**Bioassay.** A Langendorff guinea pig heart preparation paced at 260 beats/min via the left atrium served for assays of $A_1AR$ and $A_2AR$ agonist activity. The perfusion buffer consisted of (mM) NaCl (120), $NaHCO_3$ (27), KCl (3.7), $KH_2PO$ (1.3), $MgSO_4$ (0.64), $CaCl_2$ (1.3), pyruvate (2), and glucose (5). The buffer was saturated with 95% $O_2$-5% $CO_2$, equilibrated at 37 °C in a heat exchanger and delivered at a pressure equivalent to 55 mmHg. Continuous drainage of the left ventricle by means of a catheter inserted across the mitral valve insured that this cardiac chamber did no external work. An electrode in the right ventricle monitored the electrocardiogram. Time collections of cardiac effluent in a graduated cylinder during the steady-state phase of the flow responses to analogue administration measured total coronary flow, which was also monitored by an in-line electromagnetic flowmeter in the aortic perfusion cannula. The rate of the nucleoside infusion was increased stepwise until the appearance of second degree heart block. The quotient of the ratio of nucleoside influsion (mol/min) divided by coronary flow rate (L/min) equals agonist concentration in the perfusate. The $EC_{50}$ of prolongation of the stimulus-QRS interval, the concentration of agonist needed to prolong the interval by 50% of the maximum response,[8] reflects activity at the $A_1AR$. Logit transformation of the coronary flow data and solution of the regression of logit (coronary flow) on log [analogue] for logit = 0 yielded an estimate of $EC_{50}$ of coronary vasodilation, an index of $A_2AR$ activity. Table II reports the mean ± SEM of the -log $EC_{50}$ values from assays in four or more hearts. The quotient of the $EC_{50}$ of stimulus-QRS prolongation divided by the $EC_{50}$ of coronary vasodilation provided an index of selectivity. Values of the index <1 indicate selectivity for the $A_1AR$ and values >1 selectivity for the $A_2AR$. Table II reports the mean ± SEM of the $A_1/A_2$ activity ratios of individual experiments.

**Registry No.** 1 (R' = $Me_2$), 24639-06-3; 1 (R' = OEt), 56720-43-5; **2**, 50257-82-4; **3**, 131865-78-6; **4**, 131865-79-7; **5**, 131865-80-0; **6**, 131865-81-1; **7**, 131865-82-2; **8**, 131865-83-3; **9**, 131865-84-4; **10**, 131865-85-5; **11**, 131865-86-6; **12**, 131865-87-7; **13**, 131865-88-8; **14**, 131865-89-9; **15**, 131865-90-2; **16**, 131865-91-3; **17**, 131865-92-4; **18**, 131865-93-5; **19**, 131865-94-6; **20**, 131865-95-7; **21**, 131865-96-8; **22**, 131865-97-9; **23**, 131865-98-0; **24**, 131865-99-1; **25**, 131866-00-7; **26**, 131866-01-8.

# Inhibition of Human Placental Aromatase by Novel Homologated 19-Oxiranyl and 19-Thiiranyl Steroids

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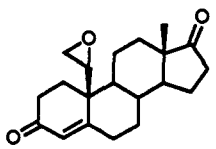
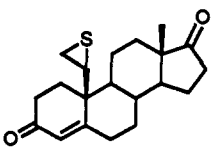
Novel homologated 19-oxiranyl- and 9-thiiranylandrost-4-ene-3,17-diones (**8a,b** and **9a,b**, respectively) have been synthesized. The configuration and conformation of compound **8a** have been established by X-ray crystallographic analysis. All four compounds have been shown to be competitive inhibitors of human placental aromatase. The thiiranes were more potent inhibitors than the corresponding oxiranes, and the 2'*S* isomers (**8b** and **9b**) were better inhibitors than the 2'*R* (**8a** and **9a**) diastereomers in each series. Spectroscopic studies with purified human placental aromatase suggest that the oxiranyl oxygen and thiiranyl sulfur of 2'*S* compounds **8b** and **9b** coordinate to the enzyme's heme iron.

Aromatase is a cytochrome P-450 enzyme complex which is responsible for the important transformation of androgens (1) to estrogens (2) (Scheme I). Inhibitors of aromatase may be valuable as therapeutic agents in the treatment of estrogen-sensitive breast tumors and as possible antifertility agents, and a number of competitive inhibitors has been reported in recent years.[1,2]

Previous work from our laboratories showed that 10β-oxiranyl- and 10β-thiiranylestr-4-ene-3,17-diones (**3** and **4**, respectively) were potent competitive inhibitors of human placental aromatase.[3-5] Furthermore, stereoselectivity was observed, with the 19*R* diastereomers (**3a**, **4a**)



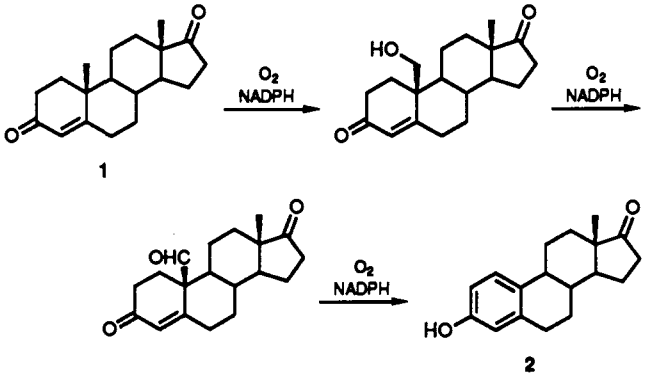
**3a** (19*R*, $K_i$ = 7 nM)
**3b** (19*S*, $K_i$ = 75 nM)



**4a** (19*R*, $K_i$ = 1 nM)
**4b** (19*S*, $K_i$ = 75 nM)

being 10-75 times more potent inhibitors than the 19*S* isomers (**3b**, **4b**). Spectroscopic studies with purified enzyme demonstrated that in the case of the 19*R* isomers, the oxiranyl and thiiranyl heteroatom coordinated with the enzyme's heme iron. This coordination, combined with the inherent binding selectivity of the steroid nucleus, endowed these inhibitors with high specificity.

