

THE SYNTHESIS OF DEUTERIUM LABELLED METABOLITES OF
WARFARIN AND PHENPROCOUMON

BY

L. D. Heimark, S. Toon, L. K. Low, D. C. Swinney
and W. F. Trager

Department of Medicinal Chemistry
School of Pharmacy
University of Washington
Seattle, Washington 98195

SUMMARY

The synthesis of deuterium labelled 4'-,6-,7- and 8-hydroxy metabolites of warfarin and phenprocoumon is described. The pentadeuterio labelled 6-,7- and 8-hydroxyphenprocoumons were prepared via alkylation of the respective 6-, 7- and 8-methoxy-4-hydroxycoumarins with 1-(phenyl-d₅)-1-bromopropane and subsequent cleavage of the methyl protecting group with boron tribromide. The synthesis of 1-(pentadeuteriophenyl)-1-bromopropane and the 6-, 7- and 8-methoxy-4-hydroxycoumarins are also presented. The pentadeuterio labelled 6-, 7- and 8-hydroxywarfarins were obtained by reaction of 4-(phenyl-d₅)-3-buten-2-one with the respective 6-, 7- and 8-hydroxy-4-hydroxycoumarins in methanol followed by hydrolysis of the intermediate cyclic methyl ketals in aqueous acid. 4-Hydroxycoumarin-5,6,7,8-d₄, prepared from phenyl-d₆ and tetradeuteriomalonic acid, was reacted with 1-(p-hydroxyphenyl)-1-propanol and 4-(p-hydroxyphenyl)-3-buten-2-one to yield labelled 4'-hydroxyphenprocoumon and 4'-hydroxywarfarin respectively.

Key Words: warfarin, phenprocoumon, metabolites, deuterated internal standards.

INTRODUCTION

The metabolic profiles of the coumarin oral anticoagulants warfarin, 1, and phenprocoumon, 2, (Figure 1) have received considerable attention as a means of studying various aspects of drug metabolizing systems. Both compounds are hydroxylated in either the 4', 6, 7, or 8 position by rat hepatic microsomal preparations (1,2). This conversion of a single substrate to multiple products has been exploited to probe not only the multiplicity of the cytochrome P-450 isozymes in various microsomal preparations (3,4) but also the mechanism of aromatic hydroxylation as catalyzed by cytochrome P-450 (5). More recently research has focused on the in vivo metabolism of these two drugs in man (6,7). Despite their close structural similarity substantial differences in their pharmacokinetics and particularly their susceptibility to drug interactions have been noted. These differences appear to be metabolic in origin. To facilitate both in vitro and in vivo metabolic studies we have developed gas chromatography/mass spectrometry (GC/MS) assays for the 4'-, 6-, 7- and 8-hydroxy metabolites of these two drugs (8,9). In this paper we report the synthesis of the deuterium labelled metabolites of warfarin and phenprocoumon for use as internal standards in the quantitative analysis of these metabolites by GC/MS.

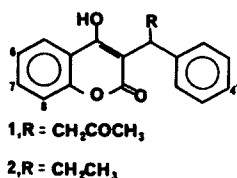


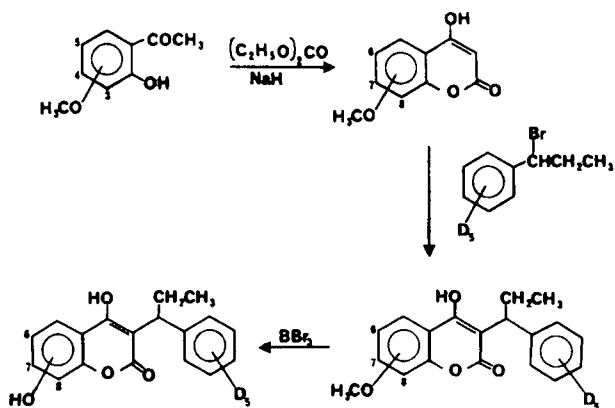
Figure 1. Structures of warfarin, 1 and phenprocoumon, 2.

