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Synthesis and Analysis of the Opioid Analgesic [^{14}C]-Fentanyl

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SUMMARY

The synthesis of [^{14}C]-fentanyl, the radiolabelled congener of the potent opioid analgesic chosen for utilization in drug disposition studies, is described. [^{14}C]-Labelling was achieved in the first of two steps, a room temperature reduction of the *in situ* generated Schiff base from 1-phenylethyl-4-piperidone and [UL-^{14}C]-aniline hydrochloride with sodium triacetoxyborohydride. A nearly instantaneous production of fentanyl was accomplished at room temperature with the addition of propionyl chloride. The overall radiochemical yield was 18%. The method described is efficiently adaptable for submicromolar scale while yielding a product of sufficient specific activity for *in vivo* studies. Our solvent system for thin layer chromatography was superior to the USP system reported for chromatographic analysis of fentanyl. This is the first reported preparation of [^{14}C]-fentanyl with the radiolabel in the aniline benzene ring.

Key Words: [^{14}C]-fentanyl, [UL-^{14}C]-aniline hydrochloride, sodium triacetoxyborohydride, room temperature, Micro Product V-Vial§

INTRODUCTION

Fentanyl {2, N-phenyl-N-[1-(2-phenylethyl)-4-piperidinyl]-propanamide} is a potent analgesic with a faster onset and shorter duration than morphine (1,2). In order to investigate the distribution of this drug as well as metabolites, we required a radiolabelled form. Presently, the ability to monitor the metabolic fate of fentanyl is not without complications. The only radiolabelled form of fentanyl that is commercially available offers the presence of tritium in the phenylethyl

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§Micro Product V-Vial™, trade mark the property of Wheaton, 1000 N. 10th St., Millville, NJ 08332.

benzene ring. However, N-dealkylation at the piperidine nitrogen is a common and extensive route of biotransformation in the mammalian metabolism of fentanyl (3). Thus if a radioisotope was incorporated in the anilidopiperidine substructure, virtually all known metabolic reactions would yield a product bearing this fragment. Incorporation of tritium in the anilino benzene ring is feasible via commercially available [^{3}H]-aniline, however metabolic hydroxylation would result in loss of radiolabel. Therefore, [^{14}C]-aniline should provide the optimal tracer as this choice offers labelled sites that would be resistant to metabolic extraction.

EXPERIMENTAL

The synthesis of 1 was carried out according to the procedure of Abdel-Magid and Maryanoff, except acetic acid was not included (4). In addition, in the workup portions of the syntheses of 1 and 2, care was taken to insure a pH>9 for complete extraction of the free base product. [UL-^{14}C]-Aniline hydrochloride (specific activity of 15.5 mCi/mmol) was purchased from Sigma Chemical Company. All other chemicals were obtained from Aldrich Chemical Company. Tetrahydrofuran (THF) was dried by distillation from sodium benzophenone ketyl anion. All other solvents were of HPLC grade. All reactions were carried out in a clear 3.0 mL capacity Wheaton Micro Product V-Vial with silicon/teflon disc closures. Vigorous stirring was accomplished via a magnetic stirring vane. Dry nitrogen was vented inward by means of stainless steel 22 gauge X 1.5 cm hypodermic needles attached to the nitrogen source by tygon tubing. Analytical TLC was performed using 250 μm silica gel GHLF coated 10 cm X 20 cm glass plates (Analtech) with radioactivity measurements made with a Bioscan System 200 Imaging Scanner. Preparative TLC was carried out using 1.0 mm preadsorbent silica gel GH coated 20 cm X 20 cm plates (Analtech). Spots

on TLC plates were visualized by iodine and 254 nm ultraviolet light. Bands after preparative chromatography were visualized with the latter illumination. The HPLC system for analysis consisted of a Keystone Scientific 4.6 cm X 250 mm Hypersil 5 μm column, a Milton Roy LDC CM4000 solvent delivery system, a Waters Model 712B WISP auto-sampler, and a Kratos 783 UV detector. HPLC radioactivity measurements were done with a Radiomatic IC Flo-one Beta detector. Preliminary experiments to test the feasibility for this small scale synthetic proposal were performed with unlabelled aniline hydrochloride. Identification of products was accomplished with spectra generated by a Bruker AC-300 nuclear magnetic spectrometer using $CDCl_3$ as solvent and TMS as internal standard. Radioactivity measurements were carried out with a Beckman Model LS7800 liquid scintillation counter using Beckman Ready Safe scintillation cocktail as medium.

