J = 11 Hz, 2 H, 2′-H), 3.67 (s, 3 H, CO_2CH_3), 3.80 (br s, 1 H, 5′-H); GC–MS (Me_3Si derivative) 372 (M^+). Anal. ($C_{16}H_{28}O_5$) C, H.

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Registry No. 1, 71117-51-6; 2, 67441-39-8; 3, 67441-40-1; 4, 67441-43-4; 5, 67441-56-9; 6, 67441-41-2; 7, 67441-44-5; 8, 68688-91-5; 9, 67441-45-6; 10, 67441-42-3; 11, 67441-62-7; 12, 67441-75-2; 13, 67441-57-0; 14, 67473-14-7; 3′-α-15, 67441-46-7; 3′-β-15, 67463-69-8; 17, 67473-13-6; 18, 67441-58-1; 19, 71117-50-5; 20, 95864-61-2; 21, 95864-62-3; 22, 80583-30-8; 23, 67441-47-8; 24, 67441-50-3; 25, 67441-49-0; 26, 67441-51-4; 27, 67441-71-8; 28, 67441-60-5; 29, 67441-61-6; 30, 95975-90-9; 31, 67441-52-5; 32, 67441-53-6; 33, 67441-54-7; 34, 67441-55-8; 35, 67441-69-4; 36, 67473-12-5; 37, 67441-70-7; 38, 67441-67-2; 39, 67441-59-2; 40, 67441-65-0; 41, 67441-66-1; 42, 95975-91-0; 43, 95975-92-1; 44, 95864-63-4; 45, 95864-64-5; 46, 95864-65-6; 46 (1′-methyl ester), 95864-66-7; 47, 77878-16-1.

7α-Substituted Derivatives of Androstenedione as Inhibitors of Estrogen Biosynthesis

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In an effort to obtain more information on the structure–activity relationship among the 7α-(phenylthio)androstenedione inhibitors of the enzyme aromatase, a series of compounds containing both electron-donating and electron-withdrawing ring substituents was synthesized and tested for aromatase inhibitory activity. No linear correlation between substituent electronic effects and inhibitory activity was observed. The halogen-containing compounds, particularly 8, appeared to be quite potent inhibitors. The ^{125}I analogue of 8 was synthesized in order to evaluate the possibility of side-chain elimination under the assay conditions. Approximately 90% of [^{125}I]-8 remained intact for up to 1 h under assay conditions.

Agents that control estrogen biosynthesis may be of therapeutic usefulness. Estrogens have been implicated in the development or maintenance of endometrial and mammary carcinoma.[2] Moreover, correlations have been made between estrogen receptor levels and the responsiveness of breast tumors to hormone therapy.[3] Particularly important may be the effect of peripheral extraglandular estrogen production on metastatic carcinoma in post menopausal women.[4]

Because of this association of estrogens with various disease states, interest has focused on the synthesis of agents that will competitively inhibit the enzyme aromatase. This enzyme is responsible for the conversion of androstenedione into estrone, the last step in estrogen biosynthesis. In particular, Brodie's group[5] has demonstrated the effectiveness of a number of analogues of androstenedione as inhibitors of aromatase. For example, 4-hydroxyandrostene-3,17-dione and its acetylated derivative 4-acetoxyandrostene-3,17-dione were shown to be competitive inhibitors of placental aromatase and also caused the regression of 7,12-dimethylbenzanthracene-induced mammary tumors in rats.[6,7]

Complementing these investigations has been the search for irreversible inhibitors of aromatase. Covey et al.[8] have developed compounds that may act as mechanism-based irreversible inhibitors of aromatase. Other potential mechanism-based irreversible inhibitors have been synthesized by Marcotte and Robinson[9] and by Metcalf et al.[10] Moreover, it has recently been found that some of the competitive inhibitors such as 4-hydroxyandrostenedione have an irreversible component associated with their inhibition of aromatase.[7,11,12]

Previous work from our laboratory has demonstrated the effectiveness of various 7α-phenylthio derivatives of androstenedione as inhibitors of aromatase. In particular 7α-[(4-aminophenyl)thio]androst-4-ene-3,17-dione (1) was found to be one of the most potent in vitro inhibitors of aromatase reported to date with a K_i of 18 nM.[13,14] Replacing the *p*-amino substituent with *p*-methoxy or hy-

