Synthesis of 2-{5-(4-[^{123}I/^{131}I]Iodophenyl)Pentyl}-Oxirane-2-Carboxylic Acid.

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SUMMARY

A method is described for the synthesis, purification and radiolabelling of 2-{5-(4-iodophenyl)pentyl}oxirane-2-carboxylic acid (I-POCA). This new compound was synthesized from 5-phenyl-pentylbromide (1), prepared via 5-phenyl-1-pentanol and subsequently converted to diethyl 5-phenylpentylmalonate (2). The latter on alkaline hydrolysis yielded ethyl 5-phenyl-pentylmalonate (3). Para-substitution of iodine on the phenyl moiety of the monoester (3) was accomplished by reacting compound (3) with thallium trifluoroacetate (TTFA) and subsequently with KI. Oxidation of ethyl 7-(4-iodophenyl)-2-methyleneheptanoate (5), which was synthesized from the monoester, ethyl 5-(4-iodophenyl)pentylmalonate (4) yielded ethyl 2-{5-(4-iodophenyl)pentyl}oxirane-2-carboxylate (6). The radiolabelling procedure was based on a Cu(I)Cl-assisted, isotopic exchange reaction, which produced a no-carrier-added and regiospecific radioiodination with a 32-52% radiochemical yield.

Key words: I-POCA , nucleophilic , radioiodination , Cu(I).

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INTRODUCTION

Several substituted alkanoic acids are known to inhibit gluconeogenesis by inhibiting fatty acid oxidation in liver mitochondria (1-4) and to cause a decrease in blood glucose concentration in vivo (5). Tutwiler et al. (6) reported on the pharmacology of methyltetradecylglycidate, which is a more effective long-chain alkyloxirane carboxylic acid, with a similar site of action. In 1982, several substituted derivatives of 2-(phenylalkyl)oxirane-2-carboxylic acid and 2-(phenoxyalkyl)oxirane-2-carboxylic acids were synthesized and exhibited marked blood glucose-lowering activities in fasting rats (7). Sodium 2-{5-(4-chlorophenyl)pentyl}oxirane-2-carboxylate (POCA) and sodium 2-{6-(4-chlorophenoxy)hexyl}oxirane-2-carboxylate (Etomoxir) were the most potent compounds of this group. These compounds also were found to be powerful inhibitors of β-oxidation of long-chain fatty acids at the stage of carnitine palmitoyltransferase-I (CPT-I) (8,9). CPT-I is localized on the outer surface of the mitochondrial inner membrane and catalyzes the formation of long-chain acyl carnitine (LCACarn) from long-chain acyl Coenzyme A (LCACoA) and free carnitine (10). LCACoA and LCACarn accumulate in the myocardium during the early phase of ischemia (11,12). Additional findings show that POCA prevents the accumulation of LCACarn and Coenzyme A esters during ischemia and significantly improves the recovery of cardiac output after ischemia and reperfusion in isolated, perfused rat hearts (13).

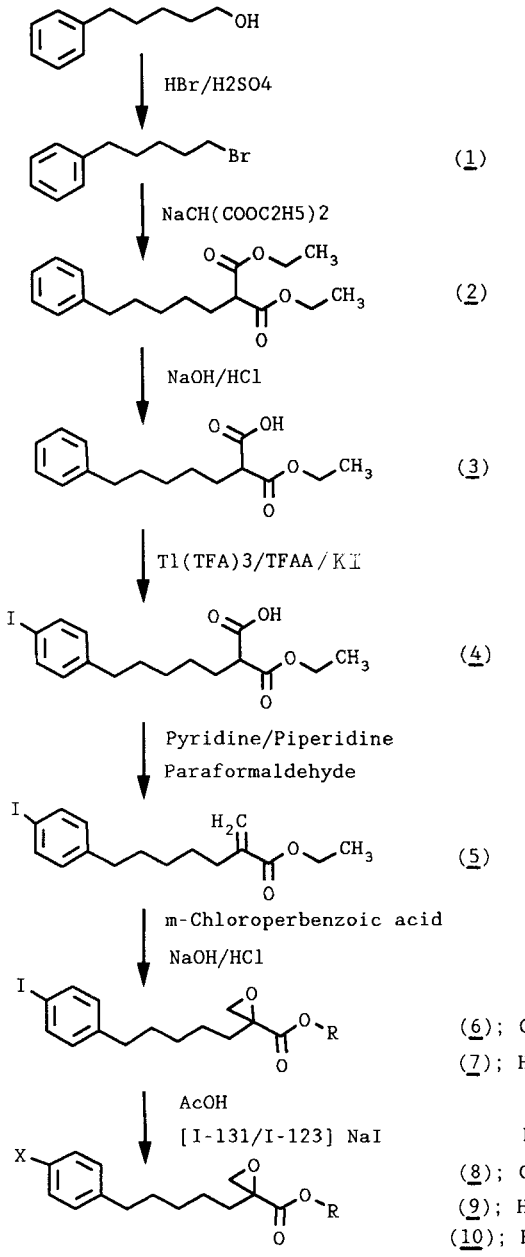
Our goal was to develop procedures for the synthesis, radiolabelling and HPLC purification of a new class of radiopharmaceuticals for use as diagnostic tools in nuclear medicine. Therefore, POCA, which possesses valuable pharmacological properties mentioned above, was labelled with

