product which corresponded to authentic 8 by TLC. The activity of the material was 1.5 mCi (30% incorporation) and the specific activity was Ca. 750 μCi/mg.

Aromatase Inhibition Assay. The screening assay procedure is the same as that described in our previous work.[21]

Stability Assay. The stability assays were performed under essentially the same conditions as the inhibition screening assay, except that no substrate or unlabeled inhibitor was added to the assay tubes. Radiolabeled inhibitor was prepared in ethanol and then as concentrated propylene glycol–buffer solutions (1:8). This solution was added to each test tube (60 μL). Each tube contained approximately 390 000 dpm. Cofactors in buffer (0.44 mL) were added to the tubes. The enzyme (0.1 mg of microsomal protein/sample) in buffer (0.5 mL) was added, and the samples were incubated at 25 °C for either 15 min or 1 h (assays run in triplicate). The assay was quenched with 1 mL of ethyl acetate and extracted twice with 1 mL of ethyl acetate. Organic layers were combined, evaporated to dryness, and counted (95% recovery of activity in first extraction). Extracts were applied to TLC plates (3 × 6 drops of acetone) and developed in benzene–ethyl acetate (80/20). TLC plates were scanned for radioactivity and compared to nonradioactive standards included on each plate. The appropriate regions of the plates were cut and counted.

Acknowledgment. This investigation was supported in part by a postdoctoral fellowship (M.V.D.) from the Damon Runyon–Walter Winchell Cancer Foundation, an NIH predoctoral traineeship (J.A.L.) under Grant T 32-GM-07767, and USPHS Grant CA-08349. We also thank G.D. Searle and Co. for their generous gift of steroid materials.

Registry No. 1, 67340-72-1; 1 diazonium salt, 95590-01-5; 2, 95589-90-5; 3, 67340-80-1; 4, 95589-91-6; 5, 95589-92-7; 6, 67340-74-3; 7, 95589-93-8; 8, 95589-94-9; ^{125}I-8, 95590-00-4; 9, 95589-95-0; 10, 95589-96-1; 11, 95589-97-2; 12, 95589-98-3; 13, 67340-75-4; 14, 95589-99-4; SH-*p*-PhNH$_2$, 1193-02-8; SH-*p*-PhNEt$_2$, 4946-24-1; SH-*p*-PhOMe, 696-63-9; SH-*p*-PhMe, 106-45-6; SH-*m*-PhNH$_2$, 22948-02-3; SHPh, 108-98-5; SH-*p*-PhF, 371-42-6; SH-*p*-PhI, 52928-01-5; SH-*p*-PhCl, 106-54-7; SH-*p*-PhBr, 106-53-6; SH-*p*-PhC(O)CH$_3$, 3814-20-8; SH-C$_6$H$_{11}$, 1569-69-3; SHCH$_2$Ph, 100-53-8; androst-4,6-diene-3,17-dione, 633-34-1; sodium iodide-125, 24359-64-6; iodine-125, 14158-31-7; aromatase, 9039-48-9.

Design, Synthesis, and Evaluation of ω-Iodovinyl- and ω-Iodoalkyl-Substituted Methyl-Branched Long-Chain Fatty Acids[†,‡]

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The synthesis of a new methyl-branched fatty acid, (*E*)-19-iodo-3(*RS*)-methyl-18-nonadecenoic acid (**19**), is described. Methyl branching has been introduced at the 3-position to inhibit β-oxidation and radioiodide has been attached as a *trans*-vinyl iodide. Preparation of **19** involved a 15-step sequence of reactions climaxing with formation of the methyl ester **18** by iododestannylation of methyl (*E*)-19-(tri-*n*-butylstannyl)-3(*RS*)-methyl-18-nonadecenoate (**17**) resulting from the reaction of *n*-Bu$_3$SnH with methyl 3(*RS*)-methyl-18-nonadecynoate (**16**). Methyl branching was introduced at an early stage by Friedel–Crafts acylation of thiophene with 3(*RS*)-methyl-4-carbomethoxybutanoyl chloride (**3**) generated from 3-methylglutaric anhydride. The new agent, [^{125}I]-**19**, showed high myocardial uptake (5 min, 4.89% dose/g; 30 min, 3.32% dose/g), good heart/blood (H/B) ratios (5 min, 5.4/1; 30 min, 4.3/1), and significantly greater myocardial retention in fasted rats than the corresponding straight-chain analogue 19-[^{125}I]-iodo-18-nonadecenoic acid (5 min, 3.52% dose/g, H/B = 4.8/1; 30 min, 1.19% dose/g, H/B = 1.6/1). Excellent myocardial images were obtained in rats after administration of [^{123}I]-**19** and confirmed the slow myocardial washout over a 60-min period. These data suggest that **19** is a good candidate for evaluation of heart disease involving aberrations in fatty acid metabolism by use of imaging techniques such as single photon emission computerized tomography (SPECT) where redistribution or washout should be minimized.

The major energy requirements of the normal myocardium are met by the oxidation of long-chain fatty acids. Regional differences delineated by mapping myocardial uptake and retention of structurally modified radiolabeled fatty acids could potentially be an accurate and elegant means of detecting subtle differences in regional metabolism. Iodine-123 is an attractive radionuclide for radiolabeling fatty acids since it has excellent radionuclidic properties (159-keV photon, 13.3-h half-life) and there are a wide variety of chemical methods available for attaching iodine to fatty acids.[1] Iodine-123-labeled terminal iodoalkyl and iodophenyl fatty acids have been used for the clinical evaluation of ischemic myocardial disease by determining differences in regional release rates of inorganic iodide or radioiodinated metabolites from normal and diseased myocardium.[2-8]

For applications where an evaluation of the regional uptake may be more desirable than a measurement of the rate of washout, ^{123}I-labeled fatty acids could be used to measure regional distribution where aberrations in fatty acid metabolism are reflected by differences in uptake. To

[†]Research supported by the Office of Health and Environmental Research, U.S. Department of Energy, under Contract DE-AC05-840R21400 with Martin Marietta Energy Systems, Inc.

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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" to reflect the initial distribution pattern. The identification of structural features that would significantly increase the residence time of radiolabeled fatty acids in the myocardium is therefore of considerable interest. These agents could potentially be used to measure 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 that results from rapid myocardial metabolism of fatty acids, we sought to introduce a structural feature into the fatty acid molecule that would not decrease uptake from the plasma, but that would interfere with subsequent metabolism within the myocyte. 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 extracted by the myocardium. Our early studies involved the use of fatty acids containing stable, nonradioactive tellurium to interfer with β-oxidation to "trap" the fatty acid in the myocardium with iodine-123 attached as either an iodoalkyl, iodophenyl, or iodovinyl group. A model agent, 15-(*p*-iodophenyl)-6-tellurapentadecanoic acid (TPDA), was prepared via introduction of radioiodide by decomposition of a triazene intermediate[9] and showed the expected high heart uptake and prolonged retention in rats.[10] We have also developed and evaluated a variety of telluraoctadecenoic acid analogues in which the radioiodide is stabilized as a vinyl iodide.[11,12] The position of the Te heteroatom is an important factor and 18-iodo-5-tellura-17-octadecenoic acid shows high myocardial uptake, high heart/blood ratios, and prolonged retention as observed with TPDA.[10]

More recently we have investigated the use of methyl branching in the 2- and 3-positions of the alkanoic acid chain to inhibit β-oxidation and prolong myocardial retention. Our initial synthetic strategy for the preparation of 14-(*p*-iodophenyl)-2(*RS*)-methyltetradecanoic acid and 15-(*p*-iodophenyl-3(*RS*)-methylpentadecanoic acid (BMIPP) involved the use of substituted oxazolines as the substrates for carbon–carbon bond formation.[13] The effect of methyl branching at the 2- and 3-positions on myocardial retention in rats was assessed by a comparison of the distribution of the methyl-branched agents with the myocardial uptake of the corresponding straight-chain analogues 14-(*p*-[^{125}I]iodophenyl)tetradecanoic acid and 15-(*p*-[^{125}I]iodophenyl)pentadecanoic acid (IPP). The increased myocardial uptake and retention of radioactivity following injection of [^{125}I]BMIPP in comparison to the 14-carbon fatty acids and the unbranched analogue [^{125}I]IPP suggested that methyl branching at the 3-position would be an effective means of inhibiting myocardial metabolism of radioiodinated phenyl fatty acids.