RESULTS AND DISCUSSION

The synthesis of the warfarin and phenprocoumon metabolites has been reported previously (10,11,12). The preparation of their deuterated analogs follows the same basic synthetic strategy but with some important modifications which improves overall yield and makes more efficient use of deuterated reagents.

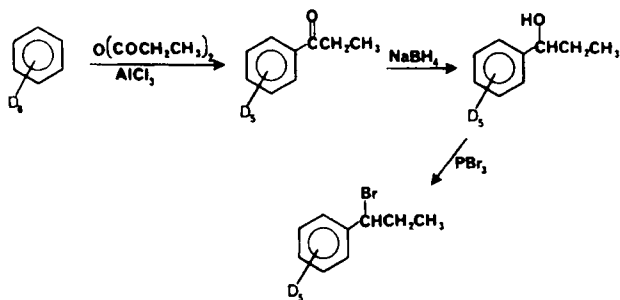
The basic structural unit common to both 1 and 2 is the 4-hydroxycoumarin moiety. Alkylation of the 3-position of 4-hydroxycoumarin with 4-phenyl-3-buten-2-one or 1-phenyl-1-bromopropane yields 1 and 2 respectively. In a similar manner the 6-, 7- and 8-hydroxy metabolites of 1 and 2 may be obtained by reaction of the appropriate electrophile with the respective 6-, 7- and 8-hydroxy derivatives of 4-hydroxycoumarin.

The preparation of the 6-, 7- and 8-hydroxy derivatives of 4-hydroxycoumarin is accomplished by reaction of the appropriate isomeric dihydroxyacetophenones with diethyl carbonate in the presence of sodium hydride (Scheme 1). In the reaction the non-ortho hydroxy group must be protected. The previously reported procedure (10) utilized a benzyl protecting group however much better results were obtained in this step and subsequent steps by use of a methyl protecting group. The 2'-hydroxy-3'-methoxyacetophenone is commercially available and the 2'-hydroxy-4'-methoxy- and 2'-hydroxy-5'-methoxyacetophenones may be readily prepared from the 4-methoxy- and 5-methoxysalicylic acids respectively by reaction with methyl lithium.



Scheme 1.

The deuterium label is incorporated into the 6-, 7- and 8-hydroxy metabolites of 1 and 2 by first labelling the respective electrophiles with deuterium then reacting them with the various hydroxy derivatives of 4-hydroxycoumarin.



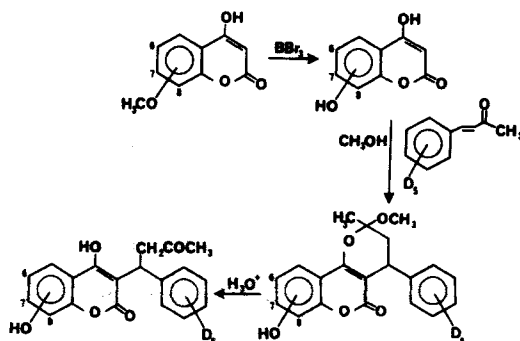
Scheme 2.

The synthesis of 4-(phenyl- d_5)-3-buten-2-one has been previously described (13). The synthesis of 1-(phenyl- d_5)-1-bromopropane, 10, (Scheme 2) begins with a Friedel-Crafts acylation of hexadeuteriobenzene using propionic anhydride. The resultant 1-(phenyl- d_5)-1-propanone is reduced to the alcohol with sodium borohydride and the final product, 1-(phenyl- d_5)-1-bromopropane, obtained by reaction of the alcohol with phosphorous tribromide.

Subsequent reaction of 10 with the phenolic derivatives of 4-hydroxycoumarin also requires protection of the phenolic positions. The methyl protecting group is then removed in the final step by the action of boron tribromide to yield the pentadeuterio labelled 6-, 7- and 8-hydroxyphenprocoumons (Scheme 1).

