THE SYNTHESIS OF C-2 ISOTOPICALLY LABELED OPTICALLY PURE WARFARIN AND PHENPROCOUMON

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SUMMARY

Optically pure isotopically labeled phenprocoumon was prepared from resolved phenprocoumon via ring opening and decarboxylation followed by recarboxylation with suitably labeled (^{13}C, ^{14}C) diethyl carbonate and ring closure. A similar procedure was followed for the preparation of optically pure isotopically labeled warfarin after protection of the side chain carbonyl group by formation of an ethylene dithioketal derivative. The protecting group was quantitatively removed after incorporation of label by treatment with mercuric acetate.

Key Words: Warfarin, Phenprocoumon, Carbon-13, Carbon-14

INTRODUCTION

Warfarin (3-(1-phenyl-3-oxobutyl)-4-hydroxycoumarin, 1) is an extensively used and clinically effective oral anticoagulant. The drug exists in two enantiomeric forms and it is known that the S isomer is five to six times more potent than the R isomer in both the rat (1) and man (2), although the clinically available form of the drug is the racemate. Recent evidence has demonstrated that in man, the two enantiomeric forms of the drug are metabolized differently (3) and, it has been further demonstrated that prior administration of other drugs, e.g., phenylbutazone, will quantitatively affect these metabolic pathways to different degrees(3).

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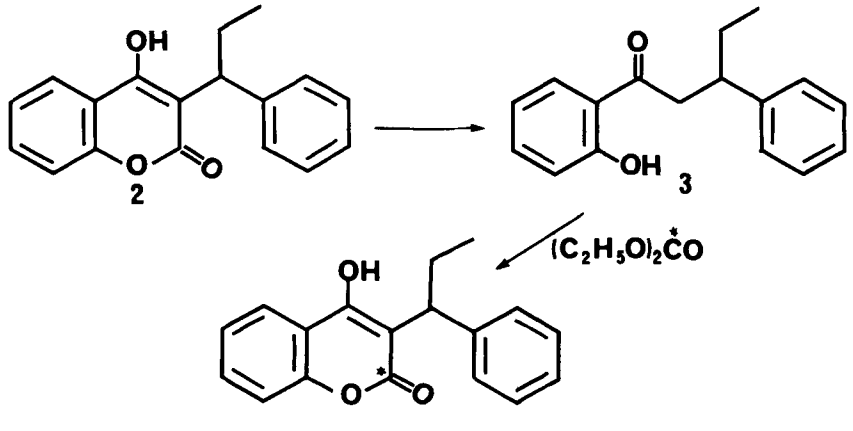
In order to probe the possible involvement of differences in metabolic fate as a cause of the observed pharmacological variability and to gain insight into both the structural and stereochemical factors governing the microsomal transformations of these and other coumarin anticoagulants, the ready accessibility of suitability labeled materials was necessary. We therefore initiated a study to develop a synthetic scheme that would allow us to efficiently incorporate either a radioactive or stable isotope label into a specific position of a common intermediate. Moreover, since most of the drugs we wished to study were optically active, we sought a labeling technique which could also be applied to previously resolved materials. The use of a stable labeled optically active drug would then allow the elucidation of the metabolism of one enantiomer in the presence of its optical antipode by mass spectrometric techniques, a situation which mimics the actual clinical use of the drug.

RESULTS AND DISCUSSION

Goding and West (4) reported the synthesis of R-(+)- and S-(-)-phenprocoumon-2-^{14}C (3-(1-phenylpropyl)-4-hydroxycoumarin) 2 from the previously resolved drug via ring opening, decarboxylation, recarboxylation with suitably labeled diethyl carbonate, followed by ring-closure SCHEME 1.