An undesired property accompanying the pronounced myocardial uptake of terminal phenyl fatty acids is high blood radioactivity that results in low heart/blood ratios (~2:1). Our recent studies with a model iodovinyl fatty acid 18-[^{125}I]iodo-17-octadecenoic acid,[14] show initial high heart uptake (1 h, 1.90–2.28% dose/g) with high heart/blood ratios (7.3–7.8:1). In order to evaluate the effects of β-methyl branching in a model agent in which radioiodide has been stabilized as a terminal *trans*-vinyl iodide, 19-[^{125}I]iodo-3(*RS*)-methyl-18-nonadecenoic acid has now been prepared and studied in rats in comparison with the corresponding straight-chain analogue 19-[^{125}I]iodo-18-nonadecenoic acid. These studies are an extension of our earlier investigations demonstrating that methyl branching leads to high myocardial uptake and prolonged retention.[13] The availability of fatty acid analogues that exhibit myocardial extraction and uptake similar to natural fatty acids but more prolonged retention offers a unique opportunity to evaluate aberrations in fatty acid metabolism that may occur when the coronary arteries are normal and delivery (flow) is not impaired. Such examples include hypertensive heart disease (vide infra) and cardiomyopathies.

Results

Chemistry. The route chosen for the construction of the methyl-branched fatty acid skeleton involved chain extension of a 3-methyl-branched dicarboxylic acid by sulfur extrusion of an elaborated 2,5-dialkyl thiophene precursor. With use of this approach, the substituents introduced into the 2- and 5-positions of the thiophene ring can be selected and thus provide a variety of 3-methyl-branched fatty acids of various chain lengths for structure activity studies. The key substrate 17-iodo-3(*RS*)-methylheptadecanoic acid (**14**) was prepared as described in Scheme I. Methyl branching was introduced into the 2-position of the thiophene ring by acylation of the half ester acid chloride **3**. 3(*RS*)-Methyl-4-carbomethoxybutanoyl chloride (**3**) was prepared from commercially available 3-methylglutaric anhydride (**1**) by treatment with methanol followed by thionyl chloride as previously reported.[15] Friedel–Crafts acylation of **3** using $SnCl_4$ gave the thienyl ketone **5**. Wolff–Kishner reduction of **5** followed by treatment with CH_2N_2 afforded the methyl-branched 2-substituted thiophene **7**. 8-Methoxyoctanoyl chloride **10** was used for introduction into the 5-position of the thiophene ring. Functionalization of the terminal methylene group with the chemically inert methoxy function allowed subsequent displacement with iodide.

The acyl chloride **10** was prepared by treatment of 8-bromooctanoic acid (**8**) with sodium methoxide followed by thionyl chloride. The methyl ester **7** was subjected to Friedel–Crafts condensation with the acyl chloride **10** to afford 2-[3(*RS*)-methyl-5-methoxypentanoyl]-5-(8-methoxy-1-oxooctyl)thiophene (**11**). Wolff–Kishner (Huang–Minlon) reduction of the keto ester **11** gave 2-[3(*RS*)-methyl-5-hydroxypentanoyl]-5-(8-methoxyoctyl)thiophene (**12**). Raney nickel was used for ring opening and desulfurization of **12**. Although the sulfur from the thiophene ring was readily extruded by treatment with Raney nickel, the desired terminal methoxy methyl-branched acid **13** was obtained in addition to an olefinic product, which was presumably formed as a result of poisoning of the catalyst.

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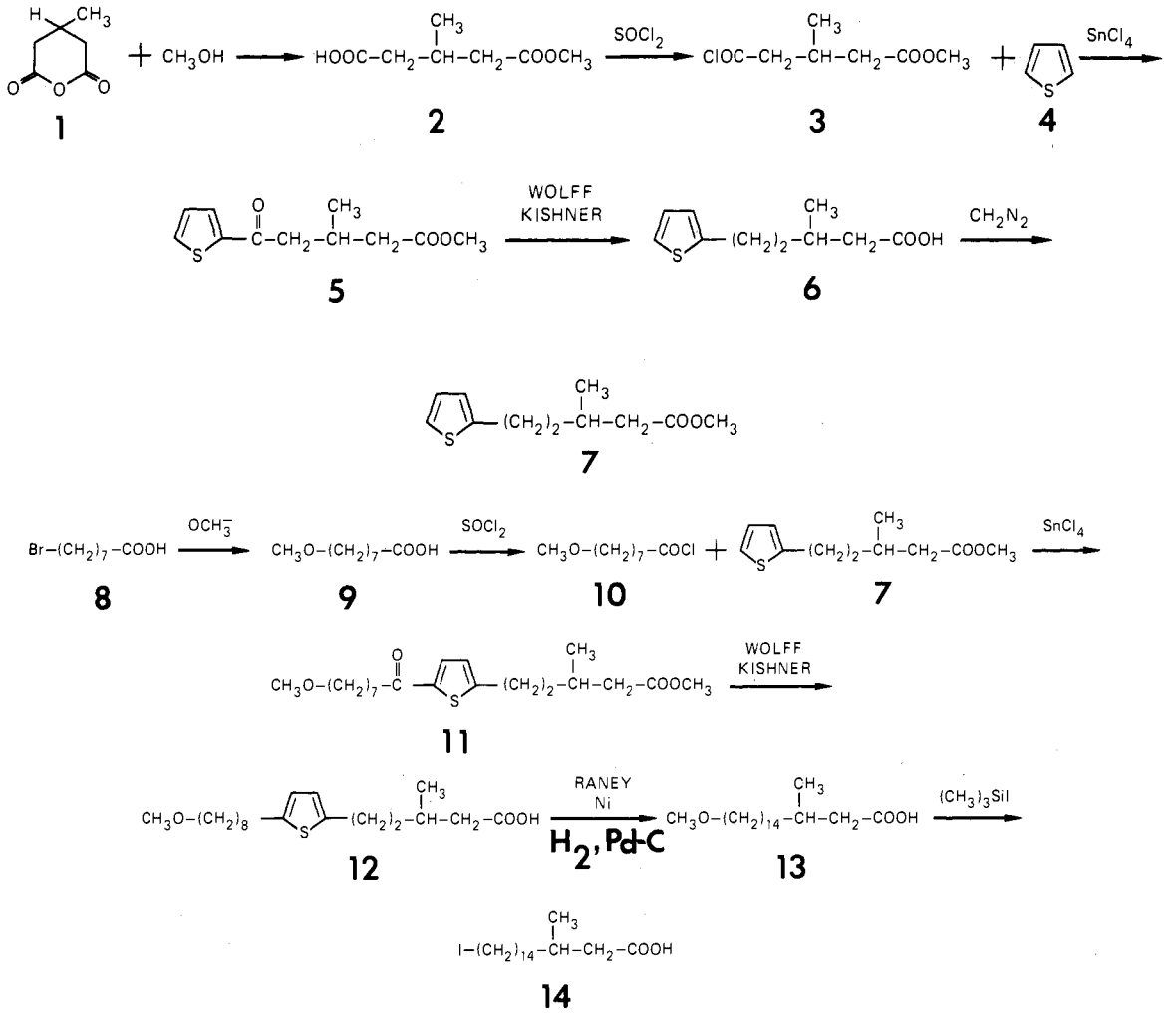
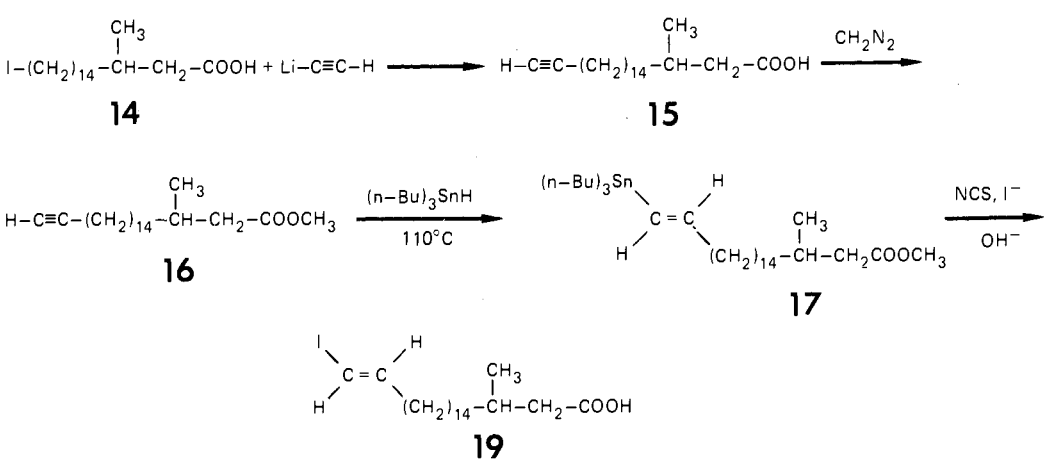
Scheme I



Scheme II



The formation of symmetrical olefins by reductive desulfurization of 2,5-dialkylated thiophenes has been observed.[16] The uncharacterized component was presumably $CH_3O(CH_2)_9CH{=}CH(CH_2)_3CH(CH_3)CH_2COOH$ and the mixture of fatty acids was reduced with H_2–Pd/C to give **13**, followed by subsequent treatment with $(CH_3)_3SiI$, to give the terminal iodinated 3-methyl-branced fatty acid 17-iodo-3(*RS*)-methylheptadecanoic acid (**14**).

