Synthesis of Androst-5-en-7-ones and Androsta-3,5-dien-7-ones and Their Related 7-Deoxy Analogs as Conformational and Catalytic Probes for the Active Site of Aromatase

Mitsuteru Numazawa,* Ayako Mutsumi, Mii Tachibana, and Kumiko Hoshi

Tohoku College of Pharmacy, 4-1 Komatsushima-4-chome, Aobaku, Sendai 981, Japan

Received January 19, 1994®

A series of androst-5-en-7-ones and androsta-3,5-dien-7-ones and their 7-deoxy derivatives, respectively, were synthesized and tested for their abilities to inhibit aromatase in human placental microsomes. All the steroids inhibited the enzyme in a competitive manner with K_i's ranging from 0.058 to 45 μM. The inhibitory activities of 17-oxo compounds were much more potent than those of the corresponding 17β-alcohols in each series. Steroids having an oxygen function (hydroxy or carbonyl) at C-19 were less potent inhibitors than the corresponding parent compounds having a 19-methyl group. 3,5-Dien-7-one **24** and its 19-hydroxy and 19-oxo derivatives (**12** and **13**) as well as 19-oxo-5-en-7-one **3** caused a time-dependent inactivation of aromatase only in the presence of NADPH in which the k_{inact} values of 19-als **3** and **13** (0.143 and 0.189 min^{-1}, respectively) were larger than those of the corresponding 19-methyl (**23** and **24**) and 19-hydroxy (**1** and **12**) steroids, respectively. 19-Nor-5-en-7-one **4** but not its 3,5-diene derivative **14** also inactivated the enzyme in a time-dependent manner. In contrast, 7-deoxy steroids **21** and **27**, having a 19-methyl group, did not cause it. The inactivations were prevented by the substrate androstenedione, and no significant effects of L-cysteine on the inactivations were observed in each case. The results suggest that oxygenation at C-19 would be at least in part involved in the inactivations caused by the inhibitors **23** and **24**. The conjugated enone structures should play a critical role in the inactivation sequences.

The conversion of 4-en-3-one androgens to the phenolic estrogens represents the last step in the multienzyme transformations of cholesterol to the female sex steroids. Aromatase is a unique cytochrome P-450 enzyme complex which is responsible for this conversion.[1] Aromatization of the androgens appears to involve three oxygenation steps, eventual loss of the angular methyl group at C-19, and the elimination of the 1β- and 2β-hydrogens, resulting in the aromatization of the A-ring of the androgen molecule to form estrogen.[2] Two of these oxygenations appear to occur at the C-19 position, while it is presently unknown whether the third oxygenation takes place at this position or at a separate site, such as the C-2β position.[3]

In the absence of a 19-methyl group, the oxygenative requirements for the aromatization of estr-4-ene-3,17-dione (19-norandrostenedione, **29**) by placental aromatase must be different from the above. However, a single enzyme system seems to aromatize both androgen and 19-norandrogen in the stallion testis,[4] and the aromatization of 19-norandrogen involves also stereospecific loss of the 1β-[5] and 2β-hydrogens.[6]

Inhibitors of aromatase may be valuable as therapeutic agents in the treatment of estrogen-sensitive breast tumors and as possible antifertility agents.[7] Present knowledge of the mechanism of aromatization had led to the successful design and development of a wide variety of suicide substrates of aromatase.[7] The known suicide substrates primarily have made use of the oxygenation of the 19-angular methyl of a 4-en-3-one steroid in the inactivation process. We have previously reported that a C_{19} steroid having a unique α,β-unsaturated ketone, a 4-en-6-one,[8] instead of the 4-en-3-one, or a 4-ene system[9] efficiently blocks the aromatase activity in a reversible manner, even though there is no oxygen function at the C-3 position. The 4-ene steroids are the most potent competitive inhibitors among those reported so far. On the other hand, 5-en-7-one steroid **23**, another α,β-unsaturated keto steroid, and its 19-hydroxy derivative **1** inactivate the enzyme in a mechanism-based manner.[10]

To gain further insight regarding the structure-activity relationships of aromatase inhibitors having a 5-en-7-one structure, we synthesized a series of 5-en-7-one and 3,5-dien-7-one steroids, with or without a 7-carbonyl function, including 19-oxygenated and 19-nor analogs. All the steroids examined inhibited human placental aromatase in a competitive manner in which a 17-carbonyl group is necessary for a tight binding to the active site of aromatase. Moreover, 3,5-dien-7-one derivatives **12**, **13**, and **24** as well as 5-en-7-oxo steroids **3** and **4** were proved to be mechanism-based inactivators of the enzyme. The 7-oxo function is essential for the irreversible inactivations by the 5-ene and 3,5-diene steroids.

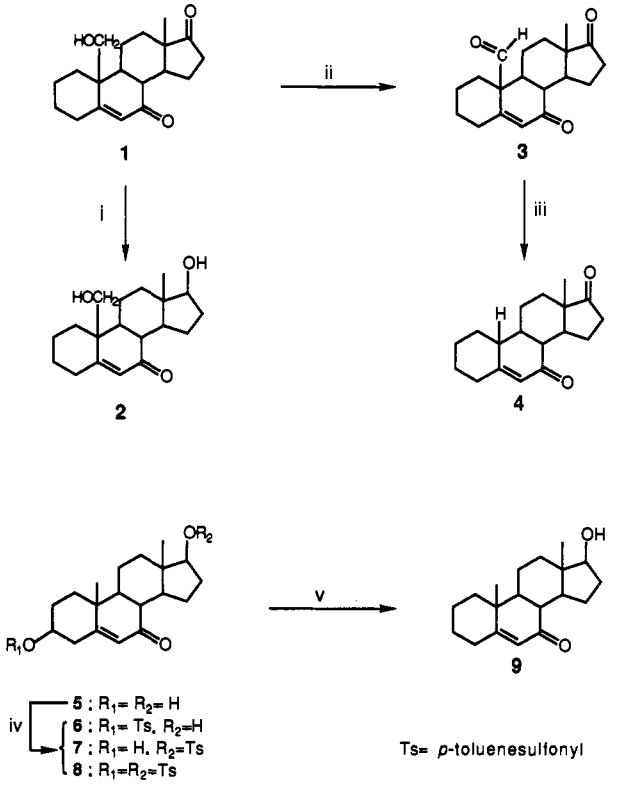
Results

Chemistry. We initially focused on preparation of 5-en-7-oxo steroids (Scheme 1). Treatment of 19-hydroxyandrost-5-ene-7,17-dione (**1**),[10] which was previously synthesized, with a limited amount of $NaBH_4$ in chilled MeOH yielded the 17β-reduced product **2** (60%) whereas oxidation of it with pyridinium dichromate (PDC) gave 19-oxo steroid **3** (20%). Reaction of the 19-al **3** with KOH in aqueous MeOH afforded the 19-nor derivative **4** (21%). The conformation of a proton at C-10 was determined to be β based on the previous findings[11] reported for the similar reaction with 19-oxo-4-en-3-one steroids. Treatment of 3β,17β-dihydroxy-5-en-7-one **5**[12] with *p*-toluenesulfonyl (tosyl) chloride in pyridine gave 3β-tosylate **6** as well as its 17β-isomer **7** and 3β,17β-ditosylate **8**. Reductive

* Abstract published in *Advance ACS Abstracts*, June 1, 1994.

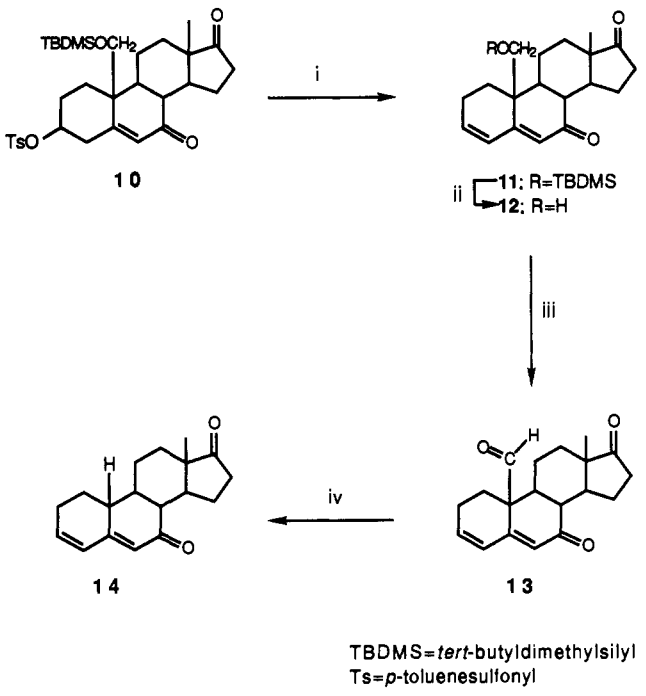
0022-2623/94/1837-2198$04.50/0

Scheme 1[a]



[a] Reagents: (i) $NaBH_4$, MeOH; (ii) pyridinium dichromate, CH_2Cl_2; (iii) KOH, aqueous MeOH; (iv) Ts-Cl, pyridine; (v) Zn powder, NaI, $(CH_2OMe)_2$.

