C(10)–C(19) Bond Cleavage Reaction of 19-Oxygenated Androst-4-ene-3,6-dione Steroids under Various Conditions

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C(10)–C(19) bond cleavage reaction of 19-hydroxy- and 19-oxoandrost-4-ene-3,6,17-triones (5, 6) was explored under various conditions. Treatment of steroids 5 and 6 with KOH in MeOH gave the A-ring aromatized product 6-oxoestrone (11) in a fair yield, respectively, in contrast, the treatment with a weak base yielded 4-methyl steroid 17 (20%) in the case of 19-alcohol 5 or 19-nor-$\Delta^{5(10)}$-steroid 9 (12—67%) along with compound 11 (6—27%) in the case of 19-aldehyde 6. Reaction of compound 6 with HCl in MeOH produced 3-methyl ethers of 6-oxoestrone and Δ^{6}-estrone, compounds 12 and 14 (*ca.* 20% each). Thus, 6-oxosteroids 5 and 6 showed unique C(10)–C(19) bond cleavage reactions with a base or acid.

Key words aromatase inhibitor; 6-oxoandrostenedione; 19-oxygenated steroid; C(10)–C(19) bond cleavage

The androgen androst-4-ene-3,17-dione (androstenedione, **1**) is converted into the estrogen estrone (**10**) through its 19-hydroxy and 19-oxo derivatives (**2**, **3**) by action of the enzyme aromatase (Fig. 1).[1] 6-Oxoandrostenedione (**4**) that is clinically used for treatment of estrogen-dependent breast cancer, is one of the earliest discovered suicide substrates of aromatase.[2—4] The mechanism for aromatase inactivation by inhibitor **4** involves the two initial hydroxylations at the C-19 position, producing the 19-oxo derivative **6** through the 19-hydroxy intermediate **5**, followed by an epoxidation of the 4,5-double bond.[5,6] In this sequence, a part of the 19-oxo intermediate is aromatized to yield 6-oxoestrone (**11**).[7] On the other hand, we previously have reported that treatment of the 19-oxygenated steroids with a nucleophile, a thiol compound, gives the corresponding 1,4-Michael adduct, a 4α-thio analog, respectively, where 5(10)-ene steroid **9**, the C(10)–C(19) bond cleavage reaction product, is also obtained from the 19-oxo steroid **6** in a low yield.[8] The 1,4-addition reaction does not occur in the reaction with corresponding 6-deoxy steroids **2** and **3**.

Taken together, we were of interest to know effect of introducing a carbonyl group at C-6 of the 19-oxygenated steroids **2** and **3** on the C(10)–C(19) bond cleavage reaction in relation to the biological aromatization. This paper describes reactions of 19-oxygenated 6-oxo steroids **5** and **6** under basic and acidic conditions.

Results and Discussion

We initially examined the C(10)–C(19) bond cleavage of 19-hydroxy-6-oxo steroid **5** under a basic condition. Treatment of 19-ol **5** with a strong base, KOH, in MeOH at room temperature yielded the aromatized product 6-oxoestrone (**11**) in 65% yield (Fig. 2). In contrast, the treatment with a weak base, $NaHCO_3$, in MeOH under reflux produced 4-methyl-6-oxoestrone (**17**) in 20% yield along with the recovered substrate. Although there is no evidence, the production mechanism of compound **17** is thought as follows: migration of the 10β-hydroxy methyl group to the 4β-position, yielding 4β-hydroxymethyl-5(10)-ene-3,6-dione intermediate (**15**), followed by dehydration and a sequential isomerization of the C-4 exocyclicmethylene double bond introduced may give the aromatized product **17** (Fig. 2). It is known that in the reaction of a 19-hydroxy steroid having no carbonyl group at C-6, compound **2**, with the strong base, the aromatized product is not formed but a 19-nor derivative, 4-ene[9] or 5(10)-ene steroid **7**,[10] is only produced, indicating that the C-6 carbonyl group plays a critical role in the base-catalyzed aromatization reaction. Treatment of the 19-ol **2** with $NaHCO_3$ yielded neither the 19-nor steroid **7** nor the aromatized product **10**.