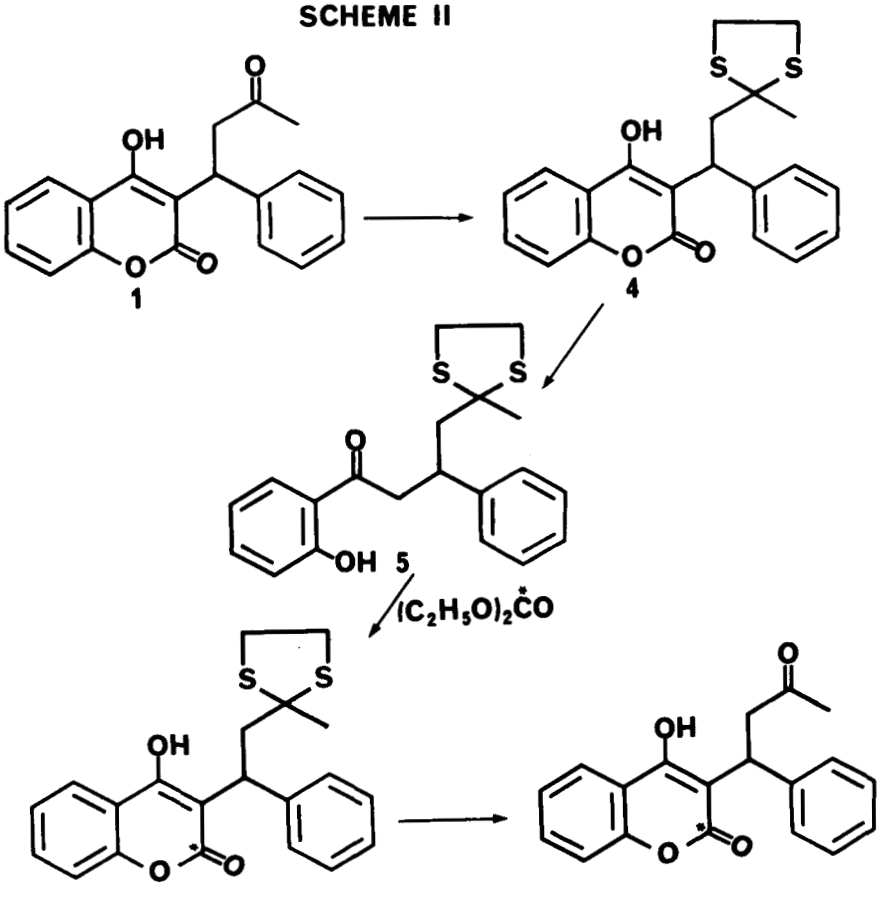
SCHEME I



Their overall yield was not reported, but from the data given (5), the recovery of radioisotope was less than 3%.

Since the synthesis of ^{13}C-labeled drugs with specific degrees of enrichment in the quantities required for metabolic studies demands a more economical recovery of the isotopic label, the entire synthetic sequence used by Goding and West was reinvestigated to determine if better reaction conditions could be obtained. The reaction sequence has now been improved and extended to the preparation of optically active labeled warfarin, the preparation of which had been reported to be unsuccessful (4,5) SCHEME 2.

SCHEME II



Recarboxylation of both 3 and 5 proceeds most efficiently when two equivalents of diethyl carbonate are used. If a single equivalent of diethyl carbonate is used poor yields result due to the more rapid formation of the phenolic ethyl carbonate ester compared to addition of a carboethoxy group to the methylene position adjacent to the carbonyl group (6). The phenolic ethyl carbonate ester of 3 will not cyclize to generate 2 (6). What appears to be necessary for cyclization to occur efficiently is to generate conditions in which a free phenolic anion can attack the carbonyl ester moiety of the carboethoxy group once it has become bonded to the methylene position of 3 (6). Thus use of two equivalents of diethyl carbonate apparently lead to the formation of an intermediate in which two carboethoxy groups have been added to 3, one in the methylene position and one to the phenolic oxygen. Upon completion of the reaction the reaction mixture is treated with 1N NaOH. Base treatment hydrolyzes the phenolic carbonate at a faster rate than the carboethoxy group and thus ring closure occurs.

Removal of the ethylene dithioketal protecting group from 1 proceeds cleanly and consistently provided that at the end of the reaction the mixture is treated with base. An unidentified soluble organomercury compound(s) formed in low yield inhibits cleanup and isolation of 1 if it is not removed (6). Base treatment converts this unknown contaminant to an insoluble precipitate that can be effectively removed by filtration. Upon isolation, compound 1 can be purified by recrystallization from 70% acetone-water or quantitatively converted to the methyl ketal by methanol and hydrochloric acid (7), recrystallized from methanol and converted back to pure 1 via hydrolysis with acidic aqueous dioxane. The latter procedure is particularly useful for purification of small quantities.