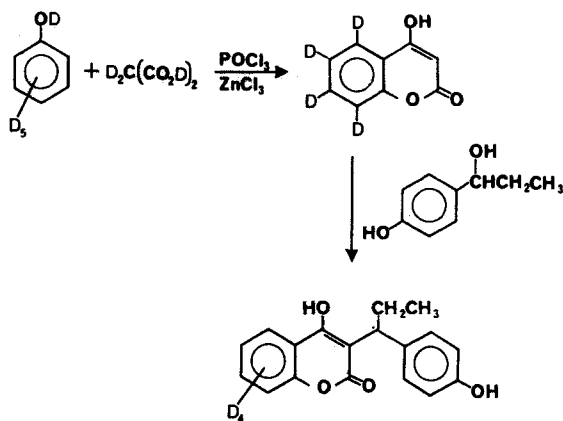
In the case of the 6-, 7- and 8-hydroxywarfarins the methyl protecting group is cleaved prior to reaction with 4-(phenyl- d_5)-3-buten-2-one. Reaction of the dihydroxycoumarins with 4-(phenyl- d_5)-3-buten-2-one in methanol (Scheme 3) initially yields the cyclic methyl ketals which are then hydrolyzed with aqueous acid to the phenyl- d_5 ,6-,7- and 8-hydroxywarfarins.

The 4'-hydroxy metabolites of 1 and 2 require a different method of introduction of the deuterium label. In this case the label was incorporated into the 4-hydroxycoumarin moiety by preparation of 4-hydroxycoumarin-5,6,7,8- d_4 from hexadeuteriophenol and tetradeuteriomalonic acid in the presence of phosphorous oxychloride and zinc chloride (Scheme 4). The tetradeuterio-4-hydroxycoumarin was then reacted with 1-(p-hydroxyphenyl)-1-propanol to give 3-(1-phenylpropyl)-



Scheme 3.

4-hydroxy-(5,6,7,8-tetradeuterio)-coumarin. Reaction of tetradeuterio-4-hydroxy-coumarin with 4-(p-hydroxyphenyl)-3-buten-2-one in methanol and acid hydrolysis of the resultant cyclic methyl ketal yields the corresponding tetradeuterio-labelled 4-hydroxy metabolite of warfarin.



Scheme 4.

EXPERIMENTAL

Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. The NMR spectra were recorded on a Varian EM 360A spectrometer using tetramethylsilane as the reference standard. Mass spectra were recorded on a VG 7070 mass spectrometer.

The 3-methoxysalicylic acid, 5-methoxysalicylic acid, 2'-hydroxy-4'-methoxy-

acetophenone, hexadeuteriophenol, hexadeuteriobenzene, and tetradeuteriomalonic acid were purchased from Aldrich Chemical Co. The 1-(p-hydroxyphenyl)-1-propanol was prepared from p-hydroxypropiophenone (Aldrich Chemical Co.) according to a literature procedure (11). 4-(Phenyl-d₅)-3-buten-2-one was prepared as previously described (13). Low halide methyllithium in diethyl ether, boron tribromide, sodium hydride dispersion in mineral oil and anhydrous aluminum chloride were purchased from Alfa Products. All other solvents and reagents were of reagent purity and obtained from readily available commercial sources.

2'-Hydroxy-3'-methoxyacetophenone(3) - To a dry three neck 2 liter round bottom flask equipped with an efficient condenser, N₂ inlet, magnetic stir bar and addition funnel was added 20 g (0.12 moles) 3-methoxysalicylic acid dissolved in 1 liter of anhydrous tetrahydrofuran (THF). After purging the system with N₂, 0.4 moles of low halide methyllithium/ether solution were transferred under strictly anhydrous conditions to the addition funnel and added to the stirred THF solution at a rate which maintained a gentle reflux. External heat was applied with a heating mantle after the addition of the methyllithium and reflux was continued 24 hours. The reaction was then cooled and rapidly added to a vigorously stirred mixture of 500 ml saturated NaCl solution and 200 g ice, acidified with 6 N HCl and the product extracted with benzene. The benzene extract was washed once with water followed by saturated NaCl solution and then dried over anhydrous Na₂SO₄. Following rotary evaporation of the solvent, recrystallization from cyclohexane yielded 15 g (76%) yellow needles mp 49-50°C [Lit. (14) 53-54°C] NMR (60 MHz, CDCl₃) δ (ppm) 2.65 (s,3H), 3.90 (s,3H), 7.12 (m,3H).