1-(2-Phenylethyl)-4-(N-[UL-^{14}C]-phenyl)piperidine (1).

[UL-^{14}C]-Aniline hydrochloride (17 mg), 113 mg of unlabelled aniline hydrochloride (0.97 mmol), and 1-phenylethyl-4-piperidone (203 mg, 1.0 mmol) were successively transfered to a 3.0 mL Wheaton V-vial. Dry THF (1.0 mL) was then added with a calibrated pipet. Sodium triacetoxyborohydride (233 mg, 1.0 mmol) was added and the reaction was vigorously stirred at room temperature under a nitrogen atmosphere for 14 hr. Analysis by TLC ($CHCl_3$-CH_3OH-NH_4OH, 95:5:0.5) showed trace amounts of starting materials, a prominent spot that co-chromatographed with an authentic sample of unlabelled 1, and the emergence of an iodine-inactive, UV-active spot just behind 1. The reaction was quenched with saturated aqueous $NaHCO_3$ (ca. 1 mL). Volatiles were removed under a nitrogen stream and then a few drops of 12 N NaOH was added to insure basification (pH>9). The concentrate was extracted with successive additions of ethyl acetate followed

by sustained vigorous stirring for 15 min after each addition of solvent (total extract volume of 8 mL). The combined extracts were applied to the top edge of the preadsorbent area of six preparative chromatographic plates. Separation of desired product 1 from the closely trailing by-product was achieved on elution with $CHCl_3$-CH_3OH-NH_4OH (95:5:0.5). Product was extracted from silica scrapings by stirring in ethyl acetate. Yield of 1 after evaporation of solvent with a nitrogen stream was 73 mg (26%). The ^1H NMR spectrum of unlabelled product isolated in a trial run indicated the following prominent resonances: δ (ppm) 3.34 (singlet, 1H, piperidine C_4-H); 6.54-6.70 (multiplet, 3H, aniline C_2-H, C_4-H, and C_6-H); 7.05-7.32 (multiplet, 7H, remaining aromatic H). The slower by-product was isolated and its weight was found to be less than 1/10th that of 1. The ^1H NMR spectrum of the by-product showed only a multiplet at 7.04-7.52 ppm and a singlet at 2.18 ppm.

N-[UL-^{14}C]-Phenyl-N-[1-(2-phenylethyl)-4-piperidinyl]-propanamide (2).

