THE SYNTHESIS OF ^{14}C, ^{3}H AND ^{2}H LABELED 7-ETHOXYCOUMARINS

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SUMMARY

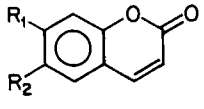
7-Ethoxycoumarins labeled in the ethoxy side chain were prepared from 7-hydroxycoumarin and the correspondingly labeled ethyl iodide or ethyl tosylate. Persulfate oxidation of 7-[1,1-$^{2}H_2$]ethoxycoumarin gave 7-[1,1-$^{2}H_2$]ethoxy-6-hydroxycoumarin. 7-[1,1-$^{2}H_2$]-Ethoxy[6-^{2}H]coumarin was prepared from 7-hydroxy[6-^{2}H]coumarin, obtained via $NaB^{2}H_4/PdCl_2$ reduction of 6-chloro-7-hydroxycoumarin.

Keywords: 7-Ethoxycoumarin, Deuterium, Tritium, Carbon-14, Labeling.

INTRODUCTION

The metabolic O-deethylation of 7-ethoxy-2H-1-benzopyran-2-one,(7-ethoxycoumarin) is a well known cytochrome P450 mediated process.[1] The kinetic mechanism of this reaction has been examined using deuterium and tritium isotope effects[2] from which the intrinsic enzymatic isotope effect, and other kinetic parameters in the processing of the substrate can be determined[3]. Moreover, deuteration of the ethyl side chain has been observed to result in metabolic switching from O-deethylation to ring hydroxylation at position 6[4], giving compound (6) as a unique metabolite of 7-[1,1-$^{2}H_2$]ethoxycoumarin. For the purposes of these studies, a series of labeled coumarins, 1-6, were prepared.

Table I



	R_1	R_2
1.	CH_3CHTO	H
2.	CH_3CD_2O	H
3.	$CH_3^{14}CH_2O$	H
4.	$CH_3^{14}CD_2O$	H
5.	$CH_3\ CD_2O$	D
6.	$CH_3\ CD_2O$	OH

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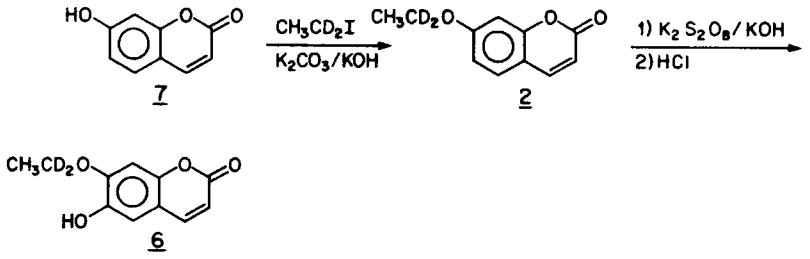
RESULTS AND DISCUSSION

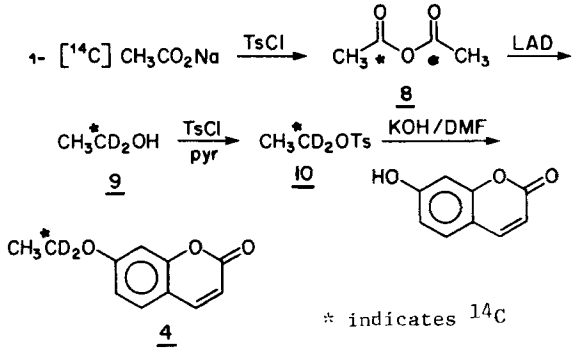
Compounds (2) and (3) were prepared by alkylation of 7-hydroxycoumarin with the appropriate ethyl iodide (scheme I). 7-ethoxy-6-hydroxycoumarin (6) was obtained via alkaline persulfate oxidation of (2)[5]. A mixture of 7-ethoxy-6-hydroxy-, and 6-ethoxy-7-hydroxycoumarin has been prepared previously but the isomers were not separated and characterized[6].

Scheme I



Compound (4) was prepared as shown in scheme II. Sodium 1-[^{14}C] acetate on treatment with p-toluenesulfonyl chloride gave [^{14}C]acetic anhydride[7]. Reduction with LiAl$^{2}H_4$ in diethylene glycol diethyl ether[8] gave [1-^{14}C-1,1-$^{2}H_2$]ethanol (9). Tosylation, followed by coupling with 7-hydroxycoumarin, gave (4).

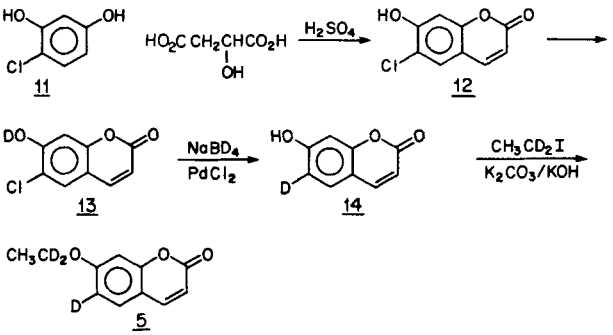
Scheme II



Compound (1) was obtained by the same route from commercially available [1-^{3}H]ethanol.