The (*E*)-vinyl iodide was introduced into the terminal position of the methyl-branched acid via the iodo-destannylation[17] of the tri-*n*-butylstannyl derivative **17**, prepared from **14** by a four-step sequence of reactions (Scheme II).

There are several advantages of fabricating an iodovinyl moiety on a carboxylic acid derivative with use of the destannylation reaction in contrast to alternative halodemetalation reactions, for example, using boron or aluminum reagents. Unlike the reaction of either catecholborane[14] or diisobutylaluminum hydride[18] with acetylenic esters, the carbomethoxy group is inert to reduction with

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Scheme III

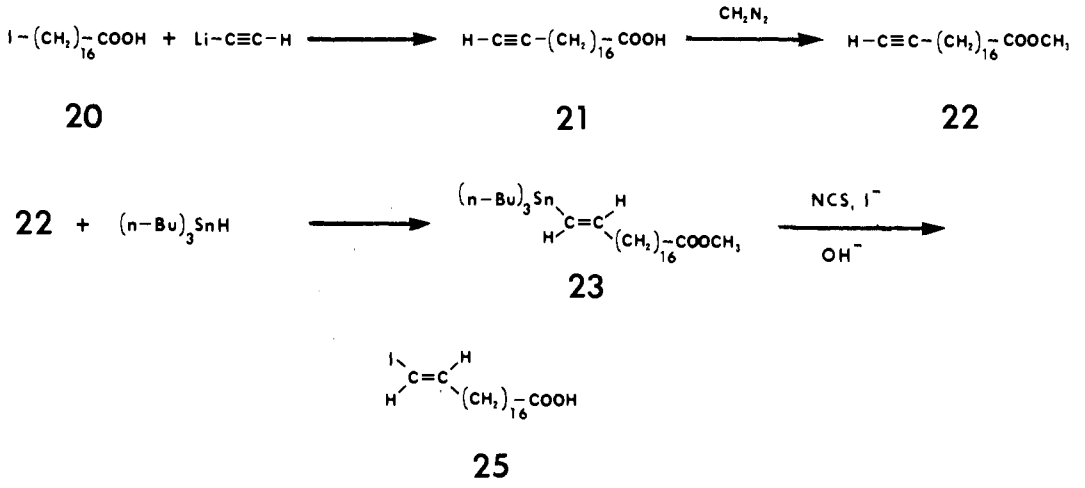

Table I. Summary of Radiochemical Yields and Specific Activities of the Various Radioiodinated Fatty Acids

fatty acid	radiolabel	% yield	sp act. Ci/mmol
14	iodine-125	28	0.269
19	iodine-123	37	4.98
	iodine-125	25	7.08
25	iodine-123	27	2.21
	iodine-125	27	1.77

trialkyltin hydrides. Secondly, in the presence of ICl the (*E*)-trialkyltin adducts undergo destannylation to yield exclusively the (*E*)- iodovinyl products. Finally, this method can be adapted to a "no carrier added" scale[19] for the preparation of high specific activity radioiodinated products, which is important for the dissolution of sparingly soluble radiolabeled fatty acids in albumin solution or serum.

The pivotal step in the synthesis (Scheme II) of the (*E*)-iodovinyl acid **19** involved hydrostannylation of the terminal ethynyl substrate **16** with $(n\text{-Bu})_3SnH$ to give the key intermediate **17**. 3(*RS*)-Methyl-18-nonadecyanoic acid **15** was prepared by treatment of the 17-iodo-3-methyl-branched acid **14** with lithium acetylide–ethylenediamine (LAEDA). The corresponding methyl ester **16** was prepared by treatment of terminal ethynyl acid **15** with diazomethane. Iododestannylation of **17** by treatment with either ICl or I^+ formed by in situ oxidation of I^- with *N*-chlorosuccinimide (NCS) followed by basic hydrolysis gave **19**. The straight-chain analogue **25** was prepared in the same manner (Scheme III), which involved iododestannylation of the substrate **23**, which was fabrictated by trialkylstannylation of the acetylene **22** generated by attachment of the ethynyl group to **20**. The iodine-125- and iodine-123-labeled fatty acids **19** and **25** were prepared in the same manner by reaction of the requisite tributylstannyl substrates with I^+ generated in situ by *N*-chlorosuccinimide oxidation of the radioiodide. The iodide-125-labeled iodoalkyl fatty acid **14** was prepared by iodide-125 exchange of the unlabeled iodoalkyl substrate. The radiochemical yields and specific activities of the various radioiodinated products are summarized in Table I.

Biological Evaluation. 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 coadministered to the same rats. These results are shown in Table IV and in Figure 2 for $[^{125}I]$-**19** and $[^{123}I]$-**25**. These results were confirmed by a duplicate study in which the radioactive labels were reversed on the two compounds (e.g., $[^{123}I]$-**19** and $[^{125}I]$-**25** mixture). Several important observations can be made from a comparison of the myocardial uptake and subsequent washout of the methyl-branched (**19**) and straight-chain (**25**) fatty acids in fasted and unfasted animals. In both the fasted and unfasted animals, the β-methyl-branched fatty acid shows higher myocardial uptake, lower blood levels, and higher heart/blood ratios (Table II and IV). These results have also been observed in similar studies where the agents

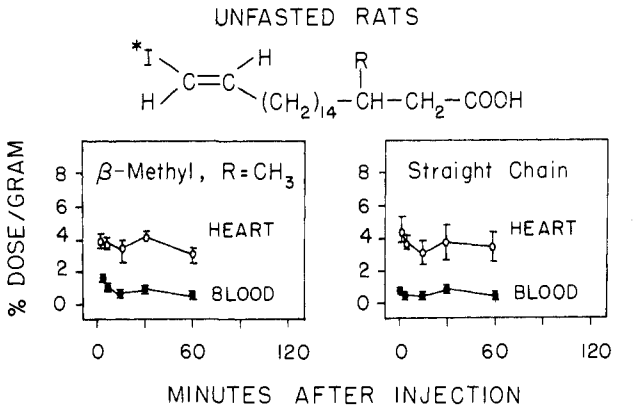

Figure 1. Comparison of the heart and blood retention of 19-[^{123}I]iodo-3(*RS*)-methyl-18-nonadecenoic acid (β-methyl, R = CH_3, **19**) and 19-[^{125}I]iodo-18-nonadecenoic acid (**25**, R = H), in groups of unfasted Fischer rats.

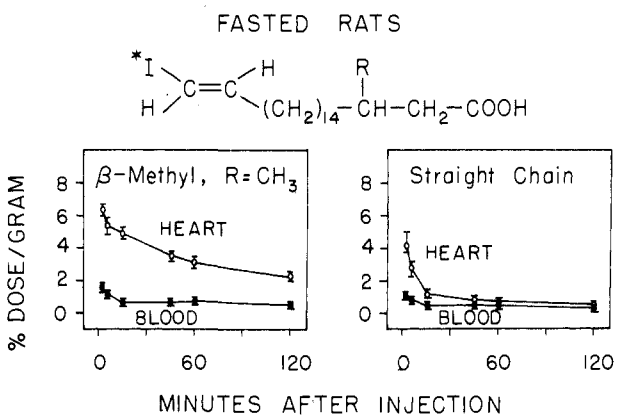

Figure 2. Comparison of the heart and blood retention of 19-[^{123}I]iodo-3(*RS*)-methyl-18-nonadecenoic acid (β-methyl, R = CH_3, **19**) and 19-[^{125}I]iodo-18-nonadecenoic acid (**25**, R = H) in groups of fasted Fischer rats.