The bond cleavage reaction of 19-al **6** was next studied under various conditions (Table 1). Treatment of compound **6** with a weak base, $NaHCO_3$ or CH_3COSK, at room temperature gave 19-nor-$\Delta^{5(10)}$-compound **9** as the major product (56 or 67%) as well as estrogen **11** (13 or 6%). In the reaction

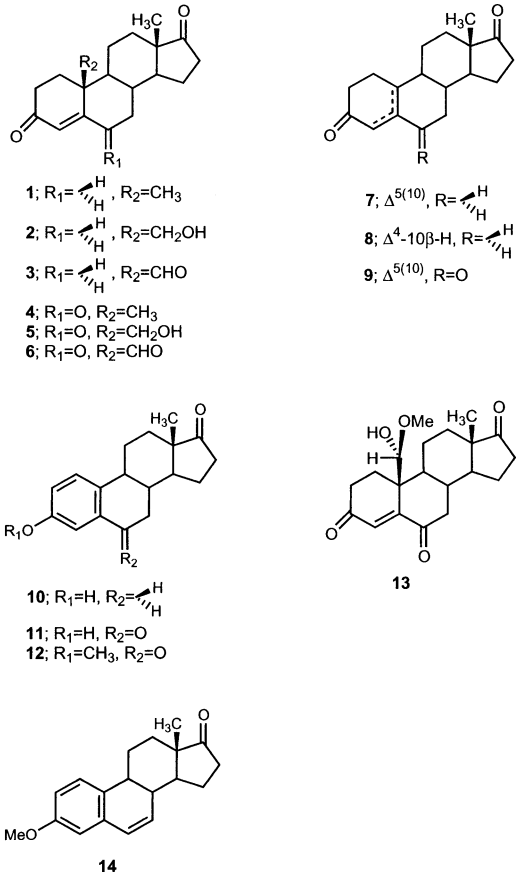

Fig. 1. Structures of Steroids

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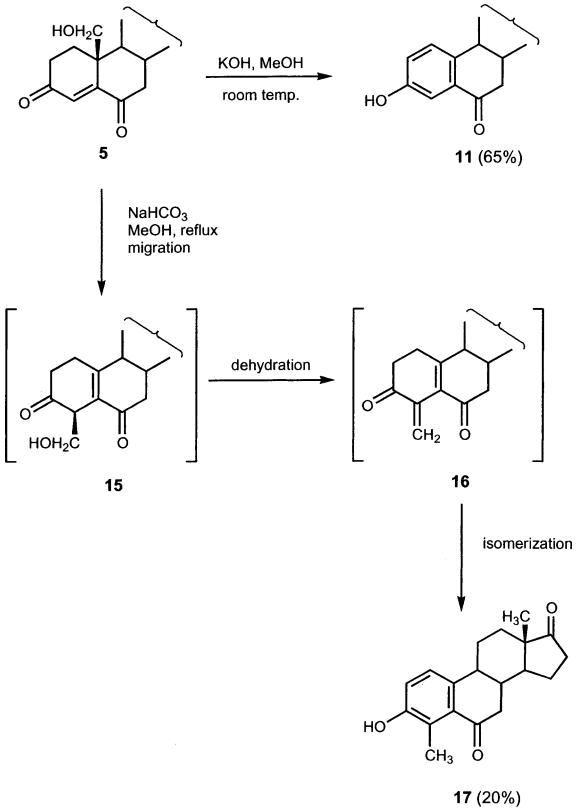

Fig. 2. Reaction of 19-Hydroxy Steroid **5** with a Base

Table 1. C(10)–C(19) Bond Cleavage of 19-Oxo Steroid **6** Under Various Conditions