**Scheme I**




X-ray crystallographic analysis of the above 10β-oxiranes showed[3] that the oxirane heteroatom in the more potent

[†] The Johns Hopkins University School of Medicine.
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[§] University of California.

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(2) Brodie, A. M. H. *Biochem. Pharmacol.* **1985**, *34*, 3213.
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(5) Kellis, J. T.; Childers, W. E.; Robinson, C. H.; Vickery, L. E. *J. Biol. Chem.* **1987**, *262*, 4421.

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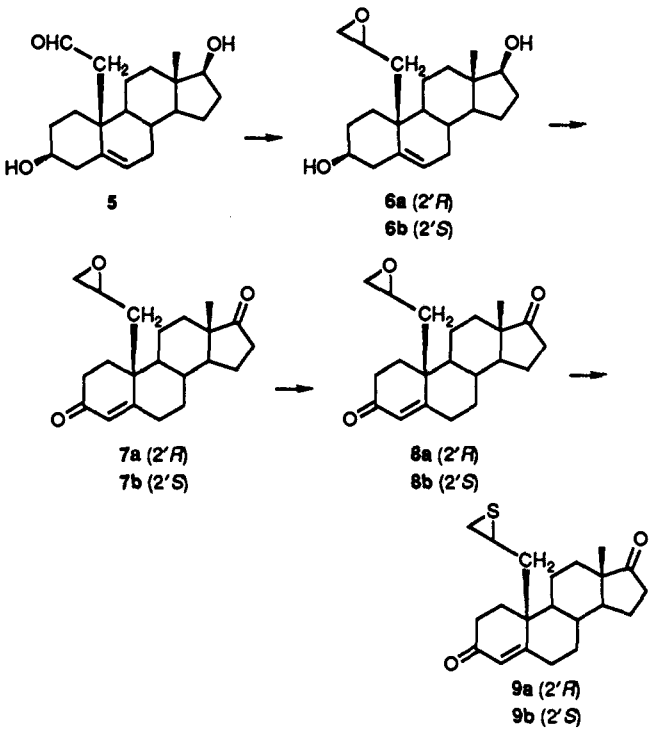
**Scheme II**




19*R* diastereomer (**3a**) is directed toward the C1 and C2 atoms in the A ring. In contrast, the oxirane oxygen in the 19*S* isomer (**3b**) is positioned over the B ring. If these crystal-structure conformations resemble the oxirane conformations when the steroids are bound at the active site, then the heme iron should be located near C1, C2, and C19. This is consistent with the conclusions of Osawa et al.[6] derived from metabolic switching studies with [19-$^3H_3$]-androstenedione and testosterone. Their results led them to postulate placement of the heme iron–oxygen equidistant from C1, C2, and C19.

These results prompted us to prepare homologated analogues of these oxiranes and thiiranes as probes of the active site around the heme moiety. Specifically, we have synthesized the homologues **8a,b** and **9a,b** (Scheme II) and have studied their enzyme-inhibitory properties as well as their spectroscopic interactions with the enzyme. It also occurred to us that oxidation by aromatase of the 19-$CH_2$ group of **8a** and **8b** to a 19-oxo group might occur, generating electrophilic $\alpha,\beta$-epoxy ketones, which might inactivate the enzyme.

## Chemistry

The methodology used to synthesize compounds **8** and **9** was similar to that used[7] for preparing the previously described 10$\beta$-oxiranyl- and -thiiranylestr-4-ene-3,17-diones. The known[8] 19-formylandrost-5-ene-3$\beta$,17$\beta$-diol (**5**) served as starting material. Reaction of **5** with either dimethylsulfonium methylide or dimethylsulfoxonium methylide gave a readily separable mixture of diastereomeric oxiranes **6a** and **6b**. Both reagents gave, in ca. 75% yield, a mixture consisting of oxiranes **6a** and **6b** in the ratio 3:1. These findings are in contrast to the results obtained[7] in the 10$\beta$-formyl system in which the two sulfur ylide reagents gave different ratios of diastereomeric epoxides. These reagents are sensitive to the steric environment of the carbonyl group, and the above results are consistent with a relatively unhindered aldehyde group in **5**.

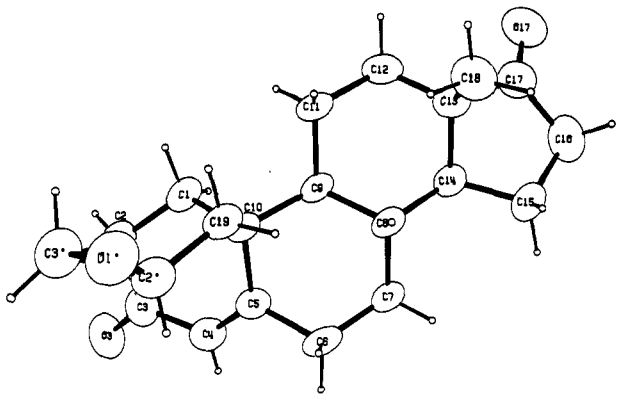


**Figure 1.** ORTEP[24] drawing showing the crystal conformation.

Epoxy diols **6a** and **6b** were separated by high-pressure liquid chromatography (HPLC) and converted separately to the desired 4-ene-3,17-diones **8a** and **8b**, respectively, by Collins oxidation followed by isomerization of the resulting 5-en-3-ones (**7a** and **7b**) with diazabicyclo[4.3.0]-nonane (DBN). X-ray crystallographic analysis of **8a** established the configuration at the 2'-epoxide carbon as (*R*) (vide infra) and consequently the diastereomer **8b** must possess the 2'*S* configuration.

