

tween π -electrons in the sandwiching planes and a carbonyl oxygen atom at one end of the molecule and an epoxide oxygen atom in the para-positioned glycidyl group. Distances between the carbonyl O21 and N3, C2, C4 of the sandwich plane are 2.88–3.11 Å, and between the epoxide O14 and the six atoms of the opposite plane range from 3.10 to 3.17 Å.

Bond distances and angles within the TGT molecules, although not specifiable with high precision because of the limited X-ray data, fall within normal ranges of values.

As stated earlier, crystals of both isomers of TGT were obtained together from solvent evaporation of a solution of what was supposedly pure α -TGT. We measured melting points of the " α -TGT" sample and of crystals of the two isomers in order to correlate the substances with previously reported characterizations. Melting points were as follows: mixture 105–108 °C, α 104–106 °C, β 148–152 °C.

Conclusions

The crystal and molecular structure determinations of α - and β -TGT have revealed the basis for their differing physical properties. Ring stacking dimeric association, facilitated by regular interactions between glycidyl groups that are possible only when their configurations are R , R , R /S, S, S, is the reason for the much lower solubility and higher melting point of the β isomer. Differences in biological properties between α - and β -TGT, e.g. greater ability of α -TGT to prolong lifespan in treated animals, are probably also attributable solely to differences in solubility, as the only distinguishing feature between R , R , R /S, S, S and R , R , R /S, S, S configurations is the propensity of the latter to form intra- and intermolecular associations.

The melting point of the mixed sample of TGT is the same as that of the α isomer, leading one to question whether some of the clinical trials were conducted with TGT mixtures, rather than pure α -TGT as reported. If

so, the major clinical problem, thrombophlebitis due to poor drug solubility, may be somewhat alleviated by the use of α -TGT only.

Because of the significant antineoplastic activity of TGT, it would be highly desirable to obtain biologically active derivatives with much greater water solubilities. In this respect it is noteworthy that no report of resolving either TGT racemate into its enantiomers has yet appeared. Analysis of the TGT crystal structures suggests that enantiomers of β -TGT should be able to adopt packing arrangements similar to the racemate, but the situation for α -TGT enantiomers could possibly be different, and therefore perhaps lead to increased solubility. Additionally, the TGT structure determinations suggest that modifications to the triazinetrione ring should be attempted, to reduce electronic π -character and hence reduce intermolecular attractions of the kinds observed in both isomers and increase solubility. One such modified compound, triglycidylurazol, has recently been undergoing clinical studies.¹²⁻¹⁴

Acknowledgment. This work was supported by PHS Grant CA15879 from the National Cancer Institute and by the Medical Research Council of Canada.

Registry No. α -TGT, 59653-74-6; β -TGT, 59653-73-5.

Supplementary Material Available: Figure showing bond lengths and angles and tables listing anisotropic thermal parameters and hydrogen atom coordinates for α -TGT and β -TGT (5 pages). Ordering information is given on any current masthead page.

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Effects of Steroid D-Ring Modification on Suicide Inactivation and Competitive Inhibition of Aromatase by Analogues of Androsta-1,4-diene-3,17-dione

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Analogues of androsta-1,4-diene-3,17-dione (**3a**) in which the D ring is modified were prepared and tested as suicide inactivators and competitive inhibitors of human placental aromatase. As long as the five-membered ring is intact, modifications of the D ring such as reduction or removal of the carbonyl group or conversion to a γ -butyrolactone cause a <6-fold decrease in affinity for and rate of inactivation of aromatase, compared to **3a**. Thus, an oxygen atom at C-17 is not required for binding of these inhibitors to aromatase, suggesting that hydrogen bonding to the D-ring oxygen does not play a major role in binding. Opening the D ring converts the cyclopentane ring to an alkyl chain and causes a >300-fold decrease in affinity; this can be partially reversed by shortening the chain length. These results are consistent with a model in which the free chain of the opened D ring adopts conformations that sterically interfere with binding of the inhibitor to the enzyme. These findings may have practical applications in drug design, by allowing the preparation of 17-deoxo analogues that have high affinity for aromatase but that are not subject to reduction of the 17-carbonyl group, which is a major mode of metabolism of **3a**.

Estrogens are linked to a number of human diseases, including carcinoma of the breast; the ability to decrease the physiologic activity of estrogens is therefore an important clinical goal. Owing to the wide anatomic distribution of aromatase,¹⁻³ the enzyme that biosynthesizes

estrogens from androgens (Scheme I, **1a** \rightarrow **1b** \rightarrow **1c** \rightarrow **2**), a pharmacologic approach is the only practical way to block

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Scheme I

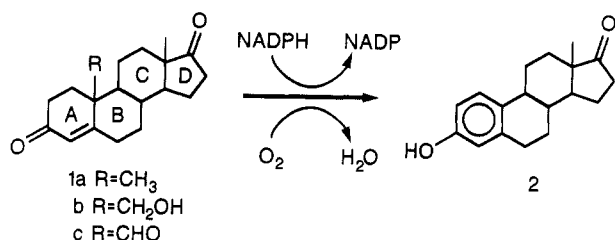
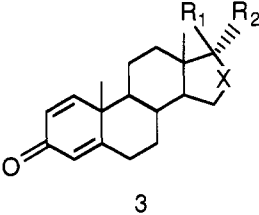
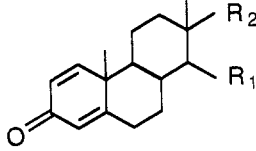


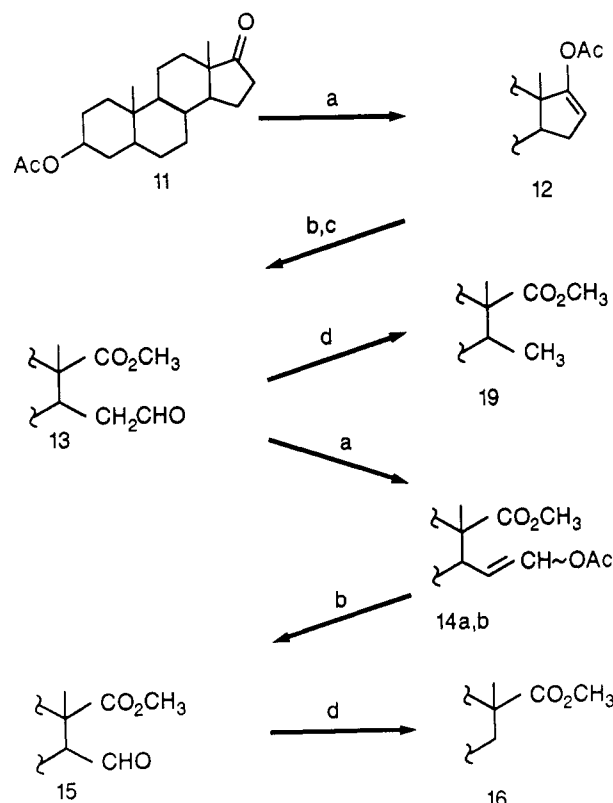
Table I. Kinetic Parameters of Inhibitors Tested

					
no.	R ₁	R ₂	X	K _i , μM	limiting t _{1/2} , min
3a		=O	CH ₂	0.26	12.6
3b	OH	H	CH ₂	1.55	12.9
3c		=O	O	0.39	6.2
3d	H	H	CH ₂	1.10	15.2

			
no.	R ₁	R ₂	enzyme t _{1/2} at 38 μM inhibitor, min
5a	CH ₃	CO ₂ CH ₃	396
5b	CH ₃	CO ₂ H	680
5c	H	CO ₂ CH ₃	587
5d	H	CO ₂ H	>2175
5e	H	CH ₂ OAc	77
5f	H	CH ₂ OH	^a
5g	H	CHO	^b
5h	H	COCH ₃	39
5i	H	COCH ₂ CH ₃	124

^a K_i = 8.56 μM; lim t_{1/2} = 21.5 min. ^b K_i = 3.98 μM; lim t_{1/2} = 12.0 min.

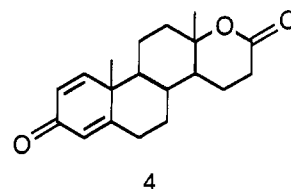
estrogen activity completely. The specific, irreversible blockade of estrogen biosynthesis via mechanism-based ("suicide") inactivation⁴ of aromatase has been intensely

Scheme II^a

^a (a) CH₂=C(CH₃)OAc/*p*-TsOH, Δ; (b) O₃/CH₂Cl₂-HOAc, -70 °C; (c) CH₂N₂/Et₂O; (d) (Ph₃P)₃Rh(I)Cl/PhCN, 155–170 °C.

