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SYNTHESIS OF ^{13}C WARFARIN LABELLED AT THE HEMIKETAL CARBON, AND ITS RESOLUTION

By Van Henry Savell, Jr.[+], Edward J. Valente[+*] and D. S. Eggleston[#]

[+]Department of Chemistry, Mississippi College, Clinton, MS 39058.

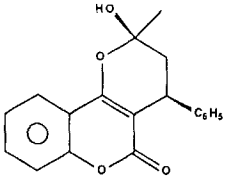
[#]Physical & Structural Chemistry, Smith, Kline & French Laboratories, King of Prussia, PA 19406

[*](To whom correspondence should be addressed.)

Summary. Warfarin (cyclic hemiketal form: 2-hydroxy-2-methyl-4-phenyl-3,4-dihydro-2H,5H-pyrano[3,2-c][1]benzopyran-5-one) is labeled with 98+% ^{13}C at the anomeric carbon (C2) and resolved into its enantiomers. Acetone-2-^{13}C(98.6%) condenses with benzaldehyde in aqueous base to produce 4-phenyl-3-buten-2-one-2-^{13}C(98+%). Michael-type addition of this to 4-hydroxycoumarin in methanol produces the labeled diastereomeric warfarin methyl ketals which on deprotection form racemic warfarin-2-^{13}C(98+%). Classical resolution of labeled warfarin with quinidine produces partly resolved (S)-(-)-warfarin-2-^{13}C(98+%). Labeled warfarin is a suitable probe for warfarin configuration for which three distinct isomeric forms are known.

Key words: Warfarin, Carbon-13, Configuration, Resolution

Introduction. Warfarin, 1, is a widely used, clinically effective oral anticoagulant and rotenticide. While the commercially available form of the drug is the sodium salt of the racemate, the enantiomeric forms are known to possess significantly different potencies in man.(1) A significant fraction of the distributed drug becomes bound to serum proteins.(2) Warfarin exists as a cyclic hemiketal in the solid state(3) and, in relatively non-polar solvents, as a mixture of two diastereomeric hemiketals, 1, 2, and an open-chain keto form 3 (4).



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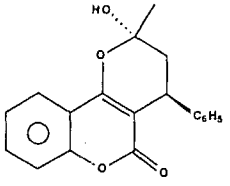

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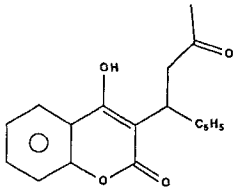

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Aspects of the biological activity and metabolic fate of warfarin have been linked to its stereochemistry and isomeric composition.(5,6)

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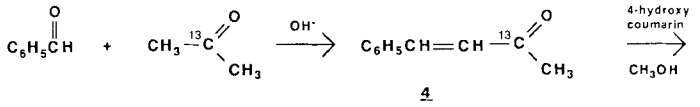
Several labelled syntheses of warfarin have been reported(7-9), but none are constituted with the label in a position particularly sensitive to the isomeric forms of warfarin as sensed by nuclear magnetic resonance spectroscopy. In the present report, we describe a synthesis and resolution of labelled warfarin in which the subject ^{13}C nuclei have well separated, configuration sensitive ^{13}Cmr resonances.

The traditional method for warfarin synthesis(10), involving a Michael-type addition, is a direct and convenient route for introduction of the ^{13}C label into the 2-position of 1 (and by implication into the corresponding positions in 2 and 3). We adapted a more recent procedure(11) which promised an improved yield. Development of this scheme required an efficient route to 4-phenyl-3-buten-2-one-2-^{13}C, and a careful method for base-catalyzed aldol condensation of benzaldehyde with labeled acetone was developed. {An alternative synthesis using $Na^{13}CN$ in several steps(12) was not used.}



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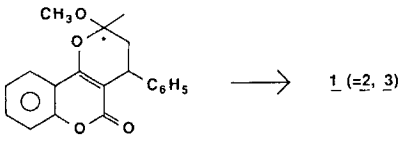

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Experimental. Solvents and reagents were obtained from commercially available sources. Freshly distilled benzaldehyde was stored under nitrogen. Labeled acetone-2-^{13}C(98.6% minimum) was from MSD Isotopes. Simple analyses were performed on Aldrich precoated tlc plates, silical gel with 254 nm fluorescent indicator. Spectroscopy: ^{13}Cmr spectra on a Bruker MSL 200 FT spectrometer, and a JEOL GX500 spectrometer operating at 125.59 MHz; mass spectra on a Nicolet FT-MS by EI and self-CI methods, and a Finnigan 3625 Quadrupole MS by CI methods.

trans-4-Phenyl-3-buten-2-one-2-^{13}C (benzalacetone-2-^{13}C) 4. Following a general procedure (13), various protocols involving ratios of acetone to benzaldehyde near unity were developed. To 8 mL water and 1.471 g (13.9 mmol) benzaldehyde in a 15 mL flask equipped for stirring add 1.00 g (17.2 mmol) acetone-2-^{13}C. After sealing the flask with a septum adaptor, stir at 22°C, and add to it 0.6 mL of 10% NaOH solution slowly by injection. An opaque emulsion forms during vigorous stirring over the next