Scheme 2[a]

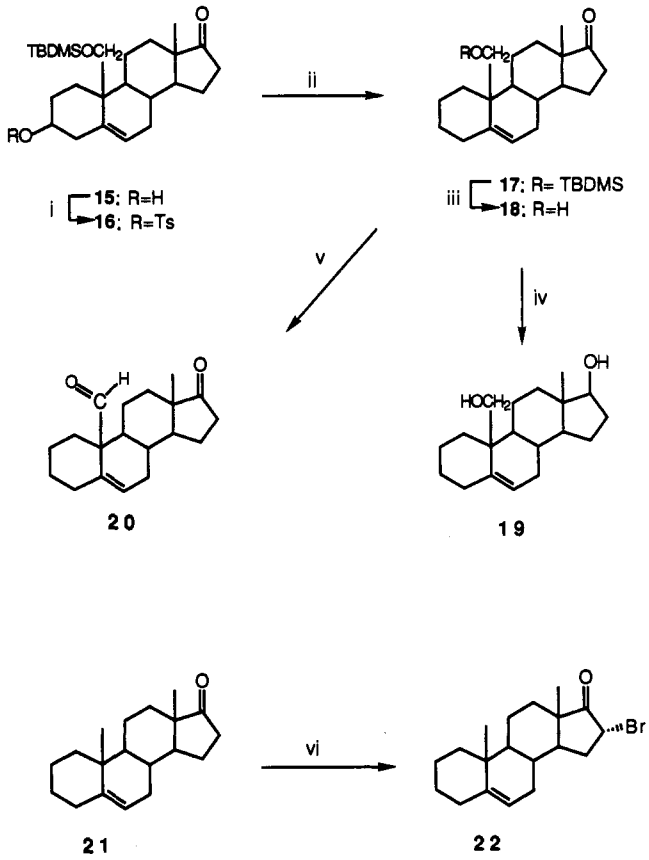


[a] Reagents: (i) *N*-methylpyrrolidone, 80 °C; (ii) $(n\text{-Bu})_4NF$, THF; (iii) pyridinium dichromate, CH_2Cl_2; (iv) KOH, aqueous MeOH.

deoxygenation of compound **6** with Zn powder in the presence of NaI[13] produced 3-deoxy compound **9** in good yield.

A series of androsta-3,5-diene-7,17-dione (**24**) derivatives were synthesized as shown in Scheme 2. Elimination reaction of the known 3β,19-dihydroxy-5-ene-7,17-dione derivative **10**,[10] which has a tosyl group at C-3 and a *tert*-butyldimethylsilyl (TBDMS) group at C-19, in basic media at elevated temperature[14] gave 19-hydroxy-3,5-diene-7,-17-dione **12** (20%) as well as its 19-TBDMS derivative **11** (65%), deprotection of which was achieved with $(n\text{-Bu})_4NF$ to also produce compound **12**. The 3,5-dien-7-one structure was confirmed by the UV (λ_{max} 280 nm) and ^1H NMR spectra [conjugated olefinic protons at δ 5.84 (1H, s) and 6.19 (2H, m)]. The 19-ol **12** was converted into 19-al **13** (30%) by the PDC oxidation. Treatment of steroid **13** with KOH in aqueous MeOH similarly gave 19-nor-3,5-dien-7-one **14** as described in the synthesis of compound **4**.

Scheme 3[a]



[a] Reagents: (i) Ts-Cl, pyridine; (ii) Zn powder, NaI, $(CH_2OMe)_2$; (iii) $(n\text{-Bu})_4NF$, THF; (iv) $NaBH_4$, MeOH; (v) pyridinium dichromate, CH_2Cl_2.

The known steroid 3β,19-dihydroxy-5-en-17-one 19-TBDMS ether **15**[15] was converted into 3-tolylate **16** and the subsequent reductive elimination reaction with Zn powder and NaI yielded 3-deoxy steroid **17** (Scheme 3), similarly as described in the synthesis of compound **9**. The 19-silyl ether **17** was hydrolyzed to afford 19-ol **18** on treatment with the fluoride reagent as described above. Reduction of the 17-one **18** with $NaBH_4$ in MeOH at 0 °C gave 17β,19-diol **19** in fair yield whereas oxidation of it with PDC yielded 19-al **20** (47%). Bromination of 17-one **21** with cupric bromide in MeOH under reflux[16] afforded 16α-bromo ketone **22** (29%).

Biochemical Properties. Reversible inhibition of aromatase activity by the 5-ene and 3,5-diene steroids with or without a 7-oxo function were initially tested in vitro by enzyme kinetics studies using human placental microsomes. The inhibition by 19-norandrostenedione (**29**, Chart 1) was also examined in our hands. 5-Ene-7,17-dione **23** and its 19-hydroxy derivative **1** and androst-4-en-17-one (**30**) are listed for comparison. The results are

Chart 1

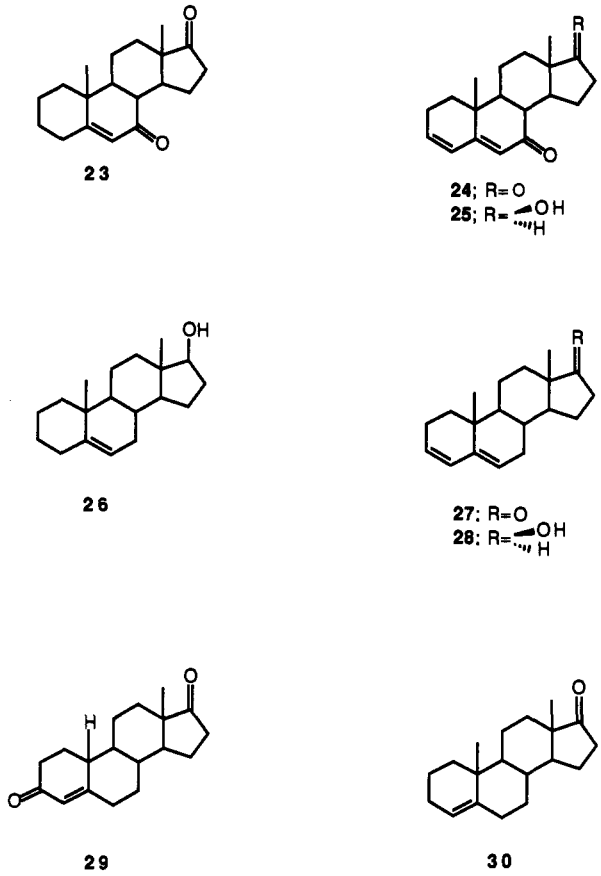


Table 1. Aromatase Inhibition by Various Androst-5-ene Derivatives

compound	IC_{50}, μM[a]	K_i, μM[b]	inhibition[c]
5-En-7-one Steroid			
17-one **23**	1.8	0.25	competitive[d]
17β-ol **9**	42	5.5	competitive
19-hydroxy-17-one **1**	56	11	competitive[d]
17β,19-diol **2**	140	45	competitive
17,19-dione **3**	60	13	competitive
19-nor-17-one **4**	28	2.4	competitive
3,5-Dien-7-one Steroid			
17-one **24**	1.8	0.22	competitive
17β-ol **25**	42	5.3	competitive
19-hydroxy-17-one **12**	60	15	competitive
17,19-dione **13**	11	1.8	competitive
19-nor-17-one **14**	33	3.0	competitive
5-Ene Steroid			
17-one **21**	0.66	0.12	competitive
17β-ol **26**	30	3.0	competitive
19-hydroxy-17-one **18**	6.9	1.0	competitive
17β,19-diol **19**	62	9.8	competitive
17,19-dione **20**	9.0	1.4	competitive
16α-bromo-17-one **22**	55	11	competitive
3,5-Diene Steroid			
17-one **27**	0.34	0.058	competitive
17β-ol **28**	18	2.0	competitive
For Comparison			
androstenedione	0.30	0.020 (K_m)	
19-norandrostenedione (**29**)	1.2	0.14	competitive
androst-4-en-17-one (**30**)	0.11	0.013	competitive[e]

[a] 300 nM of [1β-^{3}H]Androstenedione and 20 μg of protein from human placental microsomes were used. [b] Inhibition constant (K_i) was obtained by Dixon plot. [c] Inhibition type was determined by Lineweaver–Burk plot. [d] Reference 10. [e] Reference 9b.

shown in Table 1. Aromatase activity in the placental microsomes was determined essentially according to the method of Siiteri and Thompson[17] in which tritiated water released from [1β-^{3}H]androstenedione into the incubation medium during aromatization. IC_{50}'s were initially obtained under initial velocity conditions. Then, in order to characterize the nature of their bindings to the active site of aromatase, aromatization was measured at several concentrations of the inhibitors and androstenedione. The results of the studies were plotted in a typical Lineweaver–Burk plot. All the steroids studied exhibited clear-cut competitive-type inhibition. The apparent inhibition constants (K_i), an index of the affinity of the enzyme for the inhibitor, were determined by analysis of the Dixon plot. The Lineweaver–Burk plot of aromatase inhibition by compound **27** is shown in Figure 1. In these studies, the apparent K_m for androstenedione was found to be 20 ± 2 nM.