It now remained to convert the oxiranes **8a,b** to the corresponding thiiranes. The method of Chan and Finkenbine[9] employing triphenylphosphine sulfide (TPS) and trifluoroacetic acid (TFA) has been established to proceed with stereochemical inversion at the epoxide carbon. This method stereospecifically converted **8a** to thiirane **9b** and, similarly, **8b** gave **9a**, although in modest yield. We had previously shown[7] that substituting picric acid for TFA in the TPS reaction gave improved yields of thiiranes without affecting the stereochemistry of the process, and this modification again proved effective in the case of **8a** and **8b**. Treatment of oxirane **8a** with TPS/picric acid gave thiirane **9b** while **8b** gave **9a**, in 76% and 79% yield, respectively. The 2'-configurations of **9a** (*R*) and **9b** (*S*) follow from the established stereochemistry of the oxirane precursors and the known stereochemical course of the thiirane-forming reaction.

## X-ray Crystallographic Analysis of (2'*R*)-Epoxide 8a

The crystal conformation of only one molecule of **8a** is depicted in Figure 1 since the other would appear almost identical. Molecular dimensions for both and a short list of parameters for the non-hydrogen atoms as well as full refinement parameters including anisotropic thermal parameters and hydrogen parameters are available as supplemental material. The absolute configurations at C10 and C13 (both *S*) are correct for a steroid and their chiral angles (torsion angles for vectors connecting the substituents ordered in chemical priority, $\chi$, according to Ito et al.[10]) are −68.4 (2)°, −68.6 (2)° and −66.6 (2)°, and −67.1 (2)° at the two centers in the two molecules. The corresponding $\chi$ angles, referring to the ordered substituents O1', C3', C19, and H2', are +77 (1)° and +79 (1)°, respectively, and the absolute configuration at C2' in both

(6) Osawa, Y.; Yoshida, N.; Fronckowiak, M.; Kitawaki, J. *Steroids* **1987**, *50*, 11.
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(8) Marcotte, P.; Robinson, C. H. *Steroids* **1982**, *39*, 325.
(9) Chan, T. H.; Finkenbine, J. R. *J. Am. Chem. Soc.* **1972**, *94*, 2880.
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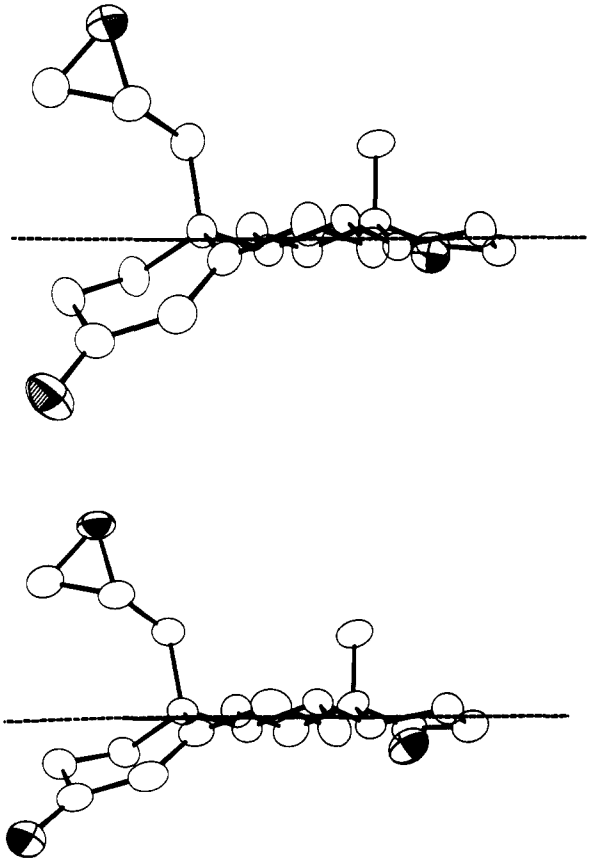

**Figure 2.** ORTEP[24] drawings of the two independent molecules projected at right angles to the least-square planes of rings A, B, and C.

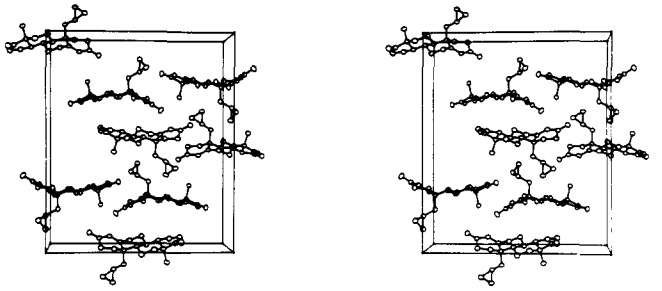

**Figure 3.** Packing diagram. The direction of projection is *a*.

molecules is thus *R* since $\chi$ is positive.

The steroid skeleton of **8a** resembles that of the related 4-androstene-3,17-dione as reported by Busetta et al.,[11] which Duax et al.[12] showed was a flexible molecule. In the present structure, flexibility is indicated by the different Altona, Geise, and Romers $\Delta$ parameters[13] for the D rings of the two independent molecules which are 34.2° and 15.1°, respectively. The D ring is thus a fairly good 14-envelope in one molecule but is intermediate between the envelope and the twist in the other. The other Altona, Geise, and Romers parameter, $\phi_m$ (the maximum torsion angle), is very similar in the two molecules, being 43.3° and 43.6°, respectively. Both molecules are depicted in Figure 2, projected at right angles to the least-squares planes of rings B, C, and D, a traditional view of steroids following Duax et al.[12] It can be seen that the A ring and the 17-keto

**Table I.** Inhibition Constants for 19-Oxiranyl and 19-Thiiranyl Steroids

| compd | 2′-configuration | $K_i$,[a] nM |
|---|---|---|
| **8a** | *R* | 753 ± 113 |
| **8b** | *S* | 86 ± 4 |
| **9a** | *R* | 206 ± 41 |
| **9b** | *S* | 22 ± 1 |

[a] $K_m$ for androstenedione in this system was 39 nM. Each compound was a competitive inhibitor, but none caused time-dependent loss of enzyme activity.

atom are oriented somewhat differently although the A rings have torsion angles which indicate similar, rather distorted half-chairs. Conformational differences can be attributed to packing forces which, as Figure 3 indicates, should be different for the two molecules given their different environments, and thus the fact that the orientations and positions of the oxirane rings of both molecules are very similar suggests that at this position the observed conformation may be close to a global energy minimum. It might appear from Figure 2 that there is considerable freedom of movement of the oxirane, but analysis of interatomic distances indicates that such is not the case. The distances C2′···C1 and C2′···C5 in the first molecule are 3.22 and 3.26 Å, respectively, and those in the second molecule are very similar, thus rotation about the C10–C19 bond would lessen one distance or the other, and the torsion angle C2′–C19–C10–C9 is effectively fixed, its values being 66.6 (2)° and 67.1 (2)° in the two molecules. A rotation of 180° about the C19–C2′ bond would bring O1′ close to the A ring and there would be close contacts of H2′1 and H3′2 en route. The actual torsion angles, C3′–C2′–C19–C10, are 168.2 (3)° and 166.4 (3)°, for the two molecules, respectively. It is also apparent that this fairly rigid system would not exist if an additional C atom were inserted between C2′ and C19.