EXPERIMENTAL

Solvents and reagents were obtained from common commercially available sources and were used without further purification unless otherwise specified. All melting point determinations were made on a Kofler hot stage and are uncorrected. NMR spectra were run on a Varian T-60 spectrometer. Infrared spectra were recorded on either a Beckman IR-5 or IR-20 spectrophotometer. Optical rotations were measured in 3 dm or 4 dm tubes in a Rudolph Polarimeter.

Eastman Chromogram tlc plates, silica gel with fluorescent indicator, were used for all analysis. Radioactivity measurements were made on a Beckman LS-230 liquid scintillation counter in Aquasol (10 ml). Isotopic compositions of ^{13}C compounds were determined by CI mass spectrometry on a modified AEI MS-9 mass spectrometer.

S-(-)-3-(1-Phenylpropyl)-4-hydroxycoumarin (S-(-)-Phenprocoumon, 2a(9)). - This material was prepared by fractional crystallization of the quinidine salt according to the method of Preis, West and Link (8). $[\alpha]_D^{27} = -125.3° \pm 0.2°$ (C=2, 95% ethanol) mp 172°-173°C [lit. (8) $[\alpha]_D^{28} = -122° \pm 0.5°$ mp 170-171°C].

R-(+)-3-(1-Phenylpropyl)-4-hydroxycoumarin (R-(+)-Phenprocoumon, 2b). - The volume of the filtrate from the previous experiment was reduced under vacuum and the residue partitioned between 5% NaOH and chloroform. The basic solution was acidified with 5N HCl, the precipitate was collected by suction filtration and recrystallized from 70% aqueous ethanol. The precipitate was dissolved in isopropyl alcohol and mixed with a solution of isopropyl alcohol containing an equimolar amount of cinchonidine (Baker, recrystallized from aqueous ethanol). Hexane was added to the isopropyl alcohol solution and the salt crystallized. The precipitate was collected and recrystallized to constant specific rotation: $[\alpha]_D^{25} = 26.8° \pm 0.5°$ (C=2, 95% ethanol). The pure cinchonidine salt was partitioned between equal volumes of 5% NaOH and chloroform. The aqueous phase was separated and acidified to a Congo Red end point with 5N HCl. The precipitate was recrystallized from 70% aqueous ethanol. The ir spectrum was identical to that of the S-(-) isomer: Specific rotation: $[\alpha]_D^{25} = +122.1° \pm 0.3°$ (C=2, 95% ethanol): mp 172-173°C.

S-(+)-1-(2-Hydroxyphenyl)-3-Phenylpentan-1-one, 3a. - This compound was prepared by a modification of the procedure of Goding and West (10). To 35 ml of a saturated sodium borate solution diluted to 10% and 3.2 ml (16 mmol) 5N NaOH was added 4.5 g (16 mmol) of R-(+)-phenprocoumon, 2b. The mixture was warmed gently to give a clear solution and transferred to a stainless steel bomb. The bomb was purged with N_2, heated in an oil bath at 155°C for 24 hr, and then cooled slowly to room temperature. The contents of the bomb were partitioned between 25 ml of dichloromethane and 25 ml of water. The organic phase was extracted with 25 ml of

a saturated sodium borate solution diluted to 20% and dried over magnesium sulfate. The solvent was removed in a stream of N_2. Yield: 3.66 g (90%); ir (neat) 1610 (C=O, H-bonded), 1580 cm^{-1} (Ar); pmr (CCl_4) δ 0.8 (t, 3, $-CH_3$) 1.7 (m, 2, $-CH_2-$) 3.2 (m, 3, $-CHCH_2-$) 6.6 - 7.7 (m, 9, ArH). $[\alpha]_D^{27} = +47.5° \pm 0.1°$ (C = 5.1, 95% ethanol). The product is a colorless oil.

R-(-)-(2-Hydroxyphenyl)-3-Phenylpentan-1-one, 3b. - This compound was prepared by the above method from 2a. The ir spectrum of the product was identical to that of 3a. $[\alpha]_D^{27.5} = -49° \pm 0.1°$ (C=5.3, 95% ethanol). The product is a colorless oil.