2'-Hydroxy-5'-methoxyacetophenone (4) - By the same procedure used for the preparation of 3, compound 4 was obtained from 5-methoxysalicylic acid in 72% yield. The product was recrystallized from ethanol /H₂O to give pale yellow crystals; mp 49-50°C [Lit. (14) 52°C].

4-Hydroxy-8-methoxycoumarin (5) - To a stirred solution of 3.0 g (90 mmoles) NaH (50% in mineral oil) and 17.7 g (150 mmoles) diethyl carbonate in 300 ml dry benzene under a N₂ atmosphere was added from an addition funnel over 10 minutes 5.0 g (30 mmoles) 3 in 30 ml dry benzene. The mixture was then refluxed 2 days

and worked up by cooling to ambient, stirring overnight with 30 ml 1 N NaOH, separating the two phases and precipitating the product from the aqueous phase with 6 N HCl. The product was filtered, washed with H₂O and after drying 4.0 g (70%) of off-white solid was obtained. Recrystallization from 95% EtOH gave white needles mp. 252-4°C. NMR (60 MHz, d₆-DMSO) δ (ppm) 3.92 (s,3H), 5.61 (s,1H), 7.33 (m,3H). Anal. Calcd. for C₁₀H₈O₄: C, 62.5%; H, 4.17% Found: C, 62.62%; H, 4.25%.

4-Hydroxy-7-methoxycoumarin (6) - Using the same method that was used in the preparation of 5, compound 6 was prepared from 2'-hydroxy-4'-methoxyacetophenone in 78% yield. Recrystallization from acetone/H₂O gave white crystals; mp 255-257°C. NMR (60 MHz, DMSO-d₆) δ (ppm) 7.70 (d, 1H, J=10 Hz), 6.88 (m, 2H), 5.48 (s, 1H), 3.87 (s, 3H). Anal. Calcd. for C₁₀H₈O₄: C, 62.5%; H, 4.17% Found: C, 62.72%; H, 4.14%.

4-Hydroxy-6-methoxycoumarin (7) - In the same manner compound 7 was prepared from compound 4 in 65% yield. Recrystallization from acetone/H₂O gave white crystals; mp 266-268°C. NMR (60 MHz, DMSO-d₆) δ (ppm) 7.24 (m, 3H), 5.62 (s, 1H), 3.82 (s, 3H). Anal. Calcd. for C₁₀H₈O₄: C, 62.5%; H, 4.17% Found: C, 62.42%; H, 4.41%.

1-(Phenyl-d₅)-1-propanone (8) - Anhydrous aluminum chloride (91.77g, 0.69 moles) was added along with 100 g of dry hexadeuteriobenzene to a 3-neck, 500 ml round bottom flask. The flask was cooled in an ice bath and to the stirred reaction mixture was added over 2.5 hr a solution of 35.75 g (0.28 moles) propionic anhydride in 25 ml of hexadeuteriobenzene. After addition was complete the flask was heated at 60°C for 2 hr then cooled and the reaction mixture carefully poured over ice. With vigorous stirring 150 ml of concentrated hydrochloric acid was added and the aqueous and benzene layers separated. The aqueous phase was extracted with benzene (2 X 25 ml). The combined benzene fractions were extracted with H₂O (30 ml) and saturated sodium chloride solution (30 ml) then dried over anhydrous magnesium sulfate. After filtering the benzene solution the solvent was removed under reduced pressure and the product distilled; bp 102°C (18 mm Hg) [Lit. (14) 91.6°C (10 mm Hg)]; yield 25.5 g (65.4 % based on the amount of propionic anhydride), MS m/z 139 [(M+5)⁺], m/z 82 [(M+5) - C₃H₅O]; isotopic purity 93.2% (M+5), 5.8% (M+4), 1.0% (M+3); 93.1% [(M+5) - C₃H₅O], 5.9% [(M+4) - C₃H₅O].

