SYNTHESIS OF ^{123m}Te LABELED FATTY ACIDS

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SUMMARY

Four ^{123m}Te labeled fatty acid analogues were synthesized: 17-tellura-9-octadecenoic acid, 17-tellura-9-nonadecenoic acid, 18-methyl-17-tellura-9-nonadecenoic acid and 3-tellura-nonadecanoic acid.

Tellurium-123m metal was solubilized in water with $NaBH_4$. The inorganic nucleophiles produced, NaHTe or Na_2Te_2 were reacted with 16-bromo-9-hexadecenoic acid in a one or two step procedure to produce the sodium alkyl telluride. To this was added either methyl iodide, ethyl bromide or isopropyl bromide and the resulting 17-tellura-fatty acids isolated and formulated. Tellurium-123m labeled 3-tellura-nonadecanoic acid was synthesized via the dihexadecanyl ditelluride or the sodium hexadecanyl telluride route.

Key Words: ^{123m}Te labeled fatty acids, 17-tellura-9-octadecenoic-^{123m}Te acid, 17-tellura-9-nonadecenoic-^{123m}Te acid, 18-methyl-17-tellura-9-nonadecenoic-^{123m}Te acid, 3-telluranonadecanoic-^{123m}Te acid.

INTRODUCTION

The demand for noninvasive methods for myocardial perfusion imaging has led to the development of various analogues of fatty acids containing ^{131}I, ^{11}C, ^{77}Br, and ^{34m}Cl (1-5). Fatty acids have a high affinity for myocardial cells through active transport whereby they are rapidly utilized as a major source of energy (6). Myocardial cells derive as high as 67% of their energy needs from the catabolism of fatty acids.

Virtually all of the fatty acid radiopharmaceuticals so far available

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possess major disadvantages (5,7,8) such as fast dehalogenation of the aliphatic radioactive halogen, fast metabolism by the myocardium, the use of positron emitting radionuclides e.g. ^{11}C, thus limiting their clinical use for myocardial scanning. Consequently, there is a need for fatty acid analogues containing a "stable" radionuclide which possesses suitable characteristics for imaging.

The incorporation of ^{123m}Te, a radionuclide with excellent gamma imaging characteristics (84% of 159 keV) and $t_{\frac{1}{2}}$ of 119 days, into fatty acids has been attempted by two groups of researchers. Knapp and co-workers introduced ^{123m}Te into the middle of long chain fatty acids (9); our laboratory (10) first reported on a successful displacement of an ω -halogen on a fatty acid with the CH_3Te-moiety. Since then we have been attempting to stabilize the tellurated fatty acids by synthesizing various analogues.

The synthesis of these ^{123m}Te fatty acids is via a single reaction vessel utilizing our developed method of ^{123m}Te metal dissolution with $NaBH_4$ in water (11,12). This method is capable of forming two inorganic nucleophiles: Sodium hydrogen telluride (NaHTe) and disodium ditelluride (Na_2Te_2) by altering reaction parameters. The non-radioactive tellurium containing fatty acids were characterized by PMR, Mass Spectroscopy, and Atomic Absorption, and their chromatographic properties were identical to the ^{123m}Te labeled fatty acids.

RESULTS AND DISCUSSION

Fatty acids labeled with gamma emitting nuclides have been shown to be useful as myocardial imaging agents. Poe and Robinson (2,4), Machulla, et. al. (5) studied the extraction rates and kinetics of utilization for fatty acids labeled with ^{11}C, ^{123}I, ^{34m}Cl, and ^{77}Br in the myocardium of animals. The latter workers concluded that ω-halofatty acids were extracted more efficiently than α-halofatty acids and that an odd-carbon fatty acid, 17-iodoheptadecanoic acid, showed an even better extraction equal to that of 1-^{11}C-palmitic acid except that it deiodinated at an extremely fast rate.

We embarked on the synthesis of fatty acids labeled with a more stable covalently bound radionuclide, ^{123m}Te. We synthesized 17-tellura-9-octadecenoic acid an even 18 carbon unsaturated fatty acid analogue with ^{123m}Te replacing car-

bon-17; 17-tellura-9-nonadecenoic acid an odd 19 carbon unsaturated fatty acid analogue with ^{123m}Te replacing carbon-17; 18-methyl-17-tellura-9-nonadecenoic acid an even carbon terminally branched unsaturated fatty acid analogue with ^{123m}Te replacing carbon-17 and 3-telluranonadecanoic acid an odd 19 carbon saturated fatty acid analogue with ^{123m}Te replacing carbon-3, the β-carbon.

