Synthesis and Study of a Methoxyhydroperoxide–Androstenedione Derivative; Analogue of a Potential Aromatase Intermediate

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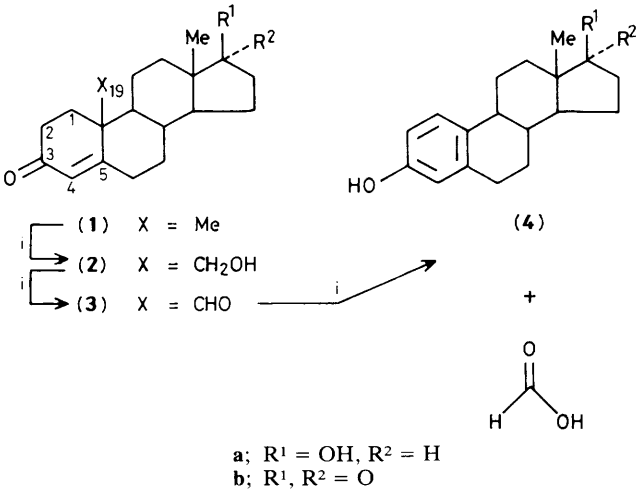
A methoxyhydroperoxide analogue (**12**) to a proposed aromatase intermediate has been synthesized by ozonolysis of methoxyvinyl compound (**7**) in the presence of methanol; it has been shown not to produce estrone with or without acetylation, but the free hydroperoxide apparently undergoes a facile, stereospecific intramolecular epoxidation.

The mechanism of aromatase has been intensively studied over the past several years. It involves the transformation of androgens [testosterone (**1a**), androstenedione (**1b**)] to estrogens [estradiol (**4a**), estrone (**4b**)] as depicted in Scheme 1.

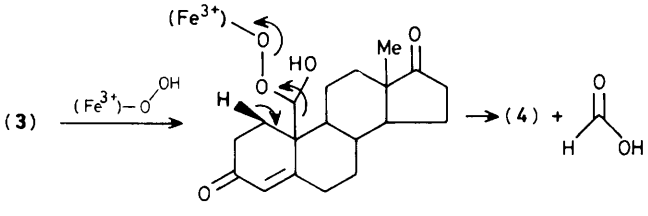
Several features of this transformation have been elucidated:[1] (i) three molar equivalents of O_2 and NADPH are required; (ii) 19-ol and 19-al, (**2**) and (**3**), serve as intermediates; (iii) the C-19 atom is lost as formic acid with the retention of one of the three original hydrogen atoms; (iv) the oxygen atoms in the formic acid are derived from the first and third equivalents of oxygen consumed; (v) the A-ring 1β,2β-hydrogens are incorporated into the aqueous medium. Although the nature of the third oxidation step has not been discovered, several mechanisms have been demonstrated to be improbable. Included among these are Baeyer–Villiger type oxidation,[2] C-2 hydroxylation of the 19-aldehyde,[1] and any hydrolytic pathway that involves incorporation of oxygen from water into formic acid. One of the most interesting proposals for the third oxidation, made by Akhtar, is the formation of a geminal hydroxy-haem iron peroxide intermediate as depicted in Scheme 2.[2d]

The iron would presumably activate the peroxide oxygen distal to C-19 enhancing its potential as a leaving group. Although 'sigmalogous' elimination is depicted, Akhtar has also proposed a hydride transfer mechanism.[2b] A similar mechanism has been suggested for 14α-demethylation in cholesterol biosynthesis.[3] In order to evaluate Akhtar's proposal, it was envisaged that an analogue of the form (**5**) might be chemically synthesized and tested as an estrone precursor. While geminal hydroxyhydroperoxides vary in stability,[4] a variety of alkoxyhydroperoxides have recently been synthesized under mild, high yield conditions and found to be quite stable.[5] Ozonolysis of an olefin in the presence of methanol results in a carbonyl at one carbon terminus and a methoxyhydroperoxide at the other. Fragmentation of the primary ozonide may occur in two modes with carbonyl oxide preferentially formed at the more highly substituted terminus,[6] and presumably the intermediate carbonyl oxide is then attacked by methanol. When the olefin (**6**)[7] was subjected to ozonolysis [4:1 CH_2Cl_2:MeOH, $NaHCO_3$(anh.)–buffer, −78 °C] regioselective cleavage of the C-19 vinyl group occurred, in preference to that at the electron-poor 4,5-double bond. Aldehyde (**3b**) was formed directly without reductive workup (53% yield), however, suggesting that primary ozonide fragmentation occurred in the undesired direction.