1-(Phenyl-d₅)-1-propanol (9) - An aqueous solution of sodium borohydride was prepared by first dissolving 2.74 g (0.072 moles) NaBH₄ in 6 ml of 2N NaOH and then adding 30 ml of H₂O. This solution was then added over the period of 1 hr to a stirred solution of 25.2 g (0.181 moles) compound 8 in 180 ml of methanol. After stirring an additional hour the excess methanol was removed under reduced pressure. The residue was partitioned between H₂O (100 ml) and ether (60 ml). After separation of the phases the aqueous phase was extracted with ether (2 X 60 ml). The combined ether fractions were washed with H₂O (30 ml) and saturated sodium chloride solution (30 ml) then dried over anhydrous magnesium sulfate. After filtering the ether solution, the solvent was removed under reduced pressure to give a quantitative yield (25.6 g) of product. The product, a clear, colorless liquid, was used without further purification.

1-(Phenyl-d₅)-1-bromopropane (10) - Following the procedure of Pines and Schappell (15) the product was obtained from compound 9 in 76% yield. bp 98°C (12 mm Hg) [Lit. (15) bp 89.5° (6 mm Hg)]; MS m/z 203, 205 [(M+5)]; isotopic purity 93.1% [(M+5)-C₂H₆Br], 5.9% [(M+4)-C₂H₆Br], 1.0% [(M+3)-C₂H₆Br].

3-(1-(Phenyl-d₅)propyl)-4-hydroxy-8-methoxycoumarin (11) - The reaction mixture consisting of 0.96 g (5 mmoles) of 5, 1.1 g (5.5 mmoles) of 10 and 0.82 g (10 mmoles) anhydrous sodium acetate in 25 ml dimethylformamide was heated at 80°C with stirring under a N₂ atmosphere. After 24 hr the reaction was terminated by pouring into 100 ml of ice water. The pH was adjusted to 12 with 4N NaOH and the solution extracted with three 50 ml portions of CH₂Cl₂. The aqueous solution was then acidified with 6 N HCl precipitating the product which was filtered and dried, furnishing 615 mg (41%) of solid. Recrystallization from acetone/H₂O yielded white needles mp 231-3°C; MS m/z 315 [(M+5)⁺]; isotopic purity 93.1% (M+5), 5.9% (M+4), 1.0% (M+3).

3-(1-(Phenyl-d₅)propyl)-4-hydroxy-7-methoxycoumarin (12) - Following the same procedure described for the preparation of 11, compound 12 was prepared from compound 6 in 35% yield. mp 161-163°C; MS m/z 315 [(M+5)⁺]; isotopic purity 92.8% (M+5), 5.4% (M+4), 1.8% (M+3).

3-(1-(Phenyl-d₅)propyl)-4-hydroxy-6-methoxycoumarin (13) - Following the procedure described for preparation of 11, compound 13 was prepared from compound 7 in 39% yield. mp 196-198°C; MS m/z 315 [(M+5)⁺]; Isotopic purity 93.1% (M+5), 5.9% (M+4), 1.0% (M+3).

3-(1-(Phenyl-d₅)propyl)-4,8-dihydroxycoumarin (14) - A stirred suspension of 84 mg (0.27 nmoles) of 11 in 5 ml of dry CH₂Cl₂ under a N₂ atmosphere was cooled to -70°C in a Dry Ice/acetone bath. In a dropwise manner 270 mg (1.16 nmoles) of boron tribromide was added to the stirred solution. After the addition was completed the reaction was stirred for 1 hr at -70°C then at room temperature for 16 hr. The reaction mixture was next poured into 20 ml of ice water, the phases separated and the aqueous phase extracted twice with 10 ml portions of CH₂Cl₂. The organic extracts were combined and partitioned with an equal volume of 1 N NaOH. After separation of the phases the product was precipitated by acidifying the aqueous phase with 6 N HCl, then filtered, washed with water and dried to yield 78 mg (98%) of a white solid; mp 175-177°C [Lit. (16) 177-178°C]. MS m/z 301 [(M+5)⁺]; isotopic purity 93.1% (M+5), 5.9% (M+4), 1.0% (M+3).