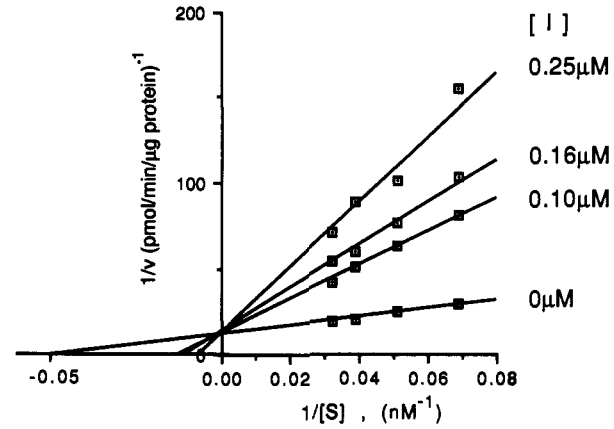


Figure 1. Lineweaver–Burk plot of inhibition of human placental aromatase by 3,5-dien-17-one **27** with androstenedione as a substrate. Each point represents the mean of two determinations which varied by less than 10% of the mean. The inhibition experiments with all the other steroids examined gave essentially similar plots to Figure 1 (data not shown).

A series of the 5-en-7-ones (**3** and **4**) and the 3,5-dien-7-ones (**12**-**14**, and **24**) were then tested for their abilities to cause a time-dependent inactivation of aromatase. All the inhibitors examined, except 19-nor-3,5-dien-7-one **14**, showed the time-dependent inactivation when they were incubated in the presence of NADPH, whereas the 19-nor steroid **14**, at concentrations employed (1.5, 3.0, and 4.5 μM), did not cause it. Pseudo-first-order kinetics were obtained during the first 12 min of the incubation of the inhibitors when the kinetic data were analyzed according to the method of Kitz and Wilson[18] (Figures 2 and 3). Double-reciprocal plots of k_{obs} versus inhibitor concentration gave k_{inact}'s and K_I's, respectively, for the inhibitors (Table 2). 19-Nor-4-en-3-one **29**, which is a regioisomer of compound **4**, inhibited the aromatase activity in a competitive manner but did not cause the time-dependent inactivation (data not shown).

NADPH was essential for the time-dependent activity loss by the irreversible inhibitors (Figure 4A). The substrate androstenedione blocked the inactivation while a nucleophile, L-cysteine, had no significant effect on it in every case (Figure 4B,C).

On the other hand, the 5-ene steroids **18**, **20**, and **21** and the 3,5-diene steroid **27**, without a carbonyl function at C-7, at concentrations employed (0.7, 1.4, and 2.8 μM for **18** and **20**, 0.12 and 0.24 μM for **21**, and 0.06, 0.12, and 0.24 μM for **27**), did not cause a time-dependent inactivation of aromatase (data not shown).

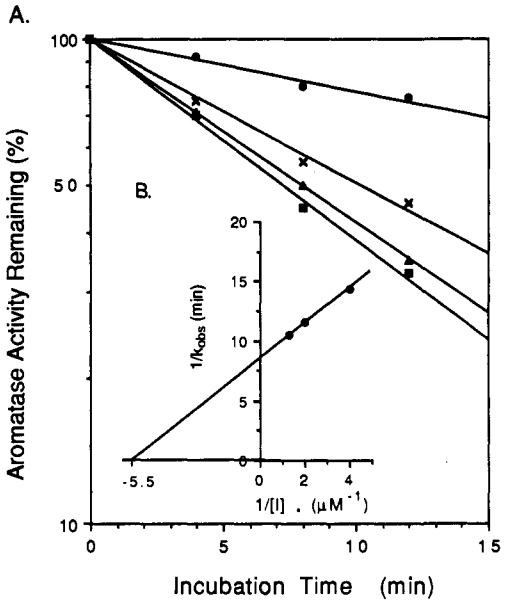


Figure 2. Time-dependent inactivation (A) and concentration-dependent inactivation (B) by 3,5-dien-7-one **24** in the presence of NADPH in air. Concentrations of the inhibitor: control (0 μM), ●; 0.25 μM, ×; 0.50 μM, ▲; 0.75 μM, ■. Each point represents the mean of two determinations which varied by less than 10% of the mean.

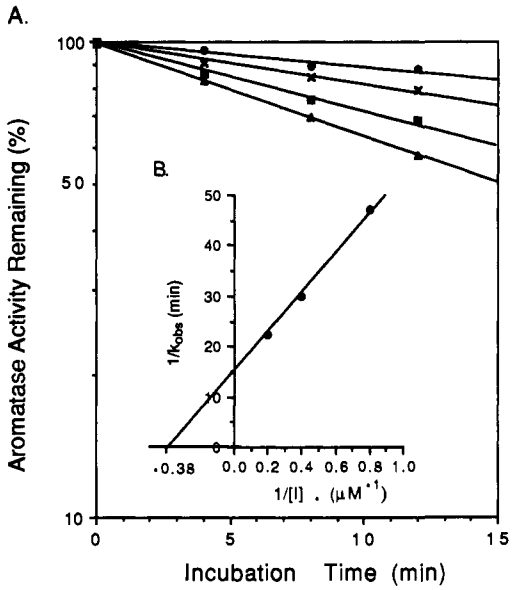


Figure 3. Time-dependent inactivation (A) and concentration-dependent inactivation (B) of human placental aromatase by 19-nor-5-en-7-one **4** in the presence of NADPH in air. Concentrations of the inhibitor: control (0 μM), ●; 1.25 μM, ×; 2.5 μM, ■; 5.0 μM, ▲. Each point represents the mean of two determinations which varied by less than 10% of the mean. The time-dependent inactivation experiments with compounds **3**, **12**, and **13** gave essentially similar plots to Figures 2 and 3 (data not shown).

Discussion

The 5-en-17-one steroid **21** and the 3,5-diene-7,17-dione steroid **24** are good competitive inhibitors of human placental aromatase, of which K_i values (0.12 μM for **21** and 0.22 μM for **24**) are comparable to that of the 5-ene-7,17-dione **23** (K_i = 0.25 μM) reported previously.[9b] An introduction of a double bond at C-3 of compound **21** markedly enhanced affinity to the active site of aromatase in which K_i value (0.058 μM) of the 3,5-diene derivative **27** is similar to that of the natural substrate androstenedione (K_m = 0.020 μM). Conformational change of the A,B-ring system and/or stereoelectronic effect may be involved in the increased affinity, although there is no evidence of that at present. The inhibitory activities of the 17-alcohols are, respectively, weaker than those of the corresponding 17-oxo analogs [K_i, μM: 45 vs 11 (**2** vs **1**), 5.5 vs 0.25 (**9** vs **23**), 9.8 vs 1.0 (**19** vs **18**), 5.3 vs 0.22 (**25** vs **24**), 3.0 vs 0.12 (**26** vs **21**), and 2.0 vs 0.058 (**28** vs **27**)], in analogy with series of 4-en-3-one[1b,1d,19,20] and 3-deoxy-4-ene[9b,c] steroids.

Table 2. Kinetic Analysis of Time-Dependent Inactivation of Aromatase Caused by Androst-5-ene-7,17-dione Derivatives[a]

compound	K_I, μM	k_{inact}, min^{-1}
5-En-7-one Steroid		
19-methyl steroid **23**	0.15	0.069[b]
19-ol **1**	11	0.058[b]
19-al **3**	20	0.143
19-nor steroid **4**	2.6	0.068
3,5-Dien-7-one Steroid		
19-methyl steroid **24**	0.18	0.119
19-ol **12**	7.0	0.088
19-al **13**	2.9	0.189
19-nor steroid **14**		NT[c]

[a] Apparent K_I and k_{inact} were obtained by Kitz–Wilson plot. [b] Reference 10. [c] NT: the time-dependent inactivation was not observed.

An introduction of a carbonyl group at C-7 of the 5-ene steroid series lowered the affinity [K_i, μM: 0.25 vs 0.12 (**23** vs **21**), 5.5 vs 3.0 (**9** vs **26**), 11 vs 1.0 (**1** vs **18**), 45 vs 9.8 (**2** vs **19**), and 13 vs 1.4 (**3** vs **20**)]. The similar structure–activity relationships are also observed in the 3,5-diene steroid series [K_i, μM: 0.22 vs 0.058 (**24** vs **27**) and 5.3 vs 2.0 (**25** vs **28**)]. These results indicate that a conjugated carbonyl group is not necessary for tight bindings of the 5-ene and 3,5-diene steroids to the active site.

