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The Design and Biological Properties of Iodine-123 Labeled β-Methyl-Branched Fatty Acids

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INTRODUCTION

The major energy requirements of the normal myocardium are met by the metabolic oxidation of long chain fatty acids. Regional differences in myocardial uptake of radiolabeled fatty acids could reflect not only differences in regional perfusion, but could also potentially be an accurate and elegant means of detecting subtle differences in regional metabolism. Iodine-123 is the most attractive radionuclide for radiolabeling fatty acids since it has excellent radionuclidic properties (159 keV photon; 13.3 hour half-life) and there are a wide variety of chemical methods available for attaching iodine to fatty acids (Knapp et al, 1984c). Iodine-123 labeled 17-iodoheptadecanoic acid (^{123}I-HDA) was developed by the Julich group and has been widely used to evaluate regional myocardial metabolism by analysis of the regional time activity curves (Freundlieb et al, 1980; Feinendegen et al, 1978; Feinendegen et al, 1980; Feinendegen et al, 1983; Dudczak et al, 1982 and 1984). Other workers have used HDA, iodine-123-labeled 16-iodohexadecanoic acid and 16-iodo-9-hexadecenoic acid in the same manner (Van der Wall et al, 1981).

For some applications where an evaluation of regional myocardial uptake may be more desirable than a measurement of the rate of washout, rapid myocardial clearance must be minimized. High blood levels of radioactivity caused by in vivo deiodination and clearance of labeled metabolites result in only modest heart:blood ratios requiring special subtraction procedures for differentiation between myocardial uptake and blood pooled within the cardiac chambers. If the problems associated with high blood levels of radioactivity and rapid washout from the myocardium could be overcome, ^{123}I-labeled fatty acids could also be used to measure regional distribution where aberrations in fatty acid metabolism are reflected by differences in uptake.

To minimize radioiodide loss, iodine has been chemically stabilized by attachment to the para-position of the phenyl ring of 15-phenylpentadecanoic acid (Machulla et al, 1980a,b, 1981). This agent (^{123}I-IPP) also exhibits significant myocardial "washout" but has been used successfully in humans (Dudczak et al, 1982 and 1983a,c; Reske et al, 1982a,b,c and 1983), where regional release rates have been used in the same manner as 17-^{123}I-HDA to evaluate alterations in regional metabolism.

To measure absolute regional uptake by planar imaging techniques, interference from radioactivity in the blood pool within the cardiac chambers should be minimized and the initial distribution pattern of the fatty acid should be "frozen". Such a pattern will reflect the initial distribution which is a function of both regional blood flow and fatty acid metabolism and errors introduced from redistribution during the imaging period could be overcome. The characteristics of an ideal agent of this type would therefore include rapid and pronounced myocardial uptake, and very slow washout. The identification of structural features which would significantly increase the residence time of radiolabeled fatty acids in the myocardium is therefore of considerable interest. These agents could be used to measure perfusion, but more importantly, aberrations in fatty acid metabolism under normal flow conditions where regional fatty acid uptake may be correlated with some aspect of regional metabolism.

To overcome the problems caused by significant myocardial clearance of the radiolabel which results from rapid myocardial metabolism of fatty acids, we sought to introduce a structural feature into the fatty acid molecule which would not affect or decrease its uptake from the plasma, but which would interfere with its metabolism. Such a goal represented both a conceptual and synthetic challenge, because drastic structural modifications could lead to a molecule that would no longer resemble a fatty acid and thus would not be

efficiently extacted by the myocardium. Our early studies involved inserting the tellurium-123m (^{123m}Te) radioisotope into the fatty acid chain (Knapp et al, 1979; Knapp et al, 1981). Tellurium-123m in the molecule had two important functions since it was a convenient radiolabel with an attractive gamma photon for imaging (159 keV) and it would interfere with β-oxidation and potentially "trap" the fatty acid in the myocardium. The ^{123m}Te-labeled fatty acid shows outstanding trapping properties in animal studies and excellent myocardial images were obtained in rats (Knapp et al, 1979; Knapp et al, 1981; Elmaleh et al, 1981) and dogs (Okada et al, 1982). Other telluraheptadecanoic acid analogues in which the Te heteroatom was inserted into various positions of the chain were also evaluated (Knapp et al, 1980 and 1981).

These studies demonstrated for the first time that radiolabeled fatty acids having drastic structural modifications still exhibited significant myocardal uptake. More importantly, these were the first studies to demonstrate that modified fatty acids would show prolonged myocardial retention, because of some special property of the Te heteroatom in the fatty acid chain. Prolonged retention with minimal redistribution is important for the use of single-photon-emission computerized tomography (SPECT) which requires extended imaging periods for image reconstruction. The Te fatty acids continue to be important research tools because of their unique "trapping" which may result from intracellular oxidation of the tellurium to form an insoluble polymeric oxidation product (Kirsch, Goodman and Knapp, 1983).

Although ^{123m}Te labeled fatty acids were crucial for developing the experimental concept of "trapping," this radioisotope is not ideal for potential routine studies since it has a long physical half-life (120 days), a high production cost, and a low specific activity. Our efforts were then directed

toward synthesizing fatty acids containing stable, non-radioactive tellurium which would result in the unique "trapping" and attaching iodine-123 for imaging. Inserting nonradioactive tellurium and attaching iodine-123 to the terminus of the modified fatty acid required some difficult, sophisticated chemistry. The synthetic approaches involved stabilizing iodine-123 on model tellurium fatty acids as either an iodophenyl or iodovinyl group (Figure 1). A model agent, 15-(p-iodophenyl)-6-tellurapentadecanoic acid (TPDA), was prepared via introduction of radioiodide via decomposition of a triazene intermediate (Goodman and Knapp, 1982b) and showed the expected high uptake and prolonged retention in rats (Goodman and Knapp, 1982c). This unique trapping property suggests that such agents may be useful tools to evaluate the salvage of threatened myocardium by dual tracer techniques following reflow in a canine model study (Bianco et al, 1984). We also developed the chemistry and evaluated a variety of telluraoctadecenoic acid analogues in which the radioiodide was stabilized as a vinyliodide (Knapp and Goodman, et al, 1983, 1984a). The position of the Te heteroatom was found to be important and the 18-iodo-5-tellura-18-octadecenoic acid analogue showed high myocardial uptake, high heart:blood ratios and prolonged retention as observed with TPDA (Knapp and Srivastava, et al, 1984), and this agent is being studied further. Although we demonstrated the unique retention properties of the Te fatty acids, the special precautions required for the synthesis and handling of these compounds has led us more recently to pursue the development of radioiodinated iodophenyl- and iodovinyl-substituted fatty acids containing methyl-branching as a structural perturbation that would potentially mimic the Te fatty acids. In this paper we review the synthetic strategy, synthesis, preclinical evaluation and potential clinical applications of 3-methyl-branched iodophenyl- and iodovinyl-substituted fatty acids.

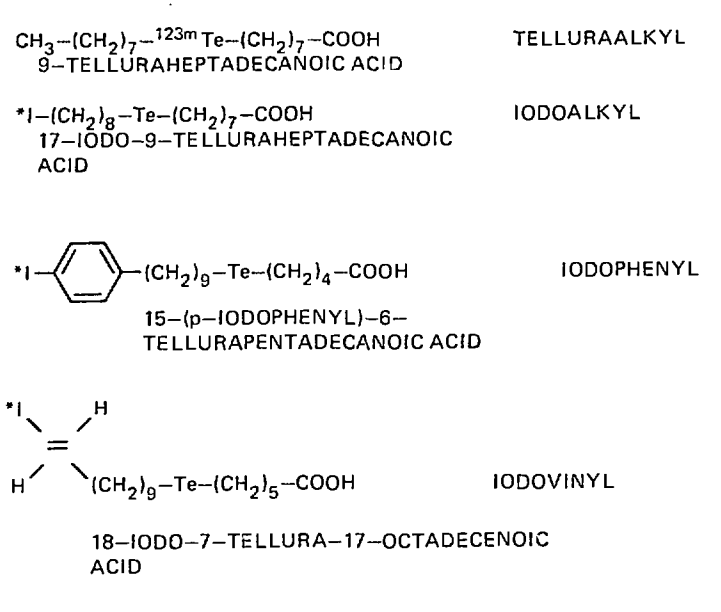


Figure 1. Structures of various tellurium fatty acids.