3-(1-(Phenyl-d₅)propyl)-4,7-dihydroxycoumarin (15) - Following the same procedure used to prepare 14, compound 12 was converted to compound 15 in 78% yield; white solid, mp 78-80°C; MS m/z 301 [(M+5)⁺]; isotopic purity 92.8% (M+5), 5.4% (M+4), 1.8% (M+3).

3-(1-(Phenyl-d₅)propyl)-4,6-dihydroxycoumarin (16) - In the same manner compound 13 was converted to compound 16 in 84% yield; white solid, mp 85-87°C [Lit (11) mp 81-83°C]; MS m/z 301 [(M+5)⁺]; isotopic purity 93.1% (M+5), 5.9% (M+4), 1.0% (M+3).

(5,6,7,8-Tetradeuterio)-4-hydroxycoumarin (17) - By the method of Shah (17) 1.0 g (10.6 nmoles) hexadeuteriophenol, 1.04 g (10.6 nmoles) tetradeuteriomalonic acid, 4 g anhydrous zinc chloride and 2.8 ml phosphorus oxychloride were heated at 65°C for 36 hr. Recrystallization from EtOH/H₂O furnished 640 mg (37%) of white needles mp 208-210°C [Lit. (17) 213-4°C]. MS m/z 166 [(M+4)⁺]; isotopic purity 81.9% (M+4), 9.2% (M+3), 6.2% (M+2), 2.4% (M+1), 0.3% (M).

3-(1-para-Hydroxyphenylpropyl)-4-hydroxy-(5,6,7,8-tetradeterio)-coumarin (18) -

Following the procedure of Pohl (11) 0.34 g (2.1 mmoles) 17 and 1.13 g. (7.5 mmoles) 1-(p-hydroxyphenyl)-1-propanol were allowed to react to yield 223 mg (36%) of crude product. Recrystallization from EtOH/H₂O gave a white crystalline solid mp 156-158°C [Lit. (11) 162-163°C]. MS m/z 300 [(M+4)⁺]; isotopic purity 81.9% (M+4), 9.2% (M+3), 6.2% (M+2), 2.4% (M+1), 0.3% (M).

4,8-Dihydroxycoumarin (19) - A stirred suspension of 960 mg (5 mmoles) of 5 in 50 ml of anhydrous CH₂Cl₂ under a dry N₂ atmosphere was cooled to -70°C in a Dry Ice/acetone bath. Using a dry syringe 5.0 g (20 mmoles) boron tribromide were then cautiously added to the reaction mixture. After stirring an hour at -70°C, the Dry Ice bath was removed and stirring continued at ambient temperature overnight. The reaction was poured into 100 ml of ice/water; the precipitated product filtered and recrystallized from acetone/H₂O, yielding 705 mg (80%) white needles, mp dec. above 260°C. NMR (60 MHz, d₆-DMSO) δ (ppm) 5.63 (s, 1H), 7.20 (m, 3H). Anal. Calcd. for C₉H₆O₄: C, 60.67%; H, 3.37% Found: C, 60.73%; H, 3.37%.

4,7 Dihydroxycoumarin (20) - By the same procedure used to convert compound 5 to compound 19, compound 20 was prepared from compound 6 in 89% yield. Recrystallization from acetone/H₂O gave a white solid mp 252-255°C. NMR (60 MHz, DMSO-d₆) δ (ppm) 7.67 (d, 1H, J=9Hz), 6.78 (m, 2H), 5.42 (s, 1H).

4,6-Dihydroxycoumarin (21) - In the same manner compound 7 was demethylated to give compound 21 in 86% yield. Recrystallization from acetone/H₂O gave a white solid mp 268-270°C. NMR (60 MHz, DMSO-d₆) δ (ppm) 7.14 (m, 3H), 5.66 (s, 1H).