radioiodine to study its pharmacological properties and to determine whether this new class of radiopharmaceutical may be useful as a diagnostic tool in nuclear medicine.

MATERIALS AND METHODS

Chemistry. Scheme 1 shows the synthetic route for the synthesis of [^{131}I/^{123}I] 2-{5-(4-iodophenyl)pentyl}oxirane-2-carboxylic acid. Compound (1), 5-phenylpentylbromide, synthesized according to the method of Kamm and Marvel (14), was converted to (2) using the method described by Marvel (15). The monoester (3) and the 2-methylene carboxylate (5) were synthesized by the method of Stetter and Kuhlmann (16). Iodination of the phenyl moiety of the monoester (3) was achieved with TTFA and aqueous KI as described by Taylor et al. (17). Oxidation of the 2-methylene carboxylate (5) with m-chloroperbenzoic acid (7) yielded the desired 2-oxirane carboxylate (6). Radiolabelling was achieved by a nucleophilic exchange in acetic acid using CuCl as a catalyst.

General methods. Flash chromatographic separations were carried out as described by Still et al. (18) on a 230-400 mesh Silica gel 60 column. HPLC separations were conducted as described by Narce et al. (19) on an HPLC system (Waters Associates) consisting of a solvent delivery system (Waters 600E), UV detector (Model 441), differential refractometer (Model R401) and a LiChrosorb RP 18 column (7μ). Melting points were determined on an Electrothermal apparatus and are uncorrected. Proton NMR spectra were consistent with assigned structures and were obtained with a Brucker model WP-80 using $CDCl_3$ as a solvent. Chemical shifts (δ) were reported in ppm downfield from tetramethylsilane as the internal standard. Elemental analyses were performed by the Division of Central



Scheme 1. Synthesis Of [I-131/I-123] 2-{5-(4-Iodophenyl)-Pentyl}Oxirane-2-Carboxylic Acid

Chemical Analyses, KFA Jülich. Thin layer chromatography (TLC) was performed on silica gel 60 plates (F254, E.Merck). Radiochromatograms were analysed using an automatic TLC linear

analyzer tracemaster 20 (Berthold, Wildbad, FRG). The radiolabelled compounds were characterized by HPLC and TLC using different eluents, as described. All solvents and reagents were analytical grade; [^{131}I]NaI was purchased from Amersham Buchler and [^{131}I]NaI was procured from Cygne, Netherlands.

Synthesis and radiolabelling

5-Phenylpentyl bromide (1): To a mixture of 108.5 g (0.63 mol) of hydrobromic acid (47%) and 33 g of concentrated sulfuric acid were added 54.2 g (0.33 mol) of 5-phenyl-1-pentanol and the mixture refluxed gently for 16 hours. The resulting solution was cooled to room temperature, diluted with water and the lower layer containing the brominated compound separated. The latter layer was washed with cold concentrated sulfuric acid, then with water and dilute sodium carbonate solution. Further purification by distillation in vacuo yielded 47.1 g (62.8% yield) of (1), bp, 73-77°C (4 Pa). ^{1}H-NMR ($CDCl_3$), 60 MHz : 1.57-2.33 [m, 6H, 3-CH_2-, -($\underline{CH_2}$)$_3$-] 2.40-2.73 (t, 2H, -$\underline{CH_2}$-Aryl); 3.41-3.77 (t, 2H, -$\underline{CH_2}$-Br); 7.32 (s, 5H, H-Aryl). Anal. Calc. for $C_{11}H_{15}Br$: C, 58.17; H, 6.66; Br, 35.18. Found: C, 58.17; H, 6.59; Br, 35.1.

