SYNTHESIS OF [^{14}C]-LABELLED 3-[N-(4-BROMOPHENYL)CARBAMOYL]-7-CHLORO-4-HYDROXYCOUMARIN AND 3-[N-[5-(TRIFLUOROMETHYL)-1,3,4-THIADIAZOL-2-YL]CARBAMOYL]-7-CHLORO-4-HYDROXYCOUMARIN.

Byung H. Lee*, and Michael F. Clothier
Upjohn Laboratories
The Upjohn Company
Kalamazoo, Michigan 49001

SUMMARY

The novel [^{14}C]-labelled 3-carbamoyl-4-hydroxycoumarins were prepared in two steps from 4-chloro-acetylsalicyloyl chloride (**3**). The isotope was incorporated by the reaction of diethyl malonate-1,3-^{14}C with 4-chloro-acetylsalicyloyl chloride (**3**). Subsequent condensation of the resulting 3-ethoxycarbonyl-4-hydroxy-7-chlorocoumarin (**5a**) with 4-bromo-aniline gave 3-[N-(4-bromophenyl)carbamoyl]-7-chloro-4-hydroxycoumarin (**1a**) with a specific activity of 0.7 mCi/mmol. Condensation of (**5a**) with 2-amino-5-trifluoromethyl-1,3,4-thiadiazole gave 3-[N-[5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl]carbamoyl]-7-chloro-4-hydroxycoumarin (**2a**) with a specific activity of 0.6mCi/mmol.

Key words: Anthelmintic, Coumarin, Malonate, Radiolabelled, Synthesis

INTRODUCTION

The anthelmintic activity of 3-carbamoyl-4-hydroxycoumarins has been reported.[1] Synthetic studies (SAR) on this class of compounds resulted in the preparation of **1** and **2**, which likewise display antiparasitic properties. In order to easily follow the *in vivo* kinetics of absorption of these compounds by parasites (e.g., *Ascaris suum* and *Haemonchus contortus*), the ^{14}C-labelled compounds, **1a** and **2a**, were synthesized. In addition to absorption kinetics, the radiolabelled compounds were also needed to identify the tissue distribution (% of compound in specific tissues) within *A. suum* and to determine if these compounds were metabolized by the parasite.

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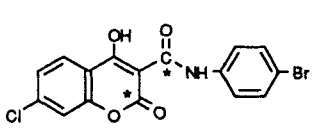
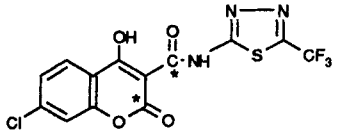