METHYL-BRANCHED IODINE-123-LABELED FATTY ACIDS

Studies with the Te fatty acids demonstrated for the first time the ability to "trap" such agents in the myocardium by an unknown mechanism and have served as a stimulus for our investigations with the methyl-branched agents which are easier to prepare. A number of early studies clearly demonstrated that methyl-branching in the α- or β-position would inhibit β-oxidation. In the rat, 2,2-dimethylstearic acid was readily absorbed through the intestine when administered in olive oil (Bergstrom et al, 1954). The presence of geminal dimethyl substitution at the α-position resulted in ω-oxidation and 2,2-dimethyladipic acid was isolated from the urine. These studies were also performed with unlabeled and ^{14}C-labeled 2,2-dimethylstearic acid in man (Tryding, 1957). Analysis of the thoracic lymph indicated the presence of

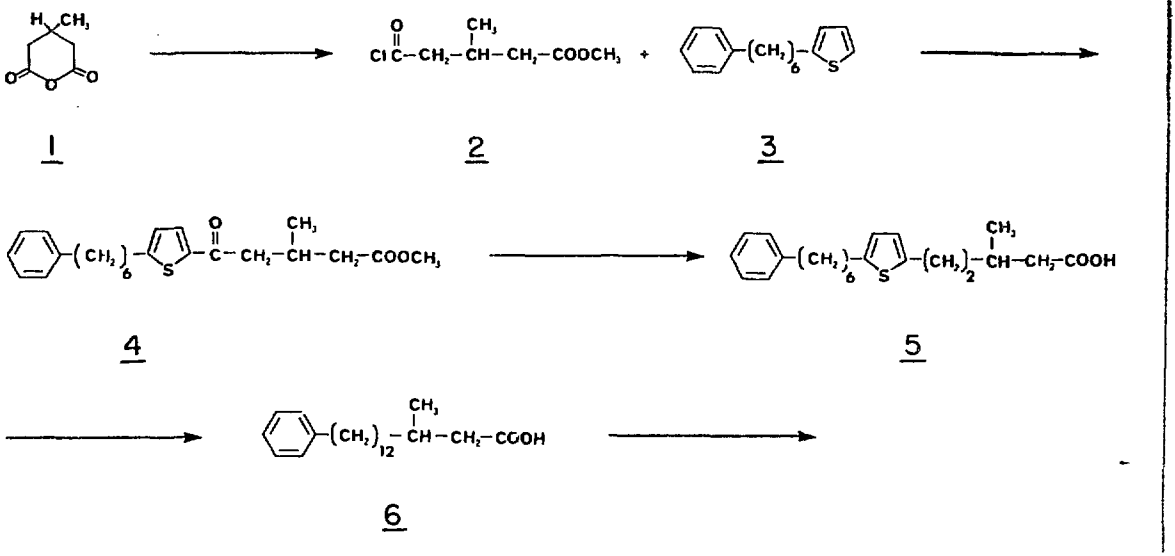
both free 2,2-dimethyl succinic acid, and incorporation into glycerides and phospholipids. These early studies thus demonstrated that although β-oxidation is inhibited by dimethyl-substitution in the α-position, activation still occurs with incorporation of the structurally modified fatty acid into storage products (glycerides) and complex lipids. As with the rat studies, both 2,2-dimethyl-adipic acid and small amounts of 2,2-dimethylsuccinic acid were identified in the urine. A similar dimethyl-branched fatty acid, 2,2-dimethylnonadecanoic acid, was also evaluated in rats (Tryding and Westoo, 1956) and humans (Tryding and Westoo, 1957b). The analogous ω-oxidation products, 2,2-dimethyl glutaric acid and 2,2-dimethylpimetic acid, were isolated from the urine. These results demonstrate that the unnatural 2,2-dimethylstearic acid is well absorbed from the intestine and that lymphatic transport is similar in comparison to naturally occurring unbranched fatty acids. The major difference is, of course, the mechanism of oxidative degradation, since the dimethyl-branched fatty acids cannot undergo β-oxidation via the usual initiation from the carboxyl terminus. A further extension of these studies resulted in the synthesis (Stallberg-Stenhagen, 1950) and evaluation (Tryding and Westoo, 1957a) of 2,2,17,17-tetramethylstearic acid, a modified fatty acid containing methyl-branching at both ends of the molecule to inhibit both β- and ω-oxidation. This agent was well absorbed by the intestinal lymph and was isolated as an unmetabolized ester. With the ^{14}C-labeled material, greater than 85% of the unmetabolized fatty acid was recovered in the feces within one week. A small amount of 2,2-dimethyl glutaric acid and 2,2-dimethylpimilic acid were also isolated. Thus, the fatty acid molecule can apparently be substantially modified by methyl-branching and still show albumin binding properties and lymphatic transport similar to natural unbranched analogues.

A similar dimethyl-substituted fatty acid, 3,3-dimethyl-14-phenylmyristic acid (e.g. 3,3-dimethyl-14-phenyltetradecanoic acid), pertinent to our studies summarized in this paper, was prepared and evaluated (Goodman and Steinberg, 1958). A phenyl ring was introduced into this fatty acid to inhibit ω-oxidation. This modified fatty acid bound well to albumin, indicating that these structural modifications do not interfere with binding and exhibited very slow clearance from the plasma. The slow clearance correlates with our more recent observation of the persistent moderate blood levels observed with the 3-methyl-branched fatty acids (vide infra). In addition, the fatty acid was not metabolized, supporting the proposition that methyl-branching in the 3-position inhibits β-oxidation. Our interest in the use of β-methyl-branched radioiodinated fatty acids was further stimulated by the reported metabolic inhibition and subsequent myocardial retention of 3-R,S-[^{11}C]methylheptadecanoic acid (Elmaleh et al, 1983; Livni, et al, 1982). Our strategy for developing a model methyl-branched long-chain fatty acid involved the synthesis of the 3-R,S-methyl-branched analogue of 15-(p-iodophenyl)pentadecanoic acid (IPP) since this agent has been used in a variety of clinical studies (Reske et al, 1982a,b,c,d, 1983; Dudczak et al, 1932a, 1983a,b,c, and 1984).

Our first synthetic approach for the preparation of 15-(p-iodophenyl)-3-R,S-methylpentadecanoic acid (BMIPP) involved the use of substituted oxazolines as the substrates for carbon-carbon bond formation (Goodman et al, 1982a and 1984a). The initial stages of this investigation entailed the synthesis of 14-phenyl-2-(R,S)-methyltetradecanoic acid. The synthetic approach for the preparation of this racemic methyl-branched fatty acid involved homologation of a masked acetic acid via a 2-substituted oxazoline. We chose this novel homologation technique