Diethyl 5-phenylpentylmalonate (2): Sodium (2.62 g, 0.114 g-atom) was added gradually to 130 ml of absolute ethanol while stirring until the reaction had gone to completion. The flask was warmed to 50°C and 18.26 g (0.114 mol) of diethyl malonate were added in a steady stream and stirred for 1 hour. To this mixture 25.89 g (0.114 mol) of compound (1) were added slowly and the resulting solution refluxed for 18 hours. The alcohol was removed in vacuo to leave a residue which was treated with water (400 ml) and then extracted with ether (3x80 ml). The

combined ether extracts were concentrated under reduced pressure and the unreacted compound (1) and diethyl malonate were removed by fractional distillation in vacuo. Further purification by flash chromatograghy and vacuum distillation yielded 25.64 g (73.4% yield) of (2), bp 135-152°C (0.1 Pa). ^{1}H-NMR ($CDCl_3$), 80 MHz : 1.1-1.32 (t, 6H, 2-$\underline{CH_3}$-Ester); 1.12-2.10 [m, 8H, 4-CH_2-, -$(\underline{CH_2})_4$-]; 2.43-2.76 (t, 2H, -$\underline{CH_2}$-Aryl); 3.14-3.45 (t, 1H, -$\underline{CH}$-); 3.98-4.44 (q, 4H, 2-$\underline{CH_2}$-Ester); 7.35 (s, 5H, $\underline{H}$-Aryl). Anal. Calc. for $C_{18}H_{26}O_4$: C, 70.56; H, 8.55; O, 20.89. Found: C, 70.1; H, 8.26; O, 20.6.

Ethyl 5-phenylpentylmalonate (3): To a solution of 36.31 g (0.1185 mol) of (2) in 90 ml of ethanol was added dropwise while stirring a solution of 6.65 g (0.1185 mol) of KOH in 120 ml of ethanol at room temperature and the reaction mixture then stirred for 16 hours. The solvent was evaporated in vacuo and, upon cooling, 250 ml of H_2O was added to the residue and the solution extracted with ether (2x60 ml) in order to remove unreacted compound (2). The aqueous phase was acidified with HCl and extracted with ether (2x70 ml). The combined ether extracts were dried over Na_2SO_4, filtered and the filtrate concentrated in vacuo to yield 27.7 g (84%) of (3) as a viscous oil which was used in the next synthetic step without further purification. ^{1}H-NMR ($CDCl_3$), 80 MHz : 1.06-1.33 (t, 3H, -$\underline{CH_3}$- Ester); 1.12-2.11 [m, 8H, 4-CH_2-, -$(\underline{CH_2})_4$-]; 2.46-2.75 (t, 2H, -$\underline{CH_2}$-Aryl); 3.22-3.50 (t, 1H, -$\underline{CH}$-); 4.04-4.41 (q, 2H, -$\underline{CH_2}$-Ester); 7.29 (s, 5H, $\underline{H}$-Aryl). Anal. Calc. for $C_{16}H_{22}O_4$: C, 69.04; H, 7.97; O, 22.99. Found: C, 68.19; H, 7.72; O, 23.4.