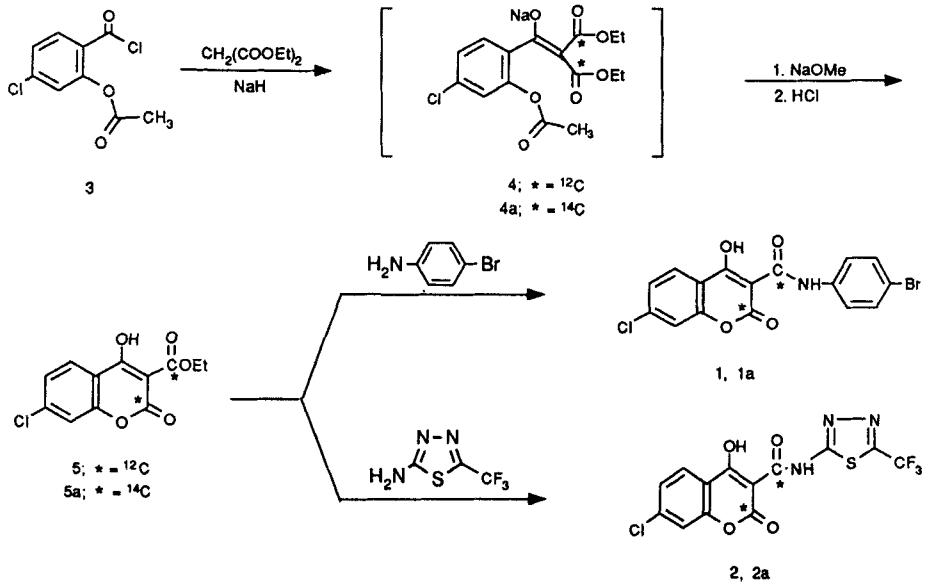
1; * = ^{12}C
1a; * = ^{14}C



2; * = ^{12}C
2a; * = ^{14}C

DISCUSSION

The following modification of a procedure developed by Wilson[2] was used for the preparation of the coumarin esters **5** (and **5a**). Addition of 4-chloro-acetylsalicyloyl chloride (**3**) to the anion of diethyl malonate (or diethyl malonate-1,3-^{14}C), which was generated with sodium hydride, gave intermediate **4** (or **4a**). Cyclization of **4** (or **4a**) on treatment with sodium methoxide in refluxing THF provided **5** (or **5a**) in 80% yield.[1c] Subsequent



3

4; * = ^{12}C
4a; * = ^{14}C

5; * = ^{12}C
5a; * = ^{14}C

1, 1a

2, 2a

condensation of **5** (or **5a**) with 4-bromoaniline gave **1** (or ^{14}C-labelled compound **1a**), while condensation with 2-amino-5-trifluoromethyl-1,3,4-thia- diazole yielded **2** (or ^{14}C-labelled compound **2a**). The purity of **1a** and **2a** was checked by HPLC comparison with **1** and **2.** The final specific activities were 0.7 mCi/mmol for **1a** and 0.6 mCi/mmol for **2a.**

EXPERIMENTAL

Diethyl malonate-1,3-^{14}C was purchased from Sigma Chemical Co. (St. Louis, MO). NMR spectra were obtained on a Bruker Aspect 3000 nuclear magnetic resonance spectrometer at 300 MHz. HPLC assays were carried out on a Varian Vista Series Model 5000 HPLC (Varian, Walnut Creek, CA) equipped with a LDC/Milton Roy (Riviera Beach, FL)

SpectroMonitor D variable wavelength UV detector, a Varian Model 9090 autosampler, and a Radiomatic Flow 1/Beta Model CR radiodetector (Radiomatic, Meriden, CT).

Standard HPLC curves for **1** and **2** were established by dissolving each compound in acetonitrile, diluting with RPMI-1640 (cell culture medium purchased from GIBCO) and adjusting the final acetonitrile concentration to 50% v/v. The concentration ranged from 0.02 to 10 μg/ml. A 50 μl injection was analyzed using a Zorbax SB-C8, 250 x 4.6mm column with a 1.5 cm guard column (Mac-Mod Analytical, Inc., Chadds Ford PA). The mobile phase was 35% 37 mM monobasic sodium phosphate adjusted to pH 7.0 with triethylamine and 65% methanol. Detection was by UV absorbance at 324 nm. The retention times were: **1**, 9.5 min.; **2**, 9.1 min. Compounds **1a** and **2a** were checked for purity using the same HPLC protocol except detection was via radiodetection. In these assays, Radiomatic Flo-Scint (Radiomatic, Meriden, CT) scintillation cocktail at a ratio of 4:1 with the HPLC mobile phase was used.

The tetrahydrofuran (THF), xylene, and dimethyl formamide (DMF) were stored over 4A-molecular sieves.

3-Ethoxycarbonyl-4-hydroxy-7-chloro-coumarin (5).

A slurry of NaH (60% oil dispersion, 47 mg, 1.2 mmol) in THF (5 mL) at 0°C under N_2 was treated dropwise with freshly distilled diethyl malonate (0.08 mL, 0.5 mmol). After 30 min a solution of 4-chloro-acetylsalicyloyl chloride (**3**) (0.11 g, 0.47 mmol) in THF (3 mL) was added dropwise at 0°C and stirred for 45 min. Sodium methoxide (28 mg, 0.5 mmol) was added at room temperature, and the reaction mixture refluxed for 30 min, allowing approximately one-half of the total volume of solvent to distill. After cooling to ambient temperature, the reaction mixture was diluted with H_2O (25 mL) and acidified with conc HCl until ppt formed. The resulting white precipitate was collected, washed with H_2O, and dried to give **5** (0.11 g, 87%) as an off-white solid: mp 145.1°C; ^{1}H NMR ($CDCl_3$) δ 1.47 (t, 1H), 4.50 (q, 2H), 7.33 (s, 2H), 7.95 (dd, 1H), 14.80(s, 1H). A small sample was recrystallized from EtOH: mp 160.4°C, lit[2] mp 159-162°C.