hour. Then, as the mixture begins to take on a yellowish hue, cool quickly to $0^{o}C$, unseal and extract with 25ml ether, wash the ether solution with 30 mL of saturated $NaHSO_3$ solution. The cooled aqueous reaction mixture can be allowed to warm to ambient temperature and may be extracted and washed twice more over the next several hours. Reduce the volume of the (combined) ethereal solution at $0^{o}C$ with a stream of dry nitrogen and distill in a thin-film (Kugelrohr) apparatus at reduced pressure. Observing no forerun, colorless 4 distills at 353-368K, 4 torr; 1.047 g (42% based on acetone). ^{13}C-nmr ($CDCl_3$): δ(ppm relative to TMS) 198.1 ($^{*}\underline{C}$=O, C2) strong; 27.3 ($\underline{C}H_3$) J_{12} = 42.5 Hz; 127.0,134.3 (H$\underline{C}$=, C3 & C4) J_{23} = 54.0 Hz; 143.2, 128.1, 128.8, 130.4 (phenyl C1', C2', C3', C4', resp.) A very small amount of yellow dibenzalacetone, 1,5-diphenyl-1,4-pentadien-3-one-3-^{13}C, remains; 0.070 g (2%). ^{13}C-nmr ($CDCl_3$): 188.8 ($^{*}\underline{C}$=O, C3) strong; 125.4 (H$\underline{C}$=*C=O, C2/4) J_{12} = 56.5 Hz; other vinyl & phenyl C's as in (4).

(+)-2-Hydroxy-2-methyl-4-phenyl-3,4-dihydro-2**H**,5**H**-pyrano[3,2-c][1]-benzopyran-5-one-2-^{13}C, (racemic warfarin-2-^{13}C) 1-2-^{13}C. In 10 mL of dry methanol, dissolve 1.047 g (7.1 mmol) 4 and 1.153 g (7.11 mmol) 4-hydroxycoumarin and gently reflux the mixture in a Soxhlet extractor with the thimble charged with 3A molecular sieves. Intermediate warfarin methyl ketals, 5, crystallize from the reaction solution after 8 h, but allow the reaction to proceed to 90+% completion (tlc) after 80 h, at which time cool and let 5 crystallize fully. {Most of this material is the major (trans) diastereomer: ^{13}C-nmr ($CDCl_3$) δ ppm (vs. TMS): enhanced signal 101.3 (*C2); 22.2 (natural abundance *C2-$\underline{C}H_3$) $J_{2\text{-}CH3}$ = 48.0 Hz; 43.4 (natural abundance $\underline{C}H_2$-*C2) $J_{2\text{-}CH2}$ = 43.2 Hz; all other nat. abundance C's as in (4,14).} Draw off the solvent, dissolve the solid in 50 mL acetone and add 50 mL 5N HCl; stir for 4 h at $37^{o}C$ upon which a white precipitate forms. Evaporate the acetone, filter the solid and wash well with cold water, recrystallize from acetone:water. Yield 1.62 g 1-2-^{13}C, 31% based on ^{13}C-acetone, mp 432-435 K. ^{13}C-nmr (CDCl3) δ ppm (vs. TMS): 3 enhanced signals, cyclic 1 (major) 99.0 (*C2), cyclic 2 (minor) 100.5 (*C2), open 3 (minor) 212.5 ($^{*}\underline{C}$=O); 27.6, 28.1, 31 (natural abundance CH_3's for 1, 2 & 3, resp.), for 1 $J_{2\text{-}Me}$ = 47.0 Hz, for 2 $J_{2\text{-}Me}$ = 48.0 Hz; 40.0, 42.5, 45.5 (natural abundance CH_2's for 1, 2, & 3, resp.), for 1 $J_{2\text{-}CH2}$ = 41.4 Hz, for 2 $J_{2\text{-}CH2}$ = 41.3 Hz; all other nat. abundance C's as in (4,14). MS: EI (@130°C) 309 (M^{+}); 265 (M-44, corresponding to loss of CH_3-^{13}CO), 187, 121 {assignments as in (15)}; self-CI - ionization with -50 eV beam at 130°C, 500 ms delay during which usually protons transfer to neutrals - calc. for $^{12}C_{18}{}^{13}CH_{16}O_4$ 310.0997 amu, obs. 310.1169 (parent, MH^{+}), 265, 188.

Resolution of Labeled Warfarin (1-2-^{13}C). Modifying a literature procedure (16), dissolve 0.138 g (0.45mmol) racemic 1-2-^{13}C and 0.145 g (0.45mmol) of anhydrous (+)-quinidine in 1.4 mL of acetone and cool to -30^{o}C. Overnight, crystals of the less soluble (S)-1-2-^{13}C·quinidine salt separate. Filter and partition the salt between $CHCl_3$ and 0.5N NaOH solution. Filter the aqueous alkaline solution while dripping into a stirred solution of 2N HCl at 5^{o}C; the (S)-1-2-^{13}C precipitates as a white solid. Wash well with water, filter and recrystallize from acetone:water forming colorless crystals, mp 426.5-430K. MS: (Direct probe CI - CH_4, 310(MH^+), 251(MH-59), 163(parent), 148($C_6H_5CH^+CH_2{}^*C[O]CH_3$); Direct probe CI - NH_3, 310(MH^+), 147(M-162), enrichment based on ratio m/e 310/309 98.8%. CD: 4.6mg/mL in CH_3OH; $[\theta]_{218} = -2.3 \times 10^{-4}$, 22% ee (17). The more soluble (R)-1-2-^{13}C·quinidine salt remains enriched in the original acetone solution. After evaporation of the solvent, similar treatment produces the (R)-1-2-^{13}C.

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