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-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 5(10)-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 through the 19-hydroxy intermediate, 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, 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, NaHCO₃, 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-hydroxy-5(10)-ene-3,6-diene intermediate (15), followed by dehydration and a sequential isomerization of the C-4 exocyclic methylene 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-ene9) or 5(10)-ene steroid 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 NaHCO₃ 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, NaHCO₃ or CH₃COSK, at room temperature gave 19-nor-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](image-url)
with CH₃COSK, 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 ¹H-NMR spectrum. This along with the ¹³C-NMR spectrum revealed that compound 13 is not a mixture of two stereoisomers at the C-19 position. Semiempirical 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 nuclophile 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 (19S)-19-hemiacetal 13, as seen in the NaBH₄ reaction of 19-al 3.¹²¹³ 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 9.⁵ When the 19-al 6 was treated with aqueous pyridine or MeOH under reflux gave the two compounds 9 (12 or 45%) and 11 (27 or 13%) in each case. Treatment of the Δ⁵(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 Δ⁵(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 Δ²(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 Δ⁶-estrone 3-methyl ether (14) in low yields. When Δ⁵(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 Δ⁵(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 Δ⁶-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 steroid 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.¹⁶¹⁷ 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). ¹H- and ¹³C-NMR spectra were obtained in
for 9 h, giving compound action mixture was allowed to stand at room temperature for 2 d. After this time, the mixture was neutralized with cone. 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 (Na2SO4). 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-oxo-6-oxoacetone (8 mg, 20%). mp 165—167 °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). 13C-NMR δ 13.2, 13.6, 21.4, 25.5, 31.2, 35.8, 38.5, 43.0, 47.7, 50.6, 119.5, 123.2, 126.0, 137.0, 158.3, 219.6. UV λmax (e): 222 (20000), 255 (8100), 322 (2900) nm. IR (KBr) νmax = 1741 and 1672 cm−1. HR-MS Found: 298.1560 Calcd for C19H22O2: 298.1559.

Reaction of 19-Hydroxy Steroid 5 with KOH or NaHCO3 (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 15 ml of MeOH and the mixture was stirred at room temperature for 3.5 h. After this time, the reaction mixture was diluted with AcOEt, washed with 5% NaHCO3 solution, NaCl solution, and dried (Na2SO4). 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-oxo-6-oxoacetone (11 mg, 50%). mp 165—167 °C (decomp.). 1H-NMR δ 0.93 (3H, s, 18-Me), 7.10 (1H, d, 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 NaHCO3: 13.2 mg of NaHCO3 (0.16 mmol) was added to a solution of compound 5 (50 mg, 0.175 mmol) in 2.5 ml of AcOEt and the reaction mixture was stirred at room temperature for 2 d. 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 h. 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.

References