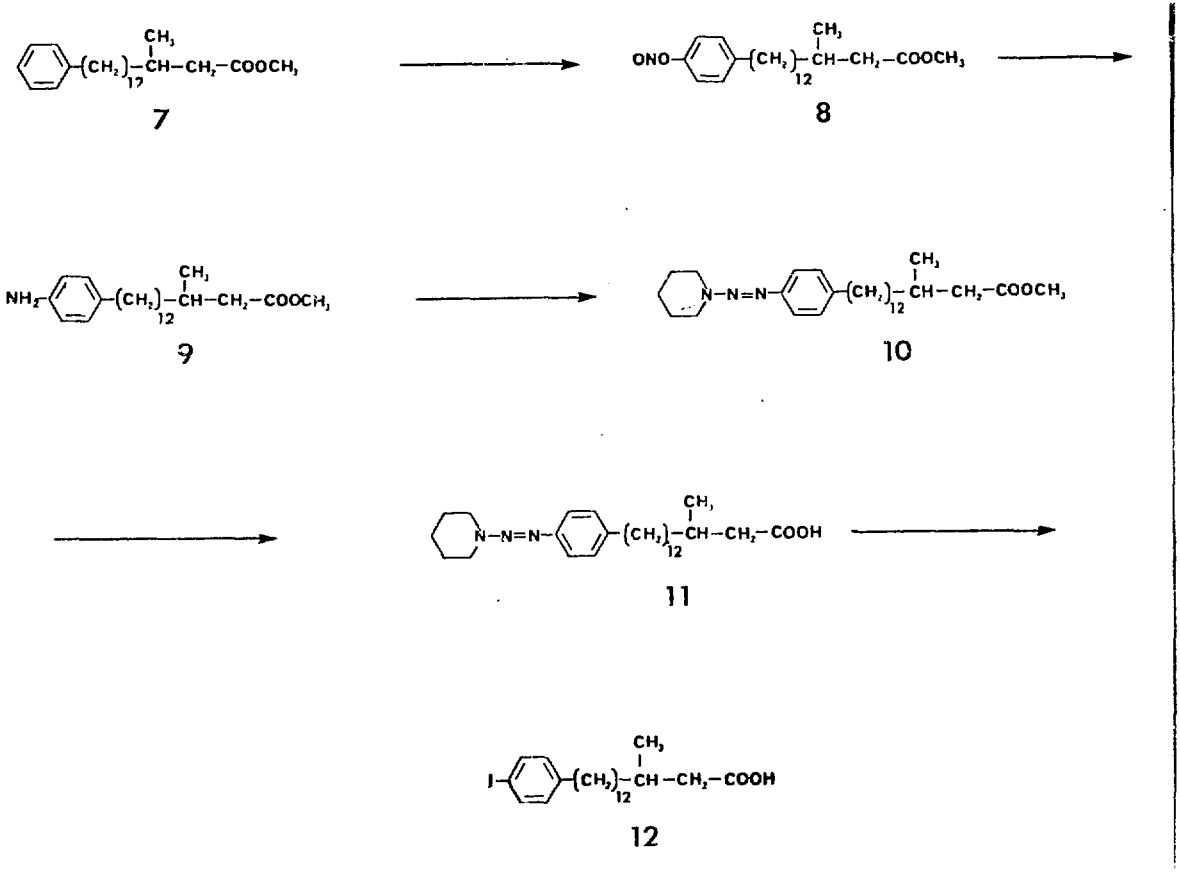
because of the mild reaction conditions for alkylation of the 2-substituted oxazoline. The details of the preparation of the methyl-branched fatty acids by this method have been described elsewhere (Goodman, Kirsch and Knapp, 1984a). The pivotal step in the synthesis of this compound involved introduction of the methyl branching via alkylation of the anion of 2-ethyl-4,4-dimethyl-2-oxazoline. The α-methyl-branched fatty acid, 14-phenyl-2-(R,S)-methyltetradecanoic acid, was then converted to 15-phenyl-3-(R,S)-methylpentadecanoic acid by a seven-step sequence of reactions. Iodide was introduced into the para-position of the terminal phenyl ring by a thallation-iodination route. The effect of methyl-branching at the 3-position on myocardial retention in rats was assessed by a comparison with the myocardial uptake of the straight chain analogue, 15-(p-[^{125}I]iodophenyl)pentadecanoic acid (IPP). The increased myocardial retention of radioactivity following injection of 15-(p-[^{125}I]iodophenyl)-3-(R,S)-methylpentadecanoic acid in comparison to the unbranched analogue, 15-(p-[^{125}I]iodophenyl)pentadecanoic acid, suggested that methyl branching at the 3-position would be an effective means of inhibiting myocardial metabolism of radioiodinated phenyl fatty acids. The availability of the 14-carbon α-methyl-branched fatty acid afforded us the opportunity to evaluate the effect of the position of methyl-branching and chain length on myocardial uptake and retention. Tissue distribution studies of 14-(p-[^{125}I]iodophenyl)-2-(R,S)-methyltetradecanoic acid in rats showed that the α-methyl-branched acid exhibited slower plasma clearance and significantly lower heart uptake than the 3-methyl-branched acid. The radioiodinated unbranched analogue 14-(p-[^{125}I]-iodophenyl)tetradecanoic acid showed considerably higher myocardial uptake in comparison to the α-methyl-branched acid, but lower uptake and retention than IPP. These preliminary results strongly suggested that 3-methyl-branching and a 15-carbon chain length are the optimum structural features for methyl-branched fatty acids.

Because the terminal phenyl-3-methyl-branched fatty acids are not commercially available, a new synthetic method applicable for the large scale preparation of 15-phenyl-3-(R,S)-methylpentadecanoic acid (6) has been more recently developed utilizing the thiophene synthesis of long chain fatty acids as described in Scheme I (Goodman, Knapp et al, 1984a). By the selection of substituents introduced into the 2- and 5-positions of the thiophene ring, a variety of 3-methyl-branched fatty acids of various chain lengths can be prepared. Using this synthetic approach, 2-(6-phenylhexyl)thiophene (3) and 3-(R,S)-methyl-4-carbomethoxybutanoyl chloride (2) were subjected to Friedel Crafts acylation to afford 2-([3-(R,S)-methyl-1-oxo-5-methoxypentanoyl])-5-(6-phenylhexyl)-thiophene (4). The methyl ester (4) was then reduced under Wolff-Kishner (Huang-Minlon) conditions to give 2-[3-(R,S)-methyl-1-hydroxypentanoyl]-5-(6-phenylhexyl)thiophene (5). The pivotal step in this method involved the Raney-Nickel desulfurization and reduction of the acid (5) to yield the desired 3-methyl-branched acid, 15-phenyl-3-(R,S)-methylpentadecanoic acid (6).



Scheme I. Synthesis of 15-phenyl-3-R,S-methylpentadecanoic acid (6).

Iodide was regiospecifically introduced (Scheme II) into the para position of the terminal phenyl ring of (6) via the HI decomposition of the piperidyl triazene derivative (11) which yields exclusively the para-iodophenyl products. This method (Goodman et al, 1984c) is an attractive alternative to electrophilic routes which give mixtures of the ortho and para isomers requiring high pressure chromatographic separation of the isomers. In the IPP series, the ortho iodo isomer has different biodistribution properties and metabolism than the desired para isomer (Beckurts et al, 1984). The key intermediate required for the regiospecific radioiodination of 15-phenyl-3-(R,S)-methylpentadecanoic acid by the triazene route (Goodman and Knapp, et al, 1984b) was 1-[4-(13-(R,S)-methyl-15-hydroxypentadecanoyl)phenyl]-3,3-(1,5-pentanediyl)triazene (11). The synthetic approach for the preparation of this triazene involved introduction of a nitroso group into the para position of the terminal phenyl group via aromatic thallation followed by a four step reaction sequence outlined in Scheme II. Aromatic thallation of the methyl ester (7) with thallium(III)trifluoroacetate in trifluoroacetic acid followed by treatment with nitrosyl chloride afforded methyl 15-(p-nitrosophenyl)-3-(R,S)-methylpentadecanoate (8). Reduction of the nitroso compound (8) with $NaBH_4$ and 10% palladium on charcoal in methanol gave methyl 15-(p-aminophenyl)-3-(R,S)-methylpentadecanoate (9). Diazotization of the aromatic amine (9) at 0°C followed by treatment with aqueous piperidine afforded 1-[4-(13-(R,S)-methyl-15-methoxypentadecanoyl)phenyl]-3,3-(1,5-pentanediyl)triazene (10). Basic hydrolysis of the triazene methyl ester gave the desired triazene, 1-[4-(13(R,S)-methyl-15-hydroxypentadecanoyl)-phenyl]-3,3-(1,5-pentanediyl) triazene (11). The triazene (11) was rapidly converted to radioiodinated, 15-(p-iodophenyl)-3(R,S)-methylpentadecanoic acid (12). This agent shows higher myocardial uptake and longer retention than the straight chain analogue IPP (See "Biological Evaluation Section," p. 14). The