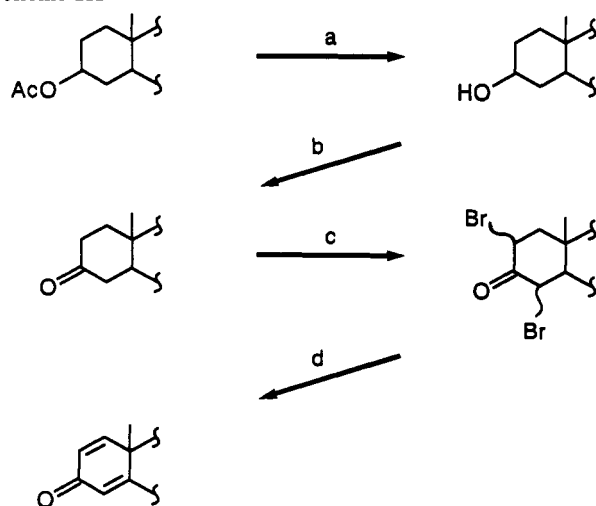
pursued with the goal of developing practical clinical drugs.⁵ Androsta-1,4-diene-3,17-dione (3a) is a prototypical mechanism-based inhibitor of aromatase;⁶ the Δ^{1,2} double bond is responsible for inactivation, although the mechanism is unknown.⁶

Various analogues of 3a with substituted A and B rings are also mechanism-based inactivators of aromatase;^{5,7} however, only one analogue with a modified D ring, testolactone (4), has been examined for inactivating ability.



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- (4) For reviews on suicide inhibitors, see: (a) Walsh, C. *Tetraedron* **1982**, *38*, 871–909. (b) Abeles, R. H. *Chem. Eng. News* **1983**, Sept. 19, 48–56. (c) Walsh, C. *Ann. Rev. Biochem.* **1984**, *53*, 493–535. (d) Rando, R. R. *Pharmacol. Rev.* **1984**, *36*, 111–142. (e) Ortiz de Montellano, P. R.; Reich, N. O. In *Cytochrome P-450: Structure, Mechanism, and Biochemistry*; Ortiz de Montellano, P. R., Ed.; Plenum Press: New York, 1986; pp 273–314.

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Scheme III^a

^a (a) HCl/MeOH, 45 °C; (b) H₂CrO₄/Me₂CO, 0 °C; (c) Br₂/HOAc; (d) Li₂CO₃/LiBr/DMF, 100 °C.

It has a lower apparent affinity for aromatase than **3a** does (apparent $K_i = 35 \mu\text{M}$ vs $0.32 \mu\text{M}$ for **3a**), and it inactivates the enzyme more slowly ($k_{\text{inact}} = 3.6 \times 10^{-4} \text{ s}^{-1}$ vs $9.1 \times 10^{-4} \text{ s}^{-1}$).⁶ We prepared analogues of **3a** with modified D rings and tested them as mechanism-based and competitive inhibitors of aromatase, to explore the effects of D-ring modification on the kinetics of inactivation of aromatase and on the affinity for the enzyme. It was hoped also that modifying the D ring might inhibit the known reduction of the 17-keto group,⁸ which could alter in vivo potency.

Results and Discussion

Synthesis of Compounds. Two categories of compounds were prepared (Table I): those with the D ring intact (**3a–d**), and those with the D ring opened (**5a–i**). In the first class, **3a** and **3b** were obtained commercially, and **3c** and **3d** were prepared by literature methods (see the Experimental Section). In the second class, the compounds were synthesized from 3 β -acetoxy-5 α -androstan-17-one (**11**), by introducing the specific D-ring modification (Scheme II), followed by the A-ring 1,4-diene-3-one system (Scheme III). The aldehyde **13** was prepared from **11** through conversion to the enol acetate,⁹ ozonolysis, hydrolysis to the free acid, and esterification with diazomethane. Decarbonylation¹⁰ of **13** with Wilkinson's catalyst¹¹ gave the 14-methyl compound **19**, the precursor for compounds **5a** and **5b**. Alternatively, conversion of **13** to the enol acetates **14** (a mixture of *E* and *Z* isomers), followed by ozonolysis, provided the 14-formyl compound **15**. It was decarbonylated to give **16**, the precursor to compounds **5c–i**.

The A-ring dienone system was introduced through a bromination¹²–dehydrobromination¹³ sequence, starting from the A-ring ketones; these were obtained by hydrolysis of the 3 β -acetate and chromic acid oxidation¹⁴ (Scheme

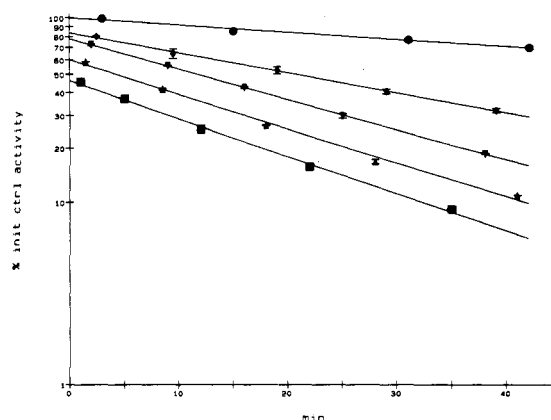


Figure 1. Inactivation of placental aromatase with **3d**. Key: ●, control; ◆, $0.61 \mu\text{M}$; ▼, $1.57 \mu\text{M}$; ▲, $3.84 \mu\text{M}$; ■, $7.69 \mu\text{M}$.

III). Acids **5b** and **5d** were prepared by basic hydrolysis of **5a** and **5c**. Acetate **5e** was prepared from hydroxy ester **17** through reduction with DIBAH, Stevens oxidation¹⁵ of the resulting diol (**22**), acetylation to give **24**, and bromination–dehydrobromination. Basic hydrolysis of **5e** gave **5f**, from which aldehyde **5g** was prepared by oxidation with pyridinium chlorochromate.¹⁶ The ketones **5h** and **5i** were prepared from the acid chloride of **5d** by reacting it with the appropriate alkyl phenylthiocuprate.¹⁷

Testing of the Compounds as Aromatase Inhibitors.

Both categories of compounds were tested as mechanism-based inactivators of aromatase. By use of our previously described method,⁶ various concentrations of putative inhibitor are preincubated with human placental microsomes and NADPH for various times, tritiated androstenedione is added, and the mixture is assayed 5 min for remaining aromatase activity.¹⁸ Control incubations contained all the components except the putative inhibitor. Duplicate determinations were made at each of four concentrations of putative inhibitor, and the results were averaged. The androstenedione concentration is sufficient to protect the enzyme from further inactivation during the assay. In the cases in which inactivation occurs (**3a–d**; **5g,h**), the enzyme is protected by the addition of $0.3 \mu\text{M}$ androst-4-ene-3,17-dione to the medium, suggesting that the inactivators require active-site access. Moreover, inactivation requires the presence of cofactor (NADPH), suggesting that enzyme turnover is required, making these compounds mechanism-based inhibitors rather than affinity labels.

Plots of log (percent initial control activity) vs time show a first-order loss of aromatase activity, as exemplified by the results from incubations of **3d** (Figure 1). Control incubations show a much slower first-order loss of activity, which contributes to the apparent loss of activity observed in the presence of inhibitors. The data are corrected by dividing the observed (apparent) loss of activity due to inhibitor at a given time point by the control activity at that time, and multiplying by 100%. This gives the line that represents the loss of enzyme activity specifically due to the inhibitor. The slope of the corrected line is $k_{\text{app}}(C)$, the apparent rate constant for enzyme inactivation at inhibitor concentration *C*. After at least four different in-

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Table II. K_i Values from Competitive Inhibition Experiments

no.	R ₁	R ₂	X	K_i , μ M	K_m , μ M	K_i/K_m	$\Delta\Delta G^a$
3a		=O	CH ₂	0.12 \pm 0.03	0.076 \pm 0.011	1.6	0.3
3b	OH		CH ₂	0.22 \pm 0.05	0.021 \pm 0.006	10.6	1.4
3c		=O	O	0.41 \pm 0.10	0.064 \pm 0.013	6.5	1.1
3d	H		CH ₂	0.69 \pm 0.20	0.079 \pm 0.002	8.8	1.3
no.	R ₁	R ₂		K_i , μ M	K_m , μ M	K_i/K_m	$\Delta\Delta G^a$
5a	CH ₃	CO ₂ CH ₃		103 \pm 23	0.039 \pm 0.006	2600	4.8
5b	CH ₃	CO ₂ H		1198 \pm 390	0.050 \pm 0.012	23234	6.2
5c	H	CO ₂ CH ₃		130 \pm 25	0.068 \pm 0.008	1898	4.6
5d	H	CO ₂ H		893 \pm 402	0.048 \pm 0.001	18651	6.0
5e	H	CH ₂ OAc		13 \pm 3	0.048 \pm 0.005	273	3.4
5h	H	COCH ₃		45 \pm 6	0.068 \pm 0.001	670	4.0
5i	H	COCH ₂ CH ₃		97 \pm 7	0.056 \pm 0.010	1833	4.6

^a Kilocalories/mole.

hibitor concentrations had been examined in duplicate, apparent K_i and k_{inact} (the rate constant of inactivation at saturation with inhibitor) were determined from a plot of reciprocal $k_{\text{app}}(C)$ vs reciprocal inhibitor concentration.¹⁹ Instead of k_{inact} , we report the limiting $t_{1/2}$, which is the half-life of enzyme inactivation in the presence of saturating concentrations of inhibitor. It is related to k_{inact} by $\lim t_{1/2} = \ln 2/(k_{\text{inact}})$. Our kinetic results are shown in Table I.