Compound (5) was prepared as shown in scheme III, from 7-hydroxy[6-^{2}H]coumarin (14) which was obtained from $NaB^2H_4/PdCl_2$ reduction of the corresponding 6-chloro-7-hydroxycoumarin (13) in turn available from Pechmann cyclization of 4-chlororesorcinol with malic acid[9].

Scheme III



Reduction of the deuterium exchanged coumarin, (13), was effected with a large excess of NaB^2H_4 in MeO^2H by a procedure similar to that described for p-chlorobenzoic acid[10]. The yield was variable on repeated runs (17-50%) with control of reaction temperature a critical factor (optimum temperature -8°C). Generally, the reaction was stopped at 50% consumption of (13), and (14) was then isolated by reverse phase HPLC. The conversion could be effected in slightly lower yields in ethanol or DME, with the use of less NaB^2H_4.

EXPERIMENTAL

Mass spectra were recorded on either a Finnegan 3700 or LKB 9000 mass spectrometer. ^{1}H NMR spectra were recorded on either a Varian EM360A or SC300.

Infrared spectra were obtained on a Beckman 421 spectrophotometer. Radioactivity measurements were made with a Packard Prias PLD scintillation counter. $LiAl^{2}H_4$ (98 atom % ^{2}H) was obtained from Aldrich.

Sodium [1-^{14}C]acetate, [1-^{3}H]ethanol, and [1-^{14}C]ethyl iodide were obtained from New England Nuclear.

7-[1,1-$^{2}H_2$]Ethoxycoumarin (2) -- 7-Hydroxycoumarin (3.9 g) was dissolved in 200 ml of ethanol. [1,1-$^{2}H_2$]Ethyl iodide (4.5g) was added followed by potassium carbonate (6.72 g) and 5M KOH (2 ml). The reaction was refluxed overnight, allowed to cool and then evaporated to dryness. The residue was partitioned between CH_2Cl_2 (100 ml) and 0.5 N NaOH (100 ml). The aqueous layer was extracted with 2 x 100 ml CH_2Cl_2 and the combined organic layers washed with 0.5 N NaOH, and water. The organic layer was dried over Na_2SO_4 and evaporated to dryness. The residue was recrystallized from methanol/water to give 3.65 g (79%) of (2). ^{1}H NMR (acetone-d_6) 0.7 (S, 3H), 5.55 (d, 1H, J = 10Hz), 6.2 (d, 1H, J = 2Hz), 6.3 (dd, 1H, J = 9Hz,2Hz), 6.95 (d, 1H, J = 9Hz), 7.25(d, 1H, J = 10Hz) MS, m/z 192 (M^{+}).

7-[1-^{14}C]Ethoxycoumarin (3) -- 7-Hydroxycoumarin (11.7 mg) and K_2CO_3 (18 mg) were added to a small screw cap vial containing a micro stir bar. [1-^{14}C]Ethyl iodide, (0.69 mg, 0.25 mCi) was then transferred to the vial with 1.5 ml of a solution of ethyl iodide (9.5 mg) in ethanol. Two drops of 0.5 N KOH were added, the vial sealed with a cap and heated at 80°C overnight. The reaction was allowed to cool, and evaporated to dryness. The residue was partitioned between CH_2Cl_2 (2 ml) and 0.5 N NaOH (2 ml), and the aqueous layer extracted further with CH_2Cl_2 (2 x 0.5 ml). The CH_2Cl_2 layers were washed with 1 ml of H_2O, and evaporated to give 11 mg of residue. This was purified by HPLC, (Partisil ODS M9, MeOH/H_2O(75/25) 3 ml/min). Yield: 8 mg (64%), 81 uCi of [1-^{14}C]ethoxycoumarin, radiochemical purity 99.3%.

[1-^{14}C 1,1-$^{2}H_2$]Ethanol (9) -- $LiAl^{2}H_4$ (268 mg) was suspended in 3.5 ml of dry diethylene glycol diethyl ether, cooled in ice, and [1-^{14}C]acetic anhydride[7] (163 mg, 14 mCi) added dropwise in 3.5 ml of solvent. The reaction was stirred under N_2 overnight, then cooled in ice and phenoxyethanol, (5.5 ml) added dropwise. The flask was then heated at 100°C for 2 hrs. while passing a stream of N_2 through the solution and the distillate collected in dry ice/acetone. Yield 117 mg (76%), 10.8 mCi. GC analysis indicated only trace impurities (1 m 3% OV17 40°C for 3 min, 25°/min to 170°C).

[1-^{14}C-1,1-$^{2}H_2$]Ethyl tosylate (10) -- [1-^{14}C-1,1-$^{2}H_2$]Ethanol (111 mg, 10.24 mCi) was dissolved in 4 ml of dry pyridine, p-toluenesulfonyl chloride (880 mg) was added and the reaction was left in the refrigerator overnight. The pyridine was removed from the pyridine hydrochloride crystals and added to 10 ml of water. This was extracted with 3 x 10 ml of ether, and the ether layers washed with 1N NaOH (2 x 10 ml), 1 N HCl (10 ml) and H_2O (10 ml). The ether was dried over Na_2SO_4, and evaporated to give 270 mg of oil (58%), which was used directly.