Based on previous studies,[8] it was supposed that the methoxyvinyl derivative (**7**) might undergo cleavage in the desired mode. Consequently, compound (**7**) was synthesized as shown in Scheme 3 from the protected aldehyde[7] (**8**) by methoxy-Wittig reaction affording (**7**) as a 1:1 mixture of double bond isomers after deprotection and Oppenauer oxidation (57% overall yield after flash chromatography).† When the 1:1 mixture of (**7**) was ozonised under the conditions described above, starting material was quickly consumed (t.l.c.). Although the reaction mixture showed strong u.v. absorption when first spotted on an analytical t.l.c. plate, no u.v. absorbing components were observed after the plate was eluted. This suggested the initial formation of an unstable adduct still containing the conjugated ketone moiety.



Scheme 1. i, NADPH, O_2.

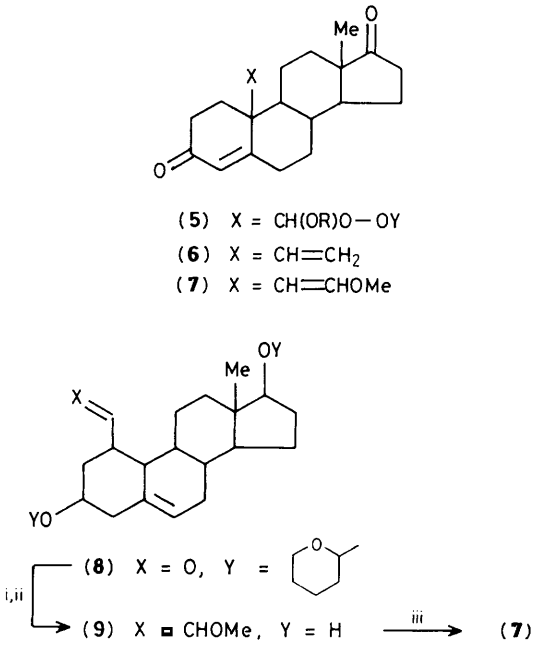


Scheme 2

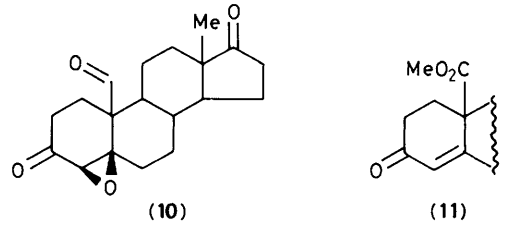
† For purposes of characterization, the *E*/*Z* diols were separated from each other by h.p.l.c. and individually oxidized. The compounds, *cis*-, *trans*-(**9**) gave appropriate ^{1}H n.m.r., mass, i.r., and u.v. data. All known compounds produced in new reactions gave appropriate ^{1}H n.m.r., mass, i.r., u.v., h.p.l.c., and t.l.c. data.

Compound *cis*-(**7**): ^{1}H n.m.r. ($CDCl_3$) δ 5.99 (1 H, d, *J* 7.2 Hz), 5.77 (1 H, s), 4.17 (1 H, d, *J* 7.2 Hz), 3.50 (3 H, s), 0.88 (3 H, s); i.r. ($CHCl_3$) 1735, 1660, 1610 cm^{-1}; u.v. (MeOH) 240 nm (ε 15 000 cm^{-1} mol^{-1} dm^3); calcd. for $C_{21}H_{28}O_3$ *m*/*z* 328.2038 found: 328.2029.

Compound *trans*-(**7**): ^{1}H n.m.r. ($CDCl_3$) δ 6.12 (1 H, d, *J* 13.0 Hz), 5.87 (1 H, s), 4.83 (1 H, d, *J* 13.0 Hz), 3.55 (3 H, s), 0.88 (3 H, s); i.r. ($CHCl_3$) 1735, 1660, 1610 cm^{-1}; u.v. (MeOH) 237 nm (ε 14 000 cm^{-1} mol^{-1} dm^3); calcd. for $C_{21}H_{28}O_3$ *m*/*z* 328.2038 found: 328.2045.