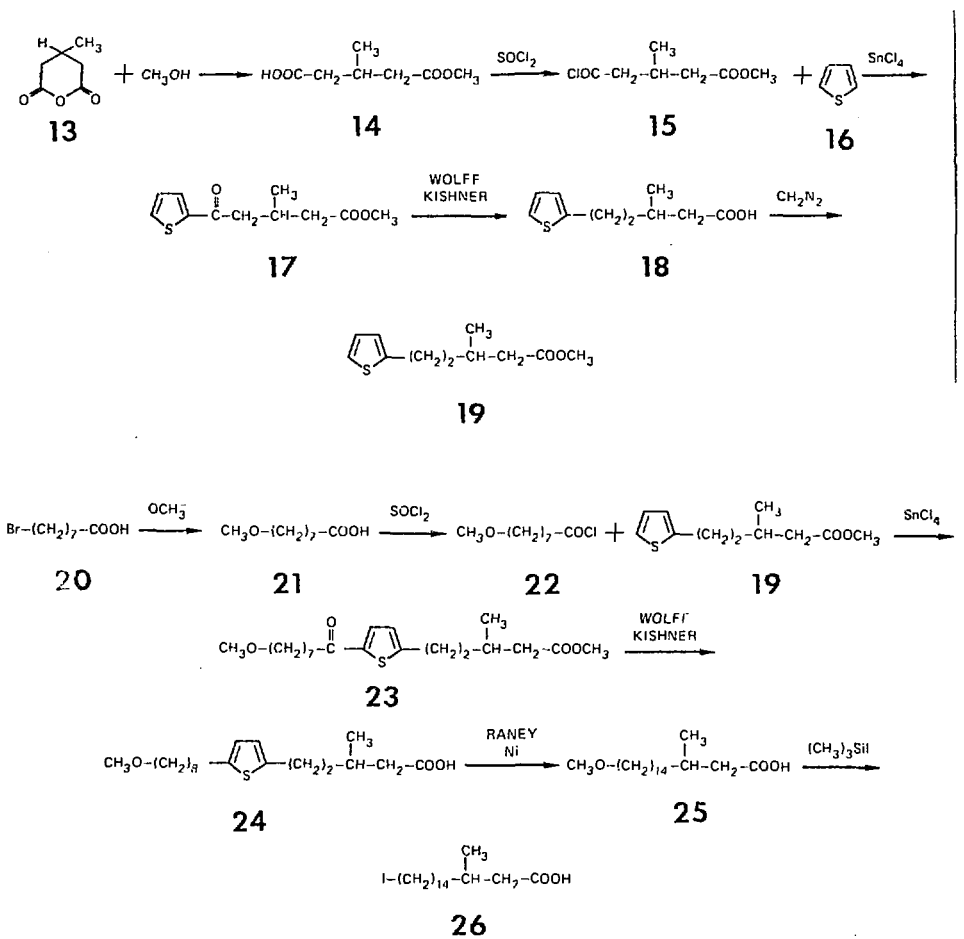


Scheme II. Synthesis of 15-(p-iodophenyl)-3-R,S-methylpentadecanoic acid (12) by HI decomposition of the triazene (11).

synthesis, radioiodination and myocardial uptake and retention of a similar 14-carbon agent, 14-(p-iodophenyl)-3-R,S-methyltetradecanoic acid, have also been reported (Elmaleh et al, 1982).

A new type of methyl-branched fatty acid has also been recently developed with radioiodide stabilized by attachment as a terminal vinyliodide moiety (Knapp and Goodman, et al, 1984d). These studies are an extension of our earlier investigations that demonstrated iodovinyl-substituted tellurium (Te) fatty acids (Knapp et al, 1983, 1984b) and 18-[^{125}I]-iodo-17-octadecenoic acid (Knapp and Goodman, et al, 1984a) showed only moderate in vivo deiodination. The route chosen for the construction of the methyl-branched fatty acid skeleton

involved a thiophene chain elongation synthesis as described earlier for the iodophenyl fatty acids (Schemes I and II). Using this approach, the substituents introduced into the 2- and 5-positions of the thiophene ring can be selected and can provide a variety of 3-methyl-branched fatty acids of various chain lengths for structure activity studies. The key substrate, 17-iodo-3-(R,S)-methylheptadecanoic acid (26), was prepared as described in Scheme III. The 3-(R,S)-methyl-4-carbomethoxybutanoyl chloride (15) was

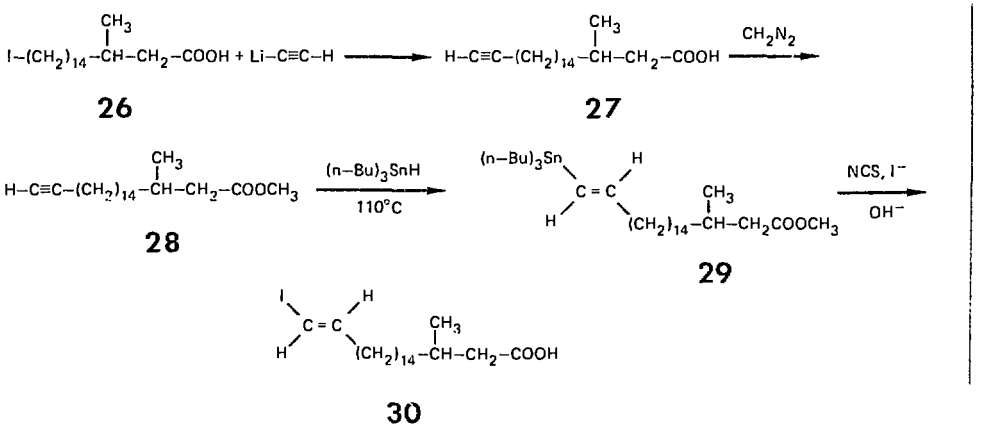


Scheme III. Synthesis of 17-iodo-3-R,S-methylheptadecanoic acid (14).

prepared from commercially available 3-methyl glutaric anhydride (13). Friedel Crafts acylation of (15) using $SnCl_4$ gave the thienyl ketone (17). Wolff-

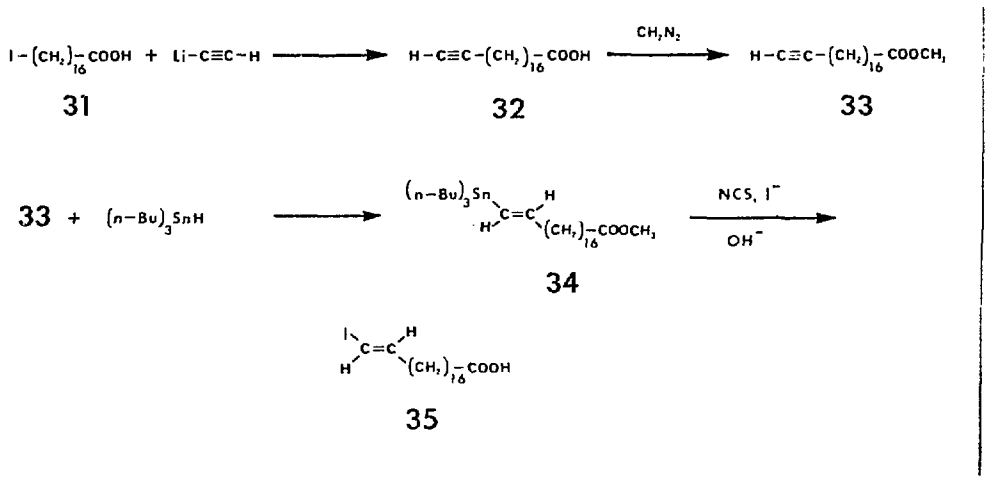
Kishner reduction of (17) followed by treatment with diazomethane afforded the methyl-branched 2-substituted thiophene (19). The methyl ester (19) was subjected to Friedel Crafts condensation with 8-methoxyoctanoyl chloride (22) to afford 2-([3-(R,S) methyl-5-methoxypentanoyl])-5-(8-methoxy-1-oxo-octyl)-thiophene (23). Following Wolff Kishner reduction of the keto ester (23) to the acid (24), and Raney nickel desulfurization of (24), treatment with trimethylsilyl iodide gave 17-iodo-3-(R,S)-methylheptadecanoic acid (26). The availability of this key methyl-branched 17-iodo intermediate may provide an opportunity to gain further insight into the mechanism of in vivo deiodination in terminal radioiodinated long chain fatty acids.