Our synthesis was dependant of the facile solubilization of ^{123m}Te into a solvent suitable for the reaction conditions of fatty acids e.g. water or alcohol. This was accomplished by the use of sodium borohydride in various mole ratios and different pH ranges (11,12).

Two inorganic ^{123m}Te nucleophiles were utilized: NaHTe and Na_2Te_2. The formation of these required an N_2 atmosphere, reflux conditions of the tellurium metal in water and the addition of excess sodium borohydride to produce NaHTe (Eqn. 1):

$$2Te + 4NaBH_4 + 7H_2O \xrightarrow{Heat,N_2} 2NaHTe + Na_2B_4O_7 + 14H_2 \quad \text{(Eqn. 1)}$$

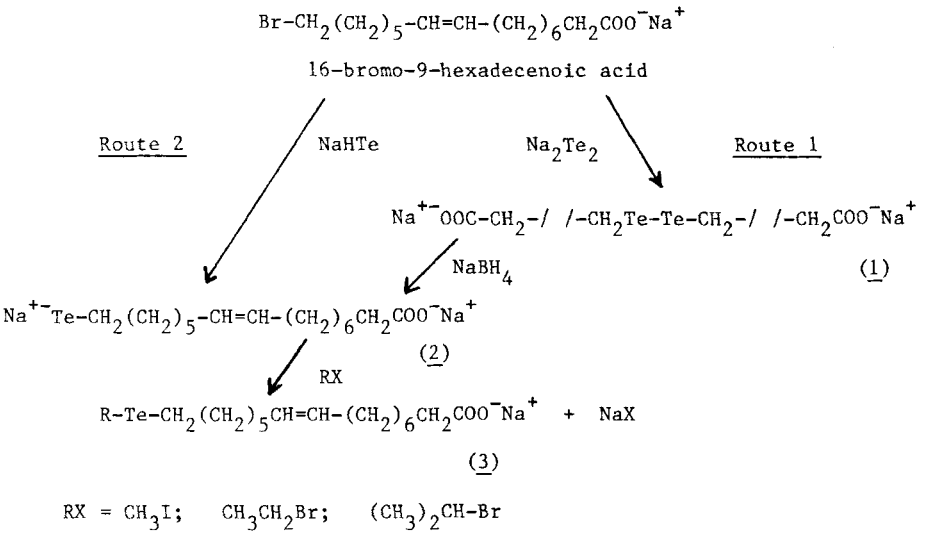
This solution is ready for further reaction because the by-product $Na_2B_4O_7$ does not interfere in subsequent reactions. If another mole equivalent of ^{123m}Te metal slurried in a basic aqueous solution is added to the above reaction, a deep-violet purple solution (Na_2Te_2) is obtained with complete dissolution of the ^{123m}Te metal (Eqn. 2):

$$NaHTe + Te + NaOH \xrightarrow{Heat,N_2} Na_2Te_2 + H_2O \quad \text{(Eqn. 2)}$$

This nucleophile is ready for further *in situ* reactions.

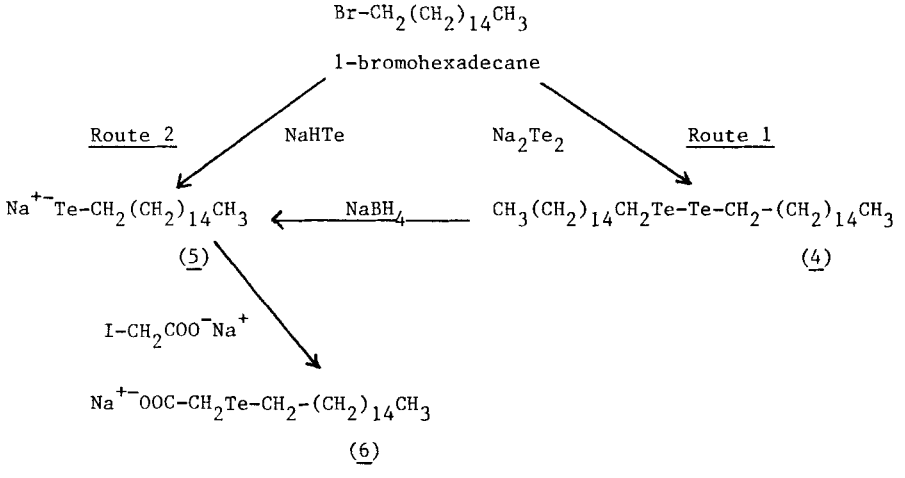
The tellurated fatty acids were synthesized by two different routes both having a final common intermediate: the sodium alkyl telluride of the fatty acid: $Na^{+-}OOC\text{-}CH_2(CH_2)_xCH_2Te^-Na^+$.