Entry	Conditions	Product, %		
		Aromatized, **11**, **12**, or **14**	$\Delta^{5(10)}$ **9**	Hemiacetal **13**
1	$NaHCO_3$–MeOH-r.t.[a)]	23 (**11**)	56	—
2	Pyridine–H_2O-reflux	37 (**11**)	22	—
3	MeOH–H_2O-reflux	18 (**11**)	55	—
4	CH_3COSK–MeOH-r.t.	6 (**11**)	67	8
5	NaOH–MeOH-r.t.	93 (**11**)	—	—
6	HCl–MeOH-r.t.	18 (**12**), 17 (**14**)	—	—

a) r.t.: room temperature.

with CH_3COSK, 19-hemiacetal **13** (8%) was produced in addition to the two products. The 19-hydrogen atom of compound **13** was observed as a singlet peak at 4.38 ppm in the ^{1}H-NMR spectrum. This along with the ^{13}C-NMR spectrum revealed that compound **13** is not a mixture of two stereoisomers at the C-19 position. Semiemprical molecular orbital PM3 calculations indicate that the 19-carbonyl group of steroid **6** favors the over-A-ring conformation among the possible three.[11)] This suggests that the nucleophile methoxide anion would approach the carbonyl carbon from the less hindered over-A-ring side rather than the crowded over-C-ring side, thereby giving streoselectively (19*S*)-19-hemiacetal **13**, as seen in the $NaBH_4$ reaction of 19-al **3**.[12,13)] In this reaction, the 1,4-addition product 4α-acetylthio ether was not isolated, although the reaction with benzylmercaptan gives the addition product 4α-benzylthio ether as well as compound **9**.[8)] When the 19-al **6** was treated with aqueous pyridine or MeOH under reflux gave the two compounds **9** (12 or

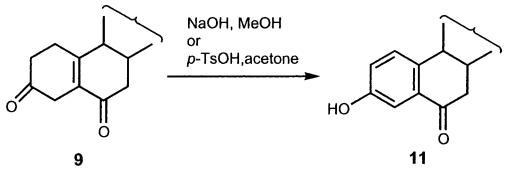

Fig. 3. Aromatization Reaction of $\Delta^{5(10)}$ Steroid **9** Under a Basic or Acidic Condition

45%) and **11** (27 or 13%) in each case. Treatment of the $\Delta^{5(10)}$-steroid **9** with NaOH in MeOH at room temperature afforded the aromatized product **11** in a good yield, suggesting that the aromatization reaction would proceed, at least in part, through the $\Delta^{5(10)}$-steroid. Based on the result, it is presumed that the conversion of 19-ol **5** into the aromatized product **11** by treatment with KOH in MeOH, described above, may proceed through 19-al **6** produced by an air-oxidation of 19-ol **5** followed by the C(10)–C(19) bond cleavage. It has previously been reported that treatment of the 6-deoxy derivative **3** with pyridine produces the $\Delta^{5(10)}$-derivative **7** but not the aromatized product **10**.[9)] The 6-oxo function would be essential for the aromatization reaction of a 5(10)-en-3-one steroid.

Reaction of 19-al **6** with HCl in MeOH at room temperature gave 3-methyl ether of the aromatized product **11**, compound **12**, as well as Δ^6-estrone 3-methyl ether (**14**) in low yields. When $\Delta^{5(10)}$-steroid **9** was treated with HCl or *p*-TsOH, the aromatized product **11** was obtained in 16% or 20% yield, but the production of compounds **2** and **14** was not detected by TLC analysis of the reaction products in each case. These results indicate that the $\Delta^{5(10)}$-steroid **9** is aromatized to produce compound **11** under not only a basic condition but also an acidic condition and this is not an intermediate in a sequence of the HCl-catalyzed production of compounds **12** and **14** from 19-al **6**. 6-Oxoestrone (**11**) was not converted into the 3-methyl ether **12** or the Δ^6-estrone analog **14** by the treatment with HCl in MeOH, indicating that the production of compound **12** or **14** does not proceed through the 6-oxo steroid **11**.

