PREPARATION OF ω-^{123}I-LABELLED FATTY ACIDS BY ^{123}I-FOR-Br EXCHANGE: COMPARISON OF THREE METHODS

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SUMMARY

Long chain ω-^{123}I-labelled fatty acids such as 16-I-hexadecanoic-, 17-I-heptadecanoic-, and 16-I-hexadec-9-enoic acid have been prepared without adding carrier by nucleophilic ^{123}I-for-Br exchange in acetone, in the melt, and by use of phase transfer catalysts in non-polar solvents. While preparation in refluxing acetone required reaction times of more than 2 hours to reach saturation yields, halogen exchange in molten bromofatty acids at temperatures of about 150 °C gave rise to yields of about 80% of radiochemically pure 17-^{123}I-heptadecanoic acid within only 5 minutes. Comparable yields were also obtained using Kryptofix 221 in refluxing toluene after 10 minutes.

Key words: ^{123}I-Radiopharmaceuticals, ^{123}I-Labelled Fatty Acids, High Pressure Liquid Chromatography, Halogen Exchange in Melt

INTRODUCTION

Fatty acids labelled with iodine-123 in the terminal position have recently been used successfully in clinical diagnosis of myocardial infarction and ischemia as well as in determining the metabolic turn-over rates of fatty acids within distinct myocardial regions (1,2,6). This development was possible by the observation

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that among fatty acids labelled with various radionuclides in different positions the ω-^{123}I-heptadecanoic acid showed a pharmacokinetic and biochemical behaviour almost identical to that of [1-^{11}C]-palmitic acid and that the final catabolic fate of the label is its rapid appearance as free halide (3-5) which can be corrected for (2,6). Improved methods allowing a fast introduction of the ^{123}I-label into aliphatic compounds such as fatty acids with high yields and high specific activities are required. Isotopic exchange does not provide the high specific activities which are necessary for the final medical in-vivo application. Instead, non-isotopic nucleophilic halogen exchange is generally the method of choice. We have already pointed out the advantages of the halogen exchange in the melt when compared with that in solution (7). In any case, to achieve a quantitative separation of the labelled compound from the inactive halofatty acid used as starting material the application of an efficient chromatographic procedure such as radio high pressure liquid chromatography (8) is also essential, since high specific activities can be obtained, a requirement for preparing injectable solutions. In this paper we compare the Br-for-I exchange in solution (3,10,11) with the corresponding exchange in the melt and that by means of phase transfer catalyst in non-polar solvents.

EXPERIMENTAL

Materials

Iodine-123 was prepared via the ^{124}Te(p,2n)^{123}I-process bombarding 99.9% enriched ^{124}Te as TeO_2 with 24 MeV protons of the CV-28 compact cyclotron or via the ^{127}I(d,6n)^{123}Xe(ß$^+$,EC)^{123}I-reaction using the Isochronous Cyclotron JULIC (9). The radioiodine was

generally obtained in aqueous solution of about 100 mCi ^{123}I-iodide in 1 to 2 ml water.

Substrates such as ω-Br-heptadecanoic acid and 16-Br-hexadec-9-enoic acid were supplied by EMKA-Chemie, Markgröningen-Talhausen, FRG, and ALDRICH-Chemie, respectively. Quaternary ammonium- and phosphonium compounds were obtained from FLUKA AG, Buchs, Switzerland. Kryptofix 221 was supplied by MERCK, Darmstadt, FRG.

A. Preparation of ω-^{123}I-fatty acid in acetone solution:

The Na^{123}I-solution brought to a pH of 8-10 and containing 1 mg of sodium sulfite or 10 µl of 25% hydrazine (to prevent oxidation of the iodide) was frozen with liquid nitrogen in a 25 ml vessel. The solvent was evaporated on a vacuum line by gentle heating with a fan. Thereafter 2 ml acetone solution of 5 mg 17-Br-fatty acid were added and the mixture then refluxed up to 110 minutes, depending on the type of halofatty acid. The solvent was then evaporated at a small vacuum line and the residue dissolved in 2 ml eluent for the final hplc-separation.

B. Preparation in the melt:

The Na^{123}I-solution was prepared and evaporated as described above. After adding the solution of 5 mg 17-Br-heptadecanoic acid in 2 ml acetone the solvent was evaporated in the same manner as the water. The closed reaction vessel was then kept in an oil bath at 150 °C for the complete reaction period which was 5 min for a routine preparation. The reaction mixture was extracted twice with 1.5 and 0.5 ml hplc-eluent, respectively, and the whole solution injected onto the column.

C. Preparation via phase transfer catalysts:

The Na^{123}I-solution was prepared and evaporated as described above. A solution of 5 mg 16-Br-hexadecanoic acid or 17-Br-heptadecanoic

acid in 1 ml benzene was added; the stated amounts of the various quaternary ammonium- or phosphonium salts (see below Table III) were added to dissolve the $^{123}I^-$ in benzene. The mixture was heated under reflux (80 °C) for the time stated in Table III. After this period the reaction mixture was directly injected onto the hplc column. The reaction was further optimized: After evaporation of $Na^{123}I$, a solution of 10 mg 17-Br-heptadecanoic acid and 1 mg of either tetra-n-butylammonium bromide or Kryptofix 221 in 1 ml toluene was added. This mixture was heated at reflux in toluene (140 °C) for 10 min. After this period the mixture was directly injected onto the hplc-column.