Ethyl 5-(4-iodophenyl)pentylmalonate (4): A solution of 3.34 g (0.012 mol) of (3) in 10 ml of trifluoroacetic acid (TFAA) was added slowly to a solution of 9.48 g (0.018 mol) of TTFA in 50

ml of TFAA and stirred for 5 days at room temperature in the dark. Evaporation of excess TFAA, followed by three co-evaporations with 1,2-dichloroethane, yielded a solid arylthallium trifluoroacetate, which was then suspended in 120 ml of water and reacted with 10.2 g (0.06 mol) of potassium iodide under vigorous stirring. The suspension was refluxed gently for 30 minutes and sodium metabisulfite (1.2 g) was added to reduce the iodine. After stirring for an additional 30 minutes, the precipitated thallium iodide was removed by filtration, washed with acetone and filtered. The filtrate was extracted with ether (3x80 ml) and the combined ether extracts dried over Na_2SO_4 and concentrated in vacuo yielding 2.4 g (50%) of a crude product as a viscous oil. This was purified further by HPLC using acetonitrile : water (93:7 v/v) as an eluating solvent system. (4) was obtained as a colourless amorphous solid with a mp 98°C. ^{1}H-NMR ($CDCl_3$) 90 MHz : 1.12-1.72 (m, 11H, 4-CH_2-, -$(CH_2)_4$- + -CH_3-Ester); 2.34-2.65 (t, 2H, -CH_2-Aryl); 3.14-3.39 (t, 1H, -CH-); 3.99-4.33 (q, 2H, -CH_2-Ester); 6.73-7.53 (2d, 4H, H-Aryl).

Ethyl 7-(4-iodophenyl)-2-methyleneheptanoate (5): To 8.85 g (0.0219 mol) of (4) was added pyridine (3.68 ml), piperidine (0.245 ml) and paraformaldehyde (0.788 g). The mixture was stirred, heated to 50°C and refluxed until CO_2 evolution ceased. Upon cooling 3.5 ml of ice cold water were added, acidified with 6N HCl and extracted with ether (3x17 ml). The combined ether extracts were washed with a dilute $NaHCO_3$ solution, dried over sodium sulfate, filtered and the filtrate concentrated. Distillation in vacuo yielded 2.68 g (32.9%) of (5), bp 65-78°C (0.1 Pa). IR: 3075 Cm^{-1} ($=CH_2$).

Ethyl 2-{5-(4-iodophenyl)pentyl}oxirane-2-carboxylate (6) : Compound (6) was prepared by the reaction of (5) (2.05 g, 5.51

mmol) with metachloroperbenzoic acid (2.25 g, 11.0 mmol) in 20 ml of CH_2Cl_2. The solution was refluxed at 50°C for 24 hours, cooled to room temperature and filtered to remove precipitated m-chloroperbenzoic acid and the filtrate combined with other subsequent CH_2Cl_2 washings. The precipitate was washed with CH_2Cl_2 (5 ml) and the combined filtrates were evaporated *in vacuo*. The resulting residue was treated with acetone (15 ml), a saturated solution of $NaHCO_3$ (10 ml) and water (10 ml). After the mixture was stirred for 30 minutes it was extracted with ether (3x15 ml). The combined ether extracts were dried over sodium sulfate and concentrated to yield 1.36 g (63.6% yield) of a crude oil. This oil was purified further by preparative HPLC using acetonitrile : H_2O (93:7 v/v) as an eluating solvent system. ^{1}H-NMR ($CDCl_3$) 80 MHz : 1.15-1.91 [m, 11H, 4-CH_2-, -($\underline{CH_2}$)$_4$- + -$\underline{CH_3}$-Ester]; 2.18-2.47 (m, 4H, 2-CH_2-, -$\underline{CH_2}$-Aryl + -$\underline{CH_2}$-Oxirane); 3.86-4.23 (q, 2H, -$\underline{CH_2}$-Ester); 6.94-7.65 (2d, 4H, $\underline{H}$-Aryl).

2-{5-(4-Iodophenyl)pentyl}oxirane-2-carboxylic acid (7): To compound (6) (32 mg, 0.082 mmol) dissolved in 0.2 ml of tetrahydrofuran were added 90 µl of a 1N solution of NaOH and the resulting mixture stirred for 2 hours. The solvent was then removed *in vacuo* and the residue dissolved in 1.5 ml of H_2O. The resulting solution was chilled in an ice bath, acidified with 170 µl of 0.5N HCl, extracted with ether (2x5 ml) and then concentrated *in vacuo*. The product was precipitated by first dissolving in acetone and then adding sufficient n-heptane for precipitation. Further purification by preparative HPLC using CH_3CN : H_2O (93:7 v/v) as eluating solvent system yielded 15 mg (50.8%) of (7), mp 65-67°C. ^{1}H-NMR ($CDCl_3$) 90 MHz : 1.18-1.95 [m, 8H, 4-CH_2-, -($\underline{CH_2}$)$_4$-]; 2.12-2.45 (m, 4H, 2-CH_2-, -$\underline{CH_2}$-Aryl + -$\underline{CH_2}$-Oxirane); 6.78-7.67 (2d, 4H, $\underline{H}$-Aryl).