In the first category of compounds (3a–d), 3a has an apparent K_i of 0.26 μ M and a limiting $t_{1/2}$ of 12.6 min, in good agreement with our earlier-determined values (0.32 μ M and 12.7 min).⁶ Inhibitors 3b–d have apparent K_i and limiting $t_{1/2}$ values reasonably close to that of 3a (Table I): K_i ranges from 0.39 to 1.10 μ M, and the limiting $t_{1/2}$ ranges from 6.2 to 15.2 min. Thus, modifications of the 17-keto group of 3a, such as reduction to a 17 β -hydroxyl group (3b), introduction of oxygen to produce a γ -butyrolactone (3c), or deoxygenation to form a methylene group (3d), cause only modest decreases (<5-fold) in apparent affinity, with little effect on the limiting $t_{1/2}$ values. The relatively minor difference in the apparent affinities of 3a and the deoxo compound 3d suggests that any hydrogen bonding that may occur between an active-site residue and the 17-keto group of enzyme-bound 3a does not make a significant net contribution to the overall apparent binding energy. The difference between the apparent free energy of binding of 3a and that of any other inhibitor may be estimated from

$$\Delta\Delta G = -RT \ln [K_i(\text{inhib})/K_i(3a)] \quad (1)$$

with $R = 1.98 \times 10^{-3}$ kcal/mol-deg, and $T = 310$ K. These calculations show the apparent binding energies of 3b, 3c, and 3d to differ from that of 3a by only 1.1, 0.25, and 0.89 kcal/mol, respectively. Since the energy of a hydrogen bond is typically 3–6 kcal/mol,²⁰ it is likely that the changes in apparent affinity are due to factors other than hydrogen bonding.

Opening of the D ring causes a marked decrease in the rate of enzyme inactivation, as seen from a comparison of the kinetic data for 3a or 3d with that of 5i or 5c (Table I). The rate of inactivation is inversely proportional to the chain length at C-13, increasing from a three-atom chain (5a, 5c, and 5i) to a two-atom chain (5h) to a one-atom substituent (5f and 5g). The apparent exceptions to this trend are the carboxylic acids 5b and 5d, which probably bind poorly due to ionization, and acetate 5e, which may undergo hydrolysis by microsomal esterases to give alcohol

5f, which appears to bind reasonably well.

Because inhibitors 5a–e, 5h, and 5i inactivate aromatase too slowly to obtain accurate parameters from kinetic experiments, competitive inhibition assays were done to determine K_i . Aromatase assays were carried out for 1–5 min in the presence of five concentrations of androstenedione (0.05–1.0 μ M) and varying concentrations of inhibitor, and K_i values were determined.²¹ The average K_i from two determinations at each of two inhibitor concentrations is shown in Table II, along with the average deviation. Included are the average K_m of androstenedione determined in each experiment and the ratio K_i/K_m . This ratio can be used to estimate directly the difference between the apparent free energy of binding (in kilocalories/mole) of androstenedione and that of the inhibitor of interest (Table II).

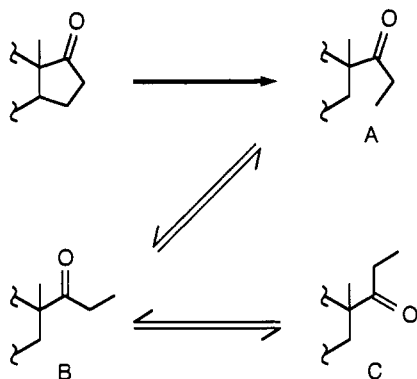
The same trend in affinity seen in the time-dependent inactivation experiments is seen in the competitive inhibition experiments: as long as the D ring is intact, modification or even removal of the D-ring carbonyl group causes relatively minor changes in affinity (<6-fold change in K_i and <5-fold change in $\Delta\Delta G$ of binding compared to androstenedione). This again suggests that hydrogen bonding to the carbonyl does not play a major role in binding of these inhibitors. The difference in the free energy of binding between 3a and 3d is ca. $1.3 - 0.3 = 1.0$ kcal/mol. As noted above, the typical energy of a hydrogen bond is 3–6 kcal/mol; thus, we believe that the differences in affinity are not due to differences in the degree of hydrogen bonding, although we cannot rule out the possibility of weak hydrogen bonding. It is clear, however, that the carbonyl group is not necessary for inhibitor binding.

Opening of the D ring causes a marked decrease in affinity, as can be seen most clearly by comparing the K_i of 3a (0.12 μ M) with that of 5i (97 μ M), or the K_i of 3c (0.41 μ M) with that of 5c (130 μ M). Again, the affinity appears to be inversely proportional to the chain length (compare 5h with 5i); it is decreased slightly by introduction of a neighboring methyl group (compare 5a and 5c). The carboxylic acids (5b and 5d) again exhibit considerably weaker binding, probably due to ionization, and the K_i of acetate 5e seems anomalously low, possibly due to hydrolysis to the alcohol (which has a higher affinity).

The large drop in affinity on opening the D ring may be due to several factors. Since K_i reflects the ratio of the rate constants for binding and dissociation ($k_{\text{on}}/k_{\text{off}}$), a decrease in K_i may reflect slower formation of the enzyme-inhibitor complex (decreased k_{on}), faster dissociation of the complex (increased k_{off}), or both. A decrease in k_{on} would result from any factors that increase the free energy of activation of complex formation; in the cases shown

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Scheme IV



here, we believe that two such factors are probably operating. First, the D-ring-opened inhibitors will have greater ground- and transition-state entropy than will the D-ring-intact analogues, since opening the ring removes the rotational restrictions imposed by the cyclopentane ring (Scheme IV) and allows chain conformations not available in an intact D ring. This reduces the probability that the chain will adopt a cyclopentanoid conformation (conformation A in Scheme IV), if that is important for binding.

Second, some of the conformations now available to the chain may interfere sterically with binding. From inspection of molecular models, it is evident that repulsive 1,3-diaxial interactions between the C-ring axial hydrogen atoms and the chain would be minimized if the chain were to adopt the extended conformations B or C (Scheme IV). Each of these conformations increases the effective radius of the steroid. These conformers might therefore bind less tightly than the cyclopentanoid conformer A, because of steric repulsion between the chain and the enzyme. Since these conformations would be of lower energy than most of the other possible conformations, they would be heavily populated and would therefore contribute more to K_i than other conformations would. This model explains why the aldehyde (5g) appears to bind better than the methyl ketone (5h), which binds better than the ethyl ketone (5i), because extending the chain length would increase the severity of steric interactions upon binding.

We have already argued on energetic grounds that one possible stabilizing interaction, hydrogen bonding, is unlikely to account for the change in K_i seen on opening the ring. Since complete removal of the C-17 oxygen results in only a ca. 6-fold decrease in affinity (compare 3a with 3d), the much larger falloff in affinity seen on opening the D ring cannot be due simply to adoption of non-hydrogen-bonding conformations by the side chain. We believe it is due instead to the combination of entropic and steric effects introduced upon opening the D ring.

An incidental outcome of this study is the opportunity to gauge the effect of the $\Delta^{1,2}$ double bond on affinity. From Table II, K_i/K_m for 3a is 1.6, corresponding to a $\Delta\Delta G$ of 0.3 kcal/mol. Thus, introduction of the $\Delta^{1,2}$ double bond into androstenedione increases the binding energy by only ca. 0.3 kcal/mol, suggesting that this is a minor structural modification with regard to binding to aromatase.

In conclusion, we have shown (1) as long as the five-membered ring is intact, modifications of the D ring cause only minor (<6-fold) changes in affinity for and rate of inactivation of aromatase, (2) an oxygen atom at C-17 is not required for relatively tight binding of an inhibitor to aromatase, suggesting that hydrogen bonding does not play a major role in binding of these inhibitors, (3) opening the D ring causes a >300-fold falloff in affinity, which can be partially reversed by shortening the length of the resultant

chain, (4) derivatives containing a carboxyl group appear to neither bind nor inactivate aromatases, and (5) the $\Delta^{1,2}$ double bond of 3a causes only a minor decrease in affinity (<2-fold; $\Delta\Delta G = 0.3$ kcal/mol) compared to androstenedione.

In addition, the finding that complete removal of the C-17 oxygen atom causes only minor changes in binding and rate of inactivation may have practical implications in drug design. Since reduction of the 17-carbonyl group is a major mode of metabolism of 3a in humans,⁸ removal of the carbonyl will defeat this metabolic path without a significant sacrifice in affinity or rate of inactivation; this would be expected to result in a pharmacologically important increase in the physiologic half-life of this class of inhibitors. Modifications of this type should be applicable to other classes of aromatase inactivators,⁵ such as the 10 β -propynylestranes, the 6-ketoandrostenediones, and the 4-hydroxyandrostenediones.

