The Preparation of 5'-Iodo-^{125}I-Δ^8-THC; A Radioligand for the Radioimmunoassay of Cannabinoids

Colin G. Pitt, Herbert H. Seltzman, Susan R. Setzer, and David L. Williams

Research Triangle Institute
P. O. Box 12194
Research Triangle Park
North Carolina

SUMMARY

Nucleophilic exchange of 5'-iodo-Δ^8-THC and sodium iodide-125 afforded 5'-iodo-^{125}I-Δ^8THC with a maximum specific activity of 42 Ci/mmole. The specific activity could be increased to at least 100 Ci/mmole by iodide-125 displacement of the corresponding 5'-tosylate. A hapten 5'-carboxy-^{14}C-Δ^9-THC, for use in conjunction with this radioligand, was synthesized from 5'-bromo-Δ^8-THC.

Key Words: Iodine-125, RIA, Cannabinoids

INTRODUCTION

We have previously described[1] the preparation of carrier-free Δ^8-THC-4',5'-$^3H_2$, 50 Ci/mmole, and this compound has been shown[2,3] to be a useful radioligand for the RIA of cannabinoids. However, an iodine-125 labeled radioligand offers the potential advantages of simplicity of preparation, higher specific activity, and more convenient and economical counting procedures. No useful iodine labeled radioligands for the RIA of cannabinoids have been prepared, although their synthesis has been addressed.[4] Here we describe the synthesis of 5'-iodo-^{125}I- Δ^8-THC and the hapten 5'-carboxy-^{14}C-Δ^9-THC, which together form the basis of a

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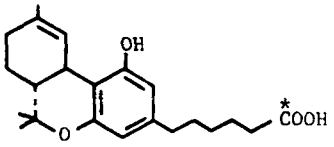
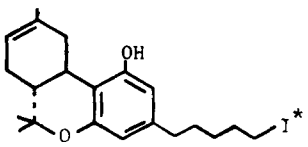
RIA for the detection of Δ^9-THC and its metabolites in physiological fluids.[2,3]



5'-Carboxy-^{14}C-Δ^9-THC



5'-Iodo-^{125}I-Δ^8-THC

RESULTS

The first method of preparation of 5'-iodo-^{125}I-Δ^8-THC evaluated was iodide exchange,[5] an SN2 displacement process which is facilitated

$$\text{THC-I} + \text{NaI}^* \rightleftharpoons \text{THC-I}^* + \text{NaI}$$

by both the nucleophilicity and ease of nucleophilic displacement of iodide. Assuming complete equilibration, the specific activity is determined by the ratio of the total radioactivity and the sum of organic and inorganic iodide.

Carrier-free sodium iodide (20 mCi, 8.1 nmole) in 0.1M NaOH (51 µl) was partly neutralized with 0.1M hydrochloric acid (48 µl), to eliminate hydroxide induced chemistry, e.g., hydrolysis. The water was evaporated in vacuo (300 Torr) and the residue was dissolved in acetone (140 µl) containing 5'-iodo-Δ^8-THC (25 µg, 57 nmole) and heated at 40-45°C. After 22 hr, tlc radioscans showed 76% of the radioactivity was associated with 5'-iodo-Δ^8-THC, equivalent to 86% exchange and a specific activity of 280 Ci/mmole. However, the specific activity of the isolated product was only 8 Ci/mmole. This large discrepancy was traced to loss of 95% of the radioactivity from the reaction medium, either by absorption on the glassware or volatilization as elemental iodine. Silanization of

the glassware was without effect. When the neutralization step was omitted, to retard any oxidation of iodide to iodine, the radio recovery increased to 45% and the specific activity to 42 Ci/mmole. This value was not improved when the amount of 5'-iodo-Δ^8-THC was reduced to 2.8 nmoles (calcd. to increase the specific activity by a factor of 1.8), and was the maximum achieved by this method.

The possibility of obtaining 5'-iodo-^{125}I-Δ^8-THC by displacement of the bromo group of 5'-bromo-Δ^8-THC with radio-iodide was considered:

$$RBr + NaI^{*} \rightarrow RI^{*} + NaBr$$

A priori, this non-reversible SN2 displacement reaction will give 5'-iodo-^{125}I-Δ^8-THC with the same specific activity as the sodium iodide-125 employed. Unfortunately, trial experiments with unlabeled materials established that this displacement reaction was incomplete (40% in 24 hr) under the conditions (reagent and solvent ratios) dictated by the use of iodine-125 and chromatographic separation of unchanged 5'-bromo-Δ^8-THC from 5-iodo-Δ^8-THC was not practical because of their almost identical Rf's.

Displacement of the 5'-tosylate was then examined. 5'-Tosyloxy-Δ^8-THC could not be obtained from the reaction of 5'-bromo-Δ^8-THC and silver tosylate in acetonitrile according to the method of Kornblum;[6] no reaction was observed, even at 70°C, despite the fact that n-butyl bromide was converted to n-butyl tosylate rapidly under the same conditions. Evidently, the pentyl side chain of Δ^8-THC adopts a conformation which reduces the steric accessibility of the 5'-position. When 5'-iodo-Δ^8-THC was utilized instead, a 91% yield of the 5'-tosylate was realized.