The pivotal step in the synthesis (Scheme IV) of the (E)-iodovinyl acid (30) involved hydrostannylation of the terminal ethynyl substrate (28) with tributyltin hydride to give the key intermediate (29). Compound (28) was prepared by treatment of the 17-iodomethyl-branched acid (26) with lithium acetylide-ethylenediamine (LAEDA). Iododestannylation of (29) by treatment with N-chlorosuccinimide (NCS) and iodide followed by basic hydrolysis gave (30).



Scheme IV. Synthesis of 19-iodo-3-R,S-methyl-18-nonadecenoic acid (30).

The unbranched analogue (35) was prepared in the same manner (Scheme V), which involved iododestannylation of the substrate (34) prepared from (33) which was fabricated by trialkylstannylation of the acetylene (33) generated by attachment of the ethynyl group to (31).



Scheme V. Synthesis of 19-iodo-18-nonadecenoic acid (35) from 17-iodoheptadecanoic acid (31).

BIOLOGICAL EVALUATION

The availability of the model methyl-branched radioiodinated fatty acids, 15-(p-iodophenyl)-3-R,S-methylpentadecanoic acid (BMIPP, 12) and 19-iodo-3-R,S-methyl-18-nonadecenoic acid (30) and the unbranched analogues provided an opportunity to examine in detail the effects of methyl-branching on the myocardial uptake and retention of these new modified agents. To minimize any differences that may be encountered in comparing the distribution of the β-methyl-branched and straight chain analogues in different groups of rats, experiments were designed in which an $^{123}I/^{125}I$ dual-labeled mixture of the fatty acids was administered to the same rats. These results are shown in Figures 2 and 3 for the iodophenyl series and Figures 4 and 5 for the iodovinyl

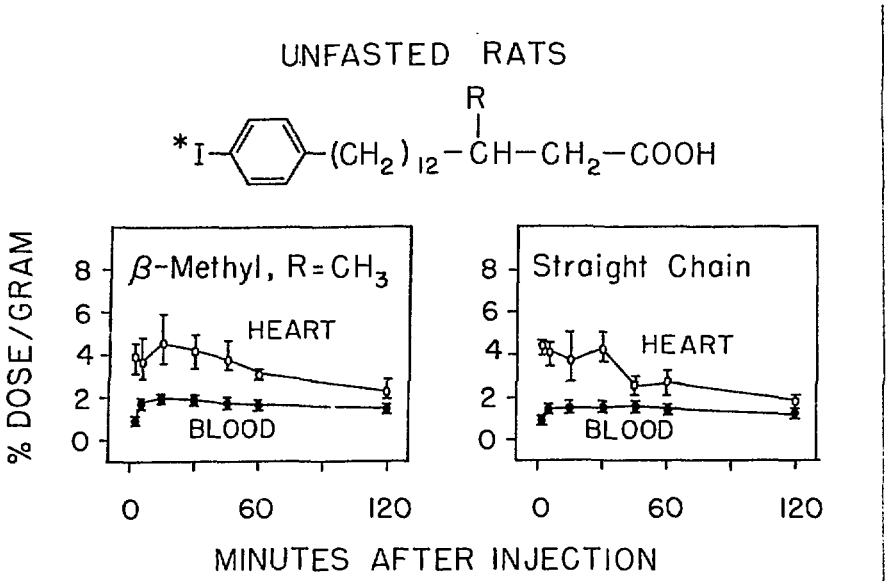


Figure 2. Comparison of the heart and blood clearance of 15-(p-[^{123}I]iodophenyl)-3-R,S-methylpentadecanoic acid (β-methyl, R=CH_3) and 15-(p-[^{125}I]iodophenyl)pentadecanoic acid (IPP, R=H) in groups of unfasted Fischer rats.

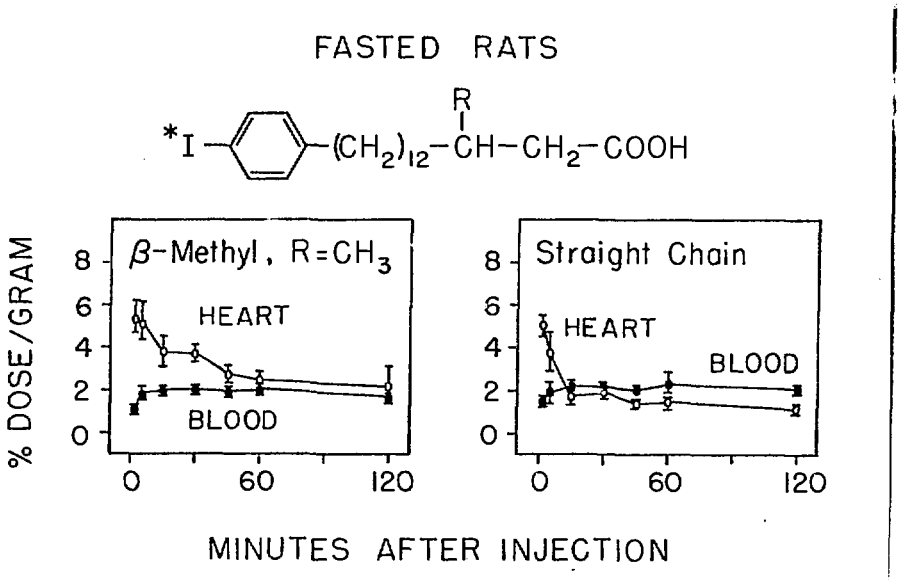


Figure 3. Comparison of the heart and blood clearance of 15-(p-[^{123}I]iodophenyl)-3-R,S-methylpentadecanoic acid (β-methyl, R=CH_3) and 15-(p-[^{125}I]iodophenyl)pentadecanoic acid (IPP, R=H) in groups of fasted Fischer rats.

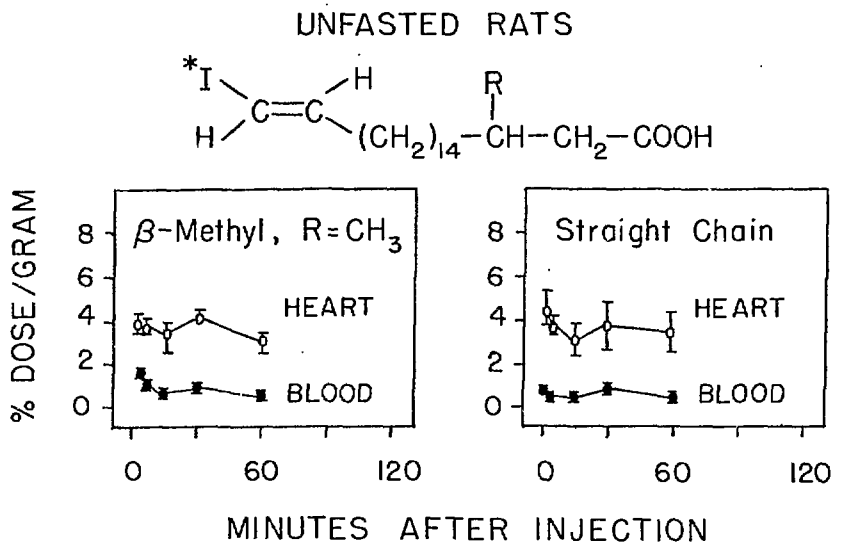


Figure 4. Comparison of the heart and blood retention of 19-[^{123}I]iodo-3-R,S-methyl-18-nonadecenoic acid (β-methyl, R=CH_3) and 19-[^{125}I]iodo-18-nonadecenoic acid in groups of unfasted Fischer rats.