It was necessary to establish the absolute configuration of one or other of oxiranes **8a** and **8b** by X-ray crystallographic analysis, which would then permit configurational assignments to the other oxirane and to the derived thiiranes **9a** and **9b**. It should be made clear that we are not attempting in this paper to relate the solid-state conformation of epoxide **8a** to the enzyme active-site arrangement.

## Biochemistry

Enzyme kinetic data were obtained with human placental microsomes, and $K_i$ values of the inhibitors were calculated by using Lineweaver–Burke analysis (Table I). All four compounds (**8a,b** and **9a,b**) were competitive inhibitors of aromatase, and the 2′*S* compounds **8b** and **9b** had lower $K_i$ values than the corresponding 2′*R* isomers **8a** and **9a**. Furthermore, (2′*S*)-thiirane **9b** was a better inhibitor than (2′*S*)-oxirane **8b**, while (2′*R*)-thiirane **9a** was superior to (2′*R*)-oxirane **8a**. The most potent compound, **9b**, which combines 2′*S* stereochemistry with a thiirane grouping, has $K_i$ = 22 nM. This apparent inhibition binding constant of 22 nM compares favorably with the $K_m$ value (40 nM) for the substrate androst-4-ene-3,17-dione. It should also be noted that the $K_i$ values for 4-hydroxyandrostenedione,[14] (19*R*)-10$\beta$-oxiranylestrenedione (**3a**), and (19*R*)-10$\beta$-thiiranylestrenedione (**4a**) are 50,[15] 7,[3] and 1 nM,[4] respectively. Thus the (2′*S*)-19-thiiranyl

(11) Busetta, B.; Comberton, G.; Courseille, C.; Hospital, M. *Cryst. Struct. Commun.* **1972**, *1*, 129.
(12) Duax, W. L.; Weeks, C. M.; Rohrer, D. C. *Topics in Stereochemistry*; Allinger, N. L.; Eliel, E. L., Eds.; Wiley: New York, 1976; Vol. 9, p 271.
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(15) (a) Brodie, A. M. H.; Garrett, W. M.; Hendrickson, J. R.; Tsai-Morris, C.; Marcotte, P. A.; Robinson, C. H. *Steroids* **1981**, *38*, 693. (b) Covey, D. F.; Hood, W. F. *Mol. Pharmacol.* **1982**, *21*, 173.

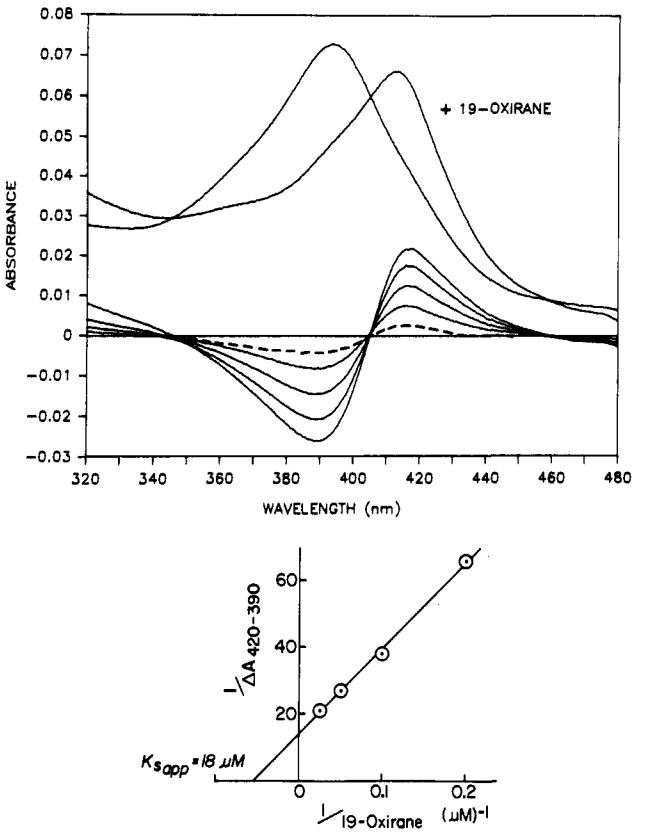


**Figure 4.** Effect of 19-oxiranes **8a** and **8b** on the spectral properties of purified P-450$_{arom}$. The sample contained 0.61 μM P-450 and 1.4 μM androstenedione; path, 1 cm. The upper panel shows absolute spectra in the absence and presence of 40 μM of (2′*S*)-19-oxirane **8b** as well as difference spectra induced by the addition of 5, 10, 20, and 40 μM of **8b** (solid curves). Also shown is a difference spectrum induced by the addition of 40 μM of (2′*R*)-19-oxirane **8a** to an identical sample (dashed curve). The lower panel shows a graphical analysis of the spectral titration with (2′*S*)-19-oxirane **8b**. The points represent the mean of duplicate determinations. The range did not fall outside the plotted symbol.