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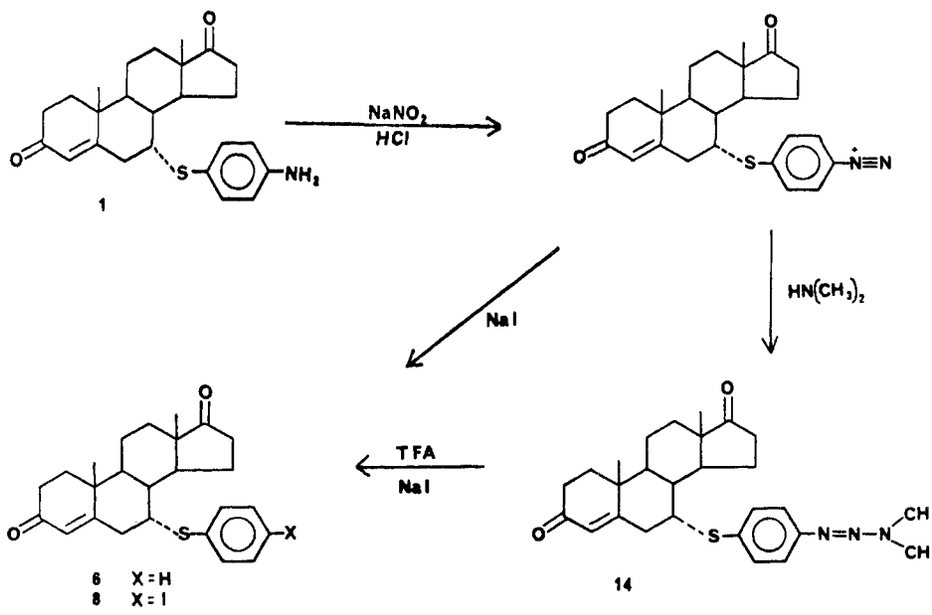
Table I. Physical Properties and Inhibitory Activity of 7α-Substituted Derivatives of Androstenedione

no.	R	formula[a]	method	recryst solvent	mp, °C	yield, %	σ value[b]	% inhibn of aromatase[c] [I] = 0.25 μM	% inhibn of aromatase[c] [I] = 1.25 μM
1	*p*-PhNH$_2$	$C_{25}H_{31}NO_2S$	A	$MeCl_2$/hexane	254–256	91	−0.66	51.63	84.96
2	*p*-PhNEt$_2$	$C_{29}H_{39}NO_2S$	A	acetone/hexane	179–181	43		11.67	53.24
3	*p*-PhOMe	$C_{26}H_{32}O_3S$	A	acetone/hexane	261–263	33	−0.268	7.93	36.66
4	*p*-PhMe	$C_{26}H_{32}O_2S$	B	acetone	246–250	34	−0.170	7.83	24.86
5	*m*-PhNH$_2$	$C_{25}H_{31}NO_2S$	A	acetone/hexane	227 dec	79	−0.16	39.88	73.26
6	Ph	$C_{25}H_{30}O_2S$	A	acetone/hexane	233–235	84	0	62.73	66.82
7	*p*-PhF	$C_{25}H_{29}FO_2S$	B	acetone	202–205	39	0.062	55.29	74.12
8	*p*-PhI	$C_{25}H_{29}IO_2S$		acetone	208–210	25	0.18	45.00	83.67
9	*p*-PhCl	$C_{25}H_{29}BrO_2S$	B	acetone	209–213	29	0.227	56.48	83.37
10	*p*-PhBr	$C_{25}H_{29}ClO_2S$	A	acetone	205–208	30	0.232	47.34	80.83
11	*p*-PhC(O)CH$_3$	$C_{27}H_{32}O_3S$	B	acetone	225–227	72	0.502	55.71	84.40
12	cyclohexyl	$C_{25}H_{36}O_2S$	B	acetone	226–229	34		18.53	32.16
13	benzyl	$C_{26}H_{32}O_2S$	A	acetone	206–208	98		33.46	69.81

[a] Anal. C, H. [b] A. J. Gordon and R. A. Ford, "A Handbook of Practical Data, Technique and References", Wiley, New York, 1972, p 145. [c] SEM < ±10%.

Scheme I



drogen caused a progressive decrease in inhibitory activity. This suggested that the inhibitory activity may have some relationship to the electron-donating capacity of the substituent.

