SYNTHESIS OF N-(2-PIPERIDYLMETHYL)-2,5-BIS(2,2,2-TRIFLUORO-ETHOXY)BENZAMIDE-CARBONYL-^{14}C ACETATE (FLECAINIDE-^{14}C ACETATE)

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SUMMARY

N-(2-Piperidylmethyl)-2,5-bis(2,2,2-trifluoroethoxy)benzamide acetate (flecainide acetate; R-818), a new antiarrhythmic agent, was labelled with carbon-14 at the carboxamide position for metabolic studies. A three-step synthetic route starting from 2,5-dihydroxybenzoic acid-carboxyl-^{14}C provided chemically pure flecainide-^{14}C acetate in 37.0% overall yield. Thin layer chromatographic analysis indicated that the carbon-14 labelled product (12.28 mCi/mmol) was at least 99.5% radiochemically pure.

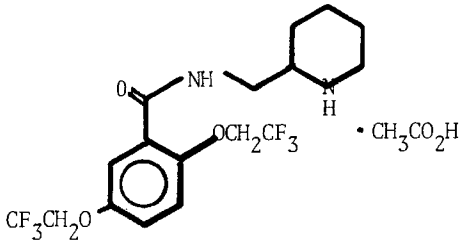
Key Words: Antiarrhythmic, Benzamide, Carbon-14, Flecainide Acetate, Fluorocarbon

INTRODUCTION

N-(2-Piperidylmethyl)-2,5-bis(2,2,2-trifluoroethoxy)benzamide acetate (R-818; flecainide acetate, USAN) is an orally active antiarrhythmic which is effective in suppressing a variety of experimentally-induced cardiac arrhythmias in laboratory animals;[1] the compound is a member of a novel series of trifluoroethoxybenzamides.[2,3] Metabolic studies required preparation of radioisotope-labelled drug. The carboxamide position was selected as an appropriate site for a carbon-14 label on the basis of the established sequence of reactions[3] for preparing R-818 and the probable metabolic stability of the label. In order to simplify the synthesis and to avoid hydrogenation of a radioactive compound, the previously reported route[3] for preparation of R-818 was modified as described herein.

0362-4803/81/050713-08$01.00

Received January 3, 1980



R-818

(flecainide acetate)

DISCUSSION

Flecainide-^{14}C acetate (4) was prepared from 2,5-dihydroxybenzoic acid-carboxyl-^{14}C (1) by the sequence of reactions shown in Scheme I. Intermediate compounds 2 and 3 were isolated and purified but were not individually characterized. The key step in this route is aminolysis of 2 with excess 2-aminomethylpiperidine. Earlier experiments demonstrated that this reaction is highly selective and occurs preferentially at the exocyclic amine function. None of the isomeric tertiary amide, resulting from reaction at the ring nitrogen, was detectable by thin layer chromatography (TLC). In the original synthesis of R-818, the aminolysis step was carried out with 2-aminomethylpyridine.[3] Catalytic hydrogenation of the resultant N-(2-pyridylmethyl)-2,5-bis(2,2,2-trifluoroethoxy)benzamide gave the non-radioactive benzamide corresponding to 3. However, prior preparation of 2-aminomethylpiperidine[4] eliminates the hydrogenation step and results in a significant simplification in the synthesis of 4. Chemically pure 4 was obtained in 37.0% overall yield by the sequence of reactions illustrated in Scheme I. IR and nmr spectra of 4 were identical to authentic R-818. Mixed mp was undepressed. Chemical purity was confirmed by elemental analysis.

The results from TLC analysis for radiochemical purity are shown in Table 1; Rf values for reference R-818 and 4 are essentially identical in each of the eight solvent systems. Radiometric analysis

of the scraped areas from TLC plates showed that the radiochemical purity of the carbon-14 labeled compound was 99.5% or greater in eight different solvent systems. The synthesis starting material, 2,5-dihydroxybenzoic acid, did not account for any of the trace radioactive impurities. However, a by-product in the synthesis, 2,5-bis-(2,2,2-trifluoroethoxy)benzoic acid, appeared to be a trace radioactive impurity (<0.1% of carbon-14) based on results with the solvent systems which resolved the compound.

The specific activity values determined in two different scintillation solvent systems were found to be essentially identical. The specific activity for 4 was found to be 12.28 mCi/mmol (mean value for 24 samples with a standard deviation of 0.11 mCi/mmol).

