particular $C_4H_7^+$ structure has a pentacoordinated carbon with the elements of square-pyramidal geometry and possesses an *exo*-methylene proton at the apical position, this could account for the observed isotopic perturbations. The bicyclobutonium cation structure has a pentacoordinated carbon which could possess this configuration.

Acknowledgment. We are pleased to acknowledge use of the Southern California Regional NMR Center facilities (Bruker WM-500 spectrometer) supported by the National Science Foundation, Grant CHE79-16324, and helpful discussions with Professor Martin Saunders of Yale University.

Registry No. 2, 92365-85-0; **4**, 92346-36-6.

Estrogen Biosynthesis. Concerning the Obligatory Intermediacy of 2β-Hydroxy-10β-formylandrost-4-ene-3,17-dione

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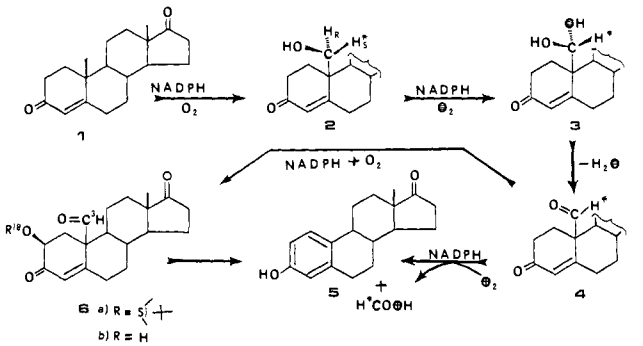
Received May 7, 1984

The transformation of an androgen (**1**) to an estrogen (**5**) by human placental aromatase was shown to involve three oxidative steps, each of which requires 1 mol of O_2 and 1 mol of NADPH.[1] The process is initiated by C-19 hydroxylation[2,3] (**2**) in the retention mode[4] and is followed by the introduction of a second C-19 hydroxyl[5] (**3**). The second hydroxylation proceeds with the stereospecific abstraction of 19-*pro-R* hydrogen atom of the 19-alcohol[6-8] **2**. The obtained diol **3** is then dehydrated with the loss of the "second" hydroxyl[9] to give the 19-aldehyde **4**. The aldehyde is subsequently aromatized with the consumption of a (third) mole each of oxygen and NADPH, and C-19 is extruded as formic acid.[5,9]

Fishman et al.[10-13] proposed that the "third" mole of oxygen and of NADPH are utilized for the enzymatic 2β-hydroxylation of a 19-aldehyde intermediate to give, e.g., 2β-hydroxy-10β-formylandrost-4-ene-3,17-dione (**6b**) (Scheme I). They proved

Scheme I



Scheme II

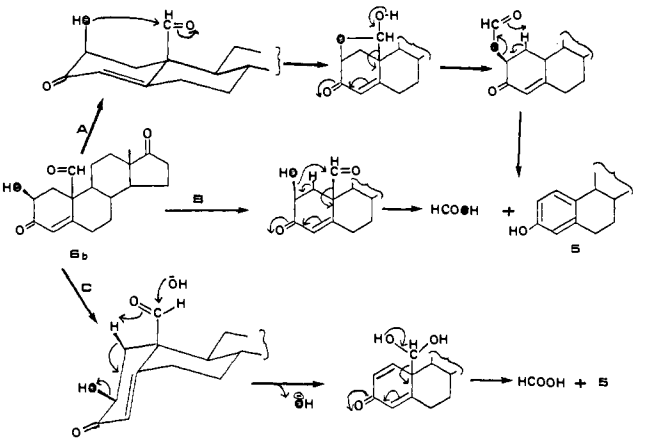


Table I. Aromatization of [2β-^{18}O,19-^{3}H]-2β-hydroxy-10β-formylandrost-4-ene-3,17-dione (**6b**): GC-MS Analyses of the Derived Benzyl Formates

source of $HCOOCH_2C_6H_5$	relative intensities of M^+ ions		
	m/z 136	*m/z* 137	*m/z* 138
(1) authentic, ref	100	9.2	0.94
(2) nonenzymatic[a] aromatization of **6b**	100	9.6	0.87
(3) enzymatic[a] aromatization of **6b**	100	11.6	0.94

[a] See text for details.

that the 2β-hydroxy-10β-formyl **6b** collapses nonenzymatically even at pH 7 with the loss of the 1β-hydrogen to give estrone and formic acid. On the basis of these and other observations,[10-13] they postulated that the last step of estrogen biosynthesis is the nonenzymatic aromatization of the presumably "not enzyme bound" **6b**. Accordingly they showed "that there is no end-product inhibition of aromatization by estrogens".[11]

The collapse of the 2β-hydroxy-10β-formyl **6b** can be rationalized in terms of the mechanisms (A, B, C) outlined in Scheme II. Pathway A provides for a "stepwise" aromatization of **6b**, while pathway B is a concerted process. It should be noted that according to mechanisms A and B, the oxygen atom of the 2β-hydroxyl of **6b** is incorporated into the formic acid produced in the aromatization process. In contrast, in mechanism C, the aromatization process is initiated by a hydroxyl group attack on the 10β-formyl moiety, and the oxygen of the 2β-hydroxyl group is eliminated as water.