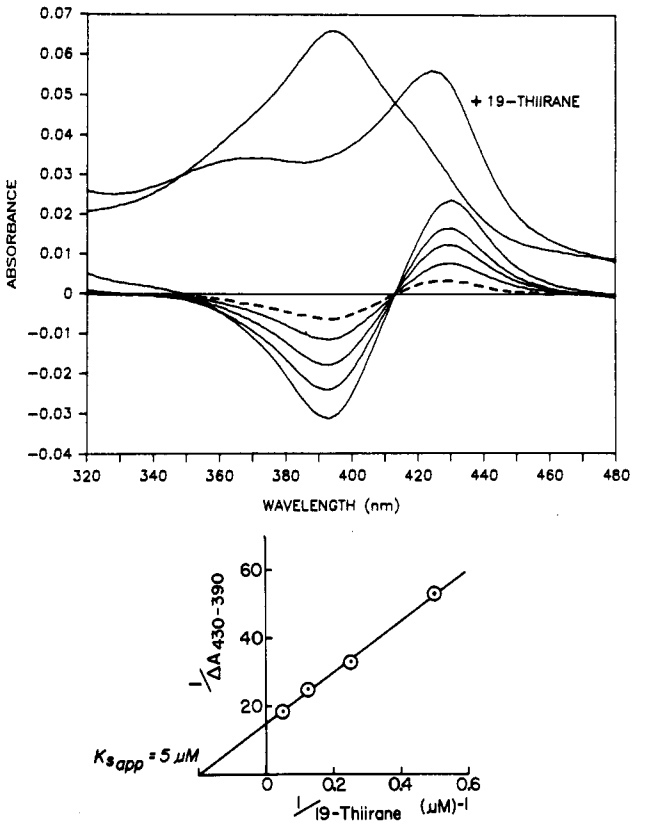


**Figure 5.** Effect of 19-thiiranes **9a** and **9b** on the spectral properties of purified P-450$_{arom}$. The sample contained 0.54 μM P-450 and 1.4 μM androstenedione; path, 1 cm. The upper panel shows absolute specta in the absence and presence of 20 μM of (2′*S*)-19-thiirane **9b** as well as difference spectra induced by the addition of 2, 4, 8, and 20 μM of **9b** (solid curves). Also shown is a difference spectrum induced by the addition of 20 μM of (2′*R*)-19-thiirane **9a** to an identical sample (dashed curve). The lower panel shows a graphical analysis of the spectral titration with (2′*S*)-19-thiirane **9b**. The points represent the mean of duplicate determinations. The range did not fall outside the plotted symbol.

steroid (**9b**) of this paper is comparable in inhibitory potency to the parent 10β-oxirane and -thiirane, as well as to 4-hydroxyandrostenedione.

None of the compounds caused time-dependent loss of enzyme activity (data not shown). Spectroscopic titrations of purified aromatase with the above inhibitors showed that binding of compounds **8b** and **9b** shifted the Soret maximum of the ferric enzyme to 413 and 424 nm, respectively (Figures 4 and 5). Addition of excess androst-4-ene-3,17-dione reversed the spectroscopic changes, producing the high-spin form of the enzyme with a Soret peak at 393 nm (data not shown). These shifts suggest that the oxygen atom of 19-oxirane **8b** and the sulfur atom of 19-thiirane **9b** are bound to the heme iron in the inhibitor complexes. The less potent 2′*R* diastereoisomers (**8a** and **9a**) also appeared to interact with aromatase in the same way, but with lower affinity.

## Discussion

We have shown (Table I) that compounds **8a,b** and **9a,b** are competitive inhibitors of aromatase. None of them caused time-dependent inactivation of the enzyme. In the case of both oxiranes and thiiranes, the 2′*S* diastereoisomer was a more powerful inhibitor than the 2′*R* isomer, while each thiirane was more potent than the corresponding oxirane.

Previous studies[5,6] indicated that the heme iron of aromatase cytochrome P-450 and C19 of androstenedione are juxtaposed in the enzyme–substrate complex. This allows the heteroatoms of 10β-oxiranyl- and 10β-thiiranylestr-4-ene-3,17-dione to occupy the sixth coordination site of the heme iron (where dioxygen normally binds during the catalytic cycle) while bound to the substrate pocket. We suspected that this might be the case with the 19-substituted (homologated) derivatives under consideration in the present study, and we therefore carried out spectroscopic studies to test this hypothesis.

The peak positions of the Soret maxima for the complexes with 19-oxirane **8b** (413 nm, Figure 4) and 19-thiirane **9b** (424 nm, Figure 5) are unusual in that they do not correspond to the substrate bound (393 nm) or substrate free (417 nm) forms of P-450$_{arom}$. This indicates a ligand-specific alteration of the coordination sphere of the P-450 heme. These spectral shifts are analogous to those produced[5] by the corresponding 10β-oxirane and 10β-thiirane derivatives and are indicative of oxygen and sulfur atom ligation to the sixth coordination position of the heme iron of P-450$_{arom}$. The less potent stereoisomer of each derivative appears to interact with P-450$_{arom}$ in the same manner, but with the expected lower affinity (Figures 4 and 5, dashed curves). Thus strong competitive inhibition of aromatase, together with heteroatom coordination to heme iron, is observed even when the oxirane and thiirane groupings are separated from C10 by a methylene group. Stereoselectivity of inhibition is also observed, as was the

case for the 10β-oxiranyl and 10β-thiiranyl systems 3 and 4. However, whereas for compounds 3 and 4 the stereochemistry at C-19 was involved, in the present cases (compounds 8 and 9) the configuration at the 2'-position of the three-carbon chain at C-10 is important. Further studies with conformationally restricted systems are planned to further explore the region between the heme system and the β-face of the steroid.

## Experimental Section

Melting points were determined on a Kofler micro hot stage and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer 710B spectrometer in $CHCl_3$ or KBr disks. Ultraviolet spectra were obtained in methanol on a Perkin-Elmer Lamba 3 instrument. Proton magnetic resonance spectra were recorded in $CDCl_3$ on IBM 80-MHz or Varian XL-400 400-MHz spectrometers, high-resolution mass spectra (HRMS) were obtained on a VG70S spectrometer, on samples purified by HPLC. The HPLC separations were carried out with a Waters Associates Model 6000 instrument with a Whatman Magnum 9 Partisil 10 (50 cm) semipreparative column (9.4 mm i.d.) or with a Dynamax Macro 60-Å silica column (30 cm, 21.4 mm i.d.). TLC was done on silica gel GF precoated plates from Machery-Nigel. Column chromatography was performed with flash silica gel (Baker) according to Still's method.[16] [1β-$^3$H]-Androst-4-ene-3,17-dione was obtained from New England Nuclear.

