SYNTHESIS OF ^{125}I-LABELED 14-IODO-9-TETRADECYNOIC ACID

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SUMMARY

The synthesis and radioiodide exchange labelling of 14-iodo-9-tetradecynoic acid (1), a potential suicide inhibitor of acyl-CoA dehydrogenase or enoyl-CoA isomerase, is presented. Tissue distribution data in dogs are reported.

Key Words: 14^{125}I-Iodo-9-tetradecynoic acid, Suicide inhibitor, Myocardial imaging, Activity distribution, Radioiodide displacement, ^{125}I.

INTRODUCTION

Radioiodinated fatty acids (RIFA) where either iodine-123 or iodine-131 is the gamma-emitting radioiodide have been used experimentally and clinically for detection of abnormalities in regional myocardial perfusion (1-3). Clinical use of RIFA is hampered by 1) short myocardial T 1/2 and 2) high blood activity levels. These problems may be related to rapid β-oxidation of the RIFA by the myocardium and liver.

One approach to solving both problems simultaneously involves mechanism based irreversible inhibition (suicide inhibition). Bloch has shown such inhibition of E. Coli dehydrase with 3-decynoic acid (4). The mechanism of this inhibition involves enzymatic conversion of the triple bond to a reactive allene which then bonds covalently to histidine at the active

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site of the enzyme. In the myocardium two enzymes, acyl-CoA dehydrogenase and enoyl-CoA isomerase, could potentially generate a reactive allene during normal enzymatic activity. If such suicide inhibition is possible in the myocardium, an RIFA containing a triple bond at an odd carbon could become covalently bound to the enzyme. Presumably, β-oxidation of the compound would cease, thus increasing its myocardial T 1/2 and decreasing the metabolic release of radioiodide into the blood.

To explore the feasibility of this approach to myocardial imaging, a radioiodinated fatty acid containing a triple bond, specifically 14-^{125}I-iodo-9-tetradecynoic acid (6), was synthesized and biologically evaluated in rats and dogs.

RESULTS AND DISCUSSION

The synthetic scheme followed is outlined in Figure 1. Direct conversion of 2 or 3 into $Br(CH_2)_4C{\equiv}C(CH_2)_7CO_2H$ or methyl ester using PBr_3/pyridine or $(C_6H_5)_3P{\cdot}Br_2$/acetonitrile failed due to bromination of the carbon-carbon triple bond and/or migration of the triple bond. Tosylation of 3 followed by iodide displacement proceeded with high yields and little or no triple bond migration.

The radioiodinated fatty acid (6) was evaluated first in rats. Myocardial uptake was low (0.08% kg dose/g at 5 min) relative to saturated long chain ω-iodofatty acids (0.39% at 5 min for $^{125}I(CH_2)_{15}CO_2H$ [5]). Since rat myocardial uptake is easily affected by carbon chain manipulations (6), 6 was also evaluated in dogs. The data obtained is contained in Table 1. For comparison, data from $^{125}I(CH_2)_{15}CO_2H$ is also tabulated. Myocardial uptake of the potential suicide inhibitor is comparable to the ω-iodo saturated sixteen carbon fatty acid. Blood values for 6 are higher however. The data show similar myocardial residence times for the two fatty acids suggesting

either that suicide inhibition is not occurring or that deiodination resulting from metabolism or hydrolysis is occurring as rapidly for 6 as for $^{125}I(CH_2)_{15}CO_2H$. In vitro enzyme assays will be undertaken to determine if suicide inhibition does occur. This initial study demonstrates that a major structural modification of an ω-iodo fatty acid, such as triple bond inclusion, does not adversely alter its myocardial extraction in dogs.

Bloch et al have shown that suicide inhibition of E. coli dehydrase was affected by fatty acid chain length and by triple bond position (8). Other iodinated fatty acids varying in chain length and triple bond position will be evaluated as possible suicide inhibitors.