The affinities of 19-ols to the active site of aromatase are significantly lower than those of the parent 19-methyl steroids in each series [K_i, μM: 45 vs 5.5 (**2** vs **9**), 15 vs 0.22 (**12** vs **24**), 1.0 vs 0.12 (**18** vs **21**), and 9.8 vs 3.0 (**19** vs **26**)]. The similar results were also obtained for 19-oxo steroids **3**, **13**, and **20** with K_i's ranging from 1.4 to 13 μM. It has been reported that in the series of androstenedione and its 16α-hydroxy[1,21] and 6-oxo[22] derivatives, which are substrates for aromatase, their 19-ols and 19-als have lower affinities for the enzyme compared to the corresponding parent steroids.

We[10] have previously reported that the 5-en-7-one **23** as well as its 19-hydroxy derivative **1** inactive aromatase in a suicidal manner. In this study, not only 19-oxo and 19-nor derivatives (**3** and **4**) of inhibitor **23** but also 3,5-dien-7-one steroids and 19-methyl (**24**), 19-hydroxy (**12**), and 19-oxo (**13**) compounds inactivated the aromatization of androstenedione in a time-dependent, pseudo-first-order manner in the presence of NADPH in air; on the other hand, 19-nor-3,5-dien-7-one **14** did not. A double-reciprocal plot[18] of the apparent rate constants for inactivation versus the concentration of each steroid was linear and gave the apparent K_I[23] and overall rate constant for inactivation (k_{inact}), respectively (Table 2). This indicates formation of a dissociable enzyme–inhibitor complex followed by unimolecular inactivation. The apparent K_I's are similar to the apparent K_i's obtained from the competitive experiments described in Table 1, suggesting that the initial binding of the inhibitors to the enzyme is not rate-limiting in the inactivation process.

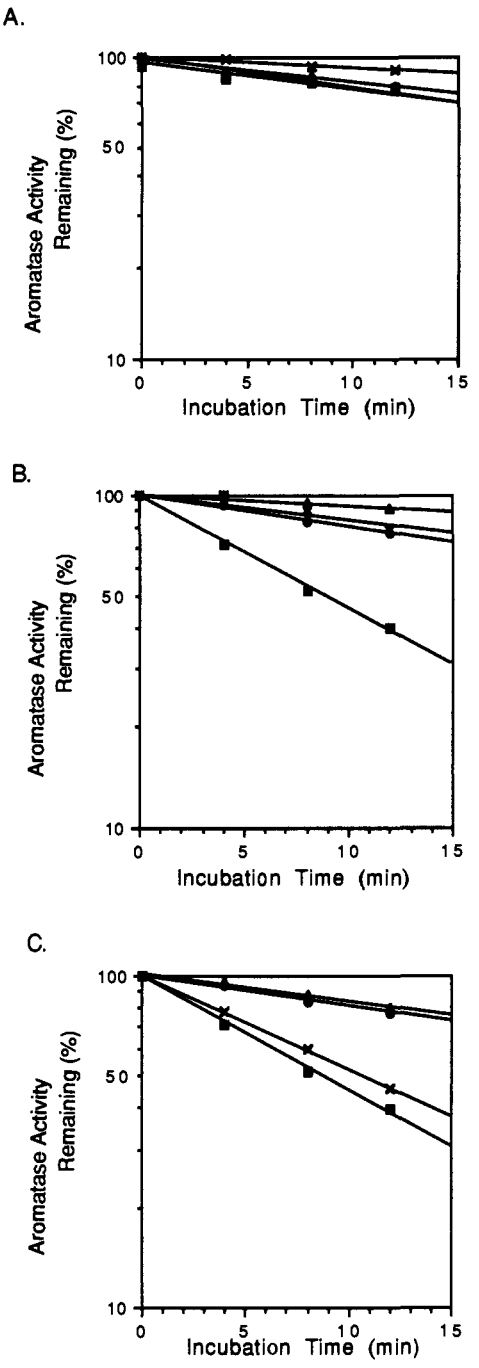


Figure 4. Inactivation of human placental aromatase by 3,5-dien-7-one **24** under various conditions. (A) In the absence of NADPH, the inhibitor at concentrations of 0.25 μM (×), 0.50 μM (▲), and 0.75 μM (■) failed to produce the inactivation. Control sample contained no inhibitor (●). (B) Androstenedione at concentration of 0.3 μM (×) protected aromatase from inactivation caused by the inhibitor (0.25 μM) (■) in the presence of NADPH. Control sample with (▲) or without (●) androstenedione contained no inhibitor. (C) In the presence (×) or absence (■) of L-cysteine (0.5 mM), a pseudo-first-order inactivation of aromatase by the inhibitor (0.25 μM) was observed. Control sample with (▲) or without (●) L-cysteine contained no inhibitor. Each point represents the mean of two determinations which varied by less than 10% of the mean. The inactivation experiments with compounds **3**, **4**, **12**, and **13** in the absence of NADPH and in the presence of androstenedione or L-cysteine gave essentially similar results to Figure 4 (data not shown).

The rate of inactivation decreased when the substrate androstenedione was included in the incubation mixture. In the nucleophile protection experiment, L-cysteine failed to protect aromatase from inactivation by the inactivators. Thus, covalent-bond formation between the enzyme and the reactive intermediate appears to occur rapidly at the active site, therefore, preventing diffusion of the activated inhibitor, a reactive electrophile, into the surrounding media.

The inactivation rates of the 19-als **3** and **13** are faster than those of the others in each series and the 19-ols **1** and **12** are also suicide substrates of aromatase. On the basis of these facts along with the relative affinities to the active site of the enzyme of the 19-oxygenated steroids and their parent 19-methyl steroids, it is suggested that the inactivation may proceed through 19-oxygenation in each series, although there is no direct evidence of this. Robinson's group[19b,24] and our group[9c] have demonstrated that the 19-methyl group of 3-deoxy-4-ene steroids having a double bond at C-2 or difluoro and acetylenic alcohol functions at C-19 are oxygenated by aromatase. In contrast, all the 7-deoxy steroids examined did not cause a significant time-dependent inactivation of aromatase, so a conjugated carbonyl structure, 5-en-7-one or 3,5-dien-7-one, should be essential for the formation of the reactive electrophile.

19-Nor steroid **4**, having a 5-en-7-one structure, unexpectedly, inactivated aromatase in a time-dependent manner only in the presence of NADPH in air whereas another 19-nor steroid **14** having a 3,5-dien-7-one structure or 19-norandrostenedione (**29**) (K_i = 0.14 μM) having a 4-en-3-one structure did not. 19-Norandrogens are aromatized far more slowly than androgens.[4,25] Thompson and Siiteri[1a] have postulated that the lack of the C-19 methyl group would result in an unfavorable orientation of the substrate with regard to the heme of aromatase, which would lead to a slowing down of the rate of oxygenation. The aromatization mechanism of 19-norandrogens is currently unknown. Compound **29** is converted to its 1β-hydroxy derivative by human placental aromatase,[5,26] although it is not clear whether the 1β-hydroxylation is involved in the aromatization mechanism. Based on these previous findings, the following mechanism is plausible for the inactivation by the 19-nor-4-en-7-one **4**. The 1β-hydroxy derivative of inhibitor **4** is initially produced by aromatase reaction, followed by its conversion to 1(10),5-dien-7-one through further oxygenation by the enzyme or nonenzymatic dehydration. The 1(10),5-diene intermediate covalently binds to the enzyme in a 1,6-addition manner to result in the irreversible inactivation of the enzyme. The 3,5-dien-7-one **14**, which is not an inactivator of the enzyme, would not be hydroxylated at C-1 principally because of the conformational changes of the A,B-ring system caused by introduction of a double bond at C-3 of compound **4**. We have currently carried out the synthesis of the 1,6-acceptor in order to clarify the inactivation mechanism.

In conclusion, 5-ene and 3,5-diene steroids with or without a carbonyl function at C-7 are good to poor competitive inhibitors of human placental aromatase in which a C-17 carbonyl function is essential for tight binding to the active site of aromatase in each series. The 7-oxo derivatives including the 19-nor steroid **4** inactivate aromatase in a suicidal manner. The present results add new aspects to structure–activity relationships of aromatase inhibitors, especially suicide substrates of aromatase. Furthermore, the inhibitors examined are promising to play an important role for understanding not only catalytic functions of aromatase as well as the mechanism

of the aromatase reaction but also conformational features of the active site.