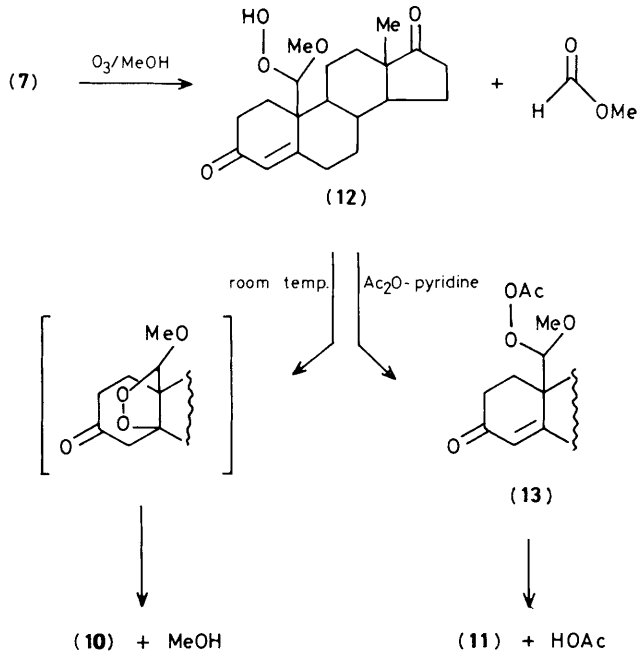
Scheme 3. *Reagents:* i, $MeOCH_2PPh_3Cl$, 1.5 M MeLi, tetrahydrofuran (THF), reflux; ii, MeOH, pyridinium toluene-*p*-sulphonate (PPTS), room temp.; iii, cyclohexanone, aluminium isopropoxide, toluene, reflux.



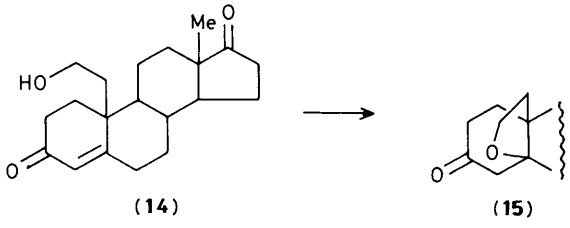
After allowing to stand at room temperature a major non-u.v. active product was isolated and proved to be 4β,5β-epoxyaldehyde[9] (**10**) (42% after flash chromatography). The 4α-isomer[9] was not present (< 2%) as judged by the ^1H n.m.r. spectrum of the crude product. In contrast, if the crude ozonolysis mixture was treated with acetic anhydride–pyridine after concentration at low temperature, the major product isolated was the methyl ester (**11**)[10] (25%) along with (**10**) (12%). In neither case was estrone (nor estrone acetate) detected by h.p.l.c.‡ Scheme 4 has been proposed to account for these results.

In the absence of acetic anhydride, the relatively unstable methoxyhydroperoxide (**12**) undergoes intramolecular Michael addition with subsequent collapse to (**10**). On the other hand, when the hydroperoxide is acetylated, the terminal oxygen is activated as a leaving group and Michael addition prevented. Thus ester (**11**) is formed. Compound (**12**) was shown to have a half-life of less than 15 minutes at room temperature.§ Variable-temperature ^1H n.m.r. spectroscopy of the ozonolysis mixture in deuteriated reaction solvent (CD_2Cl_2–CD_3OD) confirmed that methyl formate was formed. In addition, the C-4 vinyl proton signal was observed at −60 °C and gradually disappeared upon warming to room temperature with concomitant appearance of signals corresponding to epoxide (**10**). Furthermore, a crossover experiment wherein androstenedione (**1b**) was added to the ozonolysis reaction mixture at −78 °C resulted in no 4,5-epoxyandrostanedione[11] formation (<0.6% by h.p.l.c.) after warming. That formation of the epoxide (**10**) involves *intramolecular* addition of hydroperoxide (**12**) seems likely because: (i) formation of (**10**) is exceedingly rapid; (ii) the stereochemical outcome is the expected β-epoxide; (iii) an analogous Michael addition apparently occurs readily with the hydroxyethyl compound (**14**) which forms the cyclized adduct (**15**);[12] (iv) there is no crossover epoxy product upon addition of androstenedione to (**12**).

This model study suggests that removal of the 19-hydrogen of (**13**) occurs in preference to the 1β-hydrogen. A useful modification of the Akhtar mechanism might therefore involve enolization of the 3-ketone prior to elaboration of the 19-aldehyde (**3**). A driving force for aromatization would facilitate a 1β-proton abstraction. Alternatively, hydride transfer could be assisted by participation of the enol π-system.



Scheme 4



‡ The limits of detection of estrone–estrone acetate by h.p.l.c. allow for an upper limit of 0.3% yield (total estrone plus estrone acetate) under the acetylating conditions and 0.5% for the non-acetylating conditions.

§ This was determined by quenching the crude (**12**) with dimethyl sulphide at various time intervals and observing the relative amounts of (**3**) and (**10**) formed by h.p.l.c.

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