SCHEME I

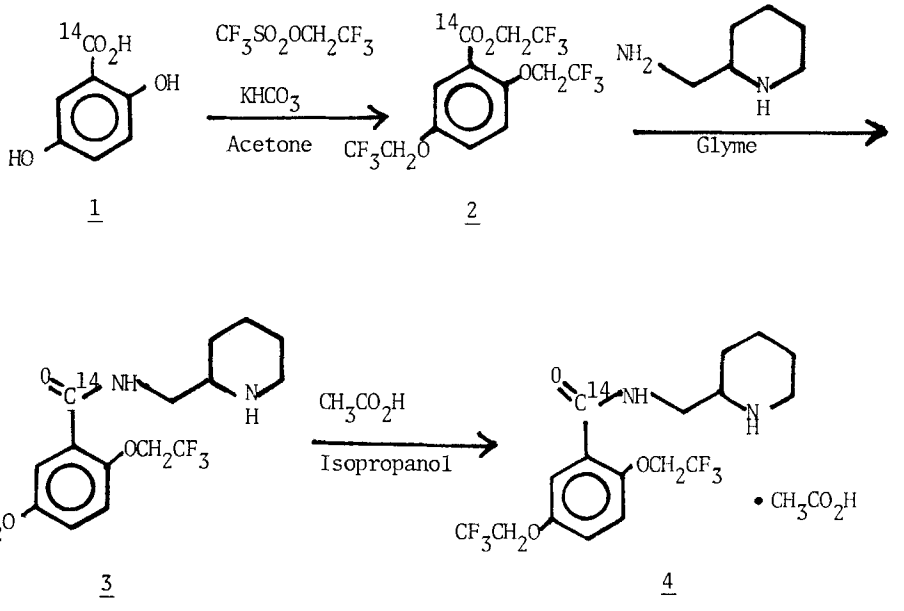


TABLE 1

TLC Analysis for Radiochemical Purity of Flecainide-^{14}C Acetate(4)

TLC Solvent System[a,b]	Rf Value of Reference R-818 (Fluorescent Quench)	Rf Value of 4 (Carbon-14 Measurement)	Rf Value of Reference 2,5-Dihydroxybenzoic Acid	Rf Value of Reference 2,5-Bis(2,2,2-trifluoroethoxy)benzoic Acid	Percent Purity of 4[d]
HOAC:MEOH:$CHCl_3$ (1:9:90)	.05	.07	.15	.51	99.8
TEA:BZ:$CHCl_3$ (10:45:45)	.16	.17	.00	.00	99.7
NH_4OH:MEOH:$CHCl_3$ (1:9:90)	.41	.40	.00	.00	99.5
HOAC:MEOH:$CHCl_3$ (5:35:60)	.49	.53	.67	.82	99.5
HOAC:MEOH:$CHCl_3$ (5:50:45)	.53	.63	.68	.78	99.7
NH_4OH:MEOH:$CHCl_3$ (2:23:75)	.69	.71	.06	.17	99.5
NH_4OH:MEOH:$CHCl_3$ (5:50:45)	.77	.77	.69	*[c]	99.8
NH_4OH:MEOH:$CHCl_3$ (5:35:60)	.84	.83	.36	.54	99.6

[a] v/v percent.

[b] Glacial acetic acid (HOAC); Benzene (BZ); Concentrated (30% NH_3) ammonium hydroxide (NH_4OH) and Triethylamine (TEA).

[c] Fluorescent quench not visible (*).

[d] Percent radiochemical purity was calculated as follows:

$$\text{Percent } 4 \text{ Purity} = \frac{\text{Total cpm in } 4 \text{ Peak (sum of segments corresponding to peak)}}{\text{Total cpm on the plate (sum of all segments scraped)}} \times 100$$

EXPERIMENTAL

Materials and Methods

2,5-Dihydroxybenzoic acid-carboxyl-^{14}C was obtained from Amersham/Searle, Arlington Heights, Illinois in two lots (386 mg, 2.500 mmol, 32 mCi/mmol and 112 mg, 0.715 mmol, 56 mCi/mmol). The two lots were combined for this synthesis (498 mg, 3.215 mmol, 37.325 mCi/mmol). Chemical identity and purity of the final product were confirmed by infrared and 100 MHz nuclear magnetic reasonance spectra, melting point (micro hot stage, uncorrected), and elemental analysis.

Synthesis

2,2,2-Trifluoroethyl 2,5-bis(2,2,2-trifluoroethoxy)benzoate-carbonyl-^{14}C(2)- A solution of 0.472 g 2,5-dihydroxybenzoic acid-carboxyl-^{14}C (1; 37.325 mCi/mmol) and 0.873 g non-radioactive 2,5-dihydroxybenzoic acid (1.345 g, 8.73 mmol total) in 15 ml acetone was added dropwise at ambient temperature to a stirred suspension of 10.0 g (0.1 mol) $KHCO_3$ and 50 ml acetone. The suspension was stirred under reflux 30 min and became very thick as potassium salts precipitated. A solution of 9.284 g (0.04 mol) $CF_3SO_2OCH_2CF_3$[5] in 10 ml acetone was then added to the refluxing mixture over a 30 min period. After the addition was complete, the mixture was stirred under reflux for 72 hrs, cooled and filtered. The filter cake was washed several times with fresh acetone. Concentration of the combined filtrates gave a brown sludge which was slurried with water and extracted two times with ether. The combined ether extracts were washed with brine, dried ($MgSO_4$), filtered and concentrated. Vacuum distillation of the residual brown oil gave 2.405 g (68.9%) of 2,2,2-trifluoroethyl 2,5-bis(2,2,2-trifluoroethoxy)benzoate-carbonyl-^{14}C as a clear, colorless liquid, bp 100°/0.2 mm.