In an effort to obtain more information on the structure–activity relationships among the 7α-(phenylthio)-androstenedione inhibitors, we have examined the various effects of electron-donating and electron-withdrawing ring substituents upon inhibitory potency. We have also looked at the effect of altering the position of ring substitution for compound 1. Of particular interest were the halogenated compounds which not only confer an electron-withdrawing effect but can be readily converted to radiolabeled probes (e.g., ^{125}I). The latter consideration was important since it is possible that these inhibitors can undergo retro-Michael elimination to form androsta-4,6-diene-3,17-dione,[15] a known competitive substrate for aromatase.[16] For this reason the *p*-^{125}I analogue was synthesized in order to ascertain the stability of these thio adducts under assay conditions.

Chemistry. All the compounds in Table I except 7α-[(4-iodophenyl)thio]androst-4-ene-3,17-dione (8) were prepared by the method of Brueggemeier et al.[13] (method A) or a slight modification thereof (method B). The corresponding thiophenols were obtained from commercial sources except *p*-acetylthiophenol, which was prepared from *p*-hydroxyacetophenone by the method of Newman and Karnes.[17]

Compound 8 was synthesized in three ways from diazotized 1 (Scheme I). Treatment with sodium iodide in aqueous solution[18] afforded a mixture of the desired iodo derivative 8 and the unsubstituted byproduct 6 in a ratio of 4:1. These products were readily separated by HPLC. A similar mixture was also obtained when the diazonium salt was treated with molecular iodine in benzene.[19] Although this latter procedure gave a better ratio of iodinated

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to unsubstituted products, this procedure was not pursued further because it was not as readily adaptable to radioiodination. The third approach involved conversion of the diazonium intermediate to the *p*-(dimethylamino)triazine derivative **14**. Treatment of **14** with trifluoroacetic acid in the presence of sodium iodide afforded compound **8** in a high yield and free from contamination by compound **6**.

The synthesis of the *m*-amino analogue **5** was uncomplicated. On the other hand, all attempts to synthesize the *o*-amino and *p*-nitro analogues proved unsuccessful. Interestingly, Campbell et al.[15,20] were able to achieve base-catalyzed conjugate addition of *o*-iodothiophenol to steroidal 4,6-dien-3-one and 1-en-3-one systems, but they were unable to add *o*-nitrothiophenol under similar conditions. They concluded that electron-withdrawing groups prevented this addition.

We attempted to add *p*-nitrothiophenol to androsta-4,6-diene-3,17-dione under a variety of base-catalyzed conditions. The presence of the 7α-thio adduct was detectable by NMR, but the product proved to be too unstable to isolate for chemical analysis and biological testing.

For the synthesis of radiolabeled inhibitors, several approaches were considered such as: (a) the use of ^{14}C or ^{3}H steroid precursors, (b) the use of sulfur-35 thiol derivatives, and (c) the introduction of the radiolabel following formation of the adduct. Since [^{14}C]- or [^{3}H]-androstadienedione is commercially unavailable and appropriate precursors are expensive, the latter two approaches were investigated.

Initial efforts focused on the synthesis of a sulfur-35-labeled adduct, but the specific activity of the final product was insufficient for use in the aromatase assay (unpublished results).

Thus, in order to obtain an adduct with the appropriate specific activity, it became necessary to employ a procedure that would introduce the radiolabel subsequent to adduct formation. Accordingly, iodine-125 was introduced by treatment of the diazonium salt of **1** or the triazine **14** with sodium iodide-125 under the appropriate conditions. Both procedures afforded radiolabeled **8** with a specific activity suitable for assay purposes.

Biochemistry. The compounds shown in Table I were assayed for inhibitory activity against aromatase by means of a tritiated water release assay[21] similar to one described by Reed and Ohno.[22] Lyophilized human placental microsomes were the source of aromatase enzyme. The substrate was [1-^{3}H]androstenedione with 83% of the tritium label in the β position. The substrate concentration was 0.25 μM, which is approximately 5 times the K_m value for the enzyme preparation. Inhibitors were assayed at concentrations of 0.25 and 1.25 μM and the results are shown in Table I.

The stability of the inhibitors was evaluated under assay conditions with the aid of the radiolabeled inhibitors. In these assays, however, no substrate or unlabeled inhibitor was added. Otherwise, the assay conditions were identical with those for the inhibition studies (i.e., the same concentration of buffer, cofactors, and enzyme). The assay was run for 15 min (inhibitor conditions) and also for 1 h. Each assay was terminated by the addition of ethyl acetate. The aqueous layers were extracted with organic solvent and the extracts chromatographed on silica gel plates. Because the stability of the sulfide linkage was the major concern, TLC conditions were designed so as to detect the presence of free thiols.