It has previously been reported that the acid-catalyzed C(10)–C(19) bond cleavage reactions of steroidal compounds having a 1,4-dien-3-one or 2,3-dihydroxy-4-ene structure produce the corresponding aromatized products *via* the dienone-phenol and related rearrangements.[16,17)] Since 19-oxygenated compounds **5** and **6** have no such structural feature, a 1,4-dien-3-one or 2,3-dihydroxy-4-ene group, required for the dienone-phenol type rearrangements, it is likely that their aromatization reactions observed in this study would not involve the rearrangements.

The present findings indicate that an introduction of the C-6 carbonyl group into 19-hydroxy and 19-oxo steroids **2** and **3** accelerates and/or gives rise to C(10)–C(19) bond cleavage reactions under acidic and basic conditions, although the exact mechanisms for the cleavage reactions are not clear.

Experimental

Melting points were measured on a Yanagimoto melting point apparatus (Kyoto, Japan) and are uncorrected. IR spectra were recorded in KBr pellets for the solid products or in neat forms for the oily products on a Perkin-Elmer FT-IR 1725X spectrophotometer (Norwalk, CT, U.S.A.), and UV spectra were determined in 95% ethanol on a Hitachi 150-20 UV spectrophotometer (Tokyo, Japan). ^{1}H- and ^{13}C-NMR spectra were obtained in

$CDCl_3$ solution with a JEOL GX 400 spectrometer (400 MHz for ^1H and 100.5 MHz for ^{13}C) (Tokyo, Japan) using tetramethylsilane as an internal standard. High-resolution mass spectra (HR-MS) were determined with a JEOL JMS-DX 303 spectrometer. Thin-layer chromatography (TLC) was performed on E. Merck precoated TLC silica gel plates (silica gel 60F-254, layer thickness 0.25 and 0.5 mm for analytical and preparation use, respectively; Darmstadt, Germany). Column chromatography was conducted with silica gel 60, 70—230 mesh (E. Merck, 70—230 mesh).

19-Hydroxyandrost-4-ene-3,6,17-trione (**5**) and androst-4-ene-3,6,17,19-tetraone (**6**) were synthesized according to the previously reported method.[6)]

Reaction of 19-Hydroxy Steroid 5 with KOH or $NaHCO_3$. (A) Reaction with KOH A solution of KOH (2.7 g, 49 mmol) in 5 ml of water was added to a solution of compound **5** (50 mg, 0.16 mmol) in 30 ml of MeOH and the mixture was stirred at room temperature for 3.5 h. After this time, the reaction mixture was neutralized with conc. HCl, then, condensed to a small volume and extracted with AcOEt (50 ml×2). The organic layer was washed with saturated NaCl solution and dried (Na_2SO_4). After evaporation of the solvent, the residue obtained was subjected to preparative TLC (hexane–AcOEt; 1 : 1). The crude product was recrystallized from acetone to give 6-oxoestrone **11** (29 mg, 65%). mp 246—248 °C (lit.[15)] mp 256—257 °C). ^1H-NMR δ 0.93 (3H, s, 18-Me), 7.10 (1H, dd, J=8.6, 2.6 Hz, 2-H), 7.33 (1H, d, J=8.6 Hz, 1-H), 7.57 (1H, d, J=2.6 Hz, 4-H).