Experimental Section

Chemistry. Materials and General Methods. Melting points were measured on a Yanagimoto melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer FT-IR 1725X spectrophotometer and UV spectra in 95% EtOH solutions on a Hitachi 150-20 spectrophotometer. ^{1}H NMR spectra were obtained in $CDCl_3$ solutions with JEOL GSX 400 (400 MHz) and JEOL EX 270 (270 MHz) spectrometers using tetramethylsilane (δ = 0.00) or $CHCl_3$ (δ = 7.26, for TBDMS derivatives) as an internal standard, and mass spectra (MS) with a JEOL JMS-DX 303 spectrometer. Thin-layer chromatography (TLC) was performed on E. Merck precoated silica gel plates (Kieselgel 60 F_{254}). Column chromatography was conducted with silica gel (E. Merck, 70-230 mesh) or alumina (E. Merck, activity II–III, 70–230 mesh). Enones **1**,[10] **5**,[12] **10**,[10] and **23**,[27] dienones **24**[28] and **25**,[29] and 5-ene compounds **26**,[30] **27**,[31] **28**,[32] and **30**[9b] were prepared according to the literature procedures. 19-Norandrostenedione (**29**) was purchased from Sigma Chemical Co.

17β,19-Dihydroxyandrost-5-en-7-one (2). $NaBH_4$ (14 mg, 0.375 mmol) was added to a chilled solution of 17-one **1** (107 mg, 0.354 mmol) in dry MeOH (7 mL) and the reaction mixture was allowed to stand at 4 °C. When the starting material completely disappeared on the basis of TLC analysis, the reaction mixture was diluted with AcOEt (100 mL), washed with 5% HCl, 5% $NaHCO_3$ solution, and water, sequentially, and dried (Na_2SO_4). After evaporation of the solvent, a crude solid product was recrystallized from acetone to give **2** (65 mg, 60%): mp 213–214 °C; UV λ_{max} (ϵ) 241 (11 400) nm; IR (KBr) ν_{max} 3482 (OH) cm^{-1}; ^{1}H NMR (270 MHz) δ 0.82 (3H, s, 18-Me), 3.65 (1H, m, 17α-H), 3.88 and 4.07 (1H each, d, J = 11.6 Hz, 19-H_2), 5.90 (1H, s, 6-H). Anal. ($C_{19}H_{28}O_3$) C, H.

Androst-5-ene-7,17,19-trione (3). Pyridinium dichromate (PDC) (152 mg, 0.40 mmol) was added to a solution of 19-ol **1** (81 mg, 0.27 mmol) in CH_2Cl_2 (4 mL) and the reaction mixture was stirred at room temperature for 4 h and then diluted with AcOEt (100 mL), washed with 1% HCl, 5% $NaHCO_3$ solution, and water, sequentially, and dried (Na_2SO_4). After evaporation of the solvent, an oily residue was purified by preparative TLC followed by recrystallization from acetone to yield **3** (16 mg, 20%): mp 159–160.5 °C; UV λ_{max} (ϵ) 241 (9100) nm; IR (KBr) ν_{max} 1741, 1723, 1668 (C=O) cm^{-1}; ^{1}H NMR (400 MHz) δ 0.82 (3H, s, 18-Me), 6.03 (1H, d, J = 1.8 Hz, 6-H), 9.87 (1H, s, 19-H). Anal. ($C_{19}H_{24}O_3$) C, H.

Estr-5-ene-7,17-dione (4). A solution of **3** (41 mg, 0.14 mmol) in MeOH (2 mL) was added to a solution of KOH (40 mg, 0.72 mmol) in MeOH (0.5 mL) and water (0.2 mL). The mixture was stirred in an ice bath for 2.75 h. After this time, benzene (20 mL) was added to the mixture, then washed with 5% $NaHCO_3$ solution and water, and dried (Na_2SO_4). Evaporation of the solvent gave the residue which was purified by preparative TLC (hexane–AcOEt, 2/1) followed by recrystallization from acetone–hexane to afford **4** (8 mg, 21%): mp 144.5-145.5 °C; UV λ_{max} (ϵ) 237 (13 000) nm; IR (KBr) ν_{max} 1737 and 1667 (C=O) cm^{-1}; ^{1}H NMR (400 MHz) δ 0.90 (3H, s, 18-Me), 5.77 (1H, d, J = 1.8 Hz, 6-H); exact MS found 272.1767, calcd for $C_{18}H_{24}O_2$ 272.1776.

Reaction of 3β,17β-Diol 5 with *p*-Toluenesulfonyl Chloride. *p*-Toluenesulfonyl chloride (0.68 mg, 3.54 mmol) was added to a stirred solution of **5** (360 mg, 1.18 mmol) in pyridine (8 mL) in an ice bath. The reaction mixture was then stirred at room temperature for 12 h, poured into water, and extracted with AcOEt (100 mL × 2). The combined organic layers were washed with 5% $NaHCO_3$ solution and water, dried (Na_2SO_4), and evaporated to give an oil which was purified by silica gel column chromatography (hexane–AcOEt, 8/1) to yield three products, **6**–**8**, each as an oil.

3β-[(*p*-Tolylsulfonyl)oxy]-17β-hydroxyandrost-5-en-7-one (6): yield 25%; ^{1}H NMR (270 MHz) δ 0.75 (3H, s, 18-Me), 1.18 (3H, s, 19-Me), 2.46 (3H, s, Ph-*Me*), 3.65 (1H, t, J = 7.8 Hz, 17α-H), 4.38 (1H, br m, 3α-H), 5.61 (1H, d, J = 2.0 Hz, 6-H), 7.35 and 7.80 (2H each, d, J = 8.1 Hz, aromatic protons); exact MS found 458.2127, calcd for $C_{26}H_{34}SO_5$ 458.2127.

17β-[(*p*-Tolylsulfonyl)oxy]-3β-hydroxyandrost-5-en-7-one (7) was obtained from the more polar fraction than that of **5** (20%): ^{1}H NMR (270 MHz) δ 0.82 (3H, s, 18-Me), 1.19 (3H, s, 19-Me), 2.45 (3H, s, Ph-*Me*), 3.65 (1H, br m, 3α-H), 4.29 (1H, dd, J = 7.6 and 8.3 Hz, 17α-H), 5.68 (1H, d, J = 1.7 Hz, 6-H), 7.38 and 7.78 (2H each, d, J = 8.0 Hz, aromatic protons); exact MS found 458.2090, calcd for $C_{26}H_{34}SO_5$ 458.2127.

3β,17β-Bis[(*p*-tolylsulfonyl)oxy]androst-5-en-7-one (8) was obtained from the less polar fraction than that of **5** (35%): ^{1}H NMR (270 MHz) δ 0.80 (3H, s, 18-Me), 1.15 (3H, s, 19-Me), 2.44 and 2.46 (3H each, s, Ph-*Me*), 4.27 (1H, dd, J = 8.6 and 9.0 Hz, 17α-H), 4.37 (1H, br m, 3α-H), 5.59 (1H, d, J = 1.6 Hz, 6-H), 7.31–7.36 and 7.75–7.80 (4H each, m, aromatic protons); exact MS found 612.2277, calcd for $C_{33}H_{40}S_2O_7$ 612.2216.

17β-Hydroxyandrost-5-en-7-one (9). Zn powder (0.25 g, 3.8 mmol), NaI (0.27 g, 1.8 mmol), and water (0.86 mL) were added to a solution of **6** (190 mg, 0.41 mmol) in ethylene glycol dimethyl ether (6 mL) and the mixture was heated under reflux for 6 h. After this time, the solid material was removed by filtration and washed with AcOEt (100 mL). The combined filtrates were washed with 5% $NaHCO_3$ solution and water, dried (Na_2SO_4), and evaporated. The residue was subjected to silica gel column chromatography (hexane–AcOEt, 4/1) and the crude product that eluted was recrystallized from acetone to afford **9** (20 mg, 17%): mp 139–140 °C; UV λ_{max} (ϵ) 239 nm (13 800); IR (KBr) ν_{max} 3431 (OH), 1665 (C=O) cm^{-1}; ^{1}H NMR (270 MHz) δ 0.77 (3H, s, 18-Me), 1.20 (3H, s, 19-Me), 3.66 (1H, t, J = 8.3 Hz, 17α-H), 5.67 (1H, d, J = 1.7 Hz, 6-H). Anal. ($C_{19}H_{28}O_2$) C, H.