Table I. Activity distribution data for ^{125}I- ω-iodo fatty acids

$^{125}I(CH_2)_4C{\equiv}C(CH_2)_7CO_2H$

Min	Heart[a]	Liver	Thyroid	Blood
5	0.49 ± 0.10[b]	0.23 ± 0.01	1.45 ± 0.69	0.20 ± 0.09
20	0.18 ± 0.04	0.20 ± 0.01	4.18 ± 0.35	0.19 ± 0.01

$^{125}I(CH_2)_{15}CO_2H$

5	0.57 ± 0.04	0.26 ± 0.02	1.05 ± 0.32	0.07 ± 0.01
20	0.17 ± 0.00	0.22 ± 0.01	7.87 ± 0.00	0.12 ± 0.01

[a] Tissues obtained from female mongrel dogs. Lung samples were $\leq 0.27 \pm 0.02$. Muscle samples were $\leq 0.08 \pm 0.01$.

[b] Activity expressed as % kg dose/g. (9)

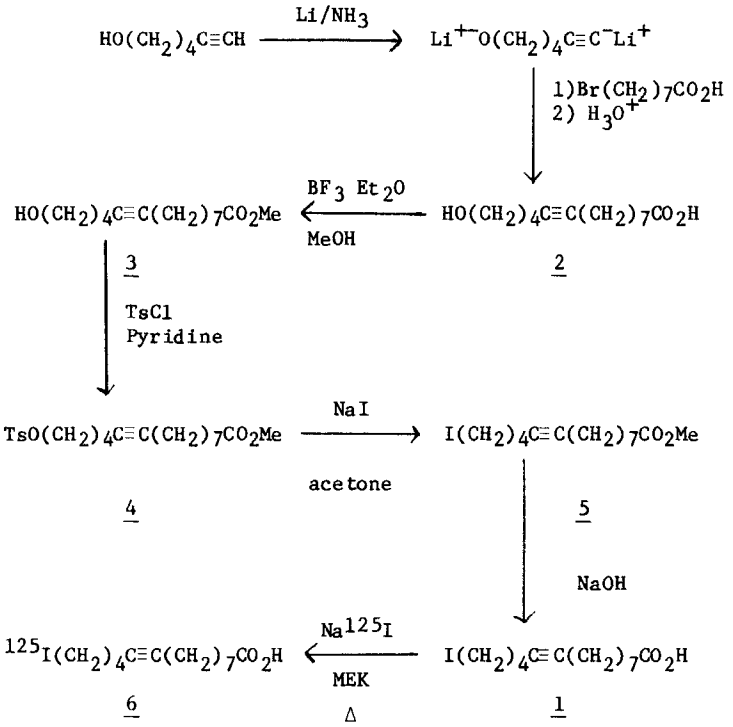

Figure 1. Outline of the synthetic route to 1 and 6.

EXPERIMENTAL

Melting points were determined on a Mel-Temp melting point apparatus and are uncorrected. NMR spectra were measured on a Varian T-60A Spectrometer and TMS used as an internal standard. IR spectra were obtained using a Beckman Acculab 8 and calibrated at 1601.8 with polystyrene. The following were obtained from commercial sources: $Na^{125}I$ in 0.1 N NaOH (Union Carbide), hexyn-1-ol (Farchan Division, Chem Samp Co, Inc.), 8-bromooctanoic acid (Pfaltz & Bauer), methylethyl ketone (Burdick and Jackson), AG1-X8 anion exchange resin (chloride form, 200-400 mesh) (Bio Rad). Elemental analyses were performed by Spang Microanalytical Laboratory, Eagle Harbor, MI.