Experimental Section

General Aspects. IR spectra were recorded on neat films and are reported as ν_{\max} , in cm^{-1} . NMR spectra (^1H at 300 MHz and ^{13}C at 75 MHz) were recorded in CDCl_3 solution; chemical shifts are reported in ppm relative to internal Me_4Si or CDCl_3 . Melting points are uncorrected. All reaction temperatures refer to the bath, unless specified otherwise. "Standard workup" denotes aqueous washing, drying over MgSO_4 , filtering, and evaporating. Androst-4-ene-3,17-dione (1a), androsta-1,4-diene-3,17-dione (3a), 17 β -hydroxyandrosta-1,4-dien-3-one (3b), and 5 α -androstan-3-one (6) were used as received from Sigma Chemical Co. 3 β -Hydroxy-5 α -androstan-17-one (7) was used as received from U.S. Biochemical Co. [1,2- $^3\text{H}_2$]Androst-4-ene-3,17-dione was purchased from New England Nuclear, Boston, MA. Microanalyses were performed by Galbraith Laboratories, Knoxville, TN. "Chromatography" refers to dry column chromatography²² on Woelm TSC silica. Flash chromatography²³ was carried out on Woelm 32-63- μm silica. High-pressure liquid chromatography (HPLC) was carried out on a Waters Associates system, using a 25 $\text{cm} \times 4.6$ mm Alltech 5- μm silica column, at a flow rate of 2.0 mL/min, with refractive index detection. Medium-pressure liquid chromatography (MPLC) was carried out on a system built in our laboratory. All chromatography was carried out with hexane-EtOAc, unless specified otherwise. Tetrahydrofuran (THF) was dried by distillation from benzophenone-sodium ketyl. Dimethylformamide (DMF) was dried over molecular sieves for several days before use, or distilled from calcium hydride. Protein assays were performed by the Bradford assay,²⁴ using a Bio-Rad protein assay kit from Bio-Rad Laboratories, Richmond, CA. Liquid scintillation counting of radioactivity was performed with Budget Solve scintillation cocktail obtained from Research Products International, Mount Prospect, IL.

Placental microsomes were prepared and stored at -70°C as described previously.²⁵ The protein content ranged from 8.9 to 16.4 mg/mL, and the specific activity ranged from 34 to 101 pmol min^{-1} (mg of protein) $^{-1}$.

Assays of aromatase activity, time-dependent inactivation studies, and competitive inhibition experiments were carried out described previously,²⁵ with the modification that the propylene glycol was incorporated into the incubation buffer (4% by weight) instead of adding it directly to each assay tube. Assay tubes were washed by soaking 24-72 h in 25% aqueous KOH and rinsing copiously with distilled water.

16-Oxaandrosta-1,4-diene-3,17-dione (3c). The following modification of the literature method²⁶ was used.

A. Bromination.¹² A solution of 10 (1.3 g, 4.5 mmol) in glacial HOAc (23 mL) was stirred at 60°C as a solution of Br_2 (0.46 mL,

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1.40 g, 8.9 mmol) in glacial HOAc (23 mL) was added during 1 min. The solution was cooled (room temperature), 4 N HBr (1.9 mL) was added, and the mixture was stirred 6.7 h and diluted with CH_2Cl_2 . Standard workup gave 2.0 g of product, which was stored at 4 °C until used in the next step.

B. Elimination.¹³ A slurry of LiBr (2.7 g, 31 mmol) and Li_2CO_3 (2.7 g, 37 mmol) in dry DMF (40 mL) was stirred at 100 °C under N_2 as the above dibromide in dry DMF (10 mL) was added during 1 min, followed by a 10-mL rinse. The mixture was stirred at 100 °C for 16.5 h, cooled to room temperature, and diluted with 1 N HCl (500 mL). Extraction (3:1 $\text{Et}_2\text{O}-\text{CH}_2\text{Cl}_2$), standard workup, and chromatography ($\text{CH}_2\text{Cl}_2-\text{Et}_2\text{O}$) on a 33 cm \times 4 cm column of silica (190 g) gave **3c** (0.7 g, 54%). Recrystallization (twice: $\text{EtOAc}-\text{hexane}$, then $\text{CH}_2\text{Cl}_2-\text{Et}_2\text{O}$) gave colorless crystals: mp 216.5–217 °C (evac capill) (lit.²⁸ mp 214–216.5 °C); IR 1773, 1661, 1626, 1605 cm^{-1} (reported²⁸ 1779, 1664, 1626, 1605); ^1H NMR δ 7.03 (d, $J = 10$ Hz, 1, C1H), 6.26 (dd, $J = 6$ and 2 Hz, 1, C2H), 6.10 (s, 1, C4H), 4.30 (dd, $J = 8$ and 7 Hz, 1, CH_2O), 4.05 (dd, $J = 11$ and 9 Hz, 1, CH_2O), 2.60–2.39 (bm, 2), 2.05–1.70 (bm, 6), 1.52 (td, $J = 12$ and 3 Hz, 1), 1.28 (s, 3, CH_3), 1.19 (s, 3, CH_3); ^{13}C NMR δ 185.66, 180.00, 166.67, 154.31, 132.03, 127.88, 124.50, 68.63, 51.68, 50.37, 43.20, 40.97, 32.76, 32.20, 31.29, 21.80, 18.73, 14.24. Anal. ($\text{C}_{18}\text{H}_{22}\text{O}_3$) C, H.

Androsta-1,4-dien-3-one (3d) was prepared as **3c** was, with the modification that the bromination was carried out at room temperature, no HBr was added, and stirring was continued 1.75 h after the addition of Br_2 . Starting from **6** (0.4772 g, 1.74 mmol) there was obtained 451 mg of dienone, which was chromatographed ($\text{CH}_2\text{Cl}_2-\text{Et}_2\text{O}$) and sublimed (80–90 °C; 0.5 mmHg) to give **3d** (325 mg, 73%) as colorless crystals: mp 80–81 °C (lit.²⁷ mp 80–81 °C); IR 1664, 1603, 1626 cm^{-1} ; ^1H NMR δ 7.07 (d, $J = 10$ Hz, 1, C1H), 6.23 (dd, $J = 10$ and 2 Hz, 1, C2H), 6.07 (t, $J = 2$ Hz, 1, C4H), 2.54–2.30 (m, 2), 2.03–1.94 (m, 1), 1.85–1.42 (m, 8), 1.30–0.84 (m, 5), 1.24 (s, 3, CH_3), 0.78 (s, 3, CH_3); ^{13}C NMR δ 186.11, 169.17, 155.86, 127.18, 123.55, 53.50, 52.64, 43.66, 40.84, 40.03, 38.31, 35.81, 33.92, 32.90, 25.63, 22.87, 20.39, 18.70, 17.44.

Methyl [1S-(1 β ,2 α ,4 α ,4 β ,10 α)]-1,2,3,4,4a,7,9,10,10a-decahydro-1,2,4b-trimethyl-7-oxo-2-phenanthrene-carboxylate (5a) was prepared as **3c** was, from **21** (3.8 g, 12 mmol), to give **5a** (2.0 g, 58%) after chromatography (twice: $\text{CH}_3\text{CN}-\text{CH}_2\text{Cl}_2$, then $\text{hexane}-\text{EtOAc}$). Recrystallization ($\text{hexane}-\text{Et}_2\text{O}$) gave colorless crystals: mp 119.5–121 °C; IR 1726, 1664, 1625, 1603, 1276, 1239, 1109, 887 cm^{-1} ; ^1H NMR δ 7.05 (d, $J = 10$ Hz, 1, C5H), 6.24 (dd, $J = 10$ and 2 Hz, 1, C6H), 6.08 (t, $J = 1$ Hz, 1, C8H), 3.66 (s, 3, OCH_3), 2.53 (bm, 2), 2.18 (bd, $J = 12$ Hz, 1), 1.85–1.42 (bm, 6), 1.22 (s, 3, CH_3), 1.16 (s, 3, CH_3), 0.802 (d, $J = 7$ Hz, 3, CH_3); ^{13}C NMR δ 185.92, 178.07, 168.42, 154.94, 127.67, 123.52, 51.87, 51.13, 47.39, 43.27, 41.51, 37.39, 36.42, 32.96, 32.74, 21.98, 18.81, 15.60, 14.56. Anal. ($\text{C}_{19}\text{H}_{26}\text{O}_3$) C, H.

[1S-(1 β ,2 α ,4 α ,4 β ,10 α)]-1,2,3,4,4a,7,9,10a-Decahydro-1,2,4b-trimethyl-7-oxo-2-phenanthrenecarboxylic Acid (5b). A solution of **5a** (213 mg, 0.703 mmol) and NaOH (0.3 g) in MeOH (10 mL) and H_2O (5 mL) was refluxed 29 h and cooled (room temperature). The solution was washed with benzene, diluted with H_2O , and acidified to pH 1 with concentrated HCl to precipitate the product. The solid was filtered, washed with H_2O , and dried to give **5b** (152 mg, 75%). Recrystallization ($\text{EtOH}-\text{H}_2\text{O}$) gave fine colorless needles: mp (evac capill) 257–9 °C dec; IR 3048, 1718, 1658, 1598, 1227, 1168, 1112, 887, 734; ^1H NMR δ 7.05 (d, $J = 10$ Hz, 1, C5H), 6.26 (dd, $J = 10$ and 2 Hz, 1, C6H), 6.09 (d, $J = 2$ Hz, 1, C8H), 2.54–2.34 (m, 1), 2.24–2.12 (m, 1), 1.90–1.40 (m, 6), 1.22 (s, 3, CH_3), 1.17 (s, 3, CH_3), 0.873 (d, $J = 7$ Hz, 3, CH_3). Anal. ($\text{C}_{18}\text{H}_{24}\text{O}_3$) C, H.