19-(*tert*-Butyldimethylsiloxy)androsta-3,5-diene-7,17-dione (11). A solution of 3β-tosylate **10** in *N*-methylpyrrolidone (4 mL) was heated under reflux for 3.5 h. After adding ether (100 mL), the mixture was washed with 5% $NaHCO_3$ solution and water and dried (Na_2SO_4). An oily residue, obtained by evaporation of the solvent, was purified by silica gel column chromatography (hexane–AcOEt, 5/1) to yield two products. The less polar one was further purified by preparative TLC (hexane–AcOEt, 8/1) to give **11** (128 mg, 65%) as an oil: UV λ_{max} (ϵ) 280 nm (17 000); IR (neat) ν_{max} 1741 and 1659 (C=O) cm^{-1}; ^{1}H NMR (400 MHz) δ −0.02 and 0.01 (3H each, s, OSiMe_2), 0.85 (9H, s, 19-OSiMe_2CMe_3), 0.94 (3H, s, 18-Me), 3.75 and 3.78 (1H each, d, J = 10.7 Hz, 19-H_2), 5.77 (1H, s, 6-H), 6.15 (2H, m, 3-H and 4-H); exact MS found 414.2624, calcd for $C_{25}H_{38}O_3Si$ 414.2590.

19-Hydroxyandrosta-3,5-diene-7,17-dione (12). The more polar product obtained from **10** was recrystallized from AcOEt to yield **12** (28 mg, 20%): mp 186–186.5 °C; UV λ_{max} (ϵ) 280 nm (17 400); IR (KBr) ν_{max} 3400 (OH), 1740 and 1645 (C=O) cm^{-1}; ^{1}H NMR (400 MHz) δ 0.97 (3H, s, 18-Me), 3.84 and 3.87 (1H each, d, J = 11.7 Hz, 19-H_2), 5.84 (1H, s, 6-H), 6.19 (2H, m, 3-H and 4-H). Anal. ($C_{19}H_{24}O_3$) C, H.

Compound **12** was also obtained by hydrolysis of the 19-silyl ether **11** as follows: a mixture of **11** (202 mg, 0.49 mmol), (n-Bu)$_4$NF (370 mg, 1.42 mmol), and THF (2.2 mL) was stirred at room temperature for 2 h and then poured into water. After extraction with AcOEt (50 mL × 2), the combined organic layers were washed with 5% $NaHCO_3$ solution and water, dried (Na_2SO_4), and evaporated to give an oil which was subjected to silica gel column chromatography (hexane–AcOEt, 3/1) and a subsequent recrystallization from AcOEt to afford **12** (75 mg, 51%): mp 186–187 °C. This was identical with the authentic sample synthesized above in every respect.

Androst-3,5-diene-7,17,19-trione (13). Compound **12** (75 mg, 0.25 mmol) was oxidized with PDC (140 mg, 0.37 mmol) essentially as described in the synthesis of **3** (CH_2Cl_2, 3.7 mL; reaction time, 5 h). The crude product was purified by silica gel column chromatography (hexane–AcOEt, 4/1) followed by recrystallization from AcOEt to yield **13** (22 mg, 30%): mp 161–162 °C; UV λ_{max} (ϵ) 281 nm (21 400); IR (KBr) ν_{max} 1734, 1717, and 1654 (C=O) cm^{-1}; ^{1}H NMR (400 MHz) δ 0.86 (3H, s, 18-Me), 5.98 (1H, s, 6-H), 6.28 (2H, m, 3-H and 4-H), 9.80 (1H, s, 19-H). Anal. ($C_{19}H_{22}O_3$) C, H.

Estra-3,5-diene-7,17-dione (14). To a mixture of KOH (50 mg, 0.89 mmol), water (0.2 mL), and MeOH (3.1 mL) was added **13** (50 mg, 0.17 mmol) at 0 °C and then the reaction mixture was stirred at 15 °C for 4 h. After addition of benzene (20 mL), the mixture was washed with water until neutral and dried (Na_2SO_4). Evaporation of the solvent gave a solid which was subjected

to silica gel column chromatography (hexane–AcOEt, 6/1) in which the product that eluted was recrystallized from benzene–ether to afford **14** (18 mg, 38%): mp 159–160 °C; UV λ_{max} (ϵ) 278 nm (21 800); IR (KBr) ν_{max} 1737 and 1654 (C=O) cm^{-1}; ^{1}H NMR (270 MHz) δ 0.93 (3H, s, 18-Me), 5.74 (1H, s, 6-H), 6.21 (1H, dd, J = 9.7 and 1.8 Hz, 4-H), 6.29 (1H, m, 3-H). Anal. ($C_{18}H_{22}O_2$) C, H.

3β-[(*p*-Tolylsulfonyl)oxy]-19-(*tert*-butyldimethylsiloxy)-androst-5-en-17-one (16). 3β-Alcohol **15** (1.08 g, 2.58 mmol) was tosylated with *p*-toluenesulfonyl chloride (1.38 g, 7.2 mmol) essentially as described in the tosylation of **5** (pyridine, 10 mL; reaction time, 22 h). Silica gel column chromatography (hexane–AcOEt, 8/1) of the product and a subsequent recrystallization from acetone yielded **16** (1.12 g, 76%): mp 139–141 °C: IR (KBr) ν_{max} 1740 (C=O), 1361 (S=O) cm^{-1}; ^{1}H NMR (270 MHz) δ 0.005 and 0.02 (3H each, s, 19-OSi*Me*$_2$), 0.84 (9H, s, 19-OSiMe$_2$C*Me*$_3$), 0.88 (3H, s, 18-Me), 2.45 (3H, s, 3-SO$_2$Ph*Me*), 3.54 and 3.70 (1H each, d, J = 10.5 Hz, 19-H$_2$), 4.32 (1H, br m, 3α-H), 5.55 (1H, br s, 6-H), 7.33 (2H, dd, J = 8.6 and 0.7 Hz, aromatic protons), 7.79 (2H, dd, J = 8.0 and 1.7 Hz, aromatic protons). Anal. ($C_{32}H_{48}O_5$-SiS) C, H.

19-(*tert*-Butyldimethylsiloxy)androst-5-en-17-one (17). Compound **16** (1.5 g, 2.6 mmol) was treated with Zn powder (2.0 g, 30.6 mmol) and NaI (2.1 g, 14 mmol) similarly as described in the synthesis of **8** (ethylene glycol dimethyl ether, 45 mL; water, 5.4 mL; reaction time, 3.5 h). The oily product was purified by silica gel column chromatography (hexane–AcOEt, 8/1) and recrystallization from acetone to give **17** (900 mg, 86%): mp 85–87 °C; IR (KBr) ν_{max} 1742 (C=O) cm^{-1}; ^{1}H NMR (270 MHz) δ 0.00 and 0.01 (3H each, s, 19-OSi*Me*$_2$), 0.85 (9H, s, 19-OSiMe$_2$C*Me*$_3$), 0.89 (3H, s, 18-Me), 3.58 and 3.79 (1H each, d, J = 10.9 Hz, 19-H$_2$), 5.48 (1H, m, 6-H). Anal. ($C_{25}H_{42}O_2Si$) C, H.

19-Hydroxyandrost-5-en-17-one (18). 3 M HCl (16 mL) was added to a solution of **17** (810 mg, 2.02 mmol) in propa-2-ol (25 mL) and THF (16 mL). The reaction mixture was stirred at room temperature for 12 h, neutralized by adding $NaHCO_3$, diluted with AcOEt (300 mL), washed with water, and dried (Na_2SO_4). Evaporation of the solvent afforded a solid which was recrystallized from acetone to yield **18** (486 mg, 84%): mp 120–124 °C; IR (KBr) ν_{max} 3500 (OH), 1740 (C=O) cm^{-1}; ^{1}H NMR (400 MHz) δ 0.94 (3H, s, 18-Me), 3.59 and 3.89 (1H each, d, J = 11.2 Hz, 19-H$_2$), 5.72 (1H, m, 6-H). Anal. ($C_{19}H_{28}O_2{}^1/_2H_2O$) C, H.

Androst-5-ene-17β,19-diol (19). $NaBH_4$ (28 mg, 0.74 mmol) was added to a solution of **18** (130 mg, 0.45 mmol) in MeOH (6 mL) at 0 °C and the mixture was allowed to stand at 0 °C for 2 h. After addition of two drops of AcOH, the solvent was removed and the residue was dissolved in AcOEt (50 mL), washed with 5% $NaHCO_3$ solution and water, and dried (Na_2SO_4). Evaporation of the solvent gave a solid which was recrystallized from AcOEt–ether to yield **19** (73 mg, 56%): mp 137–138 °C; IR (KBr) ν_{max} 3430 cm^{-1}; ^{1}H NMR (270 MHz) δ 0.82 (3H, s, 18-Me), 3.59 (1H, d, J = 11.2 Hz, 19-Ha), 3.64 (1H, t, J = 8.4 Hz, 17α-H), 3.86 (1H, d, J = 11.2 Hz, 19-Hb), 5.68 (1H, t, J = 2.6 Hz, 6-H). Anal. ($C_{19}H_{30}O_2$) C, H.