14-hydroxy-9-tetradecynoic acid (2) (7)

Hexyn-1-ol (7.84g, 0.08mol) in 100 ml anhydrous tetrahydrofuran was added to a suspension of lithium amide prepared from 1.25g lithium in 300 ml liquid ammonia and 0.14g ferric nitrate. After 30 min 8-bromooctanoic acid (3.6g, 0.016 mol) in 50 ml anhydrous tetrahydrofuran was added. The mixture was allowed to come to ambient temperature and was refluxed for 5 hr using an acetone-dry ice condenser. The reaction mixture was worked up by allowing the ammonia to evaporate, then treating the dark residual liquid with dilute HCl followed by extraction with ether three times. The ether was evaporated under reduced pressure to yield a yellow liquid which was heated at reflux for 2 hr with 25 ml 1.0 N NaOH. The cooled liquid was poured onto ice-dilute HCl. The white precipitate which formed was removed by filtration and air dried (4.1g crude yield, mp 44-46°). The solid was recrystallized from aqueous MeOH and from petroleum ether (30-60°)-ether to yield 2.72g (70%) product, m.p. 51-53°. Anal. $C_{14}H_{24}O_3$: C,H. (10) IR(CCl_4): 2940 (s), 2860 (m), 1710 (s) cm^{-1}. ^1H NMR($CDCl_3$): δ 7.03(2H,s) 3.55(2H,t,J 6Hz)

1.95-2.38 (6H,m), 1.45-1.61 (m) and 1.35 (s) (a total of 14H).

Methyl 14-hydroxy-9-tetradecynoate (3)

To 0.48g (2mmol) 2 in 10 ml absolute MeOH was added 0.3 ml boron trifluoride etherate. The solution was heated at reflux for 20 min, cooled to ambient temperature and poured onto ice-water. The mixture was extracted twice with ether. The ether layers were combined then extracted successively with 5% $NaHCO_3$, H_2O and saturated NaCl. After drying with anhydrous $MgSO_4$, the ether was evaporated under reduced pressure to yield an oil (0.44g, 87%) which was characterized by IR and NMR spectra as follows: IR(CCl_4): 2940(s), 2860(m), 1740(s) cm^{-1}. ^1H NMR(CCl_4): δ 3.61 (t,J 6Hz) and 3.46(s) (a total of 5H), 2.33 (1H,s), 1.93-2.16 (6H,m), 1.4-1.57(m) and 1.33(s) (a total of 14H).

Methyl 14-tosyl-9-tetradecynoate (4)

To 0.75g (2.9 mmol) 3 in 12ml anhydrous pyridine was added 0.9g (4.7mmol) p-toluenesulfonyl chloride at which time the solution turned yellow. The solution was refrigerated at 4° for 48 hr. The amount of precipitate present at 24hr did not increase. The reaction mixture was then poured into water and chilled in an ice bath. An oily precipitate formed which was extracted three times with ether. The combined ether layers were extracted twice with an ice cold solution of 1:1 (v:v) concentrated HCl and H_2O, then with water and then dried over anhydrous K_2CO_3-Na_2SO_4. The ether was evaporated under reduced pressure yielding a light yellow oil which was dissolved in petroleum ether (30-60°), filtered through charcoal and then chilled to -75°. The white precipitate was quickly removed by filtration and transferred to a flask. The solid liquified on warming. The tosylate, obtained in 70% yield, was characterized by IR absorbances at 1180 and 1190 cm^{-1} and by NMR as follows: IR(CCl_4): 2940(s), 2860(w), 1740(s), 1373(s), 1190(s), 1180(s) cm^{-1}. ^1H NMR (CCl_4):

δ 7.5 (4H,double d, J 8 Hz and 27Hz) 3.6 (3H,s), 3.5 (2H,t,J 6Hz), 2.43(3H,s) 1.37-2.3(m) and 1.37(s) (a total of 20H).