S-(-)-4-Hydroxy-3-(1-phenylpropyl)-2H-1-benzopyran-2-one-2-^{13}C (S-(-)-Phenprocoumon-2-^{13}C, 2a-2-^{13}C). - To a 3 neck flask fitted with a magnetic stir bar, heating mantle and an efficient fractionating column topped by a distillation assembly was added 500 ml of sodium dried benzene. Benzene 100 ml, was then distilled to insure removal of traces of water followed by a second 50 ml fraction for use in subsequent additions. A solution of 90% enriched diethyl carbonate-^{13}C (11) (78 mmol) in sodium sulfate dried cyclohexane was added and followed by the removal of another 50 ml of benzene by distillation. The distillation head was removed and the top of the fractionating column fitted with a drying tube filled with drierite. Sodium hydride (590 mg, 11.81 mmol) in a 50% mineral oil dispersion was added to the gently refluxing solution of benzene. Finally, 3b, (1.0 g, 3.94 mmol) in 50 ml benzene was added dropwise over a 15 min period. The reaction was allowed to proceed for 6 days at gentle reflux with stirring. The solution was allowed to cool to room temperature, 50 ml of 1N NaOH added and stirred overnight. The phases were separated and the aqueous phase was acidified to pH 2 with 5N HCl. The precipitate was extracted into ether, the ether dried over sodium sulfate and then removed by flash evaporation. The product was recrystallized from 70% aqueous ethanol. Yield 0.72 g (65%) $[\alpha]_D^{26} = -118° \pm 1.7°$ ir (KBr) 1672 (^{12}C=O), 1629 cm^{-1} (^{13}C=O). The isotopic composition was measured by CI mass spectrometry (methane) and was found to agree with the theoretical enrichment (90%).

R-(+)-4-Hydroxy-3-(1-Phenylpropyl)-2H-1-benzopyran-2-one-2-^{13}C (R-(+)-Phenprocoumon-2-^{13}C, 2b-2-^{13}C). - This compound was prepared from 4 mmol of 3a and 8 mmol of 90% enriched diethyl carbonate-^{13}C in a manner similar to that used

for the preparation of 2a-2-^{13}C. Yield: 0.79 g (72%). The ir spectrum was identical to that of 2a-2-^{13}C.

S-(-)-4-Hydroxy-3-(1-phenylpropyl)-2H-1-benzopyran-2-one-2-^{14}C (S-(-)-Phenprocoumon-2-^{14}C, 2a-2-^{14}C). - This compound was prepared from 3.94 mmol 3b and 7.87 mmol of diethyl carbonate-^{14}C (1.0 mCi/mmol) (11) in a manner similar to that used for the preparation of 2a-2-^{13}C. After 6 days at gentle reflux with stirring the reaction mixture was allowed to cool to room temperature and 50 ml of 1N NaOH was added. The two-phase mixture was stirred overnight and then the phases separated. The aqueous phase was washed with ether then transferred to a 200 ml 3-neck flask fitted with a N_2 inlet and an outlet connected to a trap containing a saturated aqueous solution of barium hydroxide. The solution was cautiously acidified to pH 2 with stirring and allowed to stir for an additional 1 hr at which time no further precipitation of barium carbonate-^{14}C was observed in the trap. The viscuous precipitate was extracted into ether, the ether dried over sodium sulfate and removed by flash evaporation. The residue was crystallized from 70% ethanol. Yield: 0.82 g (74%) 1.0 mCi/mmol. The product gave only one spot on tlc in 5 different solvent systems and had the same R_f as an authentic sample of 2.

R-(+)-4-Hydroxy-3-(1-phenylpropyl)-2H-1-benzopyran-2-one-2-^{14}C (R-(+)-Phenprocoumon-2-^{14}C, 2b-2-^{14}C). - This compound was prepared from 3.94 mmol 3a and 7.87 mmol of diethylcarbonate-^{14}C (1.0 mCi/mmol) in a manner similar to that used for the preparation of 2a-2-^{14}C. Yield 0.68 g (63%), 1.0 mCi/mmol. The product gave only one spot on tlc in 5 different solvent systems and had the same R_f as an authentic sample of 2.

S-(-)-3-(1-Phenyl-3-oxobutyl)-4-hydroxycoumarin (S-(-)-Warfarin, 1a). - Racemic warfarin obtained from acid treatment of the commercial sodium salt (Panwarfarin,^{R} Abbott) followed by recrystallization from aqueous acetone mp 159.5-161.5°C was resolved by the method of Preis, et al. (8) $[\alpha]_D^{25}$= -148° ± 0.4° (C=1.2, 0.5N NaOH), $[\alpha]_D^{25}$ = -117° ± 1.1° (C=1.2, 95% ethanol), mp 173.5-176.2°C (lit. (8) 172-173°C); ir (chloroform) and pmr (chloroform-d) identical to the racemate.