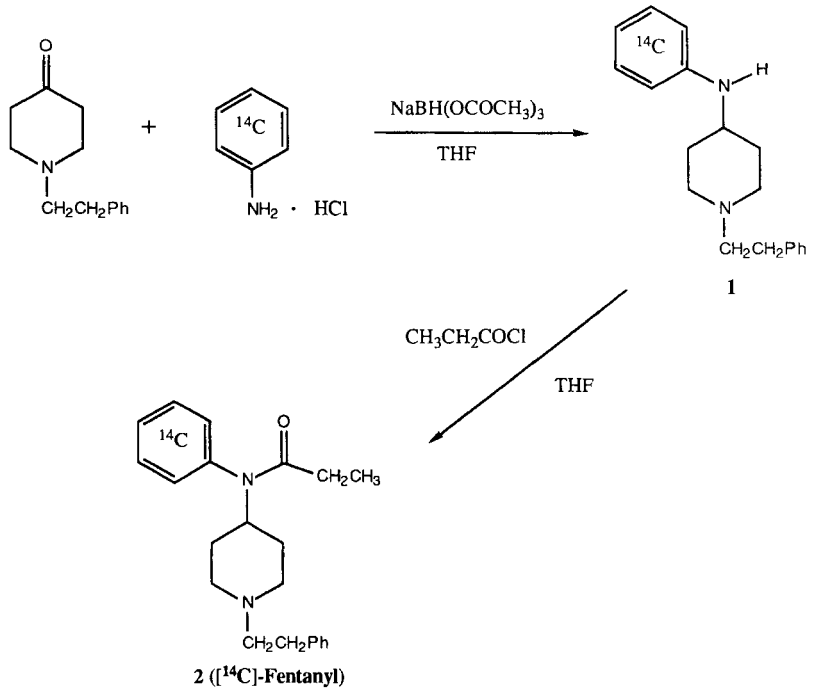
The aniline 1 was mixed with 1.0 mL of dry THF in a 3.0 mL Wheaton V-Vial. Propionyl chloride (52 μL) was added by injection which caused the near instantaneous formation of a white suspension. After 15 min, analysis by TLC showed absence of 1 and emergence of a more mobile spot. A small ice chip was added, then 1 mL of methanol and a few drops of 12 N NaOH (pH>9). The crude mixture was applied to four preparative chromatographic plates followed by elution with the usual solvent system. Yield of 2 after band extraction with ethyl acetate and concentration of solvent with dry nitrogen was 52 mg (59%). Overall chemical yield was 15%. TLC radioactivity analysis of 2 was done by the USP method (R_f = 0.21; $CHCl_3$-CH_3OH-HCO_2H, 85:15:5) and with $CHCl_3$-CH_3OH-NH_4OH (95:5:0.5; R_f 0.60). Analysis by HPLC of 2 with 60%

(CH_3OH-CH_3CN-CH_3CO_2H, 400:200:0.6) and 40% (1% ammonium acetate in CH_3CO_2H, pH = 6.0) indicated R_t values of 5.5 min (UV) and 5.7 min (radioactivity). The 0.2 min lag time was due to the transit time between the two detectors. For all three of these chromatographic systems, 2 was found to be indistinguishable from the USP standard fentanyl. The radiochemical purity of 2 was $\geq$99% with a specific activity of 2.4 mCi/mmol. The overall radiochemical yield was 18%. The ^{1}H NMR spectrum of unlabelled product isolated in a trial run indicated the following prominent resonances: δ (ppm) 1.02 (triplet, 3H, $-COCH_2\underline{CH_3}$), 1.92 (quartet, 2H, $-CO\underline{CH_2}CH_3$), 4.72 (multiplet, 1H, piperidine C_4-H), 7.08-7.44 (multiplet, 10H, aromatic H).

Results and Discussion

For our purposes, we required the final isolation of labelled drug of less than submicromolar mass of sufficient specific activity for mixing with a greater quantity of unlabelled drug for final pharmaceutical application. Historically, the rate determining step in the total synthesis of such 4-(anilido)-piperidine analgesics has been the formation of the intermediate Schiff base obtained on condensation of the aminopiperidone with the appropriate aniline. From our perspective, practical mechanics required a suitable small reaction vessel and avoidance of harsh and tedious reaction conditions, e.g., vigorous refluxing and collecting of water by-product. These requirements were satisfied with the Wheaton Micro Product V-Vial system and the convenience afforded by the recently published procedure of Abdel-Magid and Maryanoff (4). This method allowed for the *in situ* reduction of the Schiff base with sodium triacetoxyborohydride at room temperature (Scheme I). An added advantage was the ability to substitute commercially available [^{14}C]-aniline hydrochloride in lieu of unavailable free base. In agreement

SCHEME I



with the statement of the previous authors regarding their experience with sodium cyanoborohydride, we also found that reducing agent to be sluggish as well as offering no advantage in by-product formation. The application of chloroform-methanol-ammonium hydroxide in our TLC analysis was superior to the corresponding USP system that has been recommended for determination of the purity of fentanyl samples, as the latter caused moderate tailing. This account is the first reported preparation of [^{14}C]-fentanyl with the radiolabel in the anilino benzene ring.

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