[^{14}C]-3-Ethoxycarbonyl-4-hydroxy-7-chloro-coumarin (5a).

A mixture of diethyl malonate-1,3-^{14}C (0.5 mCi, sp. act. 4.2 mCi/mmol, 0.12 mmol) and diethyl malonate (57 μl, 0.38 mmol) was treated under conditions identical to those described above to give **5a** (108 mg, 85%).

3-[N-(4-Bromophenyl)carbamoyl]-7-chloro-4-hydroxycoumarin (1).

A mixture of **5** (8.0 g, 29.8 mmol) and 4-bromoaniline (5.1 g, 29.8 mmol) was refluxed in xylene (60 mL) and DMF (5 mL) for 1h. The resulting solid, which crystallized when the

reaction mixture reached ambient temp., was collected, washed with diethyl ether, and dried to yield **1** (10.4 g, 88%): mp 228.4°C; ^{1}H NMR ($CDCl_3$) δ 7.39 (d, 2H), 7.41-7.60 (m, 4H), 8.01 (d, 1H), 11.12 (brs., 1H), 14.69 (s, 1H). Anal. Calcd for $C_{16}H_9BrClNO_4$: C, 48.70; H, 2.30; N, 3.55. Found: C, 48.43; H, 2.11, N, 3.51.

[^{14}C]-3-[N-(4-Bromophenyl)carbamoyl]-7-chloro-4-hydroxycoumarin (1a).

A mixture of **5a** (50 mg, 0.19 mmol) and 4-bromoaniline (32 mg, 0.19 mmol) was refluxed in xylene (10 mL), and worked up as described above to give **1a** (65 mg, 88%); mp 225.8°C. Specific activity: 0.70 mCi/mmol; overall radiochemical yield: 50%. HPLC retention was identical to that of **1**.

3-[N-[5-(Trifluoromethyl)-1,3,4-thiadiazol-2-yl]carbamoyl]-7-chloro-4-hydroxycoumarin (2).

A mixture of **5** (6.0 g, 22.4 mmol) and 2-amino-5-trifluoromethyl-1,3,4-thiadiazole (3.7 g, 22.0 mmol) was heated at reflux in xylene (170 mL) for 1 h. Xylene (70 mL) was then distilled during the next 2 h. The reaction mixture was refluxed for an additional 17 h. The solid, which crystallized when the reaction mixture cooled to ambient temp., was collected, washed with acetonitrile (20 mL) and dried *in vacuo* to yield **2** (8.0 g, 90%): mp 246.0°C; ^{1}H NMR ($CDCl_3$ + NEt_3) δ 7.16-7.29 (m, 2H), 8.05 (d, 1H), 10.18 (brs., 1H). Anal. Calc for $C_{13}H_5ClF_3N_3O_4S$: C, 39.86; H, 1.29; N, 10.73. Found: C, 39.77; H, 1.19; N, 10.67.

[^{14}C]-3-[N-[5-(Trifluoromethyl)-1,3,4-thiadiazol-2-yl]carbamoyl]-7-chloro-4-hydroxycoumarin (2a).

A mixture of **5a** (58 mg, 0.22 mmol) and 2-amino-5-trifluoromethyl-1,3,4-thiadiazole (37 mg, 0.22 mmol) was refluxed in xylene (10 mL) and worked up as described above to give **2a** (43 mg, 50%); mp 246.7°C. Specific activity: 0.60 mCi/mmol; overall radiochemical yield: 28%. HPLC retention time was identical to that of **2**.

ACKNOWLEDGMENTS

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REFERENCES

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2. Wilson E.H., U.S. Patent No. 2,887,495 (1959).