Chromatography, Quality Control

For routine separation of the parent bromofatty acid from the practically carrier-free iodofatty acid partition chromatography is advantageous as compared to adsorption-, reversed phase- or ion exchange chromatography: the separation capacity is higher by a factor of about 100, the good solubility of the fatty acids in the mobile phase applied allows concentration in a small volume for injection, and finally, inorganic iodine remains on the column. We have used acetic acid on Lichrosorb Si-100 as stationary phase and n-heptane + 0.25 vol.% acetic acid as eluent.

The reaction mixture (substrate + product) was directly dissolved in the eluent and separated from the Na_2SO_3 suspension by filtration through a millipore filter. The column head was filled with a quartz wool filter instead of the usual frit in order to prevent closure by micro particles which can seriously disturb routine separation. Under the conditions given in Table I about 10 mg of the bromofatty acid can still be separated from the carrier-free product.

Table I

Chromatographic Data for the Separation of Halogenated Fatty Acids

Column: Lichrosorb Si-100, 5 µm, 25 x 1 cm
Eluent: n-heptane + 0.25 % acetic acid (v/v)
Flow: 10 ml/min
Detectors: RI and Well-Type Scintillation

Compound	K'	Retention (min)
16-Br-Hexadecanoic Acid	8.9	17.6
16-I-Hexadecanoic Acid	8.2	16.2
17-Br-Heptadecanoic Acid	8.8	17.4
17-I-Heptadecanoic Acid	8.1	16.1
16-Br-Hexadec-9-enoic Acid	10.7	21.2
16-I-Hexadec-9-enoic Acid	9.8	19.6

Detection of the products is achieved by RI-detector in series with a radioactivity detector (8). The radiochromatographic procedure allows chemical and radiochemical quality control during the purification step.

RESULTS AND DISCUSSION

The I-for-Br exchange in solution has been used before by Robinson and Lee (10) and Robinson (11) for preparing radioiodinated fatty acids. We first checked the iodination of 16-Br-hexadec-9-enoic acid using Robinson's (11) conditions and compared the ^{123}I-for-Br exchange in compounds such as 17-Br-heptadecanoic acid in solution, in the melt, and with phase transfer catalysts in non-polar solvents. For the iodination of the unsaturated fatty acid Robinson (11) reported saturation yields of 60 to 80% when refluxing in acetone and methylethylketone (MEK). Table II shows, however, that analysis of the labelled organic product by hplc indicates two unknown impurities.

Table II

HPLC-Analysis of 16-^{123}I-Hexadec-9-enoic Acid (I-HdeA) prepared by ^{123}I-for-Br Exchange in Acetone and MEK at different Reaction Times

Reaction Time [min]	Solvent	% Radiochemical Yield Impurity 1	Impurity 2	I-HdeA	Σ
20	Acetone	29	1	6	36
50	"	34	3	10	47
80	"	38	1	20	59
20	MEK	9	0	39	48
45	"	11	0.2	46	57
90	"	10	0.2	47	57

In acetone, one of the impurities is formed with 38% yield within 80 min whereas the desired product is obtained only with 20% yield during the same reaction period. The use of freshly distilled methylethylketone (MEK) was reported by Robinson (11) to result in a significant increase of labelled product due to the higher reflux temperature. However, the yields of Robinson (75-85%, determined by TLC) could not be confirmed by hplc. The findings clearly demonstrate the necessity of a careful analytical quality control which can easily be achieved by means of radio high pressure liquid chromatography.

In contrast to the results obtained for the iodination of the unsaturated acid the ^{123}I-for-Br exchange can be carried out more successfully in saturated fatty acids such as 17-Br-heptadecanoic acid. The observed amount of by-products is less than 10%. 17-^{123}I-heptadecanoic acid is obtained with 80% after 95 min when carrier-free ^{123}I is used in a solution of 5 mg Br-HdA in 2 ml acetone.

The dependence of yields on reaction time shows that ω-^{123}I-heptadecanoic acid can conveniently be prepared in fairly high yields by application of the ^{123}I-for-Br exchange in acetone.