N-(2-Piperidylmethyl)-2,5-bis(2,2,2-trifluoroethoxy)benzamide-carbonyl-^{14}C (3)- Ester 2 (2.356 g, 5.89 mmol) was dissolved in 5 ml glyme and the solution was added dropwise at 25° over a period of 40 min to a stirred solution of 1.343 g (11.78 mmol) 2-aminomethylpiperidine[4] in 5 ml glyme. The clear, colorless reaction mixture was stirred for 18 hrs at 25°, heated under reflux for 1.5 hrs, cooled and concentrated to a viscous yellow syrup consisting of product and water-soluble 2-aminomethylpiperidine. Trituration of this mixture with 40 ml of water caused the product to separate as an ivory solid which was collected by suction filtration and washed several times with fresh portions of water. Crude product was dissolved in 50 ml CH_2Cl_2, and the solution was dried ($MgSO_4$), filtered and concentrated. Recrystallization of the residual ivory solid (2.566 g) from 40 ml cyclohexane provided 1.8670 g (76.5%) of 3 as white crystals, mp 100-102°.

N-(2-Piperidylmethyl)-2,5-bis(2,2,2-trifluoroethoxy)benzamide-carbonyl-^{14}C Acetate (4)- A solution of 0.3576 g (5.96 mmol) CH_3CO_2H in 3 ml isopropanol was added to a suspension of 1.8670 g (4.51 mmol) of compound 3 in 15 ml isopropanol. The mixture was warmed to dissolve any residual solid and filtered. While the solution was kept warm, 55 ml of isopropyl ether was slowly added. The solution was allowed to cool to room temperature, seeded and stored at 0°. The acetate salt precipitated as a heavy, gelatinous solid which weighed 1.773 g after drying. Recrystallization of the crude salt from 18 ml CH_3CN gave 1.5110 g (70.6%) white, crystalline 4, mp 138-141°. The chemical yield from 2,5-dihydroxybenzoic acid was 37.0% and the overall carbon-14 yield was 35.8%. Anal. Calcd. for $C_{17}H_{20}F_6N_2O_3 \cdot C_2H_4O_2$: C, 48.1; H, 5.1. Found: C, 48.4; H, 5.2.

Radiochemical Purity

TLC was used to determine the radiochemical purity of flecainide-^{14}C acetate (4). Eight different solvent systems (Table 1) of varying polarity and composition (acidic and basic) were used for TLC on precoated (10 cm x 20 cm x 0.25 mm) silica gel-GF glass plates (Uniplate®, Analtech, Inc., Newark, Delaware); Rf values for R-818 (Table 1) range from 0.05 to 0.85 in these eight solvent systems.

An aqueous solution of 4 (diluted with non-radioactive R-818) was streaked across about one-half of the TLC plate; approximately 200,000 cpm of carbon-14 (and 10 µg of compound) was applied to each plate. Non-radioactive reference compounds, which included R-818, 2,5-dihydroxybenzoic acid (starting compound for synthesis), and 2,5-bis-(2,2,2-trifluoroethoxy)benzoic acid (by-product), were spotted on the opposite half of the TLC plate. After development to 15 cm, plates were air dried and the location of reference compounds was determined by fluorescent quench under UV light. Then, the portion of each TLC plate spotted with radioactive compound was divided into 0.5 cm segments of adsorbent using a mechanical guide and stainless steel scraper. Individual segments were scraped into counting vials containing 2.5 ml of methanol; after addition of 7.5 ml of toluene-based scintillation solution (PPO and dimethyl POPOP), carbon-14 was measured in a Packard Tricarb Model 3380 Liquid Scintillation Spectrometer at 4°.

Specific Activity

Liquid scintillation spectrometry was also used to determine specific activity. Accurately weighed amounts of 4 were dissolved in water, and aliquots were counted in two different scintillation solvent systems; these were: 1) 15 ml of Aquasol® (New England Nuclear, Boston, Massachusetts)

and 1 ml of water; and 2) 7.5 ml of toluene-based scintillation solution (PPO and dimethyl POPOP) and 2.5 ml of methanol. For correction of count rates to dpm, counting efficiencies were determined by addition of a carbon-14 internal standard to each sample.

ACKNOWLEDGEMENT

The authors gratefully acknowledge Mr. Gary L. Carlson for technical assistance with the radiochemical characterization and the analytical group of 3M Central Research Laboratories for its help with the chemical characterization.

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