**Enzyme Studies.** Enzyme kinetic data were obtained with human placental microsomes prepared as described by Ryan.[17] Competitive inhibition and time-dependent inactivation were assessed with [1β-$^3$H]-androst-4-ene-3,17-dione in a tritium-release assay[18] (Thompson and Siiteri) using a variation of the procedures of Marcotte and Robinson,[19] as follows: Competitive inhibition assays were performed in 5 mM aqueous potassium phosphate buffer (pH = 7.4) containing 100 mM KCl, 0.5 mM EDTA·2Na, 5 mM dithiothreitol, and 125 μM NADPH. Inhibitors were assayed at concentrations between 0.5 and 10 μM. Approximately 4.5 μg of microsomal protein (Bradford Protein Assay) was used in each assay tube. All assays were preincubated for 2 min at 37 °C, initiated by addition of placental microsomes, and then incubated at 37 °C for 5 min. Enzyme activity was then quenched by transferring an aliquot from each assay to a centrifuge tube containing chloroform and vortexing. The resulting mixtures were then centrifuged at 500 rpm for 10 min in a clinical centrifuge to insure separation of the aqueous and organic layers. Tritium release was measured by scintillation counting of an aliquot of the aqueous layer. Inhibition constant values ($K_i$ values) were calculated by using Lineweaver–Burk analysis. Substrate and inhibitors were added to the assay from stock solutions made up by dissolving steroidal samples in 0.5 mL of 95% ethanol, adding three drops of propylene glycol, evaporating the ethanol under a stream of nitrogen, and diluting to the desired concentration with distilled water. Time-dependent inactivation assays were carried out as previously described[19] over a period of 20 min at 37 °C using concentrations of inhibitors ranging from 2 to 30 μM.

Spectroscopic studies were carried out as previously described[5] using cytochrome P-450$_{arom}$ purified from human placenta.[20]

**Chemistry. 19-Oxiranylandrost-5-ene-3β,17-diols (6a,6b) from 19-Formylandrost-5-ene-3β,17β-diol (5).** To an oven-dried 250-mL three-neck round-bottom flask was added, under argon, a 50% dispersion of sodium hydride in mineral oil (250 mg, 3.5 equiv). The dispersion was washed three times with anhydrous THF to remove mineral oil, and dry distilled DMSO (20 mL) was added. The suspension was stirred at 70–75 °C under argon for 30 min. The light green solution was then cooled to 25 °C, and anhydrous THF (20 mL) was added. The stirred mixture was then cooled to 4 °C in an ice bath, and a solution of trimethylsulfonium iodide (1.05 g) in dry DMSO (20 mL) was added over 5 min. The resulting mixture was stirred at 4 °C for 10 min more, and a solution of 19-formyl compound 5 (500 mg, 1.57 mmol) in anhydrous THF (75 mL) was added. The stirred mixture was allowed to warm to 25 °C over 1 h, and was then stirred for an additional 3 h.

The reaction mixture was partitioned between methylene chloride (300 mL) and 5% aqueous sodium bicarbonate (150 mL). The aqueous layer was reextracted with methylene chloride (50 mL), and the combined organic extracts were dried ($Na_2SO_4$) and concentrated in vacuo to about 15 mL. This solution was then diluted with methylene chloride (75 mL), washed three times with water, dried ($Na_2SO_4$), and evaporated to dryness in vacuo. The crude product was purified by HPLC (ethyl acetate–hexane, 1:1) to give the 19-oxiranyl compounds (**6a, 6b**).

The less polar isomer, (2'*R*)-19-oxiranylandrost-5-ene-3β,17β-diol (**6a**, 305 mg), had mp 159–160 °C (methylene chloride–hexane); NMR (80 MHz) δ 5.56 (m, 1, vinyl-H), 3.58 (m, 2, $C_3$-H and $C_{17}$-H), 2.85 (m, 1, epoxide CH), 2.75 (m, 1, epoxide CH), 2.45 (m, 1, epoxide CH), 0.90 (s, 3, 18-$CH_3$); HRMS calcd 332.2351 ($C_{21}H_{32}O_3$), found 332.2356.

The more polar isomer, (2'*S*)-19-oxiranylandrost-5-ene-3β,17β-diol (**6b**, 98 mg), had mp 154–155 °C (methylene chloride–hexane); NMR (80 MHz) δ 5.54 (m, 1, vinyl-H), 3.55 (m, 2, $C_3$-H and $C_{17}$-H), 2.90 (m, 1, epoxide CH), 2.82 (m, 1, epoxide CH), 2.66 (m, 1, epoxide CH), 0.76 (s, 3, 18-$CH_3$); HRMS calcd 332.2351 ($C_{21}H_{32}O_3$), found 332.2353.

**19-Oxiranylandrost-4-ene-3,17-diones (8a and 8b) from the 3β,17β-Diols (6a and 6b) via the 5-Ene-3,17-diones (7a and 7b).** (A) To a stirred solution of (2'*R*)-19-oxiranylandrost-5-ene-3β,17β-diol (**6a**, 100 mg, 0.30 mmol) in dry methylene chloride (45 mL) under nitrogen at 25 °C was added Collins' reagent (977 mg, 12 equiv) in one portion. Stirring was continued for 20 min at 25 °C. The reaction mixture was then poured through a 1.5 × 12 cm column of Florisil (15 g, 60–100 mesh) and the column was eluted with a further 100 mL of methylene chloride, followed by ethyl acetate (200 mL). The ethyl acetate fraction was evaporated in vacuo, and the resulting oil was dissolved in benzene and taken to dryness, then flash chromatographed (hexane–ethyl acetate, 1:2) on silica to give (2'*R*)-19-oxiranylandrost-5-ene-3,17-dione (**7a**, 80 mg): mp 146–147 °C (methylene chloride–hexane); IR ($CHCl_3$) 1735, 1720 cm$^{-1}$; NMR (80 MHz) δ 5.62 (m, 1, vinyl-H), 2.92 (s, 2, $C_4$-H), 2.90 (m, 1, epoxide CH), 1.07 (s, 3, 18-$CH_3$); HRMS calcd 328.2038 ($C_{21}H_{28}O_3$), found 238.2044.