Methyl [2S-(2 α ,4 α ,4 β ,10 α)]-1,2,3,4,4a,7,9,10,10a-decahydro-2,4b-dimethyl-7-oxo-2-phenanthrene-carboxylate (5c) was prepared as **3c** was. Starting from **18** (8.5 g, 29 mmol) there was obtained, after flash chromatography (twice: $\text{hexane}-\text{EtOAc}$, then $\text{CH}_3\text{CN}-\text{CH}_2\text{Cl}_2$) and recrystallization ($\text{hexane}-\text{Et}_2\text{O}$), **5c** (4.0 g, 53%) as colorless crystals: mp 104.5–105.5 °C; IR 1727, 1665, 1626, 1604, 1466, 1403, 1291, 1243, 1109, 887 cm^{-1} ; ^1H NMR δ 7.00 (d, $J = 10$ Hz, 1, C5H), 6.18 (dd, $J = 10$ and 2 Hz, 1, C6H), 6.02 (t, $J = 2$ Hz, 1, C8H), 3.61 (s, 3, OCH_3), 2.45 (td, $J = 15$ and 6 Hz, 1), 2.31 (bd, $J = 15$ Hz, 1),

1.87–0.92 (bm, 9), 1.22 (s, 3, CH_3), 1.17 (s, 3, CH_3); ^{13}C NMR δ 185.90, 178.15, 168.23, 154.73, 127.55, 123.96, 51.78, 50.96, 43.13, 41.95, 41.00, 35.84, 33.51, 32.78, 31.53, 21.88, 20.52, 18.75. Anal. ($\text{C}_{18}\text{H}_{24}\text{O}_3$) C, H.

[2S-(2 α ,4 α ,4 β ,10 α)]-1,2,3,4,4a,7,9,10,10a-Decahydro-2,4b-dimethyl-7-oxo-2-phenanthrenecarboxylic Acid (5d). A solution of **5c** (3.9 g, 14 mmol) and NaOH (5.0 g, 120 mmol) in MeOH and H_2O (100 mL each) was stirred at room temperature for 3.7 h. The mixture was cooled (0 °C), acidified with 6 N HCl to precipitate the product, diluted with H_2O , cooled, and filtered. The product was washed with H_2O and vacuum dried at room temperature to give **5d** (3.6 g, 97%) as a white solid: mp 259–261 °C (evac capill); IR 1692, 1664, 1601, 1293, 1180, 1111, 884 cm^{-1} ; ^1H NMR δ 7.06 (d, $J = 10$ Hz, 1, C5H), 6.26 (dd, $J = 10$ and 2 Hz, 1, C6H), 6.10 (d, $J = 2$ Hz, 1, C8H), 2.51 (td, $J = 15$ and 6 Hz, 1), 2.37 (bd, $J = 15$ Hz, 1), 2.00–1.50 (bm, 7), 1.45–0.98 (bm, 2), 1.31 (s, 3, CH_3), 1.22 (s, 3, CH_3); ^{13}C NMR δ 186.24, 183.44, 168.73, 155.13, 127.48, 123.91, 50.91, 43.22, 41.78, 40.77, 35.83, 33.30, 32.80, 31.51, 21.89, 20.36, 18.71. Anal. ($\text{C}_{17}\text{H}_{22}\text{O}_3$) C, H.

[4aR-(4 α ,4 β ,7 β ,8 α)]-7-[(Acetyloxy)methyl]-4b,5,6,7,8,8a,9,10-octahydro-4a,7-dimethyl-2(4aH)-phenanthrene (5e) was prepared as **3c** was. From **24** (0.587 g, 1.92 mmol) there was obtained, after chromatography twice (MPLC, then dry column), **5e** (0.192 g, 35%) as a pale yellow solid. HPLC gave the analytical sample as a white solid: mp 103–105 °C; IR 1738, 1665, 1626, 1603, 1244, 1037; ^1H NMR 7.07 (d, $J = 10$ Hz, 1, C4H), 6.24 (dd, $J = 10$ and 2 Hz, 1, C3H), 6.09 (t, $J = 2$ Hz, 1, C1H), 3.74 (s, 2, CH_2OAc), 2.51 (td, $J = 12$ and 6 Hz, 1), 2.36 (bd, $J = 12$ Hz, 1), 2.06 (s, 3, CH_3), 1.92–1.82 (bm, 3), 1.62–1.40 (bm, 4), 1.22 (s, 3, CH_3), 1.01 (s, 3, CH_3). Anal. ($\text{C}_{19}\text{H}_{26}\text{O}_3$) C, H.

[4aR-(4 α ,4 β ,7 β ,8 α)]-7-(Hydroxymethyl)-4b,5,6,7,8,8a,9,10-octahydro-4a,7-dimethyl-2(4aH)-phenanthrene (5f). A solution of **5e** (0.156 g, 0.514 mmol) and NaOH (0.280 g, 7 mmol) in MeOH (10 mL) and H_2O (1 mL) was stirred 1.2 h at room temperature. Extraction (EtOAc), standard workup, and chromatography gave **5f** (0.114 g, 85%) as a pale yellow solid. HPLC gave the analytical sample as a white solid: mp 125.5–126.5 °C; IR 3428, 1660, 1618, 1601, 1041; ^1H NMR 7.08 (d, $J = 10$ Hz, 1, C4H), 6.23 (d, $J = 10$ Hz, 1, C3H), 6.08 (s, 1, C1H), 3.28 (s, 2, CH_2O), 2.51 (td, $J = 15$ and 6 Hz, 1), 2.36 (bd, $J = 12$ Hz, 1), 1.92–1.79 (bm, 3), 1.65–1.38 (bm, 4), 1.23 (s, 3, CH_3), 0.986 (s, 3, CH_3). Anal. ($\text{C}_{17}\text{H}_{24}\text{O}_2$) C, H.

[2S-(2 α ,4 α ,4 β ,10 α)]-1,2,3,4,4a,7,9,10,10a-Decahydro-2,4b-dimethyl-7-oxo-2-phenanthrenecarboxaldehyde (5g). Finely ground pyridinium chlorochromate¹⁶ (86 mg, 0.40 mmol) in CH_2Cl_2 (0.5 mL) was stirred at room temperature as **5f** (54 mg, 0.21 mmol) in CH_2Cl_2 (0.5 mL) was added, followed by NaOAc (34 mg, 0.42 mmol). The mixture was stirred 4 h under N_2 at room temperature and filtered through a 0.5 cm \times 7.5 cm column of Florisil (0.8 g). The reaction vessel and the column were washed with 1:1 $\text{Et}_2\text{O}-\text{CH}_2\text{Cl}_2$ (10 mL), and the eluate was evaporated to give 52 mg (97%) of product. HPLC gave **5g** (37 mg, 69%) as a white solid: mp 124–182 °C dec (evac capill); IR 1724, 1664, 1625, 1603 cm^{-1} ; ^1H NMR δ 9.39 (s, 1, CHO), 7.06 (d, $J = 10$ Hz, 1, C5H), 6.26 (d, $J = 10$ Hz, 1, C6H), 6.10 (s, 1, C8H), 2.60–2.45 (bm, 1), 2.38 (bd, $J = 12$ Hz, 1), 1.98–0.98 (bm, 10), 1.24 (s, 3, CH_3), 1.17 (s, 3, CH_3); MS, m/e 258.1627 ($\text{C}_{17}\text{H}_{22}\text{O}_2$ requires 258.1614).

[4aR-(4 α ,4 β ,7 β ,8 α)]-7-Acetyl-4b,5,6,7,8,8a,9,10-octahydro-4a,7-dimethyl-2(4aH)-phenanthrene (5h). **A. Preparation of Acid Chloride.** Acid **5d** (280 mg, 1.02 mmol) in dry THF (4 mL) was stirred at –95 °C under N_2 as 1.62 M ethereal methyllithium (MeLi; 0.63 mL, 1.02 mmol) was added dropwise. The bath was allowed to warm to –70 °C, and freshly distilled oxalyl chloride (90 μL , 131 mg, 1.03 mmol) was added. The mixture was stirred 15 min at –10 °C (vigorous gas evolution) and 30 min at room temperature and recooled (–70 °C) as the cuprate was prepared.