Route 2 utilized the necleophile NaHTe and the alkyl halide 16-bromo-9-hexadecenoic acid (see Scheme 1) to give the organic nucleophile sodium 16-tellura-9-hexadecenoate 2. This nucleophile can also be obtained by the sodium borohydride reduction of the ditelluride 1, a reddish-purple product formed by the reaction of Na_2Te_2 and 16-bromo-9-hexadecenoic acid, (Route 1, Scheme 1). This intermediate 2 is a near colorless oxygen sensitive compound which is immediately reacted with the desired alkyl halide e.g. methyl iodide, ethyl bromide or isopropyl bromide to give the stable ω-alkyltellura-9-hexadecenoate 3.



Scheme 1

Scheme 2 shows the synthetic routes attempted in the synthesis of 3-tellura-nonadecanoic acid. Route 2 was the choice route for the preparation of the radioactive tellurium isostere of the fatty acid and Route 1 was the choice route in the non-radioactive synthesis, because the orange-red dihexadecanyl ditelluride 4 could be isolated, purified and characterized before it was reduced and converted to sodium 1-tellurahexadecane 5. This colorless nucleophile was reacted with iodo or bromo acetate to yield the 3-tellurated fatty acid 6.



Scheme 2

Biodistribution of the four compounds produced, is underway at this time. The myocardial uptake and in vivo stability of these tellurium labeled fatty acids appear to be better than ^{11}C, ^{123}I, ^{34m}Cl, and ^{77}Br analogues produced by previous workers in this area.

EXPERIMENTAL

Stable metallic (grayish-black powder) tellurium and metallic ^{123m}Te (specific activity - 1 mCi/mg, Union Carbide) were used in the syntheses outlined below. All reactions were performed in an inert atmosphere of N_2 gas to avoid the decomposition of reactive inorganic and organic intermediate nucleophiles. 16-Bromo-9-hexadecenoic acid was purchased from Aldrich Chemical Company and recrystallized from petroleum ether to a white powder, m.p. 41-42°C. No attempts were made to elucidate the stereo chemistry of this compound. Sodium borohydride was purchased from Matheson, Coleman & Bell.

SOLUBILIZATION OF ^{123m}Te IN WATER:

Production of NaHTe-(^{123m}Te): Metallic ^{123m}Te (12.7 mg, 0.1 mmol) was weighed into a screw capped vial, assayed in a dose calibrator, and transferred quantitatively into a 25 ml 3-necked flask with about 2 ml of distilled water. The flask was fitted with a water cooled condenser. Both side arms were fitted with rubber septums, one side was used to introduce reactants while the other side was used to provide a continuous stream of nitrogen over the reaction solution, and bubbled through an I_2-KI aqueous solution to trap any released volitile ^{123m}Te compounds. Heating of the flask was started and 10 mg of $NaBH_4$ (0.25 mmol) in 1 ml of distilled water was added via a syringe through the rubber septum. Heating to reflux was continued until the stirred suspension of ^{123m}Te metal and water acquired a violet tinge which, within 15 min, turned to a colorless, clear solution. The heat was then removed and the aqueous pH 8-9 solution of NaHTe-(^{123m}Te) was ready for the next reaction.

Production of Na_2Te_2-(^{123m}Te): To 6.35 mg of metallic ^{123m}Te (0.05 mmol), weighed, radioassayed and transferred as described above, was added 4 mg of $NaBH_4$ (0.1 mmol) in 1 ml of distilled water. This stirred slurry was heated at reflux until a clear, colorless solution was obtained. To this was added a

suspension of another 0.05 mmol of ^{123m}Te (6.35 mg) in 1 ml of 1% NaOH via a syringe. Immediately, the solution acquired a violet-purple tinge as the added ^{123m}Te metal started to dissolve. Under reflux and continuous stirring within 5 min a clear dark purple solution was obtained. The species produced in situ was disodium ditelluride in a pH 12 aqueous solution.