Androst-5-ene-17,19-dione (20). Jones reagent was added dropwise to a solution of **18** (109 mg, 0.38 mmol) in acetone (6 mL) at 0 °C with stirring until the orange color of the reagent remains, and the mixture was stirred for 3 min. After this time, the mixture was poured into water and the product was extracted with AcOEt (50 mL × 2). The combined organic layers were washed with 5% $NaHCO_3$ solution and water, dried (Na_2SO_4), and evaporated to give a solid which was recrystallized from acetone to afford **20** (51 mg, 47%): mp 100–103 °C; IR (KBr) ν_{max} 1735 and 1718 (C=O) cm^{-1}; ^{1}H NMR (270 MHz) δ 0.83 (3H, s, 18-Me), 5.78 (1H, m, 6-H), 9.71 (1H, d, J = 1.0 Hz, 19-H). Anal. ($C_{19}H_{26}O_2$) C, H.

16α-Bromoandrost-5-en-17-one (22). A mixture of 17-one **21** (150 mg, 0.55 mmol), $CuBr_2$ (370 mg, 1.66 mmol), and dry MeOH (6 mL) was heated under reflux for 3 h. After this time, the cooled reaction mixture was poured into water (50 mL) and extracted with AcOEt (50 mL × 2). The combined organic layers were washed with 5% $NaHCO_3$ solution and water and dried (Na_2SO_4). A solid obtained after evaporation of the solvent was recrystallized from MeOH to give **22** (54 mg, 29%): mp 177–179 °C; IR (KBr) ν_{max} 1737 (C=O) cm^{-1}; ^{1}H NMR (400 MHz) δ 0.92 (3H, s, 18-Me), 1.02 (3H, s, 19-Me), 4.54 (1H, m, 16β-H), 5.29 (1H, d, J = 5.5 Hz, 6-H). Anal. ($C_{19}H_{27}OBr$) C, H.

Biochemical Studies. Chemicals. [1β-^{3}H]Androstenedione (24.1 Ci/mmol) (^{3}H distribution: β/α = 69.8/30.2) was purchased from New England Nuclear Corp. (Boston, MA) and NADPH from Kohjin Co., Ltd. (Tokyo, Japan).

Enzyme Preparation. Human placental microsomes (particles sedimenting at 105000g for 60 min) were obtained by the method reported by Ryan.[33] They were washed once with 0.05 mM dithiothreitol solution, lyophilized, and stored at −20 °C. No significant loss of activity occurred for 2 months.

Aromatase Assay Procedure. Aromatase activity was measured according to the procedure of Thompson and Siiteri.[17] The screening assay and time-dependent assay procedure are principally the same as those described in our previous work.[15] Briefly, 20 μg of protein from the lyophilized microsomes and 20-min incubation time for the screening assay and 20 μg of protein from the microsomes and 5-min incubation time for the kinetic assay, respectively, were employed in this study, and the assays were carried out in 67 mM phosphate buffer in the presence of NADPH in air. In the time-dependent inactivation experiment, $^1/_{20}$ of the incubation mixture was used for assay of the remaining aromatase activity.

Acknowledgment. This work was supported in part by a Grant-in-Aid for Scientific Research from The Ministry of Education, Science and Culture of Japan. We are grateful to Teikoku Hormone MFG. Co. for financial support and to Dr. Hideo Imaizumi of Imaizumi Hospital, Sendai, for generously supplying human term placenta.

References

(1) (a) Thompson, E. A. Jr.; Siiteri, P. K. The involvement of Human Placental Microsomal Cytochrome P-450 in Aromatization. *J. Biol. Chem.* **1974**, *249*, 5373–5378. (b) Kellis, J. Jr.; Vickery, L. E. Purification and Characterization of Human Placental Aromatase Cytochrome P-450. *J. Biol. Chem.* **1987**, *262*, 4413–4420. (c) Corbin, C. J.; Graham-Lorence, S.; McPhaul, M.; Mason, J. I.; Mendelson, C. R.; Simpson, E. R. Isolation of a Full-Length cDNA Insert Encoding Human Aromatase System Cytochrome P-450 and Its Expression in Nonsteroidogenic Cells. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 8948–8952. (e) Yoshida, N.; Osawa, Y. Purification of Human Placental Aromatase Cytochrome P-450 with Monoclonal Antibody and Its Characterization. *Biochemistry* **1991**, *30*, 3003–3010.

(2) Thompson, E. A.; Siiteri, P. K. Utilization of Oxygen and Reduced Nicotinamide Adenine Dinucleotide Phosphate by Human Placental Microsomes During Aromatization of Androstenedione. *J. Biol. Chem.* **1974**, *249*, 5364–5372. Meyer, A. S. Conversion of 19-Hydroxy-4-androstene-3,17-dione to Estrone by Endocrine Tissue. *Biochim. Biophys. Acta* **1955**, *17*, 441–442. Arigoni, D.; Battaglia, R.; Akhtar, M.; Smith, T. Stereospecificity of Oxidation at C-19 in Oestrogen Biosynthesis. *J. Chem. Soc. Chem. Commun.* **1975**, 185–187. Caspi, E.; Arunachalam, Y.; Nelson, P. A. Biosynthesis of Estrogens: Aromatization of (19R)-, (19S)-, and (19RS)-[19-^{3}H,^{2}H,^{1}H]-3β-hydroxyandrost-5-en-17-one by Human Placental Aromatase. *J. Am. Chem. Soc.* **1986**, *108*, 1847–1852. Akhtar, M.; Calder, M. R.; Corina, D. L.: Wright, J. N. Mechanistic Studies on C-19 Demethylation in Oestrogen Biosynthesis. *Biochem. J.* **1982**, *201*, 569–580. Akhtar, M.; Corina, D.; Pratt, J.; Smith, T. Studies on the Removal of C-19 in Oestrogen Biosynthesis Using $^{18}O_2$. *J. Chem. Soc. Chem. Commun.* **1976**, 854–856. Bednarski, P. J.; Nelson, S. D. Dissociation of 19-Hydroxy, 19-Oxo-, and Aromatizing Activities in Human Placental Microsomes Through the Use of Suicide Substrate to Aromatase. *J. Steroid Biochem.* **1989**, *32*, 309–316. Cole, P. A.; Robinson, C. H. Mechanism and Inhibition of Cytochrome P-450 Aromatase. *J. Med. Chem.* **1990**, *33*, 2933–2944.

(3) Hahn, E. F.; Fishman, J. Immunological Probe of Estrogen Biosynthesis. *J. Biol. Chem.* **1984**, *259*, 1689–1694. Caspi, E.; Dharmaratne, H. R. W.; Roitman, E.; Shakleton, C. Estrogen Biosynthesis: 2β-Hydroxy-19-oxoandrost-4-ene-3,17-dione Revisited. *J. Chem. Soc. Perkin Trans. 1* **1993**, 1191–1195.

(4) Ganguly, M.; Cheo, K. L.; Brodie, H. J. Estrogen Biosynthesis and 1β-Hydroxylation Using C_{19} and 19-Nor Steroids Precursors. *Biochim. Biophys. Acta* **1976**, *431*, 326–334. Gaillard, J.-L.; Silberzahn, P. Aromatization of 19-Norandrogens by Equine Testicular Microsomes. *J. Biol. Chem.* **1987**, *262*, 5717–5722.

(5) Townsley, J. D.; Brodie, H. J. Studies on the Mechanism of Estrogen Biosynthesis. III. The Stereochemistry of Aromatization of C_{19} and C_{18} Steroids. *Biochemistry* **1968**, *7*, 33–40.

(6) Nambara, T.; Anjyo, T.; Hosoda, H. The Stereochemistry of Enzymatic Aromatization of 19-Norsteroids. *Chem. Pharm. Bull.* **1972**, *20*, 853–854.

(7) Hervey, H. A.; Lipton, A.; Santen, R. J. Aromatase: New Perspectives for Breast Cancer. *Cancer Res. Suppl.* **1982**, *42*, 3261s–3269s. Brodie, A. M. H.; Coombes, R. C.; Dowsett, M. Aromatase Inhibitors: Their Biochemistry and Clinical Potential. *J. Steroid Biochem.* **1987**, *27*, 899–903. Henderson, D. Aromatase Inhibitors: Basic and Clinical Studies. *J. Steroid Biochem.* **1987**, *27*, 905–914. Covey, D. F. Aromatase Inhibitors: Specific Inhibitors of Oestrogen Biosynthesis. In *Steroid Biosynthesis Inhibitors: Pharmaceutical and Agrochemical Aspects*: Berg, D., Plemel, M., Eds.: Ellis Horwood Ltd.: Chichester, England, 1988; pp 534–571. Janssen, P. A. J. Is there a Case for P-450 Inhibitors in Cancer Treatment? *J. Med. Chem.* **1989**, *32*, 2231–2239. Banting, L.; Nicholls, P. J.; Shaw, M. A.; Smith, H. J. Recent Developments in Aromatase Inhibition as a Potential Treatment of Estrogen-Dependent Breast Cancer. In *Progress in Medicinal Chemistry*; Ellis, G. P., West, G. B. Eds.; Elsevier Science Publishers, B. V.: Amsterdam, 1989; Vol. 26, pp 253–298. Brodie, A. M. H.; Banks, P. K.; Inkster, S. E.; Dowsett, M.; Coombes, R. C. Aromatase Inhibitors and Hormone-Dependent Cancers. *J. Steroid Biochem. Mol. Biol.* **1990**, *37*, 327–333. Bossche, H. V. Inhibitors of P-450-Dependent Steroid Biosynthesis: From Research to Medical Treatment. *J. Steroid Biochem. Mol. Biol.* **1992**, *43*, 1003–1021.