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Table II. Distribution of Radioactivity in Tissues of Unfasted Fischer 344 Rats following Intravenous Administration of 19-[^{125}I]Iodo-3(*RS*)-methyl-18-nonadecenoic Acid ([^{125}I]-**19**)[a]

tissue	mean % injected dose/g (range) at the following times after injection				
	2 min	5 min	15 min	30 min	60 min
heart	3.93 (3.30–4.43)	3.74 (3.48–4.07)	3.47 (2.45–3.94)	4.12 (3.86–4.46)	3.08 (2.49–3.37)
blood	1.05 (0.84–1.15)	0.67 (0.54–0.81)	0.41 (0.34–0.45)	0.59 (0.54–0.62)	0.37 (0.35–0.40)
lungs	1.42 (1.19–1.76)	1.18 (1.08–1.27)	0.83 (0.79–0.85)	0.89 (0.83–0.97)	0.73 (0.66–0.79)
liver	8.32 (7.75–8.89)	7.81 (7.30–8.21)	8.44 (7.86–9.04)	5.97 (5.62–6.36)	4.73 (3.64–5.47)
kidneys	0.95 (0.92–1.03)	0.92 (0.84–1.07)	0.66 (0.61–0.70)	0.71 (0.64–0.78)	0.67 (0.63–0.75)
thyroids	7.21 (5.64–8.50)	9.49 (8.85–10.0)	16.9 (14.8–18.3)	29.4 (21.8–41.9)	65.4 (51.8–89.9)
mean heart/blood	3.7	5.6	7.4	6.9	8.3

[a] Five rats were used for each time period. Each rat received 4.47 μCi of the ^{125}I-labeled fatty acid (sp act. 4.98 Ci/mmol) administered by injection in a lateral tail vein in 6% bovine serum albumin solution (0.5 mL).

Table III. Distribution of Radioactivity in Tissues of Unfasted Fischer 344 Rats following Intravenous Administration of 19-[^{125}I]Iodo-18-nonadecenoic Acid ([^{125}I]-**25**)[a]

tissue	mean % injected dose/g (range) at the following times after injection				
	2 min	5 min	15 min	30 min	60 min
heart	4.44 (3.79–5.30)	3.72 (3.37–4.04)	3.00 (2.27–3.81)	3.79 (2.62–4.80)	3.40 (2.52–4.39)
blood	0.45 (0.37–0.50)	0.30 (0.25–0.40)	0.35 (0.24–0.45)	0.51 (0.43–0.67)	0.31 (0.29–0.33)
lungs	1.16 (1.07–1.26)	0.97 (0.89–1.05)	0.92 (0.81–1.00)	1.00 (0.93–1.08)	0.86 (0.77–0.92)
liver	6.91 (6.44–7.61)	6.45 (5.86–7.07)	5.88 (4.53–6.84)	5.72 (5.30–6.28)	3.79 (3.52–4.03)
kidneys	0.85 (0.79–0.90)	0.82 (0.70–0.88)	0.73 (0.62–0.80)	0.76 (0.67–0.81)	0.67 (0.65–0.70)
thyroids	7.91 (7.22–9.49)	8.73 (7.42–9.88)	15.7 (10.2–19.5)	21.9 (17.9–25.7)	43.5 (34.3–55.3)
mean heart/blood	9.9	12.4	8.6	7.4	10.9

[a] Five rats were used for each time period. Each rat received 1.57 μCi of the ^{125}I-labeled fatty acid (sp act. 1.77 Ci/mmol) administered by injection in a lateral tail vein in 6% bovine serum albumin solution (0.5 mL).

were administered to groups of rats separately.[26] In the unfasted animals fed normal rat chow, the relative myocardial retention of the β-methyl-branched fatty acid (Table II) and the straight-chain (Table III) analogue is similar. On the other hand, the kinetics of the myocardial washout observed in the fasted rats is much more rapid for the straight-chain agent in comparison to the β-methyl-branched analogue. Thus, under fasting conditions, which is the recommended for clinical studies since all patients are in a similar nutritional state,[20-24] the differences in myocardial clearance rates are dramatically illustrated with the β-methyl-branched agents showing considerably slower washout. These data suggest that the terminal iodovinyl methyl-branched fatty acids are excellent candidates for single-photon tomography of regional myocardial distribution of fatty acids. The same differences in the relative myocardial retention of straight-chain and 3-methyl-branched analogues in the iodophenyl series have also been found.[31]

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

The availability of radioiodinated 17-iodo-3(*RS*)-methylheptadecanoic acid (**14**) prepared as an intermediate during the synthesis of **19** (Scheme I) allowed 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 **14** would focus on the direct cleavage of the carbon–iodide bond. Preliminary tissue distribution studies of ^{125}I-labeled **14** demonstrated rapid myocardial washout and in vivo dehalogenation, indicating significant carbon–iodide cleavage in the apparent absence of β-oxidation (Table V). These results confirm our earlier studies using 17-[^{131}I]iodo-9-telluraheptadecanoic acid,[25] which exhibited high heart uptake in rats but showed significant in vivo deiodination resulting in high blood levels of activity and signficant thyroid uptake. More recently, similar results with 13- and 16-carbon analogues of **14** have been reported.[27]

Discussion

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

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Table IV. Distribution of Radioactivity in Tissues of Fasted Fischer 344 Rats following Intravenous Mixture of 19-[^{125}I]Iodo-3(*RS*)-methyl-18-nonadecenoic Acid ([^{125}I]-**19**) and 19-[^{123}I]Iodo-18-nonadecenoic Acid ([^{123}I]-**25**)[a]

tissue	mean % injected dose/g (range) at the following times after injection			
	5 min	30 min	60 min	4 h
heart				
^{125}I	4.89 (4.23–5.79)	3.32 (2.85–3.82)	2.56 (2.42–2.66)	1.38 (1.03–1.61)
^{123}I	3.52 (3.11–3.99)	1.19 (1.10–1.31)	0.78 (0.69–0.97)	0.59 (0.52–0.62)
blood				
^{125}I	0.91 (0.67–1.00)	0.77 (0.67–0.83)	0.63 (0.57–0.69)	0.53 (0.38–0.59)
^{123}I	0.74 (0.58–0.82)	0.74 (0.65–0.81)	0.64 (0.58–0.72)	0.61 (0.46–0.68)
lungs				
^{125}I	1.36 (1.19–1.45)	0.96 (0.87–1.04)	0.89 (0.76–1.01)	0.81 (0.64–0.87)
^{123}I	1.54 (1.38–1.66)	1.32 (1.22–1.46)	1.21 (1.10–1.29)	1.23 (0.99–1.36)
liver				
^{125}I	6.48 (5.45–7.56)	4.53 (4.12–4.95)	2.94 (2.75–3.15)	1.11 (0.39–1.42)
^{123}I	4.87 (4.07–5.39)	3.49 (3.12–3.85)	2.62 (2.48–2.86)	1.83 (1.71–1.98)
kidneys				
^{125}I	1.06 (0.99–1.11)	0.91 (0.78–1.10)	1.30 (0.79–3.13)	0.66 (0.51–0.78)
^{123}I	1.12 (1.00–1.21)	1.03 (0.92–1.18)	0.96 (0.94–1.01)	0.95 (0.75–1.07)
thyroid				
^{125}I	16.7 (13.7–20.4)	48.5 (35.6–58.3)	82.9 (59.9–103)	364 (285–482)
^{123}I	22.5 (20.7–26.1)	65.3 (52.4–78.1)	102 (78–127)	392 (292–484)
mean heart/blood				
^{125}I	5.4	4.3	4.1	2.60
^{123}I	4.8	1.6	1.2	0.96

[a] Five rats were used for each time period. Each rat received 2.20 μCi of the ^{125}I-labeled fatty acid (sp act. 7.08 Ci/mmol) and 10.2 μCi of the ^{123}I agent (2.21 Ci/mmol) administered by injection in a lateral tail vein in 6% bovine serum albumin solution (0.5 mL).