3-(1-Phenyl-d₅-3-oxobutyl)-4,6-dihydroxycoumarin (22) - This compound was prepared from 4,6-dihydroxycoumarin (500 mg, 2.8 mmoles) and 4-(2',3',4',5',6'-pentadeuteriophenyl)-3-buten-2-one (450 mg, 3.0 mmole) according to the procedure of Bush (12). Recrystallization from acetone/H₂O gave a white solid, mp 217-220°C [Lit. (10) 219-220°C]; yield 70%. MS m/z 329 [(M+5)⁺]; isotopic purity 91.0% (M+5), 7.6% (M+4), 1.4% (M+3).

3-(1-Phenyl-d₅-3-oxobutyl)-4,7-dihydroxycoumarin (23) - In the same manner this

compound was prepared from 20 and 4-(2',3',4',5',6-pentadeuteriophenyl)-3-buten-2-one in 70% yield; mp 206-209°C [Lit. (10) 208-210°C]; MS m/z 329 [(M+5)⁺]; isotopic purity 91.0% (M+5), 7.6% (M+4), 1.4% (M+3).

3-(1-Phenyl-d₅-3-oxobutyl)-4,8-dihydroxycoumarin (24) - In the same manner this compound was prepared from 19 and 4-(2',3',4',5',6'-pentadeuteriophenyl)-3-buten-2-one in 70% yield; mp 183-185°C [Lit. (10) 189-191°C]; MS m/z 329 [(M+5)⁺]; isotopic purity 91.0% (M+5), 7.6% (M+4), 1.4% (M+3).

3-(1-para-Hydroxyphenyl-3-oxobutyl)-4-hydroxy-(5,6,7,8-tetradeuterio)-coumarin (25) - In the same manner this compound was prepared from 17 and 4-(p-hydroxyphenyl)-3-buten-2-one in 57% yield; mp 212-214°C; MS m/z 328 [(M+4)⁺]; isotopic purity 81.9% (M+4), 9.2% (M+3), 6.2% (M+2), 2.4% (M+1), 0.3% (M).

ACKNOWLEDGEMENTS

This work was supported by PHS grant GM 25136 and GM 321565.

References

1. Barker W. M., Hermodson M. A. and Link K. P. - J. Pharm. Exp. Ther. 171:307 (1970)
2. Haddock R. E., Trager W. F. and Pohl L. R. - J. Med. Chem. 18:519 (1975)
3. Pohl L. R., Porter W. R., Trager W. F., Fasco M. J. and Fenton J. W. - Biochem. Pharmac. 26:109 (1977)
4. Porter W. R., Wheeler C. and Trager W. F. - Biochem. Pharmac. 30:3099 (1981)
5. Bush E. D. and Trager W. F. - Biochem. Biophys. Res. Comm. 104:626 (1982)
6. Toon S., Low L. K., Gibaldi M., Trager W. F., O'Reilly R. A., Motley C. H. and Goulart D. A. - submitted Clin. Pharmacol. Therap..
7. Heimark L. D., Toon S., Trager W. F., Goulart D. A. and O'Reilly R. A. - submitted to Eur. J. Clin. Pharmacol.
8. Bush E. D., Low L. K. and Trager W. F. - Biomed. Mass Spectrom. 10:395 (1983)
9. Heimark L. D. and Trager W. F. - Biomed. Mass Spectrom. in press

10. Hermodson M. A., Barker W. M. and Link K. P. - J. Med. Chem. 14:167 (1971)
11. Pohl L. R., Haddock R., Garland W. A. and Trager W. F. - J. Med. Chem. 18:513 (1975)
12. Bush E. and Trager W. F. - J. Pharm. Sci. 72:830 (1983)
13. Howald W. N., Bush E. D., Trager W. F., O'Reilly R. A. and Motley C. H. - Biomed. Mass Spectrom. 7:35 (1980)
14. Weast R. C. - CRC Handbook of Chemistry and Physics (57th Edition), CRC Press, Cleveland, 1976
15. Pines H. and Schappell F. - J. Org. Chem. 29:1503 (1964)
16. Heimark L. D. - Ph.D. dissertation, University of Washington, Seattle, 1983
17. Shah V. R., Bose J. L. and Shah R. C. - J. Org. Chem. 25:677 (1960).