(B) Reaction with $NaHCO_3$ A mixture compound **5** (50 mg, 0.16 mmol), $NaHCO_3$ (787 mg, 9.37 mmol) and MeOH (30 ml) was heated under reflux for 5 h. After this time, the reaction mixture was poured into water and extracted with AcOEt. The organic layer was washed with water and dried (Na_2SO_4). After evaporation of the solvent, the residue obtained was separated by preparative TLC (hexane–AcOEt; 1 : 1). The crude product was recrystallized from acetone to give compound **17** (9 mg, 20%). mp 167—171 °C (decomp.). ^1H-NMR δ 0.92 (3H, s, 18-Me), 2.53 (3H, s, 4-Me), 6.99 (1H, d, J=8.4 Hz, 2-H), 7.17 (1H, d, J=8.4 Hz, 1-H). ^{13}C-NMR δ 13.2, 13.6, 21.4, 25.5, 31.2, 35.8, 38.5, 43.0, 45.0, 47.6, 50.6, 119.5, 123.2, 126.0, 132.7, 139.8, 152.9, 199.6, 219.8. UV λ_{max} (ε); 260 (6800) and 330 (2400) nm. IR ν_{max}; 3400, 1728 and 1676 cm^{-1}. HR-MS Found: 298.1567; Calcd for $C_{19}H_{22}O_3$: 298.1565. The starting material **5** was also recovered in 20% yield.

Reaction of 19-Oxo Steroid 6 under Various Conditions. (A) A Basic Condition Compound **6** (50 mg, 0.16 mmol) in MeOH (15 ml) or pyridine (7.5 ml) was treated with a base for an appropriate time. The reaction mixture was diluted with AcOEt, washed with saturated NaCl solution and dried (Na_2SO_4). After evaporation of the solvent, the product was purified by preparative TLC (hexane–AcOEt; 1 : 1). 1) Reaction with NaOH: 1 mol/l NaOH solution (1.57 ml) was added to a solution of compound **6** in MeOH and the mixture was stirred at room temperature for 2 h. 6-Oxoestrone (**11**) (43 mg, 93%). ^1H-NMR δ 0.93 (3H, s, 18-Me), 7.10 (1H, dd, J=8.6, 2.6 Hz, 2-H), 7.33 (1H, d, J=8.6 Hz, 1-H), 7.60 (1H, d, J=2.6 Hz, 4-H). 2) Reaction with $NaHCO_3$: 13.2 mg of $NaHCO_3$ (0.16 mmol) was added to a solution of compound **6** in MeOH and the reaction mixture was stirred at room temperature for 3.75 h. Compound **11** (5.6 mg, 13%). Estr-5(10)-ene-3,6,17-trione (**9**) (25 mg, 56%), mp 166—168 °C (from acetone) (lit.[14)] mp 167—169 °C). ^1H-NMR δ 0.93 (3H, s, 18-Me), 2.98 and 3.25 (1H each, d, J=21.1 Hz, 4-H). 3) Reaction with CH_3COSK: CH_3COSK (22.5 mg, 0.197 mmol) was added to a solution of compound **6** and the mixture was stirred at room temperature for 3 h. Compound **11** (2.6 mg, 6%), mp 235—237 °C (from acetone) and compound **9** (30.6 mg, 67%), mp 166—168 °C. (19-*S*)19-Methoxy-19-hydroxyandrost-4-ene-3,6,17-trione (**13**) (4.3 mg, 8%); mp 98—101 °C. ^1H-NMR δ 0.95 (3H, s, 18-Me), 3.81 (3H, s, 19-OMe), 4.38 (1H, s, 19-CH), 6.08 (1H, d, J=0.7 Hz, 4-H). ^{13}C-NMR δ 13.8, 21.6, 21.8, 29.8, 31.4, 32.8, 35.1, 35.7, 38.1, 47.6, 50.2, 50.9, 52.8, 53.3, 71.7, 129.8, 157.8, 174.2, 198.8, 219.9. UV λ_{max} (ε): 237 (7400) nm. IR (KBr) ν_{max}; 3391, 1727 and 1684 cm^{-1}. HR-MS; 346.1826; Calcd for $C_{20}H_{26}O_5$: 346.1872. 4) Reaction with MeOH or pyridine: 2.5 ml of water was added to a solution of compound **6** in MeOH or pyridine and the mixture was heated under reflux for 9 h, giving compound **11** (12 mg, 27% or 6.0 mg, 13%) and compound **9** (5.5 mg, 12% or 20.5 mg, 45%).