SYNTHESIS OF ω-^{123m}Te-FATTY ACIDS:

Using the dihexadecenoic acid ditelluride route (Route 1, Scheme 1): ^{123m}Te metal (2.5 mg, 0.02 mmol, 2.5 mCi) was solubilized in water to NaHTe as previously described. To this was added another (0.02 mmol) of ^{123m}Te in 1 ml of 1% NaOH to produce the Na_2Te_2 species. This solution was cooled to room temperature and to it was slowly added 14 mg (0.04 mmol) of sodium 16-bromo-9-hexadecenoate in 2 ml of 20% ethanol (pH 8.5). The reaction mixture turned a pale reddish purple as soon as the addition was complete indicating the production of 1.

Sodium borohydride 16 mg (0.4 mmol) previously solubilized in 0.6 ml of water was added and reflux continued until a colorless solution containing a minute amount of a white precipitate was obtained. The reaction mixture was allowed to cool to room temperature and ethyl bromide 14.0 mg (0.13 mmol) in 2 ml ethanol was added. The solution was stirred for approximatly 15 min at a room temperature. The nitrogen supply was turned off, and the solution acidified with dilute HCl solution to a pH of 4. This solution was extracted three times with 10 ml of ether to yield 3.2 mCi (approximately 64%) of the free acid of 3. The ether was evaporated using mild heat and N_2 passing over the solution.

Using the sodium hexadecenoate telluride route (Route 2, Scheme 1): ^{123m}Te metal (5 mg, 0.04 mmol, 5 mCi) was solubilized in water to NaHTe as previously described. To this was added 14 mg (0.04 mmol) sodium 16-bromo-9-hexadecenoate in 1.5 ml of 20% ethanol (pH 8.5). The solution was warmed for 30 min and allowed to cool to room temperature. To this solution was added 1.6 mg (0.04 mmol) of NaOH in 1 ml of water to produce 2.

Ethyl bromide 14.0 mg (0.13 mmol) in 2 ml ethanol was added, and the solution stirred for approximately 15 min at room temperature. The nitrogen supply was turned off, and the solution acidified with dilute HCl solution

to a pH of 4. This solution was then extracted three times with 10 ml of ether. The ether extracts were combined and dried with anhydrous sodium sulfate yielding 3.8 mCi (approximatly 76%) of the free acid of 3. The ether was evaporated using mild heat and N_2 passing over the solution. A solution of 20% ethanol, 2% Tween 80 and sufficient saline to bring to volume was used to formulate the product.

The methyl and isopropyl analogues were synthesized in a similar fashion with yields of 51% and 59% using the dihexadecenoic acid ditelluride route (Route 1) and 60% and 69% using the sodium hexadecenoate telluride route (Route 2) respectively.

Crystallization of the non-radioactive 17-tellura-9-octadecenoic acid was accomplished in two different solvent systems. Ethanol was used to crystallize the product followed by recrystallization from acetone, m.p. 94-95°C. Crystallization of the non-radioactive 17-tellura-9-nonadecenoic acid was from ethanol, recrystallized from methanol, m.p. 76-77°C. Crystallization of the non-radioactive 18-methyl-17-tellura-9-nonadecenoic acid was from ethanol, m.p. 93-94°C.

The formulation of the methyl analogue was the same as for the ethyl but the isopropyl could not be formulated in this fashion. A solution of 12.5% HSA (human serum albumin) in saline was used to formulate the product.

Comparison of the radioactive and non-radioactive compounds was performed by thin layer chromatography on Silica gel (Eastman). Using the solvent chloroform:methanol (1:1 v/v) the methyl analogue had an R_f of 0.8 while 16-bromo-9-hexadecenoic acid had an R_f of 0.75. Using the solvent benzene:methanol (3:1 v/v) the ethyl and isopropyl analogues had R_fs of 0.45 and 0.4 respectively which compared to 0.5 for 16-bromo-9-hexadecenoic acid. Both the ^{123m}Te and non-radioactive compounds co-chromatographed in the above systems.

SYNTHESIS OF 3-TELLURANONADECANOIC-^{123m}Te ACID $C_{16}H_{33}$Te-CH_2COOH:

Using the dihexadecane ditelluride route (Route 1, Scheme 2): ^{123m}Te metal (9.0 mg, 0.075 mmol, 2.24 mCi) was solubilized in water to NaHTe as previously described. To this was added another (0.075 mmol) of ^{123m}Te in 1 ml of 1% NaOH to produce the Na_2Te_2 species. This solution was allowed to cool to room temperature and to it was slowly added 90 mg (0.3 mmol) of 1-bromohexadecane in

0.5 ml of peroxide-free THF via a syringe. Heat was applied to the solution whereby a color change from dark purple to brownish-red was observed. After 2 h of gentle heating and stirring, the ditelluride 4 was ready for futher reaction.