Akhtar et al.[9] proved that the third mole of oxygen, required for completion of the aromatization process, is incorporated into the formic acid derived from C-19. It follows therefore that if **6b** is an *obligatory intermediate* in estrogen biosynthesis, the oxygen (e.g., ^{18}O) atom of the 2β-hydroxyl must be incorporated into the extruded formic acid, a point that was recognized by Hahn and Fishman.[13]

To test the Fishman et al. hypothesis, we have prepared [2β-^{18}O,19-^{3}H]-2β-hydroxy-10β-formylandrost-4-ene-3,17-dione 2-(*tert*-butyldimethylsilyl) ether[14] (**6a**). The mass spectrum showed

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(1) Thompson, E. A., Jr.; Siiteri, P. K. *J. Biol. Chem.* **1974**, *249*, 5364.
(2) Meyer, A. S. *Experientia* **1955**, *11*, 99. Meyer, A. S. *Biochem. Biophys. Acta* **1955**, *17*, 441.
(3) Wilcox, R. B.; Engel, L. L. *Steroids, Suppl.* **1965**, *No. 1*, 49.
(4) Caspi, E.; Arunachalam, T.; Nelson, P. A. *J. Am. Chem. Soc.* **1983**, *105*, 6987.
(5) Akhtar, M.; Skinner, S. J. M. *Biochem. J.* **1968**, *109*, 318. Skinner, S. J. M.; Akhtar, M. *Ibid.* **1969**, *114*, 75.
(6) Arigoni, D.; Bataglia, R.; Akhtar, M.; Smith, T. *J. Chem. Soc., Chem. Commun.* **1975**, 185.
(7) Osawa, Y.; Shibata, K.; Rohrer, D.; Weeks, C.; Duax, W. L. *J. Am. Chem. Soc.* **1975**, *97*, 4400.
(8) Caspi, E.; Santaniello, E.; Patel, K.; Arunachalam, T.; Eck, C. R. *J. Am. Chem. Soc.* **1978**, *100*, 5223.
(9) Akhtar, M.; Calder, M. R.; Corina, D. L.; Wright, J. N. *Biochem. J.* **1982**, *201*, 569.
(10) Hosoda, H.; Fishman, J. *J. Am. Chem. Soc.* **1974**, *96*, 7325.
(11) Goto, J.; Fishman, J. *Science (Washington, D.C.)* **1977**, *195*, 80.
(12) Fishman, J.; Raju, M. S. *J. Biol. Chem.* **1981**, *256*, 4472.
(13) Hahn, E. F.; Fishman, J. *J. Biol. Chem.* **1984**, *259*, 1689.

0002-7863/84/1506-7282$01.50/0

Table II. Control Experiments with $^{18}O_2$

origin of formic acid	composition, % $^2HCOOCH_2C_6H_5$, m/z 137	$^2HC^{18}OOCH_2C_6H_5$, m/z 139	$^2HC^{18}O^{18}OCH_2C_6H_5$, m/z 141
(1) $^2HC^{18}O^{18}ONa$[a]	17	46	37
(2) same as 1, incubated 60 min[b]	20	41	39
(3) same as 2	19	43	38
(4) from incubation of **4**, H = ^2H, in $^{18}O_2$, 20 min	35	65	
(5) same as 4, incubated 60 min	38	62	

[a] Synthetic sample used also in Experiments 2 and 3. GC-MS analyses were carried out as described.[17] [b] For conditions of incubation, see text (4 and 5 are averages of duplicate experiments).

that at least 50% of the molecules (**6a**) were labeled with ^{18}O at the 2β-hydroxy moiety. The silyl ether was cleaved with aqueous acetic acid and, after TLC (hexane–EtOAc 2:1), homogeneous [2β-^{18}O,19-^3H]-2β-hydroxy-10β-formylandrost-4-ene-3,17-dione (**6b**) was obtained.