Displacement of 5'-tosyloxy-Δ^8-THC with sodium iodide in acetone at room temperature was slow (5% in 24 hr), but 100% conversion was achieved

at 50° in 16 hr (5 mg scale). Repetition using sodium iodide-125 (20 mCi, 100 Ci/mmole) afforded 70% of 5'-iodo-Δ^8-THC with the same specific activity. The stability was checked by tlc radioscan after 90 days storage in toluene/ethanol (9:1) at 4°C and less than 10% decomposition was detected.

5'-Carboxy-^{14}C-Δ^9-THC - This hapten was obtained from the Δ^8 isomer[1] by the method of Petrzilka et al,[7] i.e., formation of the hydrogen chloride adduct, then intramolecular elimination promoted by potassium triethylcarbinolate.

EXPERIMENTAL PROCEDURES

NMR spectra were obtained using a Varian HA-100 or EM-360 spectrometer using tetramethylsilane as the internal standard. Infrared spectra were measured using a Perkin-Elmer Model 467 spectrophotometer. Mass spectroscopic analyses were carried out using an AEI MS-902 mass spectrometer. Gas-liquid chromatographic analyses were performed using a Varian Model 1400 instrument, with columns (152 cm x 1.59 mm) containing 1.3% OV-17 on Supelcoport. Coated silica gel 60 F-254 (Merck) plates and phosphomolybdic acid/ceric sulfate reagents were employed for TLC analysis. All of the compounds were gums and their elemental compositions were determined by high-resolution mass spectroscopy after verifying purity by TLC and GLC analysis. Solvents were dried over 3 Å molecular sieves, or by distillation from either lithium aluminum hydride or calcium hydride. All reactions were carried out under a nitrogen or argon atmosphere. Radio-purity was determined using a radio-scanner (Autochrom LB2722). Silver nitrate impregnated silica gel was prepared by mixing silica gel (450 g., Mallinckrodt, 100 mesh) with silver nitrate

(125 g) in water (200 ml) and methanol (550 ml), the majority of solvent then being removed at ca. 30 Torr using a Buchler rotary evaporator. Further drying was carried out at <1 Torr. Iodine-125 was counted using a Packard Auto-Gamma Scintillation Spectrometer, Model 5135, or the carbon-14 window of a Packard Liquid Scintillation Spectrometer, Model 3375, using toluene/Omnifluor. The specific activities of iodine-125 labeled products were determined by the self displacement method.[8]

5'-Iodo-Δ^8-THC .... A solution of sodium iodide (40 mg, 0.27 mmole) and 5'-bromo-Δ^8-THC (40 mg, 0.10 mmole) in acetone (1.5 ml) was stirred at room temperature for 24 hr. More sodium iodide (20 mg) was added and after 72 hr the solvent was evaporated and the residue was partitioned between hexane and water. The product was obtained as a brown gum (40.4 mg, 90%) by elution of the organic concentrate from silica gel (2 g) with benzene; pmr ($CDCl_3$), 1.08, 1.24 (s,s,CMe_2), 1.70 (s, C=CMe), 2.46 (t, 7Hz, $ArCH_2$), 3.18 (t, 7Hz, 2H, CH_2I), 5.44 (br.s, 1H, C=CH), 6.09 (s, 1H, ArH), 6.25 (s, 1H, ArH); $\nu_{max}^{CCl_4}$ 3600 cm^{-1}(OH); m/e 440.121; calcd. for $C_{21}H_{29}O_2$, 440.121.

5'-Tosyloxy-Δ^8-THC .... Silver tosylate (16 mg, 0.058 mmole) in dry acetonitrile (0.14 ml) was added to 5'-iodo-Δ^8-THC (20 mg, 0.045 mmole) at 0° in the dark. The mixture was stirred at 70° for 4 hr, when TLC analysis showed virtually complete reaction. The mixture was diluted with iced water and extracted with ether. The organic extracts were concentrated, and the residue (20 mg, 91%) was purified by preparative TLC to give the 5'-tosylate as a brown gum (54%); pmr ($CDCl_3$) 1.05, 1.33 (s, s, CMe_2), 1.65 (s, C=CMe), 2.40 (s, 3H, ArMe), 3.97 (t, 8Hz, 2H, CH_2OTos), 5.35 (br.s, 1H, C=CH), 6.02 (s, 1H, ArH), 6.13 (s, 1H, ArH), 7.25 (d, 8Hz, 2H, $C_6H_4OSO_2$), 7.72 (d, 8Hz, 2H, $C_6H_4OSO_2$); $\nu_{max}^{CCl_4}$ 1030^{-1} ($OSO_2C_6H_5$); m/e 484.229; calcd. for $C_{28}H_{36}O_5S$, 484.229.