A stirred solution of the above product (**7a**, 100 mg) in dry methylene chloride (15 mL) was treated with diazabicyclononane (DBN, 0.075 mL) and the solution was stirred for 18 h at 25 °C. The reaction mixture was then evaporated in vacuo and the residue was flash chromatographed on silica (hexane–ethyl acetate, 1:2), giving (2'*R*)-19-oxiranylandrost-4-ene-3,17-dione (**8a**, 83 mg): mp 122–123 °C (methylene chloride–hexane); IR ($CHCl_3$) 1740, 1670, 1620 cm$^{-1}$; $\lambda_{max}$ (MeOH) 240 nm (ε 13100); NMR (400 MHz) δ 5.96 (s, 1, vinyl-H), 2.85 (m, 1, epoxide CH), 2.81 (dd, 1, $J$ = 5.7, 5.5 Hz, epoxide CH), 2.53 (dd, 1, $J$ = 3.0, 5.5 Hz, epoxide CH), 0.90 (s, 3, 18-$CH_3$); HRMS calcd 328.2038 ($C_{21}H_{28}O_3$), found 328.2043. Anal. ($C_{21}H_{28}O_3$) C, H.

(B) Using the procedures described in A above, a solution of (2*S*')-19-oxiranylandrost-5-ene-3β,17β-diol (**6b**, 100 mg, 0.3 mmol) in dry methylene chloride (45 mL) was oxidized with Collins' reagent (977 mg, 12 equiv) for 20 min at 25 °C. The crude product was purified by flash chromatography (hexane–ethyl acetate, 1:2) on silica to give (2*S*')-19-oxiranylandrost-5-ene-3,17-dione (**7b**), 73 mg): mp 127–128 °C (methylene chloride–hexane); IR ($CHCl_3$) 1740, 1715 cm$^{-1}$; NMR (80 MHz) δ 5.57 (m, 1, vinyl-H), 2.97 (s, 2, $C_4$-H), 2.95 (m, 1, epoxide CH), 2.84 (m, 1, epoxide CH), 2.55 (m, 1, epoxide CH), 0.92 (s, 3, 18-$CH_3$); HRMS calcd 328.2038 ($C_{21}H_{28}O_3$), found 328.2043.

Compound **7b** (80 mg) was then converted by the action of DBN (0.075 mL) in methylene chloride (15 mL) at 25 °C for 18 h, as in part A above, to give (2'*S*)-19-oxiranylandrost-4-ene-3,17-dione (**8b**, 65 mg): mp 142–143 °C (methylene chloride–hexane); IR ($CHCl_3$) 1735, 1765, 1615 cm$^{-1}$; $\lambda_{max}$ (MeOH) 239 nm (ε 12100); NMR (400 MHz) δ 5.94 (s, 1, vinyl-H), 2.87 (m, 1, epoxide CH), 2.80 (dd, 1, $J$ = 4.5, 4.8 Hz, epoxide CH), 2.52 (dd, 1, $J$ = 2.6, 4.5 Hz, epoxide CH), 0.90 (s, 3, 18-$CH_3$); HRMS calcd 328.2038 ($C_{21}H_{28}O_3$), found 328.2046. Anal. ($C_{21}H_{28}O_3$) C, H.

**(2'*R*)-19-Thiiranylandrost-4-ene-3,17-dione (9a).** To a stirred solution of (2'*S*)-10β-oxiranylandrost-4-ene-3,17-dione (**8b**, 50 mg, 0.15 mmol) in dry benzene (25 mL) under nitrogen at 25

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°C was added triphenylphosphine sulfide (440 mg, 10 equiv) and recrystallized picric acid (343 mg, 10 equiv). The reaction mixture was heated under reflux for 18 h under nitrogen and then was cooled to 25 °C, diluted with ether (50 mL), and washed with saturated aqueous sodium bicarbonate (four times). The organic layer was dried ($Na_2SO_4$) and evaporated in vacuo, and the residue was filtered through a Florisil column (60–100 mesh, 15 g) using methylene chloride (75 mL) as eluant to remove triphenylphosphine sulfide. Elution with ethyl acetate (75 mL) then gave steroidal product which was purified by HPLC (ethyl acetate–hexane, 1:1) to give pure (2′*R*)-19-thiiranylandrost-4-ene-3,17-dione (**9a**, 38 mg): mp 144–145 °C (methylene chloride–hexane); IR ($CHCl_3$) 1740, 1655, 1616 $cm^{-1}$; $\lambda_{max}$ (MeOH) 241 nm ($\epsilon$ 12 900); NMR (400 MHz) $\delta$ 5.97 (s, 1, vinyl-H), 2.77 (m, 1, thiirane CH), 2.63 (dd, 1, $J$ = 6.0, 6.4 Hz, thiirane CH), 2.57 (dd, 1, $J$ = 2.1, 6.0 Hz, thiirane CH), 0.89 (s, 3, 18-$CH_3$). HRMS calcd 344.1810 ($C_{21}H_{28}O_2S$), found 344.1815. Purity was established by HPLC using two different solvent systems: HPLC system A–Whatman Magnum 9 Partisil 10 column (50 cm, 9.4 mm i.d.), eluent chloroform-ethyl acetate (9:1), flow rate 4 mL/min, 254-nm UV detection, $t_R$ = 10.2 min; HPLC system B—Dynamax Macro 60-Å silica column (30 cm, 21.4 mm i.d.), eluent hexane–ethyl acetate (1:1), flow rate 15 mL/min, 254-nm UV detection, $t_R$ = 20.3 min.