Table II. Extracted Activity Separated by TLC

	TLC 1	TLC 2	TLC 3	av % of total
	time = 15 min			
origin	21648[a]	29320	26177	8.02 ± 0.74[b]
7-[(4-iodophenyl)thio]-androstenedione	303556	287547	269942	89.25 ± 1.25
4-iodothiophenol	5518	10530	9496	2.66 ± 0.50
front	184	233	281	0.07 ± 0.01
total	330906	327360	305896	
	time = 1 h			
origin	20952	36441	21908	8.05 ± 1.56
7-[(4-iodophenyl)thio]-androstenedione	315453	280047	288325	89.49 ± 2.00
4-iodothiophenol	7637	10872	5239	2.40 ± 0.48
front	269	184	148	0.06 ± 0.01
total	344311	327544	315620	

[a] Reported in dpm, uncorrected for decay. [b] SEM.

Discussion

Our previous work dealing with 7α-(phenylthio)-androstenedione derivatives suggested that their potency as inhibitors of aromatase generally increased with an increase in electron-releasing capacity of para substituents.[13] It was therefore anticipated that substitution with electron-withdrawing groups on the phenyl ring might lead to inhibitors with diminished inhibitory action. The halogen-containing compounds **7–10**, however, proved to be quite potent inhibitors. Moreover, the more strongly electron-withdrawing acetyl group also gave rise to an effective inhibitor (**11**). In addition, shifting an amino group to the meta position (**5**) only slightly diminished the inhibitory activity compared to that of **1**. Consequently, there is no apparent linear correlation between the electronic character of the substituent as represented by the Hammett σ values and inhibitory activity within this enlarged series of compounds.

These results are of particular interest in light of the studies that have been conducted with aminoglutethimide and related arylamine compounds. In this series the arylamine group appears to be important for inhibitory activity and it has been proposed on the basis of spectral studies that the amino moiety coordinates with the P-450 heme iron of the enzyme.[23] A similar interaction might explain the high potency of compound **1**. However, we have found that an amino moiety is not essential for high inhibitory activity in the androstenedione derivatives described in this paper. Work is continuing to compare the aromatase P-450 binding spectra of the *p*-amino analogue **1** with those of the other arylamine inhibitors and with those of the other potent inhibitors in this series.

The high inhibitory activity displayed by the iodine-containing analogue meant that radioiodine could be used as an appropriate tag for ascertaining the in vitro and in vivo stability of this compound. Studies with 7α-[(4-[^{125}I]iodophenyl)thio]androst-4-ene-3,17-dione ([^{125}I]-**8**) indicated that this radiolabeled inhibitor remained essentially intact when incubated for up to 1 h under assay conditions. Approximately 90% of the recovered activity analyzed by TLC coincided with the starting 7α-[(4-[^{125}I]iodophenyl)thio]androst-4-ene-3,17-dione. Less than

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3% appeared as 4-iodothiophenol, while approximately 8% was associated with material that did not migrate in our TLC system (see Table II). This may represent a more polar metabolite or material bound to protein. Unless these unidentified materials are unusually potent inhibitors of aromatase, it seems reasonable to conclude that aromatase inhibition resides with the intact molecule 8, and that such is probably the case for the other inhibitors in this series as well.

Experimental Section

Synthetic Methods. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Proton magnetic resonance spectra were obtained in $CDCl_3$ on either a Varian EM-360A spectrometer or a Bruker WM-360 spectrometer and are reported in parts per million (δ) downfield from Me_4Si. Infrared spectra were recorded in $CHCl_3$ solution on a Perkin-Elmer 281 spectrophotometer. Elemental analyses were performed by Midwest Microlab, Ltd., Indianapolis, IN. Analytical HPLC was performed on a Whatman ODS-3 reverse-phase column, and preparative HPLC was done on a Whatman ODS-3 Magnum 20 reverse-phase column. Thin-layer chromatography was done with polyethylene-backed silica gel plates obtained from Eastman Kodak, Rochester, NY. Preparative thin-layer chromatography plates were obtained from Merck. Steroids were obtained from Searle Laboratories, Skokie, IL. Other reagents were purchased from Aldrich Chemical Co., Milwaukee, WI. Radiochemicals were purchased from New England Nuclear, Boston, MA.