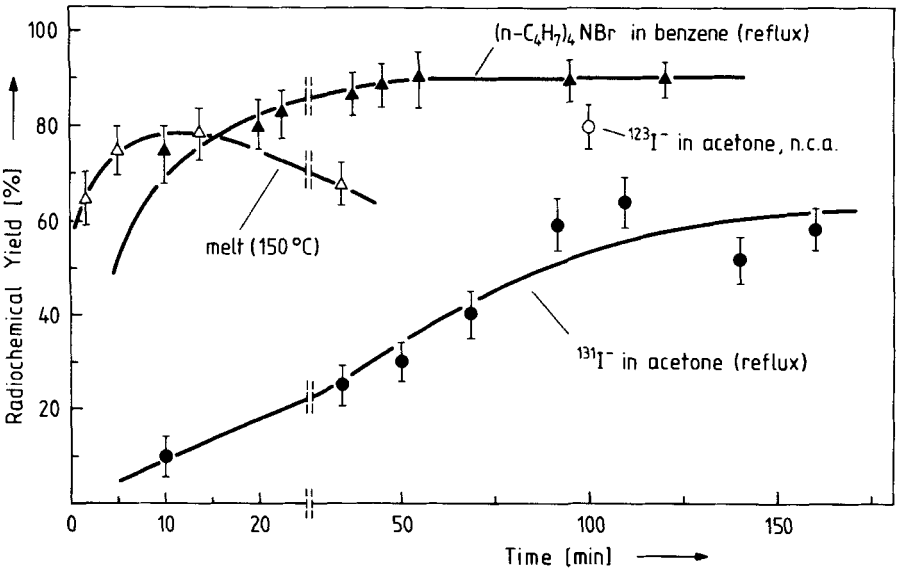

Fig. 1 Preparation of ω-I-heptadecanoic acid via I-for-Br exchange
● : $^{131}I^-$ in solution of 1 mg Br-HdA in 1 ml acetone at 57 °C
○ : $^{123}I^-$ in solution of 5 mg Br-HdA in 2 ml acetone at 57 °C
△ : $^{123}I^-$ in melt of 5 mg Br-HdA at 150 °C
▲ : $^{123}I^-$ in solution of 5 mg Br-HdA in 1 ml benzene (containing 50 µg $(n\text{-}C_4H_7)_4Br$) at 80 °C
in all experiments no carrier was added.

The data shown in Fig. 1 for the exchange reaction in acetone have been obtained using $^{131}I^-$ for convenience. They show that the reaction is rather slow and more than 2 hours are needed to reach saturation yields. With ^{123}I ($T_{1/2}$ = 13.3 h) such long reaction times are not acceptable; the reaction was only carried out for 95 min, after which time yields (79 ± 6%) were significantly higher than for $^{131}I^-$, both at 17-Br-heptadecanoic acid concentrations of 1 mg/ml and 2.5 mg/ml. Higher bromofatty acid concentrations did not increase the yield. In addition, hplc-separation from the parent compound is insufficient above 10 mg/ml.

Fig. 1 also shows that a considerably faster iodination can be achieved in the melt of the bromofatty acid itself. It can be seen that a radiochemical yield of 17-^{123}I-heptadecanoic acid up to 80% is obtained within about 5 min when the melt is kept at 150 °C. With respect to a routine production care has to be taken to minimize

losses of radioactivity during the preceding evaporation of the solution containing the ^{123}I-iodide. Losses can be avoided by lyophilizing the solution as described above.

Good yields for the ^{123}I-for-Br exchange can also be obtained in non-polar solvents using phase transfer catalysts, as seen in Table III and Fig. 1. The time needed for the reaction can be shortened to 10 min without major losses in yields. During the experiments we observed that the ionic compounds like tetra-n-butylammonium bromide slowly accumulated on the chromatographic column. This led to ion pair formation during chromatography resulting in two radioactive peaks, both corresponding to 17-^{123}I-heptadecanoic acid. We, therefore, employed the non-ionic cryptand Kryptofix 221, which did not accumulate on the chromatographic column after multiple injections.

With saturated aliphatic bromofatty acids all the three methods furnish comparable yields of ^{123}I-iodofatty acids by ^{123}I-for-Br exchange. The most rapid method is the exchange in the melt. Difficulties are encountered in the melt when the starting $^{123}I^-$ solution contains significant amounts of salts, like NaCl, from the work-up of the iodide target. In this case (see Fig. 1), the method using phase transfer catalysts is the most rapid one. Using this method significant amounts of salts can be tolerated. This is also true for the acetone method which only has the disadvantage of resulting in slightly lower yields after significantly longer reaction times.

Although the halogen exchange in acetone had been in routine use with satisfying results for the preparation of 17-^{123}I-heptadecanoic acid for some time in our laboratory, the melt method is now preferred because of its short reaction time. Quantities of 20 to 50 mCi of 17-^{123}I-heptadecanoic acid (no carrier added) are prepared twice each week.

Table III

^{123}I-for-Br Exchange in Saturated 16- and/or 17-ω-Br-Fatty Acids using Phase Transfer Catalysts in Non-Polar Solvents at Reflux

Catalyst	Solvent	Yield after Chromatography	Reaction time (min)
tetra-n-butyl-ammonium bromide 50 µg	benzene	90%	60
hexadecyl-tri-n-butyl-phosphonium bromide 50 µg	"	85%	60
tetraethyl-ammonium toluene-4-sulfonate 50 µg	"	95%	120
hexadecyl-trimethyl-ammonium bromide 50 µg	"	90%	80
benzyl-tri-n-butyl-ammonium bromide 100 µg	"	55%	80
benzyl-dimethyl-hexadecyl-ammonium chloride 50 µg	"	70%	120
tetra-n-butyl-ammonium bromide 1 mg	toluene	80%	10
Kryptofix 221 1 mg	"	82%	10

for details: see Methods

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