Ethyl 2-{5-(4-[^{131}I]iodophenyl)pentyl}oxirane-2-carboxylate(8): A dilute alkaline solution of [^{131}I]NaI (58 μCi, 2.15 MBq) was placed in a reaction tube, cooled and evaporated to dryness in vacuo. Then 100 μl of a solution of (6) (0.5 mg/100 μl) in acetone was added to the reaction tube and evaporated to dryness. Glacial acetic acid (50 μl) and 5 μl of a solution of Cu(I)Cl (0.1 mg/ml) in glacial acetic acid were added to the reaction tube, which was then tightly closed and heated at 170°C for 25 minutes. Upon cooling to room temperature, the mixture was chromatographed on a TLC plate using n-hexane: diethyl ether : acetic acid (70:30:1 v/v) as a solvent system. The radiochromatogram, obtained using an automatic TLC linear analyzer, revealed 47% radiochemical yield. Purification by HPLC yielded the radiochemically pure (>98%) (8). Radiochemical purity was ascertained using an automatic TLC linear analyzer.

2-{5-(4-[^{131}I]Iodophenyl)pentyl}oxirane-2-carboxylic acid (9): Na^{131}I (0.6 mCi, 22.2 MBq) and 100 μl of a solution of (7) (0.5 mg/100 μl) in methanol were added to a reaction tube and evaporated to dryness in vacuo. Glacial acetic acid (100 μl) and 5 μl of a solution of Cu(I)Cl (0.1 mg/ml) in glacial acetic acid were added to a reaction tube, which was then tightly closed and heated at 170°C for 30 minutes. Upon cooling, the solvent was evaporated in a stream of nitrogen. HPLC purification yielded (9) in a radiochemical yield of 52%.

2-{5-(4-[^{123}I]Iodophenyl)pentyl}oxirane-2-carboxylic acid (10): Na^{123}I (10.0 mCi, 0.37 GBq), compound (7) (0.7 mg, 1.94 μmol), 50 μl glacial acetic acid, and 5 μl of a solution of Cu(I)Cl (0.1 mg/ml) in glacial acetic acid were placed in a reaction tube and treated in a manner analogous to that employed in the preparation of compound (9) and HPLC purification afforded (10) in a radiochemical yield of 32%.

DISCUSSION

It was important that the labelling method chosen yielded a product of high radiochemical purity and chemical stability, and essential that the presence and position of a radionuclide in the radiopharmaceutical did not alter the properties of the pharmaceutical when applied *in vivo* (20). In view of these facts POCA was radiolabelled with [^{123}I]iodine for application in diagnostic nuclear medicine. An attempt at exchanging chlorine for radioiodine in the para-position of the aromatic ring was unsuccessful because the carbon-to-chlorine bond is much stronger and more stable than the carbon-to-iodine bond. Further difficulties arose because of the presence of the three-membered oxirane ring which is more reactive than the benzene ring and was more likely to break down under the iodination coditions. Therefore, using the TTFA/KI method (17), para-substitution of iodine on the phenyl moiety of the monoester (3) was achieved and the synthesis continued as illustrated in scheme 1.

Iodination *via* thallation introduced only a single iodine atom into the phenyl ring. Regiospecificity was the important goal and a monoiodocompound with para-substitution was desired for the synthesis of I-POCA; the specificity of thallation fulfilled both of these requirements and avoided the possibility of multiple substitution. Analysis of the I-POCA by ^{1}H-NMR spectroscopy revealed a symmetrical split of four aromatic protons and confirmed the para-substitution of iodine. The use of Cu(I)Cl as a catalyst and glacial acetic acid as a solvent under anhydrous conditions was of critical importance in the labelling method described above. This radiolabelling procedure is a fast and simple method, which results in a high radiochemical yield.

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