General Procedure for 7α-(Phenylthio)androstenedione Derivatives. Method A. The preparation of 7α-[(3-aminophenyl)thio]androst-4-ene-3,17-dione (**5**) is illustrative. A solution of androsta-4,6-diene-3,17-dione (1 g, 3.5 mmol) in 25 mL of dry dioxane was treated with 3-aminothiophenol (1.41 g, 11.2 mmol) and a catalytic amount of sodium metal (34 mg, 1.5 mmol). A yellow suspension formed as the solution was stirred under nitrogen for 5 days at 10 °C. The reaction mixture was poured into ice water and filtered to yield 1.14 g (79%) of a yellowish-white precipitate. This precipitate was recrystallized from acetone-hexane to yield 591 mg of product. This compound was passed through a silica gel filtration column and recrystallized several times from acetone–hexane to afford 170 mg of analytically pure material: mp 227 °C dec with complete melting at 237 °C; NMR ($CDCl_3$) δ 0.87 (s, 3 H, C-18 CH_3), 1.18 (s, 3 H, C-19 CH_3), 5.70 (s, 1 H, C-4 vinyl), 6.57–7.27 (m, 4 H, aromatic); IR ($CHCl_3$) 3400, 3005, 2950, 1740, 1667 cm^{-1}. Anal. ($C_{25}H_{31}NO_2S$) C, H, N, S.

Method B. Androst-4,6-diene-3,17-dione (2.0 g, 7.0 mmol) in 15 mL of the appropriate thiophenol was treated with a catalytic amount of sodium metal (68 mg, 3.0 mmol) and the reaction stirred under nitrogen at 60 °C for 2 days. Ether was added to the reaction and the mixture was extracted with a saturated solution of ammonium chloride. The aqueous layer was back-extracted with two portions of ether. The ether layers were combined and evaporated to dryness in vacuo. The residue was taken up in benzene, dried over anhydrous sodium sulfate, and applied to 50 g of silica gel. Flash chromatography with benzene removed the excess thiol. Flash chromatography using ethyl acetate eluted the crude product, which could be recrystallized from an appropriate solvent.

7α-[(4-Iodophenyl)thio]androst-4-ene-3,17-dione (8) from 1. 7α-[(4-Aminophenyl)thio]androst-4-ene-3,17-dione (**1**; 500 mg, 1.22 mmol) was dissolved in a mixture of acetone (50 mL) and 2 N hydrochloric acid (15 mL), cooled to 0 °C in an ice bath, and treated dropwise with a solution of sodium nitrite (176 mg, 2.55 mmol) in 4 mL of water. The solution was stirred for 30 min, at which time no starting material remained as indicated by TLC. The reaction mixture was treated with a solution of sodium iodide (2.7 g, 18.5 mmol) in 25 mL of water and immediately turned dark brown presumably from I_2 formation. The mixture was overlaid with 250 mL of ether and stirred for an additional hour. The ether layer was separated and washed twice with a saturated solution of sodium thiosulfate. The ether was evaporated in vacuo and the residue taken up in chloroform. The chloroform solution was dried over sodium sulfate, filtered, and evaporated to dryness in vacuo to yield 574 mg (90%) of crude product. The crude product was recrystallized from hexane–acetone to yield grayish-white crystals. Analytical HPLC on an ODS-3 reverse-phase column (methanol–water, 90:10) showed two distinct components in a ratio of approximately 3:1. The minor constituent coeluted with 7α-(phenylthio)androst-4-ene-3,17-dione. The two components were separated by preparative reverse-phase HPLC to yield 55 mg of the noniodinated material and 156 mg (25%) of the desired product. Analytical material was obtained by recrystallization from acetone: mp 208–210 °C; NMR ($CDCl_3$) δ 0.92 (s, 3 H, C-18 CH_3), 1.23 (s, 3 H, C-19 CH_3), 3.60 (m, 1 H, C-7 CH), 5.67 (s, 1 H, C-4 CH), 7.11 (d, J = 8 Hz, 2 H, CH ortho to sulfur), 7.64 (d, J = 8 Hz, 2 H, CH ortho to iodide); IR ($CHCl_3$) 2950, 1740, 1665, 1473, 1003, 905 cm^{-1}. Anal. ($C_{25}H_{29}IO_2S$) C, H, I. S.