Table V. Distribution of Radioactivity in Tissues of Fischer 344 Rats following Intravenous Administration of 17-[^{125}I]Iodo-3(*RS*)-methylheptadecanoic Acid ([^{125}I]-14)[a]

tissue	mean % injected dose/g (range) at the following times after injection			
	2 min	5 min	10 min	60 min
heart	2.68 (2.11–3.42)	1.93 (1.16–2.78)	1.77 (1.29–2.21)	0.95 (0.49–1.25)
blood	2.47 (1.97–2.84)	1.43 (0.93–1.65)	1.31 (1.19–1.39)	0.72 (0.58–0.78)
lungs	1.75 (1.41–2.07)	1.03 (0.60–1.31)	1.16 (0.98–1.38)	0.68 (0.61–0.80)
liver	3.93 (3.09–4.60)	2.50 (1.89–2.80)	2.54 (2.34–2.69)	0.95 (0.90–0.98)
kidneys	1.35 (1.22–1.61)	1.06 (0.78–1.23)	0.96 (0.85–1.01)	0.59 (0.52–0.66)
thyroids	22.5 (19.6–24.8)	30.2 (14.9–40.2)	43.3 (39.6–46.6)	217 (170–283)
mean heart/blood	1.09	1.35	1.35	1.32

[a] Five rats were used for each time period. Each rat received 11 μCi of the ^{125}I-labeled fatty acid (sp act. ~269 mCi/mmol) administered by injection in a lateral tail vein in 6% bovine serum albumin solution (0.5 mL).

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.[28,29] As an example, iodine-131-labeled 15-(*p*-iodophenyl)-3(*RS*)-methylpentadecanoic acid (BMIPP) was administered to normal rats and to a second group of salt-sensitive hypertensive rats.[30] Similar groups of rats were injected with thallium-201, a perfusion (flow) monitor. The distribution of the iodine-131-labeled fatty acid was determined by examining the autoradiographs of the tissue slices. These studies showed that in normal rats both the thallium-201 and the iodine-131-labeled fatty acid were homogeneously distributed in the normal rats, and in the hypertensive rats thallium-201 had a homogeneous distribution but the iodine-131-labeled fatty acid clearly showed 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 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 are 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 in the above rat studies. On the other hand, the combination of iodine-123-labeled methyl-branched fatty acids and single photon emission computerized tomography (SPECT) can potentially evaluate hypertensive disease and assess the effects of drug therapy.

Experimental Section

General Procedures. All chemicals and solvents were analytical grade and were used without further purification. The petroleum ether (pet eth) had a boiling range of 30–60 °C. The iodine-125 was purchased from New England Nuclear, Inc. (North Billierica, MA). The iodine-123 was purchased either from the Brookhaven National Laboratory [p,5n] or from RadPharm, Inc. [p,2n]. The melting points (mp) were determined in capillary tubes with a Büchi SP apparatus and are uncorrected. The thin-layer chromatographic analyses (TLC) were performed with 250-μm thick layers of silica gel G PF-254 coated on glass plates (Analtech, Inc.). The infrared spectra (IR) were recorded on a Beckman 18-A spectrophotometer with NaCl plates or KBr pellets. The low-resolution mass spectra (MS) were determined with a Kratos MS-25 instrument at 70 eV. The proton nuclear magnetic resonance spectra (NMR) were obtained at 60 MHz with a Varian 360-L instrument or at 200 MHz with a Nicolet high-resolution instrument. Samples (30–40 mg) were dissolved in $CDCl_3$ or CCl_4 and the resonances (ppm) are reported downfield (δ) from the internal tetramethylsilane standard. The elemental analyses were

determined by Galbraith Laboratories, Knoxville, TN. Commercially available incandescent red lights were used where indicated.

Animal Tissue Distribution Studies. The distribution of radioactivity was determined in tissues of 10–12-week-old female Fischer 344 rats (170–200 g) after intravenous administration of the radioiodinated fatty acids. For the unfasted experiments, the animals were allowed food and water ad libitum prior to and during the course of the experiment. Food was removed from the fasted rats 18 h prior to initiation of the experiment. The radioiodinated fatty acids were dissolved in 0.5 mL of absolute ethanol and added dropwise to a stirred solution of 6% bovine serum albumin at ~40 °C. The final ethanol concentartion was 10%. The solution was filtered through a 0.22-μm Millipore filter and injected via a lateral tail vein into the ether-anesthetized animals. After the times indicated, the animals were killed by cervical fracture, and blood samples were obtained by cardiac puncture. The organs were then removed, rinsed with saline solution, and blotted dry to remove residual blood. The organs were weighed and counted in a NaI autogamma counter (Packard Instruments). Samples of the injected radioactive solutions were also assayed as standards to calculate the percent injected dose per gram of tissue values. The thyroid glands were not weighed directly. The weight of the thyroid glands was calculated in the usual manner[29] by multiplying the animal weight by (7.5 mg/100 g).

2-[3(*RS*)-Methyl-1-oxo-5-methoxypentanoyl]thiophene (5). The crude acid chloride **3** [3(*R,S*)-methyl-4-carbomethoxybutanoyl chloride; 16 g, 0.09 mol] was prepared as described earlier[15] and added to a solution of thiophene 4 (8.4 g, 0.1 mol) in 400 mL of CH_2Cl_2. The resulting mixture was cooled to 0–5 °C and anhydrous $SnCl_4$ (52 g, 0.2 mol) was added dropwise. The mixture was stirred at 0–5 °C for 60 min and room temperature for 2 h. The resulting purple solution was then treated with 6 N HCl (300 mL). After decomposition of the $SnCl_4$ complex with HCl, a yellow solution was obtained. The CH_2Cl_2 layer was separated, then washed thoroughly with 10% HCl, H_2O, and 10% NaOH, and dried over anhydrous Na_2SO_4. The CH_2Cl_2 was removed in vacuo to give 17.4 g (85%) of the keto ester **5**: bp 120–125 °C (0.5 mm); NMR ($CDCl_3$) δ 1.1 (d, J = 6 Hz, 3 H, CH_3), 2.4 (d, J = 6 Hz, 2 H, CH_2CO_2), 2.65 (m, 1 H, $CHCH_2CO_2CH_3$), 2.86 (d, J = 6 Hz, 2 H, $C_4H_3SCOCH_2$), 7.06–7.26 (m, 1 H, aromatic), 7.63–7.83 (m, 2 H, aromatic); MS, m/z 226 (M^+, 15). Anal. ($C_{11}H_{14}SO_3$) C, H, S.

2-[3(*RS*)-Methyl-5-hydroxypentanoyl]thiophene (6). The keto ester **5** (19 g, 0.084 mol) was added to 120 mL of diethylene glycol containing KOH (34 g, 0.61 mol) and hydrazine hydrate (85%) (6.4 g, 0.1 mol) and the solution refluxed for 1 h. The resulting mixture was distilled until the liquid reached a temperature of 200 °C and then it was refluxed for 3 h. After the mixture cooled to 90 °C, 260 mL of 5 N HCl was added, and the resulting mixture was cooled to room temperature and extracted with Et_2O (4 × 100 mL). The combined ether extracts were washed with H_2O (4 × 100 mL) and dried over anhydrous Na_2SO_4, and the solvent was removed to give 15 g (90%) of the acid **6**: bp 110–113 °C (0.5 mm); NMR ($CDCl_3$) δ 0.99 (d, J = 6 Hz, 3 H, CH_3), 1.5–2.1 (m, 3 H, $H_2CCH(CH_3)CH_2CO_2$), 2.23 (d, J = 6 Hz, 2 H, CH_2CO_2), 2.9 (t, J = 8 Hz, 2 H, $C_4H_3SCH_2$), 6.7–7.1 (m, 3 H, aromatic); MS, m/z 198 (M^+, 25). Anal. ($C_{10}H_{14}SO)_2$ C, H, S.

2-[3(*RS*)-Methyl-5-methoxypentanoyl]thiophene (7). The acid **6** (15 g, 0.076 mol) was dissolved in 50 mL of ether and the resulting solution was added to an ether solution (150 mL) containing CH_2N_2, prepared from *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NMMG, 15 g). The mixture was stirred at 0–5 °C and protected from light for 12 h. The ether solution was dried over anhydrous $MgSO_4$ and the solvent concentrated in vacuo to give 10 g (63%) of the ester **7**: bp 85–86 °C (0.5 mm); NMR ($CDCl_3$) δ 0.97 (d, J = 6 Hz, 3 H, CH_3), 1.5–2.1 (m, 3 H, $CH_2CH(CH_3)CH_2CO_2$), 2.25 (d, J = 6 Hz, 2 H, CH_2CO_2), 2.8 (t, J = 8 Hz, $C_4H_3SCH_2$), 3.63 (s, 3 H, CO_2CH_3), 6.7–7.1 (m, 3 H, aromatic); MS, m/z 212 (M^+, 35). Anal. ($C_{11}H_{16}SO_2$) C, H, S.