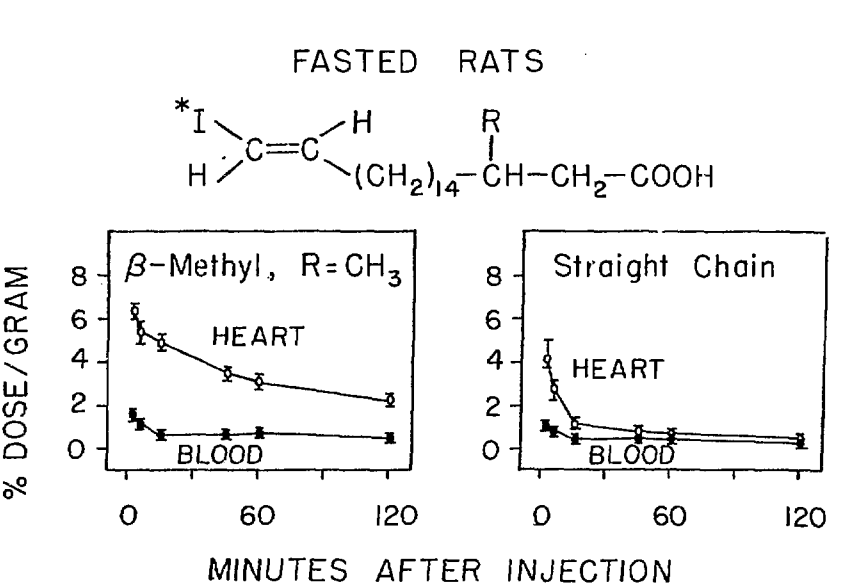


Figure 5. Comparison of the heart and blood retention of 19-[^{123}I]iodo-3-R,S-methyl-18-nonadecenoic acid (β-methyl, R=CH_3) and 19-[^{125}I]iodo-18-nonadecenoic acid in groups of fasted Fischer rats.

series. Several important observations can be made from a comparison of these two sets of experiments involving the 3-R,S-methyl-branched iodophenyl (Figures 2 and 3) and iodovinyl-substituted (Figures 4 and 5) model fatty acids in fasted and unfasted animals. In both the fasted and unfasted animals, the β-methyl-branched fatty acids show higher myocardial uptake, lower blood levels and higher heart:blood ratios. These results have also been observed in similar studies where the agents were administered to groups of rats separately. In the unfasted animals fed standard rat chow, the relative myocardial retention of the β-methyl-branched fatty acids and the straight chain analogues is similar. More importantly, the kinetics of the myocardial washout in the observed fasted rats is much more rapid for the straight-chain agents in comparison to the β-methyl-branched analogues. Thus, under fasting conditions, as has been recommended for clinical studies since all patients are in a similar nutritional state (Dudczak et al, 1983a,b,c and 1984), the differences in myocardial clearance rates are dramatically magnified with the β-methyl-branched agents showing considerably slower wash-out. These data suggest that the methyl-branched fatty acids are excellent candidates for single photon tomography of regional myocardial distribution of fatty acids. Gamma camera imaging studies with the methyl-branched iodovinyl fatty acid in fasted rats confirm the slow washout (Figure 6).

In addition to investigating the metabolism of these new methyl-branched agents, additional synthetic studies are being pursued to extensively evaluate the structural features affecting the biodistribution and metabolism of the methyl-branched fatty acids. These studies include a systematic evaluation of the strucutral features of the methyl-branched fatty acids that lead to optimal myocardial uptake and retention. These include the effects of total chain

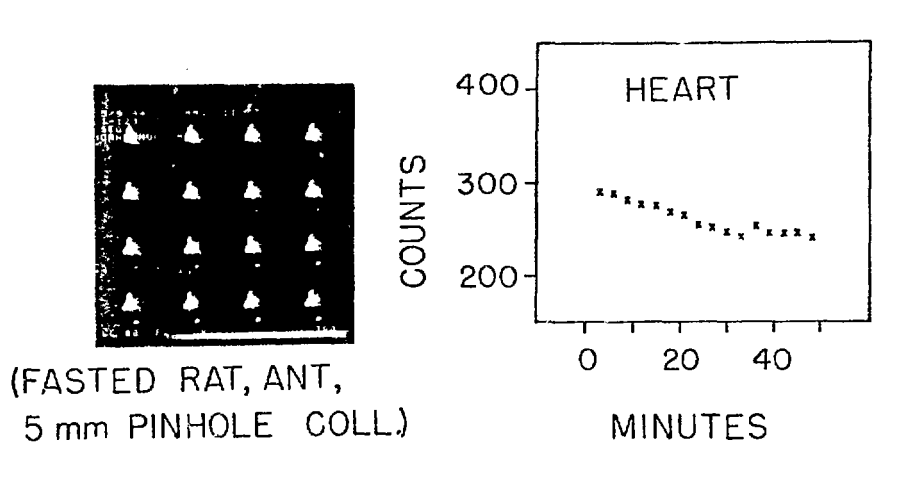


Figure 6. Sequential gamma camera images of 19-[^{123}I]iodo-3-R,S-methyl-18-nonadecenoic acid in a Fischer rat using a 5 mm pinhole collimator and accumulation of sequential 3 min images as 64 x 64 matrices.

length, the position of methyl-branching and the absolute configuration of the asymmetric methyl-branching site. In addition, the effects of dimethyl-branching at the 3-position should be pursued. Recent studies with 14-[^{125}I]iodo-3,3-dimethyltetradecanoic acid have demonstrated only low heart uptake in rats (Otto et al, 1984), although the low uptake may result from the short chain length or rapid deiodination.

The availability of radioiodinated 17-iodo-3-R,S-methylheptadecanoic acid (26, Scheme III) will allow evaluation of the extent and mechanism of iodide loss in the absence of β-oxidation. Since loss of halide from an iodinated coenzyme A product would not be expected, the evaluation of ^{125}I labeled (26) would focus on the direct cleavage of the carbon-iodide bond. Preliminary tissue distribution studies of ^{125}I-labeled (26) demonstrated rapid dehalogenation indicating significant carbon-iodide cleavage in the apparent absence of β-oxidation. These results confirm earlier studies using 17-[^{131}I]iodo-9-telluraheptadecanoic acid (Figure 1) which exhibited high heart uptake in rats, but showed significant in vivo

deiodination resulting in high blood levels of activity and significant thyroid uptake (Goodman et al, 1982c). More recently, similar results with a 13 carbon analogue of (26) have been reported (Otto et al, 1984).

POTENTIAL APPLICATIONS OF STRUCTURALLY MODIFIED FATTY ACIDS

The principal application using structurally modified agents that show slow myocardial washout include evaluation of regional differences in fatty acid uptake when the coronary arteries are normal and regional perfusion is not impaired. Structurally-modified fatty acids that show prolonged retention are also candidates for potential clinical evaluation of hypertensive heart disease as recently demonstrated in studies involving quantitative dual tracer autoradiography, a technique where the relative distribution of two different agents is monitored (Yonekura et al, 1984a,b; Yamamoto et al, 1984). As an example, 1-(^{14}C)-3-R,S-methylheptadecanoic acid was administred to normal rats which were also injected with thallium-201. The same mixture was administered to a second group of salt sensitive hypertensive rats. The autoradiographic studies showed a homogeneous distribution of the thallium-201 in the hearts of rats of both the control group and the hypertensive group. After allowing 30 days for all of the thallium-201 to decay (10 half-lives later), the distribution of the ^{14}C-labeled fatty acid was determined by examining the later autoradiographs of the same tissue slices. These studies showed that in normal rats both the thallium-201 and the carbon-14-labeled fatty acid are homogeneously distributed and in the hypertensive rats thallium-201 has a homogeneous distribution but the carbon-14-labeled fatty acid clearly shows a heterogeneous distribution. The thallium results show that the regional blood flow is normal in the hypertensive rat hearts, thus indicating that fatty acid

delivery to the heart was not impaired. However, the heterogeneous distribution of fatty acid indicates that hypertensive heart disease may have altered the ability of portions of the heart to metabolize fatty acids. These studies have recently been performed using 15-(p-[^{131}I]iodophenyl)-3-R,S-methylpentadecanoic acid which shows the same heterogeneous distribution in hypertensive rat hearts (Yamamoto et al, 1984).

These observations are very important because they suggest that a metabolic change occurs in severe hypertension before any differences in blood flow (ischemia) can be detected. These results indicate that severe hypertension and the accompanying onset of myocardial hypertrophy is associated with regional changes in fatty acid utilization. Currently, agents such as thallium-201 are widely used to detect and evaluate coronary artery disease. But, because such agents can indicate only differences in blood flow, they may not be used effectively to evaluate hypertensive heart disease, as demonstrated above in the rat studies. On the other hand, the combination of iodine-123-labeled fatty acids and Single Photon Emission Computerized Tomography (SPECT) can potentially evaluate hypertensive disease and assess the effects of drug therapy.