7-[1-^{14}C-1,1-$^{2}H_2$]Ethoxycoumarin (4) -- 7-Hydroxycoumarin (324 mg) was dissolved in 8 ml of DMF, KOH (224 mg) added, and heated at 100° for 20 mins. [1-^{14}C-1,1-$^{2}H_2$]Ethyl tosylate (270 mg) in 4 ml of DMF was added dropwise, and heating continued for 10 min. The reaction was allowed to cool, then 80 ml of H_2O was added slowly with stirring. The flask was cooled in ice for 2 hrs, and the resulting crystals (86 mg) collected. Recrystallization from methanol/water gave 66.5 mg, 1.54 mCi, radiochemical purity, 99.6%. Extraction of the aqueous reaction mixture with CH_2Cl_2 yielded, after methanol/water recrystallization, a further 49 mg. (Total yield 45%).

[1-^{3}H]Ethoxycoumarin (1) -- The tosylate of [1-^{3}H]ethanol (26.5 mg, 25 mCi) was prepared as described for (10). Compound (1) was prepared by reaction with 7-hydroxycoumarin as described for (4) to give 12.2 mg (11%), 2.5 mCi, radiochemical purity, 97.0%.

6-Chloro-7-hydroxycoumarin (12) -- 4-Chlororesorcinol (6 g) and malic acid (7.8 g) were heated in 12 ml of conc. H_2SO_4 for 25 min. at 85°C. After cooling the reaction mixture was added to 100 ml of ice water and the precipitate filtered off. Recrystallization from ethanol gave 1.27 g (16%) of (12). ^1H NMR (DMSO-d_6) 6.1 (d, 1H, J = 10Hz), 6.8 (S, 1H), 7.6 (S, 1H), 7.75 (d, 1H, J = 10Hz) MS, m/z 196 (M^+), 168 ($M^+ - H_2O$). IR (NUJOL) 3160, 1680, 1390, 1240 cm^{-1}.

7-Hydroxy[6-^2H]coumarin (14) -- 6-Chloro-7-hydroxycoumarin (400 mg) was dissolved in 150 ml of dioxane, 5 ml of ^2H_2O added and evaporated to dryness. This was repeated twice. To the residue was added 50 ml of MeOD. This was cooled to -8°C and $PdCl_2$ was added (716 mg). 1.15 g of NaB^2H_4 was then added over a 2 hr. period. The reaction was quenched after the last addition of NaB^2H_4 with 190 ml of 0.3N HCl. This was then extracted with ethyl acetate to give 590 mg of crude (14). The 7-hydroxy[6-^2H]coumarin was purified by HPLC, (Whatman ODS 3 M20 H_2O/MeOH/ACOH 50/50/0.5, 10 ml/min) to give 137 mg. Recrystallization from methanol/water gave 58 mg (17.5%) of pure 7-hydroxy[6-^2H]coumarin. MS, m/z 163 (M^+).

7-[1,1-$^2H_2$]Ethoxy[6-^2H]coumarin (5) -- This was prepared by alkylation of 7-hydroxy[6-^2H]coumarin as described for (9). The product was purified by HPLC, (Whatman ODS 3 M20, MeOH/H_2O 65/35, 10 ml/min). ^1H NMR ($CDCl_3$) 1.58 (S,3H), 6.3 (d,1H, J = 10Hz), 6.85 (S,1H), 7.4 (S,1H) 7.8 (d,1H, J = 10Hz) MS, m/z 193 (M^+), 135.

7-[1,1-$^2H_2$]Ethoxy-6-hydroxycoumarin (6) -- 7-[1,1-$^2H_2$]Ethoxycoumarin (400 mg) was dissolved in 12 ml of pyridine, cooled in ice and solutions of KOH (1.0 g) in 10 ml of H_2O and potassium persulfate (1.0 g) in 12 ml of H_2O added separately dropwise over a 3 hr. period. The reaction was stirred an additional 2 hr. at 4°C and then at room temperature for 24 hrs. The solution was adjusted to pH 4 with conc. HCl, filtered and the filtrate extracted with 3 x 50 ml of ether. The aqueous phase was treated with Na_2SO_3 (0.96 g) and conc. HCl (12.8 ml), and heated at 65°C for 1.5 hrs. It was then extracted with CH_2Cl_2 (3 x 20 ml), and the CH_2Cl_2 evaporated. The residue was recrystallized twice from glacial acetic acid to give 50 mg (11%) of (6), (m.p. 134.5-135.5°C) ^1H NMR (acetone-d_6), 1.45 (S,3H), 6.25(d, 1H, J = 10Hz), 6.95(S,1H), 7.1(S,1H), 7.86(d, 1H, J = 10Hz), IR(NUJOL), 3540, 3360, 1715, 1655, 1610, 1560, 1270, 1160 cm^{-1}, MS, m/z 208 (M^+).

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