8-Methoxyoctanoic Acid (9). A mixture of 8-bromooctanoic acid (**8**; 16 g, 0.072 mol) and sodium metal (5 g, 0.216 mol) in dry methanol (150 mL) under argon was stirred at reflux for 6 h. The resulting mixture was cooled to room temperature, carefully poured into H_2O (300 mL), acidified with 6 N HCl, and extracted several times with ether. The combined ether extracts were washed thoroughly with H_2O and dried over anhydrous Na_2SO_4, and the solvent was removed in vacuo to yield 11 g (88%) of **9**: bp 98–100 °C (0.5 mm); NMR ($CDCl_3$) δ 1.37 (s, 10 H, CH_2), 2.31 (t, J = 6 Hz, 2 H, CH_2CO_2), 3.3 (s, 3 H, OCH_3), 3.37 (t, J = 6 Hz, 2 H, OCH_2); MS, m/z 174 (M^+, 5), 156 ($M^+ - H_2O$, 60). Anal. ($C_9H_{18}O_3$) C, H.

8-Methoxyoctanoyl Chloride (10). A solution of 8-methoxyoctanoic acid (**9**; 7.00 g, 0.04 mol) and thionyl chloride (4 mL, 0.07 mol) was stirred at 80 °C for 1 h. The resulting mixture was vacuum distilled to yield 5.5 g (72%) of the acid chloride **10**: bp 80–85 °C (0.5 mm); NMR ($CDCl_3$) δ 1.43 (s, 10 H, CH_2), 2.93 (t, J = 6 Hz, 2 H, CH_2COCl), 3.33 (s, 3 H, CH_3O), 3.37 (t, J = 6 Hz, 2 H, OCH_2).

2-[3(*RS*)-Methyl-5-methoxypentanoyl]-5-(1-oxo-8-methoxyoctyl)thiophene (11). The acid chloride **10** (5.5 g, 0.029 mol) was added to 100 mL of CH_2Cl_2 containing thienyl ester **7** (6.06 g, 0.029 mol). The resulting mixture was cooled to 0–5 °C and treated dropwise with anhydrous $SnCl_4$ (15 g, 0.058 mol) and worked up as described for **5**. The dried CH_2Cl_2 layer was evaporated in vacuo to yield 7 g (66%) of **11**: bp 215–220 °C (0.5 mm); NMR ($CDCl_3$) δ 0.99 (d, J = 6 Hz, 3 H, $CH_3(CH)$), 1.37 (s, 10 H, CH_2), 1.7 (m, 3 H, CH_2CH), 2.5 (d, J = 4 Hz, 2 H, CH_2CO_2), 2.82 (t, J = 8 Hz, 2 H, $C_4H_2SCH_2$), 2.85 (t, J = 8 Hz, 2 H, $C_4H_2SCOCH_2$), 3.3 (s, 3 H, CH_3O), 3.5 (t, J = 6 Hz, 2 H, OCH_2), 3.67 (s, 3 H, CO_2CH_3), 6.87 (d, J = 4 Hz, 1 H, aromatic), 7.57 (d, J = 4 Hz, 1 H, aromatic); MS, m/z 368 (M^+, 100). Anal. ($C_{19}H_{30}SO_4$) C, H, S.

2-[3(*RS*)-Methyl-5-hydroxypentanoyl]-5-(8-methoxyoctyl)thiophene (12). The keto ester **11** (7 g, 0.019 mol) was added to 30 mL of diethylene glycol containing KOH (6.2 g, 10 mmol) and 85% hydrazine hydrate (3.2 g, 0.064 mol) and the mixture was reacted as described for **6**. The dried Et_2O extracts were concentrated in vacuo to afford an orange oil. The crude material was dissolved in 2 mL of $CHCl_3$ applied to a column containing SiO_2 (100 g) slurried in $CHCl_3$. Fractions 13–39 (25 mL in volume) were combined, and the solvent was removed in vacuo to afford 5.45 g (84%) of **12** as a colorless oil: NMR ($CDCl_3$) δ 1.00 (d, J = 6 Hz, 3 H, CH_3), 1.33 (s, 12 H, CH_2), 1.4–2.1 (m, 3 H, $CHCH_2$), 2.27 (m, J = 6 Hz, 2 H, CH_2CO_2), 2.75 (t, J = 8 Hz, 2 H, $C_4H_2SCH_2$), 3.00 (t, J = 8 Hz, 2 H, $C_4H_2SCH_2$), 3.33 (s, 3 H, OCH_3), 3.39 (t, J = 6 Hz, 2 H, OCH_2), 6.6 (s, 2 H, CH, aromatic); MS, m/z 340 (M^+, 85%).

17-Methoxy-3(*RS*)-methylheptadecanoic Acid (13). Raney nickel (50 g) and the thienyl acid **12** (5 g, 0.0147 mol) were vigorously stirred and refluxed in 500 mL of 1:1 mixture of EtOH/10% Na_2CO_3 for 18 h. The hot solution was filtered through Celite, and the cooled filtrate was carefully acidified to pH 3 with 12 N HCl and extracted thoroughly with Et_2O. The combined Et_2O extracts were washed several times with H_2O and dried over anhydrous $MgSO_4$, and the solvent was evaporated in vacuo to give 3.55 g of an oil. The NMR spectrum of the oil indicated a 1:1 mixture of **13** and an olefin. The oil was dissolved in 25 mL of absolute EtOH containing 10% Pd/C and hydrogenated at 40 psi (Parr apparatus) for 18 h. The resulting solution was filtered and the EtOH evaporated in vacuo to yield 3.45 g (75%) of **13** as a colorless oil, which crystallized on standing. An analytical sample was purified by column chromatography (25 g, SA 200, silicic acid) by elution with $CHCl_3$. Fractions 11–15 (25 mL in volume) were combined, and the solvent, was removed to yield a white solid: mp 45–46 °C; NMR ($CDCl_3$) δ 1.00 (d, J = 6 Hz, 3 H, CH_3), 1.29 (s, 26 H, CH_2), 2.27 (m, 2 H, CH_2CO_2), 3.3 (s, 5 H, CH_3OCH_2); MS, m/z 314 (M^+, 5). Anal. ($C_{19}H_{38}O_3$) C, H.

17-Iodo-3(*RS*)-methylheptadecanoic Acid (14). A mixture of the methoxy acid **13** (1.57 g, 5 mmol), sodium iodide (6.0 g, 20 mmol), and chlorotrimethylsilane (6.0 g, 20 mmol) was heated under an argon atmosphere for 18 h at reflux in 20 mL of acetonitrile. The resulting solution was cooled to room temperature, poured into 150 mL H_2O, and extracted three times with 50 mL of Et_2O. The combined Et_2O extracts were washed three times with 50 mL of 10% sodium thiosulfate, and two times with 50 mL of H_2O and dried over anhydrous Na_2SO_4. The Et_2O was removed in vacuo to afford a yellow solid. The crude product was crystallized from petroleum ether (30–60 °C) to afford 1.78

g (87%) of **14**: mp 52–54 °C; NMR ($CDCl_3$) δ 0.97 (d, J = 6 Hz, 3 H, CH_3), 1.30 (s, 28 H, CH_2), 2.27 (m, 3 H, $CHCH_2CO_2$), 3.2 (t, J = 6 Hz, 2 H, CH_2I); MS, m/z 283 (M^+ - I, 20%). Anal. ($C_{18}H_{35}O_2$) C, H, I.

3(*RS*)-Methyl-18-nonadecynoic Acid (15). Lithium acetylide–ethylenediamine (1.49 g, 16 mmol) was suspended in dry hexamethylphosphoramide (HMPA, 15 mL) and the resulting sluurry was stirred at room temperature under argon for 45 min. The reaction mixture was cooled to 0 °C and a solution of 17-iodo-3(*RS*)-methylheptadecanoic acid (**14**; 1.64 g, 4 mmol) in 5 mL of argon-purged HMPA was added dropwise. The resulting solution was stirred at 0 °C for 30 min, poured into crushed ice (10 g), acidified with 10% H_2SO_4, and extracted several times with Et_2O. The combined ether extracts were washed thoroughly with H_2O and then dried over anhydrous Na_2SO_4, and the solvent was removed in vacuo to give an amber oil. The crude product was purified by column chromatography (SiO_2) by elution with $CHCl_3$ to give 900 mg (73%): NMR ($CDCl_3$) δ 1.00 (d, J = 6 Hz, 3 H, $CHCH_3$), 1.3 (s, 26 H, CH_2), 2.0 m, 1 H C≡CH), 2.25 (m, 2 H, CH_2CO_2).