R-(+)-3-(1-Phenyl-3-oxobutyl)-4-hydroxycoumarin (R-(+)-Warfarin, 1b). - The residues from the preparation of 1a were combined and resolved by the method of Preis, et al.

(8); $[\alpha]_D^{25}$ = + 103.3 ± 0.8° (C=1.2, 95% ethanol); ir (chloroform) and pmr (chloroform-d) identical to the racemate.

4-Hydroxy-3-(3,3-ethylenedithio-1-phenylbutyl)-2H-1-benzopyran-2-one (Warfarin Ethylenedithioketal, 4). - This compound was prepared by the procedure of West, et al. (12) reported for the synthesis of the S-(-)-isomer. Yield: 80-89%; mp 192-193°C; ir (KBr) 1672, 1613, 1570, 1497 cm^{-1}; pmr chloroform-d δ 1.84 (s,3,-CH_3), 3.03 (t,2,-CH_2-) 3.33 (s,4,SCH_2CH_2S-), 4170 (m,1,-PhCH-), 7.37 (m,9, ArH), 12.0 (s,1,ArOH).

S-(-)-4-Hydroxy-3-(3,3-ethylenedithio-1-phenylbutyl)-2H-1-benzopyran-2-one (S-(-)-Warfarin Ethylenedithioketal, 4a). - This compound was prepared by the method of West, et al. (12) mp 196-198°C $[\alpha]_D^{25}$ = 108° ± 1.6° (C=1.2, 95% EtOH), (lit. (12) mp 193-195°); $[\alpha]_D^{25}$ = -127° ± 0.5° (C=1.3, 95% ethanol).

R-(+)-4-Hydroxy-3-(3-ethylenedithio-1-phenylbutyl)-2H-1-benzopyran-2-one (R-(+)-Warfarin Ethylenedithioketal, 4b). - This compound was prepared by the method employed by West, et al. (12) for the synthesis of the S-(-) isomer. $[\alpha]_D^{25}$ +111° ± 0.3° (C=1.1, 95% ethanol mp 196-198°C).

5,5-Ethylenedithio-1-(2-hydroxyphenyl)-3-phenylhexan-1-one, 5. - To 20 ml of saturated sodium borate solution diluted to 20% and 3.2 ml (16 mmol) 5N NaOH was added 6.16 g (16 mmol) of 4a. The mixture was placed in a stainless steel container which was purged with N_2 and heated in an oil bath at 155°C for 24 hr, then cooled slowly to room temperature. The product was extracted into dichloromethane, concentrated in vacuo, and the residue recrystallized from hot ethanol. Yield: 4.7 g (90%); mp 68-69°C; ir (KBr) 1645, 1490, 1449 cm^{-1}; pmr chloroform-d δ 1.65 (s,3,-CH_3), 2.48 (d,2,-CH_2-), 3.35 (s,4,SCH_2CH_2S-), 3.41 (d,2,-$COCH_2$-), 3.75 (m,1,PhCH-) 7.32 (m,9,ArH), 12.3 (s,1,ArOH···O=C). Anal. $C_{18}H_{22}O_2S_2$: Calc: C 67.0, H 6.2, S 17.9: Found: C 67.1, H 6.3, S 17.4

S-(-)-5,5-Ethylenedithio-1-(2-hydroxyphenyl)-3-phenylhexan-1-one, 5a). - This compound was prepared by the above method from 4a. The ir spectrum of the product was identical to that of the racemate. $[\alpha]_D^{25}$ = -25 ± 0.6° (C=1.1, dichloromethane), -25° ± 1.1° (C=1.8, chloroform). The product is a yellow oil.

R-(+)-5,5-Ethylenedithio-1-(2-hydroxyphenyl)-3-phenylhexan-1-one, 5b). - This compound was prepared by the above method from 4b. The ir spectrum of the product

was identical to that of the racemate. $[\alpha]_D^{25}$ = +22.1° ± 1.0° (C=0.9, chloroform). The product is a yellow oil.