B. Preparation of Cuprate.¹⁷ CuI (214 mg, 1.12 mmol) was flame-dried under vacuum and cooled under N_2 , and dry THF (0.5 mL) and PhSH (0.115 mL, 123 mg, 1.12 mmol) were added. The mixture was stirred at –70 °C as 1.62 M MeLi (0.7 mL, 1.13 mmol) was added, then 1 h at –70 °C, 5 min at 0 °C, and 5 min at room temperature. It was recooled (–70 °C) as a second portion of 1.62 M MeLi (0.63 mL, 1.02 mmol) was added, producing a solid mass. Additional THF (0.5 mL) was added, and the vessel

was briefly removed from the cooling bath and shaken vigorously to break up the mass. The cuprate was stirred at -70°C as the cold (-70°C) solution of the acid chloride was added rapidly. The mixture was shaken vigorously to break up solid and stirred 2.5 h at -70°C . The bath was allowed to warm slowly to -20°C , and the mixture was stirred 16.5 h. Saturated aqueous NH_4Cl and EtOAc were added, and the organic layer was washed with saturated NH_4Cl , 1 N NaOH , and 1 N NH_4OH and then subjected to standard workup. To remove a hydrolyzable byproduct (believed to be thioester), the crude product and NaOH (0.4 g) in MeOH and H_2O (7.5 mL each) was stirred 4 h at room temperature and the mixture was extracted with CH_2Cl_2 . Standard workup and flash chromatography (benzene- EtOAc) gave **5h** (97 mg, 35%). Recrystallization (hexane- EtOAc , twice) gave colorless crystals: mp $79-80^{\circ}\text{C}$; ^1H NMR δ 7.02 (d, $J = 10$ Hz, 1, C4H), 6.20 (d, $J = 10$ Hz, 1, C3H), 6.04 (t, $J = 2$ Hz, 1, C1H), 2.55-2.28 (m, 2), 2.09 (s, 3, CH_3), 1.90-1.40 (m, 9), 1.19 (s, 3, CH_3), 1.16 (s, 3, CH_3). Anal. ($\text{C}_{18}\text{H}_{24}\text{O}_2$) C, H.

[**4aR**-(4a α ,4b β ,7 β ,8a α)]-7-(1-Oxopropyl)-4b,5,6,7,8a,9,10-octahydro-4a,7-dimethyl-2(4aH)-phenanthrenone (**5i**) was prepared as **5h** was, from **5d** (278 mg, 1.01 mmol). The preparation of the cuprate was modified by using 2.0 mL of THF and substituting 3.76 M ethereal ethylmagnesium bromide (0.34 mL, 1.28 mmol) for the second portion of MeLi. Workup gave 275 mg (95%) of a yellow oil. Flash chromatography (benzene- EtOAc , 11 g of silica) gave **5i** (58 mg, 20%) as a white solid. Recrystallization (hexane- EtOAc) gave colorless crystals: mp $111-112.5^{\circ}\text{C}$; IR 1701, 1663, 1624, 1458, 965, 886 cm^{-1} ; ^1H NMR δ 7.04 (d, $J = 10$ Hz, 1, C4H), 6.22 (dd, $J = 10$ and 2 Hz, 1, C3H), 6.06 (t, $J = 2$ Hz, 1, C1H), 2.47 (q, $J = 7$ Hz, 2, CH_2CH_3), 2.55-2.42 (bm, 1), 2.41-2.30 (bd, $J = 15$ Hz, 1), 1.92-1.44 (bm, 5), 1.59 (s, 3, CH_3), 1.20 (s, 3, CH_3), 1.18 (s, 3, CH_3), 1.00 (t, $J = 7$ Hz, 3, CH_2CH_3). Anal. ($\text{C}_{19}\text{H}_{26}\text{O}_2$) C, H.

[**1R**-(1 α ,2 β ,3 β ,4a β ,4b α ,7 α ,8a β ,10a α)]-1-[(1,3-Dihydro-3-oxo-2H-indol-2-ylidene)methyl]tetradecahydro-7-hydroxy-2,4b-dimethyl-2-phenanthrenecarboxylic acid (**8**) was prepared by the method of Hassner et al.,^{28a} in 88% yield, from **7** (5 g, 17 mmol).

3 β -Hydroxy-16-oxaandrostan-17-one (9). The following modification of the method of Rosen and Oliva^{28b} was used to ozonize **8**. A solution of **8** (5.6 g, 13 mmol) in MeOH (250 mL) was ozonized 1 h at -70°C . The yellow-brown solution was purged 10 min with O_2 , giving a clear yellow solution, and Me_2S (3 mL, 2.5 g, 41 mmol) was added. The solution was warmed to 5°C and stirred as NaBH_4 (1.6 g, 42 mmol) was added in 10 portions, producing gas evolution and heat (final temperature 30°C). After 30 min of stirring at room temperature acetone (20 mL) was added. The mixture was acidified to pH 1 with concentrated HCl , stirred 3.5 h at room temperature, and evaporated. The residue was suspended in CH_2Cl_2 , and standard workup and chromatography gave **9** (1.8 g, 47%) as a pale orange solid. Recrystallization (aqueous MeOH , then hexane- EtOAc) gave colorless crystals: mp $184-184.5^{\circ}\text{C}$ (lit.^{28b} mp $182.5-184^{\circ}\text{C}$); IR 3422, 1780, 1457, 1349, 1078, 986 cm^{-1} ; ^1H NMR δ 4.37 (dd, $J = 8, 7$ Hz, 1, CH_2O), 3.98 (dd, $J = 11, 9$ Hz, 1, CH_2O), 3.61 (septet, $J = 5$ Hz, 1, $3\alpha\text{H}$), 1.96-1.76 (m, 2), 1.76-1.55 (m, 5), 1.52-0.95 (m, 7), 1.09 (s, 3, CH_3), 0.847 (s, 3, CH_3); ^{13}C NMR δ 181.15, 70.95, 69.18, 53.89, 51.34, 44.85, 41.02, 37.91, 36.77, 35.67, 32.65, 31.60, 31.36, 31.07, 28.07, 20.22, 14.22, 12.28.

16-Oxa-5 α -androstan-3,17-dione (10) was prepared in 89% yield by the procedure of Kierstead and Faraone,²⁶ starting from **9** (1.3295 g, 4.547 mmol).

Androst-16-ene-3 β ,17-diol Diacetate (12). A solution of tosic acid monohydrate (1.3 g), **11** (25 g, 75 mmol), and isopropenyl acetate (180 mL, 1.7 mol) was refluxed 12 h, and 55 mL of solvent was distilled during 1 h. The mixture was cooled and diluted with CH_2Cl_2 . Standard workup and recrystallization (hexane) gave **12** (15.7 g, 56%) as colorless crystals. Additional **12** (3 g, 11%) was obtained by flash chromatography of the mother-liquor evaporate, for a total of 18.7 g (67%): mp $169-70^{\circ}\text{C}$ (lit.⁹ mp $170-2^{\circ}$); IR 1752, 1732, 1615, 1365, 1248, 1210, 1197 cm^{-1} ; ^1H NMR δ 5.45 (s, 1, vinyl H), 4.69 (septet, $J = 6$ Hz, 1, $3\alpha\text{H}$), 2.15 (s, 3,

CH_3), 2.02 (s, 3, CH_3), 0.88 (s, 3, CH_3), 0.85 (s, 3, CH_3); ^{13}C NMR δ 170.47, 168.70, 159.53, 111.11, 73.58, 54.82, 54.02, 44.91, 36.57, 35.76, 34.06, 33.64, 33.41, 31.12, 28.94, 28.45, 27.49, 21.52, 21.22, 20.78, 15.65, 12.26.

Methyl [1S-(1 α ,2 β ,4a β ,4b α ,7 α ,8a β ,10a α)]-7-(Acetyloxy)-tetradecahydro-2,4b-dimethyl-1-(2-oxoethyl)-2-phenanthrenecarboxylate (13). A solution of **12** (23.7 g, 0.063 mol) in CH_2Cl_2 (700 mL) and glacial HOAc (61 mL) at -70°C was ozonized until blue. Dimethyl sulfide (9.5 mL, 8.0 g, 130 mmol) was added in small portions over several minutes. Glacial HOAc (620 mL) and H_2O (130 mL) were added, and the solution was stirred 18 h at room temperature. Standard workup, esterification with ethereal CH_3N_2 , and flash chromatography gave **13** (21.3 g, 89%) as a pale yellow solid. HPLC gave the analytical sample as a white solid: mp $71-73^{\circ}\text{C}$; IR 2717, 1732, 1244, 1074, 1033 cm^{-1} ; ^1H NMR δ 9.67 (d, $J = 2$ Hz, 1, CHO), 4.68 (septet, $J = 5$ Hz, 1, $3\alpha\text{H}$), 3.65 (s, 3, CH_3), 2.42-0.85 (bm, 18), 2.02 (s, 2, CH_2CHO), 1.12 (s, 3, CH_3), 0.792 (s, 3, CH_3); ^{13}C NMR δ 201.91, 170.53, 73.44, 53.03, 52.06, 47.43, 46.71, 44.13, 41.58, 37.39, 36.60, 36.44, 35.66, 33.85, 31.88, 28.45, 27.42, 21.56, 20.05, 15.60, 12.27. Anal. ($\text{C}_{22}\text{H}_{34}\text{O}_5$) C, H.