(B) An Acidic Condition HCl gas was bubbled for 1 min in a solution of compound **6** (50 mg, 0.16 mmol) in 15 ml of MeOH, and the resulting reaction mixture was allowed to stand at room temperature for 2 d. After this time, the reaction mixture was diluted with AcOEt, washed with 5% $NaHCO_3$ solution, NaCl solution, and dried (Na_2SO_4). After evaporation of the solvent, the residue obtained was subjected to preparative TLC (hexane–AcOEt; 1 : 1) to give two products. The less polar product was recrystallized from acetone to give 3-methoxyestra-1,3,5(10),6-tetraen-17-one (**14**) (7.8 mg, 17%). mp 117—120 °C. ^1H-NMR δ 0.92 (3H, s, 18-Me), 3.80 (3H, s, 3-OMe), 6.07 (1H, dd, J=9.7, 1.6 Hz, 7-H), 6.52 (1H, dd, J=9.7, 2.3 Hz, 6-H), 6.67 (1H, d, J=2.8 Hz, 4-H), 6.75 (1H, dd, J=8.4, 2.8 Hz, 2-H), 7.17 (1H, d, J=8.4 Hz, 1-H). ^{13}C-NMR δ 13.6, 21.5, 23.8, 31.1, 35.7, 38.2, 42.1, 48.5, 48.8, 55.3, 111.9, 112.1, 124.3, 128.6, 130.9, 131.0, 135.3, 158.3, 220.3. UV λ_{max} (ε); 226 (27000), 262 (6200) nm. IR (KBr) ν_{max} 1736 and 1601 cm^{-1}. HR-MS Found 282.16180, Calcd for $C_{19}H_{22}O_2$ 282.16178. The more polar product was recrystallized from acetone to give 3-methoxyestra-1,3,5(10)-triene-6,17-dione (**12**) (8.4 mg, 18%). mp 134—137 °C. ^1H-NMR δ 0.93 (3H, s, 18-Me), 3.85 (3H, s, 3-OMe), 7.13 (1H, dd, J=8.4, 2.9 Hz, 2-H), 7.35 (1H, d, J=8.4 Hz, 1-H), 7.58 (1H, d, J=2.9 Hz, 4-H). ^{13}C-NMR δ 13.7, 21.3, 25.2, 31.2, 35.7, 39.6, 43.0, 43.2, 47.7, 50.2, 55.5, 109.8, 121.7, 126.6, 133.3, 138.9, 158.3, 197.3, 219.6. UV λ_{max} (ε): 222 (20000), 255 (8100), 322 (2900) nm. IR (KBr) ν_{max} 1741 and 1672 cm^{-1}. HR-MS Found; 298.15690 Calcd for $C_{19}H_{22}O_3$: 298.15590.

Reaction of $\Delta^{5(10)}$-Steroid 9. (A) A Basic Condition To a solution of **9** (50 mg, 0.175 mmol) in 2.5 ml of MeOH was added 1 mol/l NaOH solution (0.17 ml, 0.174 mmol) and the reaction mixture was stirred at room temperature for 5 h. After the same workup as described above, compound **11** (25 mg, 50%) was obtained.

(B) An Acidic Condition 1) Reaction with *p*-TsOH: *p*-TsOH monohydrate (21 mg, 0.11 mmol) was added to a solution of compound **9** (50 mg, 0.175 mmol) in 2.5 ml of acetone and the reaction mixture was stirred at room temperature for 2 d. After the same workup as described above, compound **11** (10 mg, 20%) was obtained. 2) Reaction with HCl: HCl gas was bubbled for 1 min in a solution of compound **9** (50 mg, 0.175 mmol) in 15 ml of MeOH, and the reaction mixture was allowed to stand at room temperature for 2 d. After the same workup as described above, compound **11** (8 mg, 16%) was obtained but the production of the other aromatized products **12** and **14** was not detected by the TLC analysis.

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