5'-Iodo-^{125}I-Δ^8-THC A. From 5'-Iodo-Δ^8-THC .... A solution of 5'-iodo-Δ^8-THC (25 µg, 0.057 µmole) in acetone (80 ml) was added to carrier-free sodium iodide (20 mCi) in 3 µl of 0.02N sodium hydroxide contained in a combi-V-vial (New England Nuclear Corp), by injection through a Teflon lined rubber septum. The mixture was heated at 48° for 36 hr. TLC radioscan analysis (SiO_2, benzene) showed 23% of the radioactivity was associated with 5'-iodo- Δ^8-THC, (Rf 0.4), the remainder with more polar material (Rf 0, inorganic iodide or decomposition products). After an additional 20 hr, this proportion was unchanged. The theoretical proportion, based on the ratio of reagents, was 88:12 of organic to inorganic iodide.

The mixture was diluted with water and hexane, and the aqueous phase was extracted with hexane. The combined organic extracts were washed, dried (Na_2SO_4), and concentrated. The radioactivity in the organic phase was 2.2 mCi, and that in the aqueous phase was 5.9 mCi. The product was purified by preparative TLC (SiO_2, benzene) of the organic concentrate, and its specific activity determined to be 42 Ci/mmole.

B. From 5'-Tosyloxy-Δ^8-THC .... Unlabeled sodium iodide (28.4 µg, 0.189 µmole) was added to carrier-free sodium iodide (20 mCi) in 41 µl of 0.017N sodium hydroxide in a combi-V-vial. The water was evaporated in vacuo (300 mm) in a desiccator in the presence of conc. sulfuric acid (66 hr). The vial was closed with a Teflon lined septum, and 5'-tosyloxy-Δ^8-THC (287 µg, 0.593 µmole) in dry, redistilled acetone (80 µl) was introduced. After 18 hr at room temperature (<10% conversion, TLC radioscan), the mixture was heated at 48°C for 18 hr (40% conversion), then an additional 24 hr (60% conversion). The mixture was diluted with water and hexane and the aqueous phase was extracted with hexane.

Approximately 75% of the radioactivity remained in the organic phase. The hexane solution was concentrated in vacuo and the product was purified by preparative TLC (SiO_2, benzene); specific activity 100 Ci/mmole, yield 1.69 mCi. It was stored in toluene:ethanol (9:1, 750 ml) at 4°.

5'-Carboxy-^{14}C-Δ^9-THC .... 5'-Bromo-Δ^8-THC (640 mg, 1.62 mmoles) and sodium cyanide-^{14}C (145.6 mg, 2.971 mmoles, 1 mCi) in dry DMSO (15 ml) were stirred at 50° for 4 hr, when TLC indicated quantitative formation of the 5'-nitrile. The mixture was diluted with water, saturated with salt, and extracted with benzene and ether. The combined organic extracts were washed with water, dried, and concentrated. The residual oil (535 mg, 97%) and degassed ethanol (50 ml), water (4 ml), and potassium hydroxide (4 g) were refluxed for 22 hr. The mixture was then concentrated, diluted with brine, extracted with ether, acidified, and again extracted with ether. The latter extracts were dried and concentrated to give 434 mg (77%) of the known[1] 5'-carboxy-Δ^8-THC as a pure (TLC, radioscan) oil.

A solution of this product and anhydrous zinc chloride (350 mg) in methylene chloride (25 ml) was saturated with hydrogen chloride at 0°. The mixture was stirred at 25° for 15 hr, then washed with brine, aq. dipotassium phosphate (pH 6.5) and water, dried and concentrated. The residual oil (408 mg) in benzene (20 ml) was treated with 0.85M potassium triethylcarbinolate in toluene (4.8 ml) and maintained at 50° for 3 hr, then 25° for 70 hr. The pH of the mixture was reduced to 8 by addition of dry ice before washing with aq. dipotassium phosphate and drying. The solvent was evaporated and the residue was diluted with saturated aq. sodium carbonate and extracted with ether to remove triethylcarbinol. The aqueous phase was acidified and extracted with ether. The ether

extracts were dried and concentrated, to afford 273 mg of a mixture of 5'-carboxy-Δ^8-, Δ^9-, and $\Delta^{9(11)}$-THC. Elution of 120 mg from silica gel (7.5 g) impregnated with silver nitrate and mixed with celite (7.5 g) using a 2-8% acetone in benzene gradient afforded 65 mg of 5'-carboxy-Δ^9-THC as a foam, with no $\Delta^{9(11)}$-isomer and less than 10% of the Δ^8-isomer; pmr (deuterioacetone) 1.04, 1.35 (s,s, CMe_2), 1.62 (br.s, C=CMe), 2.28 (t, 7Hz, CH_2COOH), 2.44 (t, 7Hz, $ArCH_2$), 3.21 (br.d, 10Hz, 1H, 10a-CH), 6.12, 6.25 (s,s, 2H, ArH), 6.45 (br.s, 1H, C=CH); specific activity 2.1 μC/mg.

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