S-(-)-4-Hydroxy-3-(3,3-ethylenedithio-1-phenylbutyl)-2H-1-benzopyran-2-one-2-^{13}C, 4a-2-^{13}C). - This compound was prepared from 1.42 g (4.0 mmol) of 5a and 8 mmol of 90% enriched diethyl carbonate-^{13}C (11) in a manner similar to that described for the preparation of 2a-2-^{13}C. The product was recrystallized from 95% ethanol. Yield: 1.21 g (80%); mp 196-198°C, ir (KBr) 1623, 1605, 1570, 1479 cm^{-1}.

R-(+)-4-Hydroxy-3-(3,3-ethylenedithio-1-phenylbutyl)-2H-1-benzopyran-2-one-2-^{13}C; 4b-2-^{13}C. - This compound was prepared in the same manner from 5b and diethyl carbonate-^{13}C (90% enriched); Yield: 60% mp 196-198°C ir identical to 4a-2-^{13}C.

S-(-)-4-Hydroxy-3-(3,3-ethylenedithio-1-phenylbutyl)-2H-1-benzopyran-2-one-2-^{14}C, 4a-2-^{14}C. - This compound was prepared from 0.23 g of 5a (0.65 mmol) and 1.30 mmol diethyl carbonate-^{14}C (5 mCi/mmol) in a manner similar to that used for the preparation of 2a-^{14}C. In this case the reaction was run only 5 days as no starting material was left at the end of this time (tlc, toluene: acetic acid, 300:1). The product was recrystallized from 95% ethanol. Yield: 72%.

R-(+)-4-Hydroxy-3-(3,3-ethylenedithio-1-phenylbutyl)-2H-1-benzopyran-2-one-2-^{14}C; 4b-2-^{14}C. - This compound was prepared from 0.6 mmol of 5b and 1.30 mmol diethyl carbonate-^{14}C (5 mCi/mmol) in a similar manner to that used for the preparation of 4a-2-^{14}C. The product was recrystallized from 95% ethanol. Yield 70%.

S-(-)-3-(1-Phenyl-3-oxobutyl)-4-hydroxycoumarin-2-^{13}C. 1a-2-^{13}C. - A magnetically stirred solution containing 1.92 g (5.0 mmol) of 4a-2-^{13}C in 130 ml acetone was warmed and maintained at 55°C in a water bath. A solution of 3.84 g (11 mmol) of mercuric acetate in 100 ml of water was added all at once to the acetone solution and the mixture allowed to stir for 45 min. The mixture was cooled, filtered through a celite pad and washed with 20 ml portions of acetone 3 times. The filtrate was basified (pH 12) with 1N NaOH and the acetone removed by flash evaporation. The basic solution contained a yellow precipitate which was removed by filtration through a celite pad. The celite pad was washed with 10 ml of 0.1N NaOH. The filtrate was allowed to stand for 2 hr, filtered through the celite pad for a third time and the pad washed with 10 ml of 0.1N NaOH. The combined filtrate and

washing was acidified to pH 3 with 5N HCl, the precipitate collected by filtration and crystallized from 70% acetone-water. Yield: 1.15 g (75%) mp 174-175°C, ir (KBr) 1650, 1621, 1570, 1493 cm^{-1}. The isotopic composition of the product was measured by chemical ionization mass spectroscopy, using isobutane as the reagent gas, and found to agree with the calculated enrichment (90%).

R-(+)-3-(1-Phenyl-3-oxobutyl)-5-hydroxycoumarin-2-^{13}C, 1b-2-^{13}C. - This compound was obtained from 4b-2-^{13}C in a manner similar to that described for the preparation of 1a-2-^{13}C. Yield 70%; mp 174-176°C.

S-(-)-3-(1-Phenyl-3-oxobutyl)-4-hydroxycoumarin-2-^{14}C, 1a-2-^{14}C. - This compound was obtained from 4a-2-^{14}C in a manner similar to that described for the preparation of 1a-2-^{14}C. Yield 74%.

R-(+)-3-(1-Phenyl-3-oxobutyl)-4-hydroxucoumarin-2-^{14}C, 1b-2-^{14}C. - This compound was obtained from 4b-2-^{14}C in a manner similar to that described for the preparation of 1a-2-^{13}C. Yield 72%.

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