7α-[[4-(3,3-Dimethylazido)phenyl]thio]androst-4-ene-3,17-dione (14). A solution of 7α-[(4-aminophenyl)thio]-androst-4-ene-3,17-dione (1.64 g, 4.0 mmol) in 150 mL of acetone and 10 mL of 1.0 N HCl was cooled to 0 °C and treated with a solution of $NaNO_2$ (276 mg, 4.0 mmol) in 4 mL of water and stirred at 0 °C for 15 min. The reaction mixture was then treated with dimethylamine (5 mL of a 40% aqueous solution, excess) dropwise over 10 min and then poured into a mixture of 150 mL of dichloromethane and 150 mL of water. The layers were separated, and the organic layer was washed with water (3 × 200 mL) and brine (1 × 50 mL) and dried (Na_2SO_4). Rotary evaporation of the solvent afforded 1.79 g (96%) of crude product. Recrystallization from methanol afforded pure product: mp 209–210 °C; NMR ($CDCl_3$) δ 0.93 (s, 3 H, 18-CH_3), 1.24 (s, 3 H, 19-CH_3), 3.40 (s, 6 H, N (CH_3)$_2$), 3.49 (m, 2 H, 5-CH_2), 5.80 (m, 1 H, 4-H), 7.46 (m, 4 H, aromatic); IR (KBr) 1744, 2915 cm^{-1}. Anal. C, H, N, S.

7α-[(4-Iodophenyl)thio]androst-4-ene-3,17-dione (8) from 14. A solution of NaI (150 mg, 1.0 mmol) and trifluoroacetic acid (85 μL, 1.1 mmol) in 1.0 mL of distilled formic acid was treated with a solution of 7α-[[4-(3,3-dimethylazido)phenyl]thio]-androst-4-ene-3,17-dione (233 mg, 0.5 mmol) in 10 mL of benzene. The reaction mixture was stirred vigorously at room temperature for 30 min and then diluted to 20 mL with benzene. The benzene layer was removed, and the formic acid was washed with 20 mL of fresh benzene. The combined benzene solutions were allowed to stand over anhydrous K_2CO_3 for 30 min and then rotary evaporated to afford 247 mg (95%) of crude product as a pale yellow solid. Column chromatography (35 g, silica gel, 1:1 ethyl acetate–hexane as eluent) of this material afforded pure product as an off-white solid: mp 206–206.5 °C; NMR and IR spectra as above.

7α-[(4-[^{125}I]Iodophenyl)thio]androst-4-ene-3,17-dione ([^{125}I]-8). A. From Diazonium Salt of 8. A solution of sodium nitrite (17.6 mg, 0.25 mmol) in 0.4 mL of water was added dropwise to a solution of 7α-[(4-aminophenyl)thio]androst-4-ene-3,17-dione (50 mg, 0.12 mmol) in 5 mL of acetone and 1.5 mL of 2 N HCl at 0 °C. The solution was stirred for 30 min and then was treated with sodium iodide-125 (5 mCi) and 0.3 mL of 1×10^{-7} M sodium thiosulfate solution. The reaction mixture was stirred for an additional 30 min and then overlaid with 25 mL of absolute ether. Stirring was continued for 1 h, the ether layer removed, and the aqueous layer washed twice with ether. The ether extracts were combined and evaporated to dryness. The residue was dissolved in 1 mL of benzene and chromatographed on silica gel with hexane–ethyl acetate (5:2). The resulting product showed only one radiochemical component by TLC, which corresponded to authentic 7α-[(4-iodophenyl)thio]androst-4-ene-3,17-dione (**8**). Activity of the material was 864 μCi (17.3% incorporation) and the specific activity was at least 52 μCi/mg.

B. From Triazene 14. A solution of sodium iodide (75 mg, 0.5 mmol) and trifluoroacetic acid (85 μL, 1.1 mmol) in 1.0 mL of freshly distilled formic acid was prepared. A mixture of 10 μL of this solution and sodium iodide-125 (5 mCi in 10 μL of 0.1 N NaOH) was treated with a solution of **14** (2.3 mg, 5.0 μmol) in 0.10 mL of benzene, and the reaction mixture was vortexed at room temperature for 30 min. The mixture was diluted with 2 mL of benzene, and the layers were separated. The benzene solution was washed with 2 mL of saturated aqueous $NaHSO_3$, dried (Na_2SO_4), and purified by two successive preparative thin-layer chromatographies (Merck 500-μm silica gel, 20 cm × 7 cm, 3:2 ethyl acetate–hexane as eluent) to afford the radiolabeled

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.

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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_6H_{11}, 1569-69-3; $SHCH_2Ph$, 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_3SnH 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

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