(8) Numazawa, M.; Mutsumi, A.; Tsuji, M. 3β-Hydroxyandrost-4-en-6-one Derivativs as Aromatase Inhibitors. *Steroids* **1989**, *54*, 299–311.

(9) (a) Numazawa, M.; Mutsumi, A. 6α,7α-Cyclopropane Derivatives of Androst-4-ene: A Novel Class of Competitive Aromatase Inhibitors. *Biochem. Biophys. Res. Commun.* **1991**, *177*, 401–406. (b) Numazawa, M.; Mutsumi, A.; Hoshi, K.; Koike, R. 19-Hydroxy-4-androsten-17-one: Potential Competitive Inhibitor of Estrogen Biosynthesis. *Biochem. Biophys. Res. Commun.* **1989**, *160*, 1009–1014. (c) Numazawa, M.; Mutsumi, A.; Hoshi, K.; Oshibe, M.; Ishikawa, E.; Kigawa, H. Synthesis and Biochemical Studies of 16- or 19-Substituted Androst-4-enes as Aromatase Inhibitors. *J. Med. Chem.* **1991**, *34*, 2496–2504.

(10) Numazawa, M.; Mustumi, A.; Hoshi, K.; Tanaka, Y. Androst-5-ene-7,17-dione: A Novel Class of Suicide Substrate of Aromatase. *Biochem. Biophys. Res. Commun.* **1992**, *186*, 32–39.

(11) Heusler, K.; Kalvoda, J. Selective Functionalization of the Angular Methyl Group and Further Transformation to 19-Norsteroids. In *Organic Reactions in Steroid Chemistry*; Fried, J., Edwards, J A., Eds.: Van Nostrand Reinhold Co., New York, 1972; Vol. 2, pp 237–287.

(12) Schering, A.-G. Oxidation of Steroids of the Androstane and Pregnane Series. German Patent 873,699 [*Chem. Abstr.* **1958**, *52*, 7366*i*].

(13) Turecek, F.; Veres, K.; Kocovsky, P.; Pouzar, V.; Fajkos, J. Deuterium and Tritium Labeling with the Zinc-Sodium Iodide Method. *J. Org. Chem.* **1983**, *48*, 2233–2237.

(14) Henbest, H. B.; Jackson, W. R. The Use of Aprotic Solvents for Nucleophilic Substitution Reactions at C(3) and C(17) in Steroids. *J. Chem. Soc.* **1962**, 954–959.

(15) Numazawa, M.; Mutsumi, A.; Hoshi, K.; Kigawa, H.; Oshibe, M. A. Time-Dependent Inactivation of Aromatase by 19-Oxygenated Androst-4-ene-3,6,17-triones. *J. Steroid Biochem. Mol. Biol.* **1991**, *39*, 959–966.

(16) Numazawa, M.; Nagoaka, M.; Osawa, Y. Stereospecific Synthesis of 16α-Hydroxy-17-oxo Steroids by Controlled Alkaline Hydrolysis of Corresponding 16-Bromo 17-Ketones and Its Reaction Mechanism. *J. Org. Chem.* **1982**, *47*, 4024–4029.

(17) Siiteri, P. K.; Thompson, E. A. Human Placental Aromatase. *J. Steroid Biochem.* **1975**, *6*, 317–372.

(18) Kitz, R.; Wilson, I. B. Effects of Metanesulfonic Acid as Irreversible Inhibitors of Acetylcholine Esterase. *J. Biol. Chem.* **1962**, *237*, 3245–3249.

(19) (a) Laughton, C. A.; Zvelebil, M. J. J. M.; Niedle, S. A. Detailed Molecular Model for Human Aromatase. *J. Steroid Biochem. Mol. Biol.* **1993**, *44*, 399–407. (b) Oh, S. S.; Robinson, C. Mechanism of Human Placental Aromatase: A New Active Site Model. *J. Steroid Biochem. Mol. Biol.* **1993**, *44*, 389–397.

(20) (a) Schwarzel, W. C.; Kruggel, W. G.; Brodie, H. J. Studies on the Mechanism of Estrogen Biosynthesis VIII. The Development of Inhibitors of the Enzyme System in Human Placenta. *Endocrinology* **1973**, *92*, 866–880. (b) Brodie, A. M. H.; Schwarzel, W. C.; Brodie, H. J. Studies on the Mechanism of Estrogen Biosynthesis in the Rat Ovary-1. *J. Steroid Biochem.* **1976**, *7*, 787–793. (c) Sherwin, P. F.; McMullan, P. C.; Covey, D. F. Effects of Steroid D-Ring Modification on the Suicide Inactivation and Competitive Inhibition of Aromatase by Analogues of Androst-1,4-diene-3,17-dione. *J. Med. Chem.* **1989**, *32*, 651–658.

(21) Numazawa, M.; Konno, T.; Furihata, R.; Ishikawa, S. Determination of Aromatization of 19-Oxygenated 16α-Hydroxyandrostenedione with Human Placental Microsomes by High-Performance Liquid Chromatography Coupled with Coulometric Detection. *J. Steroid Biochem.* **1990**, *36*, 369–375.

(22) Numazawa, M.; Midzuhashi, K.; Nagaoka, M. Metabolic Aspects of the 1β-Proton and the 19-Methyl Group of Androstene-3,6,17-trione during Aromatization by Placental Microsomes and Inactivation of Aromatase. *Biochem. Pharmacol.* **1994**, *47*, 717–726.

(23) Silverman, R. B. Rate Constant and Dissociation Constant Terminology. In *Mechanism-Based Enzyme Inactivator; Chemistry and Enzymology*, Silverman, R. B. Ed., CRC Press; Boca Raton, FL, 1988; Vol. 1, pp 4–5.

(24) Cole, P. A.; Bean, J. A.; Robinson, C. H. Conversion of a 3-Deoxysteroid to 3-Deoxyestrogen by Human Placental Aromatase. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 2999–3003.

(25) Fishman, J. Biochemical Mechanism of Aromatization. *Cancer Res. Suppl.* **1982**, *42*, 3277s-3280s. Dintinger, T.; Gaillard, J.-L.; Moslemic, S.; Zwain, I.; Silberzahn, P. Androgen and 19-Norandrogen Aromatization by Equine and Human Placental Microsomes. *J. Steroid Biochem.* **1989**, *33*, 949–954.

(26) Ranjith, H.; Dharmarantne, W.; Kilgone, J. L.; Roitman, E.; Schackleton, C.; Caspi, E. Biosynthesis of Estrogens. Estr-5(10)-ene-3,17-dione: Isolation, Metabolism and Mechanistic Implications. *J. Chem. Soc. Perkin Trans. 1* **1993**, 1529–1535.

(27) Rubin, M.; Hipps, G. E.; Glover, D. Photodimerization of $\Delta^{4,5}$-Diene-3-keto Steroids. *J. Org. Chem.* **1964**, *29*, 68–74.

(28) Billeter, J. R.; Mieschner, K. Steroids 78. Degradation Product of Sterol Oxydation IV. Isolation of $\Delta^{3,5}$-Androstadiene-7,17-dione. *Helv. Chim. Acta* **1948**, *31*, 629–632.

(29) Weintraub, P. M.; Tiernan, P. L.; Benson, H. D.; Grunwell, J. F.; Johnston, J. O.; Petrow, V. Steroidal 3,5-Dienes. *J. Med. Chem.* **1976**, *19*, 1395–1399.

(30) Marker, R. E.; Wittle, E. L.; Tullar, B. F. Sterols. LXXXVI. Desoxotestosterone and Its Conversion to Testosterone. *J. Am. Chem. Soc.* **1940**, *62*, 223–226.

(31) Nambara, T.; Kato, M. Analytical Chemical Studies on Steroids. VII. The Zimmermann Complexes Derived from *i*-Androstanolone and Its Related 17-Oxosteroids. *Chem. Pharm. Bull.* **1965**, *13*, 1435–1439.

(32) Blickenstaff, R. T.; Foster, E. L. Seroflocculants in the Androstane Series. *J. Org. Chem.* **1961**, *26*, 5029-5032.

(33) Ryan, K. J. Biological Aromatization of Steroids. *J. Biol. Chem.* **1959**, *234*, 268–272.