To the brownish-red solution of the dihexadecane ditelluride 4 was added enough $NaBH_4$ in 1% NaOH solution to yield a colorless solution containing the nucleophile, sodium salt of the hexadecanyl telluride 5. Three fold molar excess of sodium iodoacetate (in THF-H_2O 1:1) was added to the solution and reflux continued for 1 h. The solution was cooled to room temperature and extracted with ether to remove the by-product dihexadecanyl telluride $(C_{16}H_{33})_2Te$ (1 mCi). To the basic aqueous layer was added 1 ml of 25% HSA (human serum albumin) solution and enough acetic acid to adjust the pH to 9.0. Yield 3.1 mCi (approximately 70%) of 5 as the sodium salt.

Using the sodium hexadecanyl telluride route (Route 2, Scheme 2): ^{123m}Te metal (9 mg, 0.075 mmol, 2.24 mCi) was solubilized in water to NaHTe as previously described. To this was added 1-iodohexadecane (35 mg, 0.1 mmol) in 0.5 ml of peroxide-free THF. The solution was refluxed for 15 min to produce 5. To the room temperature solution was added 0.5 ml of 1% NaOH, followed by three fold molar excess of sodium iodoacetate in (THF-H_2O 1:1) and the solution again refluxed for 1 h.

The cooled colorless solution was extracted with ether to remove the by-product dihexadecanyl telluride (1 mCi). The basic aqueous solution was cooled with ice and slowly acidified with 1% HCl solution to pH 3. A white precipitate appeared which was immediately extracted with ether, the ether layer dried over $MgSO_4$ and evaporated to dryness yielding 1.2 mCi (approximately 54%) of 6 as the free acid.

Thin layer chromatography on Silica gel (Eastman) with benzene:methanol (3:1) showed a single radioactive compound at the origin. After acidification, the R_f was 0.4 corresponding to the non-radioactive free acid reference compound.

In the non-radioactive synthesis using route 1 outlined above, the dihexadecanyl ditelluride 4 was isolated (over 90% yield) as a red liquid which solidified upon cooling and had a melting point of 48-50°C. Thin layer chromatography

using silica gel eluted with $CHCl_3$ gave an R_f of 0.9.

The sodium salt of 3-telluranonadecanoic acid 6 was recrystallized from ethanol:water to yield a white solid, m.p. 138-140°C.

The synthesized telluro fatty acids were analyzed by atomic absorption spectroscopy utilizing a tellurium lamp to confirm the presence of tellurium.

The proton NMR spectra of the Te containing fatty acids exhibited the following chemical shifts (ppm from TMS): C-16 methylene protons at δ 2.60 (triplet), C-18 methylene protons at δ 2.49 (multiplet), C-18 methine protons at δ 2.80 (multiplet), C-2 methylene protons at δ 3.20 (singlet) and C-4 methylene protons at δ 2.60 (triplet). All the above shifts are for protons adjacent to the Te atom and they agree with those reported by Knapp et al (9) on tellurium isosters of palmitoleic and oleic acids.

The mass spectra of the four title compounds exhibited multiple small M^+ peaks due to the presence of naturally occuring isotopes of tellurium, m/e 120, 122, 124, 126, and 130. The relative abundance of the $(Te)^+$ and $(TeH)^+$ ranged from 3-20% for each of the isotopes compared to 60-100% abundance for the characteristic aliphatic hydorcarbon chain fragmentation seen in fatty acids. Other prominent characteristic peaks were m/e 383 corresponding to M-15 ($-CH_3$) or M-29 ($-CH_2CH_3$) or M-43 ($-CH(CH_3)_2$); m/e 253 corresponding to M-145 (CH_3Te-) or M-159 (CH_3CH_2Te-), or M-173 ($(CH_3)_2CHTe-$); m/e 145 $(CH_3Te)^+$; m/e 159 $(CH_3CH_2Te)^+$; m/e 173 $((CH_3)_2CHTe)^+$; and m/e 188 $(TeCH_2COO)^+$.

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