There is also a need for diagnostic agents to determine the effectiveness of drug treatment after an ischemic attack by revealing changes in blood flow in the myocardium. Because the Te fatty acids have been shown to be essentially irreversibly trapped after delivery to the heart muscle, these agents are excellent candidates to examine how blood distribution after the administration of drugs differs from the distribution before therapy. The results can indicate how much of the threatened heart muscle has been salvaged. There is presently no agent available to study myocardial salvage non-invasively. The unique

ability of our Te fatty acids, such as 15-(p-iodophenyl)-6-tellurapentadecenoic acid (TPDA), to detect and characterize ischemia and changes in blood flow patterns as a result of drug therapy has recently been demonstrated in dogs (Bianco et al, 1984). Tissue counting techniques were used to compare the distribution of ^{125}I-TPDA administered after partial coronary artery occlusion to the distribution of ^{131}I-TPDA administered after resumption of normal flow. This is the first demonstration of a radionuclide technique to evaluate salvage and suggests that not only are the Te fatty acids important tools to study this crucially important problem, but also that ^{123}I-labeled methyl-branched fatty acids may be useful for the clinical evalaution of myocardial salvage.

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REFERENCES

Angelberger, P., Wagner-Loffler, M., Dudczak, R., and Hruby, R. "I-123(131)-Labelled Aliphatic and Aromatic Fatty Acids: Optimized Preparation and Biodistribution," Radioaktive Isotope in Klinik und Forschung, Gasteiner Internationales Symposium, 1982.

Beckurts, T. E., Shreeve, W. W., Machulla, H. J., and Feinendegen, L. E. "Kinetics of Different 123-I and 14-C Fatty Acids in Normal and Diabetic Rat Myocardium In Vivo," 16th International Symposium "Radioaktive Isotope" in Klinik und Forschung, Bad Gastein, Austria, January 9-12, 1984.

Bergstrom, S., Bergstrom, B., Tryding, N. and Westoo, G. "Intestinal Absorption and Metabolism of 2:2-Dimethylstearic Acid in the Rat," Biochem. J., 58, 604-608, 1954.

Bianco, J. A., Alpert, J. S., Pape, L. A., Zheng, M., Hnatowich, D., Goodman, M. M., and Knapp, F. F., Jr. "Accumulation of Radioiodinated 15-(p-Iodophenyl)-6-Tellurapentadecanoic Acid in Necrotic and Ischemic Myocardium During Acute Coronary Occlusion and Reperfusion," J. Amer. College Card., 4, 80-87, 1984.

Dudczak, R., Kletter, K., Frischauf, H., Schmoliner, R., Derfler, K., and Losert (Wien), U. "Myocardial Turnover Rates of I-123 Heptadecanoic Acid (HD) and I-123 p-Phenylpentadecanoic Acid (pIPPA)," Radioaktive Isotope in Klinik und Forschung, 15, Gasteiner Internationales Symposium 685-696, 1982.

Dudczak, R. "Myokardszintigraphie mit Jod-123-markierten Fettsauren," Wiener klinische Wochenschrift, 95 (143) 4-35, 1983a.

Dudczak, R. and Hofer, R. "Myocardial Scintigraphy with ^{123}I Labeled Fatty Acids," J. Radioanal. Chem., 79 (2), 329-336, 1983b.

Dudczak, R., Schmoliner, R., Kletter, K., Frischauf, H., and Angelberger, P. "Clinical Evaluation of ^{123}I Labeled p-Phenylpentadecanoic Acid (p-IPPA) for Myocardial Scintigraphy," J. Nucl. Med. Allied Sci., 27 (4), 1983c.

Dudczak, R., Kletter, K., Frischauf, H., Losert, U., Angelberger, P. and Schmoliner, R. "The Use of ^{123}I-labeled Heptadecanoic Acid (HDA) as Metabolic Tracer: Preliminary Report," Eur. J. Nucl. Med., 9, 81-85, 1984.

Elmaleh, D. R., Knapp, F. F., Jr., Tasuda, T., Coffey, J. L., Kopiwoda, S., Okada, R., and Strauss, H. W. "Myocardial Imaging with 9-[Te-123m]Tellura-hepadecanoic Acid," J. Nucl. Med., 22, 994-999, 1981.

Elmaleh, D. R., Livni, E., Levy, S., Schluederberg, C. J., and Strauss, W. H. "Radioiodinated Beta Methyl Omega Phenyl Tetradecanoic Acid (BMPTA): A Potential New Agent for Studies of Myocardial Fatty Acid (FA) Metabolism," Society of Nuclear Medicine 29th Annual Meeting, Miami Beach, Florida, June 15-18, 1982.

Elmaleh, D. R., Livni, E., Varnum, D., Anderton, K. and Strauss, H. W. "The Metabolic Fate of 1- C-14 Betamethylheptadecanoic Acid (BMHDA) and 1-C-14 Palmitic Acid (PA) in Mice Heart and Blood," J. Nucl. Med., Proceedings of the 30th Annual Meeting, P 120, 1983.

Feinendegen, L. F., Vyska, K., Freundlieb, C., Hock, A., Machulla, H.-J., Kloster, G. and Stocklin, G. Eur. J. Nucl. Med., 6, 191-200, 1981.

Freundlieb, C., Hock, A., Vyska, K., Feinendegen, L. F., Machulla, H.-J. and Stocklin, J. J. Nucl. Med., 21, 1043-1050, 1980.

Goodman, D. S., and Steinberg, D. "Studies on the Metabolism of 3,3-Dimethyl Phenylmyristic Acid, a Nonoxidizeable Fatty Acid Analogue," J. B. C., 233 (5), - 1066-1071, 1958.

Goodman, M. M., Kirsch, G., and Knapp, F. F., Jr. "Synthesis of Radioiodinated ω-p-(Iodophenyl)-Substituted Methyl Branched Long-Chain Fatty Acids," 4th International Symposium on Radiopharmaceutical Chemistry, Julich, West Germany, August 23-27, 1982; J. Lab. Cmpds. Radiopharm., XIX, 1316-1318, 1982a.

Goodman, M. M. and Knapp, F. F., Jr. "Synthesis of 15-(p-Iodophenyl)-6-telluraPentadecanoic Acid: A New Myocardial Imaging Agent," J. Org. Chem., 47, 3004-3006, 1982b.

Goodman, M. M., Knapp, F. F., Jr., Callahan, A. P., and Ferren, L. A. "A New, Well-Retained Myocardial Imaging Agent: Radioiodinated 15-(p-Iodophenyl)-6-Telluropentadecanoic Acid," J. Nucl. Med., 23, 904-908, 1982c.

Goodman, M. M., Knapp, F. F., Jr., Callahan, A. P., and Ferren, L. A. "Synthesis and Biological Evalaution of 17-[^{131}I]Iodo-9-telluraheptadecanoic Acid, a Potential Myocardial Imaging Agent," J. Med. Chem., 25, 613-618, 1982d.

Goodman, M. M., Kirsch, G., and Knapp, F. F., Jr. "Synthesis and Evaluation of Radioiodinated Terminal p-Iodophenyl-Substituted α- and β-Methyl-Branched Fatty Acids," J. Med. Chem., 27, 390-397, 1984a.

Goodman, M. M., Knapp, F. F., Jr., Elmaleh, D. R., and Strauss, H. W. "New Myocardial Imaging Agents: Synthesis of 15-(p-Iodophenyl)-3(R,S)-Methyl-pentadecanoic Acid by Decomposition of a Piperidinyl Triazene Precursor," J. Org. Chem., in press, 1984b.

Goodman, M. M., Cunningham, E. B., and Knapp, F. F., Jr. "Design, Synthesis and Evaluation of ω-Iodovinyl- and ω-Iodoalkyl-substituted Methyl-Branched Long-Chain Fatty Acids," 5th International Symposium on Radiopharmaceutical Chemistry, Tokyo, Japan, June 9-13, 1984, J. Label. Cmpds. and Radiopharm., in press, 1984c.

Kirsch, G., Goodman, M. M., and Knapp, F. F., Jr. "Organotellurium Compounds of Biological Interest - Unique Properties of the N-Chlorosuccinimide Oxidation Product of 9-Telluraheptadecanoic Acid," Organometallics, 2, 357-363, 1983.