Methyl 14-iodo-9-tetradecynoate (5)

To 0.3g (0.7 mmol) 4 in 10 ml acetone was added 0.5g (3.3 mmol) sodium iodide. The reaction was stirred at ambient temperature overnight during which time a precipitate formed. The mixture was poured into water, and extracted with ether twice. The combined ether layers were dried with anhydrous $MgSO_4$ and the ether was removed under reduced pressure to yield a yellow oil. The oil was purified by column chromatography on silica gel using CCl_4 as eluent to yield 0.19g (74%) of a colorless oil. Anal. $C_{15}H_{25}IO_2$: C,H,I. IR (CCl_4): 2940 (s), 2860 (m), 1740 (s) cm^{-1}. ^1H NMR (CCl_4): δ 3.55(3H,s), 3.13 (2H,t,J 6Hz), 1.35-2.36 (m) and 1.35 (s) (a total of 20H).

14-iodo-9-tetradecynoic acid(1)

A solution of 0.3g (0.82 mmol) 5 and 0.25 g (0.45 mmol) potassium hydroxide in 4 ml of 50% ethanol was stirred at ambient temperature overnight. The solution was chilled to 0° and acidified dropwise with concentrated HCl. The white precipitate was removed by filtration and recrystallized from petroleum ether (30-60°) to yield 0.22g (76%) of a white solid mp 43-44. Anal. $C_{14}H_{23}IO_2$: C,H,I. IR (CCl_4): 2940 (s), 2860 (m), 1711 (s) cm^{-1}. ^1H NMR(CCl_4): δ 11.52 (1H,s), 3.17 (2H,t,J 6Hz), 1.4-2.35 (m) and 1.4 (s) (a total of 20H).

14-^{125}I-iodo-9-tetradecynoic acid (6)

A solution of 14-iodo-9-tetradecynoic acid (1) (1.4 mg) and ^{125}I-NaI (1.1 mCi) (no carrier added) in 3 ml of methylethyl ketone was placed in a 10 ml flask and was heated at reflux (oil bath). The progress of exchange was followed by tlc (cellulose plates, n-hexane/diethyl ether/glacial acetic acid : 320/80/1: R_f iodo fatty acid = 0.89, R_f free iodide = 0.0). After approximately 1.5 hr

of reflux, the exchange was judged complete (95% displacement determined by radiochromatogram scans of tlc plates), the solvent was removed under a stream of nitrogen. The residue was formulated by dissolution in absolute ethanol-Tween 80 then diluting to volume with normal saline. For removal of free radioiodide the formulation was passed through a glass column packed with about 1.0g AG1-X8 anion exchange resin (chloride form) and washed with formulation. Removal of free radioiodide was confirmed by repeat tlc on cellulose using eluent as above (R_f values as above). Radiochemical purity was determined to be >97% by the tlc on cellulose (n-hexane/diethyl ether/glacial acetic acid, 320/80/1) R_f iodo acid = 0.89, R_f free iodide = 0.0 and on silica gel (n-hexane/diethyl ether/glacial acetic acid, 70/30/1) R_f iodo acid = 0.23, R_f free iodide = 0.0. Final specific activity obtained was 0.78 μCi/mg.

Tissue Distribution Studies

The radioiodinated fatty acid was evaluated in female Sprague-Dawley rats and in female mongrel dogs.

For tissue distribution studies, approximately 25 μCi (rats) or 100 μCi (dogs) of 6 in a physiological formulation (EtOH/Tween 80/0.9% saline: 2.5 ml/0.65 ml/22 ml) was administered intravenously. The animals (3 rats or 2 dogs per time interval) were sacrificed at prescribed time intervals (5, 10 and 20 min.). Representative 50-100mg samples of liver, lung, heart, blood, muscle and thyroid were removed and cleaned of fat and connective tissue. Duplicate samples of each tissue were weighed on a Mettler analytical balance with computer printout and prepared for counting. Samples were placed in test tubes together with distilled water and counted using an auto-gamma scintillation counter. All values are corrected for radioactive decay, counting efficiency and background. The values of resulting

tissue concentrations were normalized to a per kilogram body weight and are therefore reported as % kg dose/g (9).

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