Methyl [1R-(1 α ,2 β ,3 β ,4a β ,4b α ,7 α ,8a β ,10a α)]-7-(acetyloxy)-1-[(Z)-2-(acetyloxy)ethenyl]tetradecahydro-2,4b-dimethyl-2-phenanthrenecarboxylate (14a) and methyl [1R-(1 α ,2 β ,3 β ,4a β ,4b α ,7 α ,8a β ,10a α)]-7-(acetyloxy)-1-[(E)-2-(acetyloxy)ethenyl]tetradecahydro-2,4b-dimethyl-2-phenanthrenecarboxylate (14b) were prepared as **12 was, from **13** (14.3 g, 37.8 mmol) and *p*-tosic acid hydrate (3 g, 0.016 mol) in isopropenyl acetate (200 mL, 1.82 mol). Decolorization by chromatography on silica (530 g) gave **14a,b** (14.7 g, 92%) as a pale-yellow solid, which was a 55:45 mixture of *E* and *Z* isomers by HPLC and by ^1H NMR. The analytical samples were obtained through further chromatography and recrystallization (hexane). The *Z* isomer (**14a**) had the following: mp $149-151.5^{\circ}\text{C}$; IR 1758, 1732, 1368, 1243, 1226, 1214, 1031 cm^{-1} ; ^1H NMR δ 6.99 (d, $J = 7$ Hz, 1, $\text{CH}=\text{CHOAc}$), 4.77-4.57 (m, 2, $7\alpha\text{H}$ and $\text{CH}=\text{CHOAc}$), 3.58 (s, 3, CH_3), 2.81 (t, 1, $J = 11$ Hz, $1\alpha\text{H}$), 2.15 (s, 3, CH_3), 2.03 (s, 3, CH_3), 1.15 (s, 3, CH_3), 0.81 (s, 3, CH_3); ^{13}C NMR δ 177.50, 170.49, 168.02, 135.21, 113.75, 73.49, 52.57, 51.64, 46.81, 44.88, 44.33, 36.50, 35.93, 35.71, 35.25, 33.87, 31.83, 28.42, 27.37, 21.50, 20.81, 19.97, 15.34, 12.13. Anal. ($\text{C}_{24}\text{H}_{36}\text{O}_6$) C, H.**

The *E* isomer (**14b**) had the following: mp $171.5-172^{\circ}\text{C}$; IR 1757, 1734, 1670, 1244, 1214 cm^{-1} ; ^1H NMR δ 6.94 (d, $J = 12$ Hz, 1, $\text{CH}=\text{CHOAc}$), 5.10 (dd, $J = 12$ and 11 Hz, 1, $\text{CH}=\text{CHOAc}$), 4.69 (septet, $J = 5$ Hz, 1, $7\alpha\text{H}$), 3.65 (s, 3, CH_3), 2.21 (t, $J = 11$ Hz, 1, C1H), 2.08 (s, 3, CH_3), 2.02 (s, 3, CH_3), 1.90-0.78 (m, 15), 1.13 (s, 3, CH_3), 0.815 (s, 3, CH_3); ^{13}C NMR δ 177.62, 170.60, 167.88, 136.34, 114.52, 73.46, 52.77, 51.68, 48.05, 47.91, 44.22, 36.43, 35.86, 35.67, 34.99, 33.81, 32.46, 28.35, 27.31, 21.40, 20.66, 19.89, 15.08, 12.06. Anal. ($\text{C}_{24}\text{H}_{36}\text{O}_6$) C, H.

Methyl [1S-(1 α ,2 β ,3 β ,4a β ,4b α ,7 α ,8a β ,10a α)]-7-(Acetyloxy)-1-formyltetradecahydro-2,4b-dimethyl-2-phenanthrenecarboxylate (15). A solution of **14a** and **14b** (17.0 g, 40.5 mmol) in CH_2Cl_2 (375 mL) and glacial HOAc (35 mL) at -70°C was ozonized until blue. Dimethyl sulfide (6.3 mL, 5.3 g, 0.085 mol) was added dropwise over several minutes, and the solution was allowed to warm to room temperature over 1 h. Water (0.8 mL) was added, and the solution was stirred vigorously 45 min. Standard workup gave **15** (14.6 g, 99%) as a pale yellow solid. Recrystallization (hexane) gave colorless crystals: mp $122-123.5^{\circ}\text{C}$; IR 2861, 2724, 1731, 1245, 1032 cm^{-1} ; ^1H NMR δ 9.68 (s, 1, CHO), 4.64 (septet, 1, $J = 6$ Hz, $7\alpha\text{H}$), 3.64 (s, 3, CH_3), 2.54 (dd, $J = 11$ and 3 Hz, 1, $1\alpha\text{H}$), 1.97 (s, 3, CH_3), 1.90-0.87 (m, 19), 1.19 (s, 3, CH_3), 0.81 (s, 3, CH_3); ^{13}C NMR δ 204.44, 177.12, 170.46, 73.29, 59.79, 52.08, 44.96, 44.22, 36.41, 35.74, 33.69, 32.02, 31.96, 28.11, 27.28, 21.44, 19.66, 16.83, 12.07. Anal. ($\text{C}_{22}\text{H}_{32}\text{O}_6$) C, H.

Methyl [2S-(2 α ,4a α ,4b β ,7 β ,8a α ,10a β)]-7-(Acetyloxy)-tetradecahydro-2,4b-dimethyl-2-phenanthrenecarboxylate (16). A mechanically stirred mixture of **15** (11.4 g, 31.3 mmol) and tris(triphenylphosphine)rhodium(I) chloride (31 g, 34 mmol) in PhCN (200 mL) was heated under N_2 at $155-170^{\circ}\text{C}$ (internal temperature) for 20 h, by which time it had become clear orange. The mixture was cooled to room temperature and filtered to remove yellow solid, and the filtrate was evaporated in vacuo. The yellow solid on the filter was washed with CH_2Cl_2 , and the washings were added to the residue. The resulting mixture was

(28) (a) Hassner, A.; Haddadin, M. J.; Catsoulacos, P. *J. Org. Chem.* **1966**, *31*, 1363-9. (b) Rosen, P.; Oliva, G. *J. Org. Chem.* **1973**, *38*, 3040-6.

filtered to remove additional yellow solid which formed, and the additional solid was washed well with CH_2Cl_2 . Evaporation of the filtrates and chromatography of the residue gave 16 (8.0 g, 76%) as a pale yellow solid. Recrystallization (hexane-EtOAc) gave colorless crystals: mp 72.5–73 °C; IR 1735, 1465, 1364, 1243, 1118, 1024 cm^{-1} ; ^1H NMR δ 4.70 (septet, 1, C7H), 3.66 (s, 3, CH_3), 2.03 (s, 3, CH_3), 1.19 (s, 3, CH_3), 0.81 (s, 3, CH_3); ^{13}C NMR δ 179.07, 170.54, 53.17, 51.79, 44.49, 42.15, 42.10, 36.47, 35.45, 34.58, 34.05, 33.96, 31.45, 28.56, 27.37, 21.58, 20.54, 20.42, 12.26. Anal. ($\text{C}_{20}\text{H}_{32}\text{O}_4$) C, H.

Methyl [2*S*-(2 α ,4 α ,4 β ,7 β ,8 α ,10 α)]-Tetradecahydro-2,4*b*-dimethyl-7-hydroxy-2-phenanthrenecarboxylate (17). A solution of 16 (11.7 g, 34.8 mmol) and AcCl (1.5 mL, 1.7 g, 21 mmol) in MeOH (180 mL) was stirred 6 h at 45 °C. Evaporation and flash chromatography gave 17 (9.5 g, 93%) as a pale yellow solid. Recrystallization (hexane) gave colorless crystals: mp 89.5–91.5 °C; IR 3371, 1729, 1465, 1240, 1119, 1058 cm^{-1} ; ^1H NMR δ 3.66 (s, 3, CH_3), 3.60 (septet, 1, C7H), 1.58 (s, 3, CH_3), 1.20 (s, 3, CH_3), 0.79 (s, 3, CH_3); ^{13}C NMR δ 179.04, 71.22, 53.35, 51.72, 51.67, 44.72, 42.19, 38.14, 36.72, 35.47, 34.69, 34.11, 31.48, 31.38, 28.69, 20.47, 12.31. Anal. ($\text{C}_{18}\text{H}_{30}\text{O}_5$) C, H.

Methyl [2*S*-(2 α ,4 α ,4 β ,7 β ,8 α ,10 α)]-Tetradecahydro-2,4*b*-dimethyl-7-oxo-2-phenanthrenecarboxylate (18). A solution of 17 (8.9 g, 30.3 mmol) in acetone (200 mL) was stirred at 0 °C as Jones reagent (ca. 10 mL) was added dropwise until an orange color persisted. Excess reagent was decomposed with MeOH, the mixture was filtered to remove solid, and the filtrate was evaporated. The residue was partitioned between H_2O and CH_2Cl_2 , and standard workup gave 18 (8.6 g, 98%) as a white solid. Recrystallization (hexane-EtOAc) gave colorless crystals: mp 105.5–106.5 °C; IR 1726, 1710, 1240, 1224, 1170, 1118, 1094 cm^{-1} ; ^1H NMR δ 3.67 (s, 3, CH_3), 2.45–2.20 (m, 3), 2.15–2.00 (m, 2), 1.80–1.45 (m, 7), 1.42–1.26 (m, 5), 1.22 (s, 3, CH_3), 1.00 (s, 3, CH_3); ^{13}C NMR δ 211.56, 178.82, 52.68, 51.79, 46.42, 44.59, 42.07, 41.87, 38.11, 37.92, 35.56, 34.23, 33.94, 31.32, 28.84, 20.63, 20.47, 11.48. Anal. ($\text{C}_{18}\text{H}_{28}\text{O}_5$) C, H.