Knapp, F. F., Jr. "Tellurium and Selenium as Carbon Substitutes," Proceedings, First International Symposium on Radiopharmaceuticals: Structure-Activity Relationships, Chapter 16, pp 345-391, Grune and Stratton, 1980.

Knapp, F. F., Jr., Ambrose, K. R., Callahan, A. P., Grigsby, R. A., and Irgolic, K. J. "Tellurium-123m-Labeled Isosteres of Palmitoleic and Oleic Acids Show High Myocardial Uptake," Radiopharmacetuicals II: Proceedings of the Second International Symposium on Radiopharmaceuticals, Society of Nuclear Medicine, 101-108, 1979.

Knapp, F. F., Jr., Ambrose, K. R., Callahan, A. P., Ferren, L. A., Grigsby, R. A. and Irgolic, K. J. "Effects of Chain Length and Tellurium Position on the Myocardial Uptake of Te-123m Fatty Acids," J. Nucl. Med., 22 (11), 988-993, 1981.

Knapp, F. F., Jr., Goodman, M. M., Callahan, A. P., Ferren, L. A., Kabalka, G. W., and Sastry, K. A. R. "New Myocardial Imaging Agents: Stabilization of Radioiodine as a Terminal Vinyl Iodide Moiety on Tellurium Fatty Acid," J. Med. Chem., 26, 1293-1300, 1983.

Knapp, F. F., Jr., Goodman, M. M., Kabalka, G. W., and Sastry, K. A. R. "Synthesis and Evaluation of Radioiodinated (E)-18-Iodo-17-octadecenoic Acid as a Model Iodoalkenyl Fatty Acid for Myocadial Imaging," J. Med. Chem., 27, 94-97, 1984a.

Knapp, F. F., Jr., Srivastava, P. C., Callahan, A. P., Cunningham, E. B., Kabalka, G. W., and Sastry, K. A. R. "Effect of Tellurium Position on the Myocardial Uptake of Radioiodinated 18-Iodotellura-17-octadecenoic Acid Analogues," J. Med. Chem., 27, 57-63, 1984b.

Knapp, F. F., Jr., Goodman, M. M., Elmaleh, D. R., Okada, R. and Strauss, H. W. "The Development of Radioiodinated Fatty Acids for Applications in Nuclear Cardiology," In, Proceedings of the International Symposium on the Developing Role of Short-Lived Radionuclides in Nuclear Medical Practice, U.S. Department of Energy, in press, 1984c.

Livni, E., Elmaleh, D. R., Levy, S., Brownell, L., and Strauss, W. H. "Beta-methyl[1-^{11}C]heptadecanoic Acid: A New Myocardial Metabolic Tracer for Positron Emission Tomography," J. Nucl. Med., 23 (2), 169-175, 1982.

Machulla, H. J., Marsmann, M., Dutschka, K. "Synthesis of Radioiodinated Phenylfatty Acids for Studying Myocardial Metabolism," J. Radioanal. Chem., 56 (1-2), 253-261, 1980a.

Machulla, H. J., Marsmann, M., Dutschka, K. "Biochemical Synthesis of a Radioiodinated Phenylfatty Acid for In Vivo Metabolic Studies of the Myocardium," Europ. J. Nucl. Med., 5, 171-173, 1980b.

Machulla, H. J., Dutschka, K., van Benningen, D., Chen, T. "Development of 15-(p-^{123}I-phenyl-)pentadecnaoic Acid for In Vivo Diagnosis of the Myocardium," J. Radioanal. Chem., 65, 279-286, 1981.

Okada, R. D., Knapp, F. F., Jr., Elmaleh, D. R., Yasuda, T., Boucher, C. A., and Strauss, H. W. "Tellurium-123m-labeled-9-Telluraheptadecanoic Acid: A Possible Cardiac Imaging Agent," Circulation, 65 (2), 305-310, 1982.

Otto, C. A., Brown, L. E., and Scott, A. M. "Radioiodinated Branched-Chain Fatty Acids: Substrates for Beta Oxidation? Concise Communication," J. Nucl. Med., 25 (1), 75-80, 1984.

Reske, S. N., Biersack, H. J., Lackner, K., Machulla, H. J., Knopp, R., Hahn, N., Winkler, C. "Assessment of Regional Myocardial Uptake and Metabolism of ω-(p-^{123}I-Phenyl-)Pentadecanoic Acid with Serial Single-Photon Emission Tomography, Nucl. Med. Band XXI, Heft 6, 249-253, 1982a.

Reske, S. N., Fuchs, R., Machulla, H. J., Winkler, C. "Radiochromatographic Microanalysis of Myocardial Lipids Prelabeled with Radioiodinated Fatty Acids," Radiochem. Radioanal. Letters, 55 (4), 257-264, 1982b.

Reske, S. N., Machulla, H. J., Winkler, C. "Metabolism of 15-(p-^{123}I-Phenyl-) Pentadecanoic Acid in Hearts of Rats," J. Nucl. Med., 23 (5), P 10, 1982c.

Reske, S. N., Simon, H., Machulla, H. J., Biersack, H. J., Knopp, R., Winkler, C. "Myocardial Turnover of p-(^{123}I-Phenyl-) Pentadecanoic Acid in Patients with CAD," J. Nucl. Med., 23 (5), P 34, 1982d.

Reske, S. N. "Jod-123-phenyl-pentadecansaure - Ein neuer tracer zur Untersuchung des Myokardialen Metabolismus freier Fettsauren," Der Nuclearmediziner, 1 (6), 25-46, 1983.

Stallberg-Stenhagen, S. "Optically Active Higher Aliphatic Compounds. VII The Synthesis of (-)-3(L),6(D)-Dimethyltetracosanoic and L(+)-6-methylpentacosanoic Acids," Arkiv For Kemi, 2 (31), 431-442, 1950.

Tryding, N. "Intestinal Absorption and Metabolism of 2,2-Dimethylstearic Acid in Man," Arkiv For Kemi, 11 (32), 307-312, 1957.

Tryding, N., and Westoo, G. "Synthesis and Metabolism of 2,2-Dimethylnonadecanoic Acid," Acta Chemica Scandinavica, 10 (8) 1234-1242, 1956.

Tryding, N., and Westoo, G. "Synthesis and Metabolism of 2,2,17,17-Tetramethylstearic Acid.I," Arkiv For Kemi, 11 (31), 291-305, 1957a.

Tryding, N., and Westoo, G. "On the Metabolism of 2,2-Dimethylnonadecanoic Acid in Man," Arkiv For Kemi, 11 (33), 313-316, 1957b.

Van der Wall, E. E., Heidendahl, G. A. K., Den Hollander, W., Westera, G., Roos, J. P. "Metabolic Myocardial Imaging with ^{123}I-labeled Heptadecanoic Acid in Patients with Angina Pectoris," Europ. Nucl. Med., 6, 391-396, 1981.

Yamamoto, K., Som, P., Brill, A. B., Yonekura, Y., Goodman, M. M., Knapp, F. F., Jr., Elmaleh, D. R. and Strauss, H. W. "Comparative Dual Tracer Studies of β-Methyl-(1-C-14)Heptadecanoic Acid (BMHDA) and 15-p-(I-131)-Iodophenyl-β-Methyl-pentadecanoic Acid (BMPDA) in Hypertensive Rats," J. Nucl. Med., 25, P 31, 1984.

Yonekura, Y., Brill, A. B., Som, P., Yamamoto, K., Srivastava, Suresh C., and Iwai, J., Elmaleh, D. R., Livni, E., Strauss, H. W., Goodman, M. M., and Knapp, F. F., Jr. "Quantitative Autoradiographic Measurement of Regional Myocardial Substrate Utilization in Hypertensive Rats," Science, submitted, 1984a.

Yonekura, Y., Tamaki, N., Torizuka, K., Brill, A. B., Som, P., Yamamoto, K., Srivastava, S., Iwai, J., Elmaleh, D. R., Livni, E., Strauss, H. W., Goodman, M. M., Knapp, F. F., Jr. "Quantitative Autoradiographic Measurement of Regional Myocardial Substrate Uptake in Hypertensive Rats," J. Nucl. Med., 25, P 24, 1984b.