**(2′*S*)-10$\beta$-Thiiranylandrost-4-ene-3,17-dione (9b).** To a stirred solution of (2′*R*)-10$\beta$-oxiranylandrost-4-ene-3,17-dione (**8a**, 80 mg, 0.24 mmol) in dry benzene (45 mL) under nitrogen at 25 °C was added triphenylphosphine sulfide (717 mg, 10 equiv) and recrystallized picric acid (560 mg, 10 equiv). The reaction mixture was heated under reflux for 18 h under nitrogen, and then was cooled to 25 °C, diluted with ether (75 mL), and washed four times with saturated aqueous sodium bicarbonate. The organic layer was dried ($Na_2SO_4$) and evaporated in vacuo, and the residue was dissolved in methylene chloride and filtered through Florisil (60–100 mesh, 18 g), using methylene chloride (75 mL) to remove triphenylphosphine sulfide. Elution with ethyl acetate (100 mL) furnished steroidal product which was purified by HPLC (ethyl acetate–hexane, 1:1) to give the desired (2′*S*)-19-thiiranylandrost-4-ene-3,17-dione (**9a**, 63 mg): mp 126–127 °C (from methylene chloride–hexane); IR ($CHCl_3$) 1730, 1660, 1616 $cm^{-1}$; $\lambda_{max}$ (MeOH) 240 nm ($\epsilon$ 12 500); NMR (400 MHz) $\delta$ 5.97 (s, 1, vinyl-H), 2.80 (m, 1, thiirane CH), 2.78 (m, 1, thiirane CH), 2.53 (dd, 1, $J$ = 1.8, 6.6 Hz, thiirane CH), 0.89 (s, 3, 18-$CH_3$); HMRS calcd 344.1810 ($C_{21}H_{28}O_2S$), found 344.1808. Purity was established by HPLC using two different solvent systems: HPLC system A—Whatman Magnum 9 Partisil 10 column (50 cm, 9.4 mm i.d.), eluent chloroform–ethyl acetate (9:1), flow rate 4 mL/min, 254-nm UV detection, $t_R$ = 9.85 min; HPLC system B—Dynamax Macro 60-Å silica column (30 cm, 21.4 mm i.d.), eluent hexane–ethyl acetate (1:1), flow rate 15 mL/min, 254-nm UV detection, $t_R$ = 19.6 min.

**X-ray Crystallographic Analysis of (2′*R*)-Epoxide 8a.** Diffractometer and data collection method: CAD4, graphite monochromator, Cu K$\alpha$ ($\lambda$ = 1.5418 Å), $\theta/2\theta$. Maximum $\theta$: 74°. Variation of intensity standards: 3%, measured every 3 h. Crystal habit: Probably prismatic but having rounded edges and corners. Crystal size: 0.2 × 0.2 × 0.3 mm. Friedel symmetry: $2/m$ $2/m$ $2/m$, orthorhombic. Conditions limiting reflections: $hkl$: all, $h00$: $h = 2n$, $k00$: $k = 2n$, $00l$: $l = 2n$. Space group $P2_12_12_1$. Reflections used for lattice parameters: 22 ($21° < \theta < 26°$). Cell dimensions: $a$ = 7.362 (1) Å, $b$ = 20.079 (2) Å, $c$ = 23.406 (2) Å, $V$ = 1841.37 Å$^3$, $Z$ = 8 (two molecules in an asymmetric unit), $D_x$ = 1.261 g $mm^{-3}$, $\mu$ = 6.10 $cm^{-1}$, $F(000)$ = 1424, $T$ = 23 °C. Counter aperture: 1.3 mm. Scan width (deg): 1.25 + 0.35 tan $\theta$. Maximum (sin $\theta/\lambda$): 0.6432 Å$^{-1}$. Range of $h,k,l$: 0:9, 0:25, 0:29. Reflections measured: 4108 (2613 with $I$ $\delta(I)$). Intensity standards: 3 4 7, 0 11 1, 3 7 1. Phase problem solution MITHRIL.[21] Refinement: full-matrix least squares: H atoms isotropic, other atoms anisotropic. Anisotropic parameters: $\exp(-2\pi^2\sum i\sum j(U_{ij}h_ih_ja^*_ia^*_j))$. Function minimized: $\sum(w(|F_o| - |Fc|))^2$. Weights ($w$): $1/\sigma(F_o)$. $\sigma(F_o)$: Statistical as for Peterson and Levy.[22] Refinement program: XTL.[23] Atomic scattering factors: from XTAL. $R$ and $R_w$: 0.040, 0.037. 2.40. $(\Delta/\delta)_{max}$ at C, O positions: 0.013. Extrema of density in final difference map: −0.26, +0.22 Å$^{-3}$ at C101 and C171, respectively.

Since the crystal structure was undertaken to determine the absolute configuration and conformation of the oxirane, it was important to assign atomic identities reliably. Assignments for the oxirane atoms were based on peak heights in an $E$ map after two cycles of Fourier refinement and on the known steroid moiety for the rest. At convergence of isotropic refinement, $R$ was 0.123 and $R_w$ was 0.128 and the $U$ values were 0.069 (2), 0.074 (2), 0.059 (3), 0.061 (3), 0.083 (4), and 0.080 (3) for O1′(1), O1′(2), C2′(1), C2′(2), C3′(1), and C3′(2), respectively. When the labels at 1′ and 3′ were interchanged, the agreement factors became $R$ = 0.132 and $R_w$ = 0.135 and the corresponding thermal parameters were 0.037 (2), 0.039 (2), 0.058 (3), 0.060 (3), 0.143 (4), and 0.153 (4). THe significant increase in the $R$ factors and the change to unrealistic thermal parameters was confirmation of the original label choice. After anisotropic refinement, indications were found in a difference map for all the expected H atoms including those on the oxirane C atoms and this further substantiates the label choice since the oxirane H thermal parameters refined smoothly to realistically small values. Since some other H atom thermal parameters were rather large, the final refinement, which converged at $R$ = 0.040 and $R_w$ = 0.037, was carried out with unrefined hydrogen thermal parameters assigned from those of the heavier atoms.

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**Supplementary Material Available:** Listings of molecular dimensions and of hydrogen and non-hydrogen atomic parameters (5 pages); and of observed structure factors, calculated structure factors, and standard deviations (20 pages). Ordering information is given on any current masthead page.

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