Nitrowarfarin methyl ketal (**7**) was a pure *trans* product whereas the corresponding 4'-halo-7-nitro compounds (**8** and **9**) were almost equal mixtures of *cis* and *trans* isomers. Some conversion of *cis* to *trans* product occurred during the catalytic hydrogenation of **8** and **9** to **19** and **20**, but this was not observed for any of the other regioisomers. The formation of **1** favored the *trans* isomer whereas **2** and **3** were only *cis*. This is the only case where the presence of the halogen reversed the stereochemical outcome of the reaction. (This outcome is actually fortuitous because the conversion of **3** to 4'-aminowarfarin methyl ketal-6-^{3}H results in only *cis* product. *trans*-4'-Aminowarfarin methyl ketal co-elutes with *trans*-6-aminowarfarin methyl ketal on all TLC systems tested for use in separation of the tritiated aminowarfarin methyl ketal products. Had this isomer been formed, it would have complicated purification of the radiolabelled products. See below.) The diverse stereochemical outcomes of these reactions merit further investigation.

The preparations of the nitrodehydrowarfarins (**23**-**26**, Table 1) were undertaken using CuCl in pyridine in a flask open to air following a procedure previously described for the

preparation of dehydrowarfarin (Method E, Figure 1) (17). Formation of the cyclic methyl ketals of these compounds indicated that only the *cis* geometric isomer was formed. This was also the case reported for the parent compound (17).

Three methods (F-H, Figure 2) for the incorporation of tritium into aminowarfarins or aminowarfarin methyl ketals were tested using deuterium incorporation. Method H involves catalytic deuteration of both the nitro group and C9-C10 double bond of compounds **23**-**26** to form (after exchange of amino and α-keto deuterium atoms) aminowarfarins-9-^{2}H. This reaction proceeded to generate 50-70% yield of aminowarfarins that were 80% enriched with deuterium as determined by HPLC/MS. Additionally, some aminowarfarin alcohol by-products resulting from reduction of the carbonyl group were observed as evidenced by a peak with *m/z* 327. Despite the success of this reaction with deuterium, when tritiation was attempted using identical procedures, neither tritiated aminowarfarins nor alcohol by-products were observed. (No identification of products could be made.) The reason for the failure of this approach remains unresolved.

Method G is based on a report of specific deuterium incorporation into aryl halides using $NaBD_4$ and Pd/C in CH_3OD (18). Use of this procedure on compounds **13**, **16**, and **19** in an aprotic solvent (ethyl acetate) resulted in reaction to the dehalogenated products without any observable deuterium incorporation. It is likely that this reaction is of a radical nature and involves abstraction of hydrogen atoms from the solvent or the amino nitrogen of the substrate. Regardless of the mechanism, this method could not be used for labelling the amino warfarin methyl ketals.

Tritium labelled aminowarfarin methyl ketals were successfully prepared via catalytic tritiation of bromonitrowarfarin methyl ketals (compounds **3**, **6**, **9**, and **11**) (Method F, Figure 2). Test reactions using deuterium gave a quantitative yield of product, with over 80% incorporation of deuterium as assessed by mass spectrometry. The reaction went to completion after one hour and neither aminobromowarfarin methyl ketals nor deuterated nitrowarfarin methyl ketals, potential intermediates of this reaction, were observed. Tritiation of 5.0 mg (11.2 μmol) of an equal mixture of **3**, **6**, **9**, and **11** resulted in a product containing 200 mCi of tritium

after removal of labile tritium (59% radiochemical yield). Of this material, 93% corresponded to the tritiated aminowarfarin methyl ketal products. The regioisomers were readily purified by silica TLC. (It should be noted that the use of Baker-flex TLC sheets resulted in resolution of all four regioisomers whereas Kodak silica TLC sheets did not completely separate these compounds.) The products were pure as assessed by HPLC, and specific activity was greater than 24 Ci/mmol.

The described procedure is thus useful for the preparation of four specifically tritiated aminowarfarin methyl ketal regioisomers at one time. Preliminary work has shown that these compounds can be directly converted to the ring-opened azidowarfarins via diazotization and azide substitution. The acidic reaction conditions of the diazotization reaction are sufficient to open the methyl ketal ring. These compounds may then be used in photoaffinity studies of P450 enzymes. The use of radiolabelled photoprobes is essential for answering questions concerning the mechanism by which these enzymes bind their substrates for reaction. Additionally, they could be used to answer questions concerning the pharmacology of warfarin, e.g. identifying the pharmacological receptor for this molecule. It should also be noted that, through the diazotization reaction, tritium-labelled aminowarfarin methyl ketals may be converted not only to azidowarfarins but also to many different substituted warfarin compounds. Thus, these compounds are potentially important synthetic intermediates in the formation of tritium-labelled warfarin analogues.

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REFERENCES

1. Kaminsky L.S. - Drug Metab. Rev. 20:479 (1989)
2. Kaminsky L.S., Fasco M.J. and Guengerich F.P. - J. Biol. Chem. 255:85 (1980)
3. Guengerich F.P., Dannan G.A., Wright J.T., Martin M.V. and Kaminsky L.S. - Biochemistry 21:6019 (1982)

4. Obach R.S., Spink D.C., Chen N. and Kaminsky L.S. - Arch. Biochem. Biophys. (1992) (in press)
5. Wheeler C.R. and Trager W.F. - J. Med. Chem. 22:1122 (1979)
6. Nishimura T. - Bull. Chem. Soc. Jpn. 26:253 (1953)
7. Lutz R.E., Martin T.A., Codington J.F., Amacker T.M., Allison R.K., Leake N.H., Rowlett R.J., Smith S.D. and Wilson J.W. - J. Org. Chem. 14:982 (1949)
8. Buckle D.R., Cantello B.C.C., Smith H. and Spicer B.A. - J. Med. Chem. 18:391 (1975)
9. Karoseva M.G., Smirnova T.V. and Shirokov D.P. - Zh. Vses. Khim. Obshchest. im D.I. Mendeleeva 10:104 (1965); Chem. Abst. 62:16178g (1965)
10. Julia M. and Tchernoff G. - Bull. Soc. Chim. Fr. 779 (1952)
11. Melchiorre C., Giannella M. and Gualtieri F. - Ann. Chim. (Rome) 62:216 (1972)
12. Walton H.F. - Inorg. Prep. 154 (1965)
13. Valente E.J., Lingafelter E.C., Porter W.R., and Trager W.F. - J. Med. Chem. 20: 1489 (1977)
14. Bush E. and Trager W.F. - J. Pharm. Sci. 72:830 (1983)
15. Landesberg J.M., Katz L. and Olsen C. - J. Org. Chem. 37:930 (1972)
16. Doyle M.P., Dellaria J.F., Siegfried B. and Bishop S.W. - J. Org. Chem. 42:3494 (1977)
17. Fasco M.J., Dymerski P.P., Wos J.D. and Kaminsky L.S. - J. Med. Chem. 21:1054 (1978)
18. Bosin T.R., Raymond M.G. and Buckpitt, A.R. - Tet. Lett. 4699 (1973)