Methyl 3(*RS*)-Methyl-18-decynoate (16). The acid **15** (300 mg, 1 mmol) was added to an ether solution (100 mL) containing CH_2N_2, prepared from MNNG (1 g) as described for **7**. The dried ether was evaporated to give a yellow oil. The crude material was dissolved in 1 mL of C_6H_5 and applied to a SiO_2 column (25 g, Davidson) slurried in benzene. Fractions 18–20 (5 mL in volume) were combined, and the solvent was removed in vacuo to yield 238 mg (80%) of a colorless oil: NMR ($CDCl_3$) δ 0.90 (d, J = 6 Hz, 3 H, $CHCH_3$), 1.23 (s, 26 H, CH_2), 1.93 (m, 1 H, C≡CH), 2.19 (m, 2 H, CH_2CO_2), 3.65 (s, 3 H, CO_2CH_3). Anal. ($C_{21}H_{38}O_2$) C, H.

Methyl 19-(Tri-*n*-butylstannyl)-3(*RS*)-methyl-18-nonadecenoate (17). The ester **16** (100 mg, 0.320 mmol), tributyltin hydride (100 mg, 0.333 mmol), and azobisisobutyronitrile (5 mg) were refluxed in 2 mL of toluene under an argon atmosphere for 6 h. The reaction mixture was cooled and applied to a column of SiO_2 (25 g) slurried in petroleum ether. Fractions (10 mL) were eluted with a 1:1 mixture of petroleum ether/benzene. Fractions 8–11 were combined to give (105 mg, 54%) a colorless oil: NMR ($CDCl_3$) δ 0.83 [t, J = 6 Hz, 9 H, $(C_3H_6CH_3)_3$], 0.90 (d, J = 6 Hz, 3 H, $CHCH_3$), $(C_3H_6CH_3)_3$], 1.23 (s, 44 H, CH_2), 1.8–2.3 (m, 5 H, $CHCH_3$, CH_2CO_2, C=CCH_2), 3.67 (s, 3 H, CO_2CH_3), 5.85 (m, 2 H, HC=CH).

Methyl (*E*)-19-Iodo-3(*RS*)-methyl-19-nonadecenoate (18). The stannyl ester **17** (200 mg, 0.33 mmol) was suspended in 5 mL of CCl_4 and the resulting mixture cooled to 0–5 °C (ice bath) and stirred under red lights. Iodine monochloride (52 mg, 0.33 mmol) was added and the resulting solution mixture was removed from the ice bath and stirred until a colorless solution resulted. The CCl_4 was removed in vacuo and residue applied to a column of SiO_2 slurried in benzene, and fractions (5 mL) 12–17 were combined to afford (150 mg, 80%) as colorless oil: NMR ($CDCl_3$) δ 0.90 (d, J = 6 Hz, 3 H, $CHCH_3$), 1.23 (s, 26 H, CH_2), 2.10 (m, 2 H, C=CCH_2), 2.30 (d, J = 8 Hz, 2 H, CH_2CO_2), 3.67 (s, 3 H, CO_2CH_3), 5.80 (d, 1 H, HC=CHI), 6.40 (m, 1 H, CH=CHI).

(*E*)-19-Iodo-3(*RS*)-methyl-19-nonadecenoic Acid (19). The methyl ester **18** (45 mg, 0.1 mmol) was dissolved in EtOH (5 mL) and refluxed with 1 N NaOH (1 mL) for 60 min. The mixture was cooled, poured into H_2O (50 mL), acidified to pH 3 with 1 N HCl, and extracted (3 × 25 mL) with Et_2O. Following washing with H_2O (3 × 25 mL), the Et_2O extracts were dried over anhydrous Na_2SO_4, and the solvent was evaporated in vacuo to give **19** as a colorless oil (40 mg, 91%): NMR ($CDCl_3$) δ 0.90 (d, J = 6 Hz, 3 H, $CHCH_3$), 1.23 (s, 26 H, CH_2), 2.10 (m, 2 H, C=CCH_2), 2.30 (d, J = 8 Hz, 2 H, CH_2CO_2), 5.80 (d, 1 H, HC=CHI), 6.40 (m, 1 H, CH=CHI).

19-Nonadecynoic Acid (21). The acid **20** (1.98 g, 5 mmol)[2,3] and lithium acetylide–ethylenediamine (1.86 g, 20 mmol) in 10 mL of dry hexamethylphosphoramide were reacted as described for compound **15**, and the product obtained crystallized from petroleum ether (30–60 °C) to give 1.25 g (85%) of **21**: mp 74–75 °C; NMR ($CDCl_3$) δ 1.25 (s, 28 H, CH_2), 1.9 (m, 1 H, C≡CH), 2.17 (t, J = 6 Hz, 2 H, CH_2C≡CH), 2.33 (t, J = 6 Hz, 2 H, CH_2CO_2); MS, m/z 294 (4%).

Methyl 19-Nonadecynoate (22). The acid **21** (600 mg, 2 mmol) was added to an ether solution (100 mL) containing CH_2N_2, prepared from MNNG (1 g). The mixture was stirred as described for **7**, and the solvent was removed in vacuo to yield a white solid. The crude product was crystalized from MeOH to afford 566 mg (92%) of **22**: mp 42–43 °C; NMR ($CDCl_3$) δ 1.23 (s, 28 H, CH_2), 1.9 (m, 1 H, C≡CH), 2.17 (t, J = 6 Hz, 2 H, CH_2C≡CH), 2.23 (t, J = 6 Hz, 2 H, CH_2CO_2), 3.63 (s, 3 H, CO_2CH_3); MS, m/z 308 (M^+, 1%).

Methyl 19-(Tri-*n*-butylstannyl)-18-nonadecenoate (23). The ester **22** (100 mg, 0.325 mmol), tributylin hydride (100 mg, 0.34 mmol), and azobisisobutyronitrile (5 mg) were reacted as described for **17**. Fractions 12–15 (10 mL) were combined to give the product (175 mg, 90%) as a colorless oil: NMR ($CDCl_3$) δ 0.90 (t, 9 H, CH_3), 1.23 (s, 46 H, CH_2), 2.00 (m, CH, C=CCH_2), 2.33 (t, J = 8 Hz, CH_2CO_2), 3.67 (s, 3 H, CO_2CH_3), 5.9 (m, 2 H, HC=CH); MS, m/z 600 (M^+ - 57, 100%).

Methyl (*E*)-19-Iodo-18-nonadecenoate (24). The stannyl ester **23** (88 mg, 0.147 mmol) and iodine monochloride (26 mg, 0.162 mmol) were reacted as described for **18**. The crude product was applied to a column of SiO_2 slurried in benzene, and fractions (5 mL) 13–16 were combined to afford **24** (49 mg, 77%) as a colorless oil: NMR ($CDCl_3$) δ 1.23 (s, 28 H, CH_2), 2.10 (m, 2 H, C=CCH_2), 2.33 (t, J = 8 Hz, CH_2CO_2), 3.67 (s, 3 H, CO_2CH_3), 5.80 (d, 1 H, HC=CHI), 6.40 (m, 1 H, HC=CHI).

(*E*)-19-Iodo-18-nonadecenoic Acid (25). The methyl ester **24** (49 mg, 0.11 mmol) was heated to reflux in a mixture of 1 mL of 1 N NaOH and 5 mL of EtOH as described for **19**. Crystallization from pet eth (30–60 °C) gave **25** (31 mg, 67%) as a white solid: mp 42–43 °C; NMR ($CDCl_3$) δ 1.23 (s, 28 H, CH_2), 2.10 (m, 2 H, C=CCH_2), 2.33 (t, J = 8 Hz, CH_2CO_2), 5.80 (d, 1 H, HC=CHI), 6.40 (m, 1 H, HC=CHI).