Methyl [1*S*-(1 β ,2 α ,4 α ,4 β ,7 β ,8 α ,10 α)]-7-(acetyloxy)-tetradecahydro-1,2,4*b*-trimethyl-2-phenanthrenecarboxylate (19) was prepared as 16 was, but with heating 5 h at 95–100 °C. From 13 (5.6 g, 15 mmol) there was obtained, after chromatography (benzene-EtOAc), crude 19 (7.8 g) contaminated with PhCN. A portion (314 mg) was chromatographed (hexane-EtOAc) to give 19 (144 mg) as a white solid. Based on this recovery, the overall yield was 68%. Recrystallization (aqueous EtOH) gave colorless crystals: mp 128.5–29 °C; IR 1733, 1724, 1272, 1251 cm^{-1} ; ^1H NMR δ 4.70 (septet, J = 5 Hz, 1, C7H), 3.66 (s, 3, CH_3), 2.03 (s, 3, CH_3), 1.94–0.76 (bm, 14), 1.07 (s, 3, CH_3), 0.799 (s, 3, CH_3), 0.726 (d, J = 7 Hz, 3, CH_3). Anal. ($\text{C}_{21}\text{H}_{34}\text{O}_4$) C, H.

Methyl [1*S*-(1 β ,2 α ,4 α ,4 β ,7 β ,8 α ,10 α)]-7-hydroxy-tetradecahydro-1,2,4*b*-trimethyl-2-phenanthrenecarboxylate (20) was prepared as 17 was, in 96% yield (overall from 13) after chromatography, from 19 (7.5 g, contaminated with PhCN). Recrystallization (aqueous EtOH) gave colorless crystals: mp 174–5 °C; IR 3359, 1729, 1237, 1085 cm^{-1} ; ^1H NMR δ 3.66 (s, 3, CH_3), 3.61 (septet, J = 5 Hz, 1, C7H), 1.94–0.80 (bm, 14), 1.50 (s, 1, OH), 1.07 (s, 3, CH_3), 0.781 (s, 3, CH_3), 0.727 (d, J = 7 Hz, 3, CH_3); ^{13}C NMR δ 179.10, 71.25, 53.19, 51.85, 51.81, 47.63, 44.33, 42.22, 38.13, 37.36, 36.94, 35.63, 31.50, 31.28, 28.77, 20.38, 15.42, 14.69, 12.39. Anal. ($\text{C}_{19}\text{H}_{32}\text{O}_5$) C, H.

Methyl [1*S*-(1 β ,2 α ,4 α ,4 β ,7 β ,8 α ,10 α)]-7-oxotetradecahydro-1,2,4*b*-trimethyl-2-phenanthrenecarboxylate (21) was prepared as 18 was, in 95% yield from 20 (4.2 g, 14 mmol), with the modification that the product was isolated by precipitation with H_2O and filtering: mp 168.5–70 °C; IR 1726, 1715, 1280, 1243, 1226, 1161, 1121, 1078 cm^{-1} ; ^1H NMR δ 3.66 (s, 3, CH_3), 2.42–0.82 (bm, 2), 1.10 (s, 3, CH_3), 0.985 (s, 3, CH_3), 0.746 (d, J = 7 Hz, 3, CH_3); ^{13}C NMR δ 211.71, 178.80, 52.63, 51.84, 47.54, 46.14, 44.59, 42.03, 38.43, 38.11, 37.25, 36.74, 35.77, 30.91, 28.95, 20.56, 15.39,

14.64, 11.52. Anal. ($\text{C}_{19}\text{H}_{30}\text{O}_5$) C, H.

[2*S*-(2 α ,4 α ,4 β ,7 β ,8 α ,10 α)]-Tetradecahydro-2,4*b*-dimethyl-7-hydroxy-2-phenanthrenemethanol (22). A solution of 17 (549 mg, 1.86 mmol) in dry toluene (20 mL) at –70 °C was stirred under N_2 as 1 M diisobutylaluminum hydride (DIBALH, 8.4 mL, 8.4 mmol) in toluene was added dropwise during 7 min. The mixture was stirred 1 h at –70 °C, and EtOAc and saturated aqueous NH_4Cl (2.5 mL each) were added. The mixture was warmed to room temperature, washed with 6 N HCl and 0.1 N NaOH, and subjected to standard workup. MPLC (benzene-EtOAc) of the residue (0.518 g) on a 45 cm X 2.2 cm column of silica (44 g), eluting at 14 mL/min, gave 22 (0.449 g, 90%) as a white solid. Recrystallization (hexane-EtOAc) gave colorless crystals: mp 144–5 °C; IR 3337, 1131, 1038, 736 cm^{-1} ; ^1H NMR δ 3.60 (septet, 1, C7H), 3.26 (s, 2, CH_2O), 0.903 (s, 3, CH_3), 0.802 (s, 3, CH_3). Anal. ($\text{C}_{17}\text{H}_{30}\text{O}_2$) C, H.

[4*aR*-(4 α ,4 β ,7 β ,8 α ,10 α)]-7-(Hydroxymethyl)-1,2,3,4,4*a*,4*b*,5,6,7,8,8*a*,9,10,10*a*-tetradecahydro-4*a*,7-dimethyl-2(4*aH*)-phenanthreneone (23). The method of Stevens et al.¹⁵ was followed. A solution of 22 (533 mg, 2 mmol) in glacial HOAc (5 mL) was stirred in a 0 °C bath as 5% aqueous NaOCl solution ("Clorox", 2.7 mL) was added dropwise during 5 min. Additional 0.5-mL portions NaOCl solution were needed at 30 and 45 min. At 80 min, the mixture was stirred 5 min at room temperature and diluted with EtOAc. Standard workup and MPLC under the conditions used for 22 gave 23 (359 mg, 68%) as a white solid. Recrystallization (hexane-EtOAc) gave colorless crystals: mp 103–104.5 °C; IR 3451, 1710, 1449, 1385, 1035 cm^{-1} ; ^1H NMR δ 3.28 (s, 2, CH_2O), 2.46–2.22 (m, 3), 2.15–2.01 (m, 2), 1.68–1.13 (m, 12), 1.01 (s, 3, CH_3), 0.929 (s, 3, CH_3), 0.675 (td, J = 6 and 3 Hz, 1). Anal. ($\text{C}_{17}\text{H}_{28}\text{O}_3$) C, H.

[4*aR*-(4 α ,4 β ,7 β ,8 α ,10 α)]-7-[(Acetyloxy)methyl]-1,2,3,4,4*a*,4*b*,5,6,7,8,8*a*,9,10,10*a*-tetradecahydro-4*a*,7-dimethyl-2(4*aH*)-phenanthreneone (24). A solution of 23 (0.2526 g, 0.955 mmol) in pyridine and Ac_2O (2 mL each) was stirred at room temperature for 3.3 h. The solution was cooled (0 °C), H_2O (2 mL) was added, and the mixture was stirred 10 min. Extraction (CH_2Cl_2) and standard workup gave 24 (0.2730 g, 93%). Recrystallization (hexane-EtOAc) gave colorless crystals: mp 88.5–90 °C; IR 1742, 1713, 1244, 1035 cm^{-1} ; ^1H NMR δ 3.74 (s, 2, CH_2O), 2.46–2.22 (m, 2), 2.07 (s, 3, CH_3), 1.66–1.42 (m, 5), 1.40–1.20 (m, 5), 1.00 (s, 3, CH_3), 0.95 (s, 3, CH_3), 0.68 (td, J = 3 and 9 Hz, 1). Anal. ($\text{C}_{19}\text{H}_{30}\text{O}_3$) C, H.

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Registry No. 3*a*, 897-06-3; 3*b*, 846-48-0; 3*c*, 7785-95-7; 3*d*, 3090-99-1; 5*a*, 118228-07-2; 5*b*, 118228-08-3; 5*c*, 114549-30-3; 5*d*, 114549-32-5; 5*d* (acid chloride deriv), 118228-06-1; 5*e*, 118228-09-4; 5*f*, 118228-10-7; 5*g*, 118228-11-8; 5*h*, 118228-12-9; 5*i*, 118228-13-0; 6, 1224-95-9; 8, 6217-96-5; 9, 7785-94-6; 10, 10028-43-0; 10 (2,4-dibromo deriv), 118228-05-0; 11, 1239-31-2; 12, 1247-67-2; 13, 114549-38-1; 14*a*, 114549-40-5; 14*b*, 114549-39-2; 15, 40715-35-3; 16, 114549-26-7; 17, 114549-27-8; 18, 114549-28-9; 19, 21508-84-9; 20, 21508-85-0; 21, 21508-89-4; 22, 118228-02-7; 23, 118228-03-8; 24, 118228-04-9; aromatase, 9039-48-9.