17-[^{125}I]Iodo-3(*RS*)-methylheptadecanoic Acid ([^{125}I]-14). A mixture of the substrate **14** (4 mg, 0.01 mmol) and sodium [^{125}I]iodide (2.69 mCi, no carrier added) was refluxed for 5 h in 10 mL of 2-butanone. The mixture was cooled to room temperature, poured into 50 mL of H_2O containing 1 mL 1 N HCl, and extracted several times with Et_2O. The combined Et_2O extracts were washed thoroughly with 5% sodium bisulfite, dried over anhydrous Na_2SO_4, and evaporated under a stream of argon. The residue was applied to a column of silicic acid (25 g, acidic form) slurried in $CHCl_3$. The column was eluted with $CHCl_3$, and fractions 9–14 (25 mL in volume) were combined to give ^{125}I-labeled **14** (751 μCi, 28%). The radiochemical and chemical purity were confirmed by TLC (SiO_2-GF) ($CHCl_3/CH_3OH$, 96:4), R_f 0.43.

Radioiodinated Iodovinyl Fatty Acids. General Procedure. The stannyl ester (2.5×10^{-3} mmol) and *N*-chlorosuccinimide (5.0×10^{-3} mmol) were suspended in 3 mL of CCl_4, and the resulting mixture was cooled to 0–5 °C (ice bath) and stirred under red lights. Sodium [^{123}I]iodide (2.5×10^{-3} mmol) in 2 mL of H_2O was added, and the resulting mixture was removed from the ice bath and stirred for 30 min. The mixture initially turned yellow from the formation of I^+. The solution rapidly became lighter until a colorless solution resulted. The mixture was poured into 50 mL of 5% sodium thiosulfate and extracted several times with Et_2O. The combined Et_2O extracts were washed several times with 5% sodium thiosulfate and H_2O and dried over anhydrous Na_2SO_4. The Et_2O was evaporated by a stream of argon and the residue applied to a SiO_2 column slurried in pet eth. Fractions (10 mL) were eluted with Et_2O/pet eth (5:95). Fractions 10–11 were combined, and the solvent was removed by a stream of argon to afford the radioiodinated product. The radiochemical and chemical purity were confirmed by TLC (SiO_2-GF) in C_6H_6 (R_f 0.56).

The radioiodinated ester was suspended in a mixture of EtOH (5 mL) and 1 N (1 mL) and hydrolyzed as described for **25**. The dried Et_2O extracts were evaporated by a stream of argon (see Table I). The free acids were analyzed by TLC (SiO_2-GF) in $CHCl_3$/MeOH (94:6) (R_f 0.36).

Acknowledgment. Research sponsored by the Office of Health and Environmental Research, U.S. Department of Energy, under Contract DE-AC05-840R21400 with Martin Marietta Energy Systems, Inc., is acknowledged. We thank E. B. Cunningham and B. A. Owen for technical assistance and L. S. Ailey for typing the manuscript.

Registry No. **3**, 90046-92-7; **4**, 110-02-1; **5**, 95978-36-2; **6**,

95978-37-3; **7**, 95978-38-4; **8**, 17696-11-6; **9**, 90677-38-6; **10**, 95978-39-5; **11**, 95978-40-8; **12**, 95978-41-9; **13**, 95978-42-0; **14**, 95978-43-1; [^{125}I]-**14**, 95978-44-2; **15**, 95978-45-3; **16**, 95978-46-4; **17**, 95978-47-5; **18**, 95978-48-6; [^{123}I]-**18**, 95978-58-8; [^{125}I]-**18**, 95978-59-9; **19**, 95998-63-3; [^{123}I]-**19**, 95978-49-7; [^{125}I]-**19**, 95978-50-0; **20**, 60451-92-5; **21**, 95978-51-1; **22**, 95978-52-2; **23**, 95978-53-3; **24**, 95978-54-4; [^{123}I]-**24**, 95978-60-2; [^{125}I]-**24**, 95978-61-3; **25**, 95978-55-5; [^{123}I]-**25**, 95978-56-6; [^{125}I]-**25**, 95978-57-7; LiC≡CH, 1111-64-4; $(CH_3)_3SiI$, 18089-64-0; CH_2N_2, 334-88-3; $(n\text{-}Bu)_3SnH$, 688-73-3.

Notes

N-Phenylpiperazine Derivatives with Hypocholesterolemic Activity

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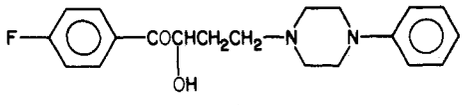
A series of new 4-(4-phenyl-1-piperazinyl)-1-(4-fluorophenyl)-2-(acyloxy)-1-butanones and 4-aryl-5-[ω-(4-aryl-1-piperazinyl)alkyl]-1,3-dioxol-2-ones were synthesized and tested preliminarily for hypolipemic activity. Plasma cholesterol-lowering activity in normal rats was found especially in several dioxolones, two of the most active compounds (**6** and **8**) being more potent than clofibrate. 4-(4-Chlorophenyl)-5-[2-(4-phenyl-1-piperazinyl)ethyl]-1,3-dioxol-2-one (**8**, LR-19,731) has been selected for clinical trials.

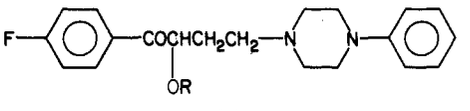
In a preceding paper we reported that α-ketols structurally related to a family of drugs widely used in the therapy of neurodisorders (haloperidol, butropipazone, fluanisone, etc.) are practically devoid of any neuroleptic activity.[1] In spite of this negative result, the research was continued in a different direction, since among the other products ketol **1** was found to possess some interesting hypocholesterolemic properties. Indeed, it was already known that the biosynthesis of cholesterol is to some extent inhibited by some butyrophenones endowed with neuroleptic activity.[2] Moreover, several *N*-phenylpiperazines have been reported to possess hypocholesterolemic activity.[3]



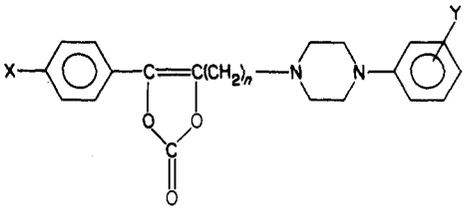
1

In this paper we report the synthesis and some pharmacological data concerning hypocholesterolemic and behavioral activities of a series of new *N*-phenylpiperazines, namely, esters **2–5** and 1,3-dioxol-2-ones **6–20** (listed in Tables I and II, respectively); in the latter, the α-ketol grouping is imbedded in the heterocyclic structure.

Chemistry. Esters **2–3** and **4–5** were obtained by acylation by ketol **1**, respectively, with the corresponding acid chloride or with phosgene in the presence of triethylamine, followed by reaction with the desired amine. The most convenient procedure for the synthesis of 1,3-dioxol-2-one derivatives **6–20** required treatment of the corresponding ketols with phosgene in the presence of triethylamine, followed by thermal cyclization of the intermediate chloroformates (method A). The use of *N*,*N*′-carbonyldiimidazole in refluxing benzene offered a useful alternative[4] (method B). Dioxolones **7** and **8** were obtained with both procedures.



2-5



6-20

Biological Results and Discussion

All the new compounds were evaluated for activities on rat plasma lipids (cholesterol) and on mouse CNS (spontaneous locomotion). Acute toxicity was tested in mice. 2-(4-Chlorophenoxy)-2-methylpropionate (clofibrate) and 4-(4-phenyl-1-piperazinyl)-1-(4-fluorophenyl)-1-butanone (butropipazone, a neuroleptic chemically related to the new compounds) were included in the biological assays as appropriate standards. The results reported in Tables I and II allow the following observations. The presence of an oxygenated function at the carbon adjacent to the carbonyl group of butropipazone caused a strong depression of the CNS activity both in esters **2–5** and in 1,3-dioxolones **6–20**. On the other hand, hypolipemic activity was shown to be present in a number of compounds, being very pronounced in the cyclic carbonates **6** and **8**. Hypolipemic activity might well be associated with the *N*-phenylpiperazine moiety; thus, in the dioxolone series, a strong depression or complete loss of the activity was found with several open chain, cycloaliphatic, as well as heterocyclic amines. However, also the α-ketol moiety may play an important role. Possibly, certain of the derivatives (**6–20**) undergo hydrolysis in vivo, releasing the α-ketol grouping, such a function being easily oxidizable to α-dicarbonyl compounds. These redox properties may interfere with enzyme systems involved in cholesterol and lipid biosynthesis.[5] Aromatic ring substitution in the *N*-phenylpiperazine

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