Two sets of experiments were then carried out. In the first experiment, **6b** [100 μg (3×10^4 dpm of ^3H) in each of five flasks] was incubated in Tris-buffer (pH 7.4) with placental aromatase for 1 h at 37 °C, under nitrogen, as previously described.[4,15] At the termination of the reaction, the contents of the flasks were rapidly combined, the mixture was acidified and frozen in liquid nitrogen, and the formic acid was recovered by lyophilization.[4,15] The derived sodium formate was then converted to benzyl formate.[16,9] The second set of experiments was carried out exactly as above[4,15] but *without placental aromatase*, and the recovered formic acid was also converted to benzyl formate. Each of the two samples of benzyl formate contained tritium (1.35×10^5 dpm), indicating that ca. 90% of the substrate **6b** was aromatized. The GC-MS[17] of the two samples were recorded, and the results are summarized in Table I. The benzyl formates showed peaks at m/z 136 and 137, but none was present at m/z 138 indicating the absence of ^{18}O.

Usually, variable amounts of endogenous [^{16}O]formic acid were recovered from the placental aromatase preparations. It is therefore of importance that the benzyl formate derived from aromatization of [^{18}O]-**6b** in the *absence of placental aromatase* gave formic acid (analyzed as benzyl formate) which contained only ^{16}O. This benzyl formate could not be contaminated with a formate of endogenous origin and must have originated solely from C-19 of the [2β-^{18}OH]-**6b** substrate.

To determine whether ^{18}O was exchanged (lost) under the experimental conditions, $^2HC^{18}O^{18}ONa$ (80% ^{18}O enrichment) was prepared,[9] and an aliquot of the salt was converted to benzyl formate.[16,9] A second aliquot was incubated with human placental aromatase (1 h, at 37 °C, in the air), and the formic acid was recovered by lyophilization of the acidified mixture.[4,15] The GC-MS analyses of the two [$^{18}O_2$]benzyl formates indicated that *no* detectable loss of ^{18}O occurred (Table II, entries 1–3).

We have also incubated [19-^2H]-10β-formylandrost-4-ene-3,17-dione **4**, H = ^2H] in an atmosphere of $^{18}O_2$. Two sets of incubations (in duplicate) were carried out for 20 and 60 min with human placental aromatase[4,15] in an atmosphere of $^{18}O_2$ (98% excess). The recovered formic acids were analyzed (as benzyl formates) by GC-MS,[17] and all four samples showed ions at m/z 139 for $^2HC^{16}O^{18}OCH_2C_6H_5$ (Table II, entries 4 and 5). These results confirm the Akhtar et al.[9] observations that the "third" mole of oxygen is incorporated into the extruded formic acid.

To evaluate the operation of pathway C (Scheme II), 19-DT aldehyde **4** was incubated with placental aromatase in $^{18}OH_2$ (30% excess of ^{18}O) in the air. The rationale of the experiment was based on the premise that, if Fishman's hypothesis is correct, the $^{16}O_2$ should be utilized for C-2 hydroxylation to give **6b**, 2β-^{16}OH, which will then aromatize with the incorporation of ^{18}O from the water into the formic acid. The recovered formic acid (60% yield) contained only ^{16}O. These results when taken together with the results on the aromatization of [^{18}O]-**6b** exclude the operation of pathway C, Scheme II.

Our results show that the oxygen atom of the [^{18}O]-2β-hydroxyl of **6b** was *not incorporated* into the extruded formic acid derived from C-19. It may therefore be concluded that the mechanism proposed by Fishman et al.[10–13] via enzymatic formation of 2β-hydroxy-10β-formylandrost-4-ene-3,17-dione (**6b**) and its non-enzymatic aromatization is *not an obligatory pathway* of estrogen biosynthesis by placental aromatase.

(14) The synthesis was carried out by treating [19-^3H]-6β-bromo-19-acetoxyandrost-4-ene-3,17-dione with $^{18}O_2$-labeled potassium acetate in glacial ^{18}O-labeled acetic acid to give [2β-$^{18}O_2$,19-^3H]-2β,19-diacetoxyandrost-4-ene-3,17-dione. Saponification followed by selective silylation of the resulting 2β,19-diol furnished 19-hydroxy-2β-(*tert*-butyldimethylsilyl) ether which on mild oxidation gave the required **6a**. The proton NMR of **6a** showed signals at δ 9.78 (1 H, s, 19-CHO), 5.85 (1 H, s, C_4–H), 3.98 (1 H, br s, 2α-H), and 0.82 (3 H, s, C_{18}–H) in accordance with published data.[10] The MS of [^{16}O]-2β-silyl ether **6a** showed ions at m/z 402 ($M^+ - 28$) and 373 ($M^+ - C_4H_9$), while the MS of [^{18}O]-2β-silyl ether **6a** showed ions at m/z 402 (49%), 404 (51%), 373 (45%), and 375 (55%).

(15) Caspi, E.; Arunachalam, T.; Nelson, P. A., manuscript in preparation.

(16) Corina, D. L. *Anal. Biochem.* **1977**, *80*, 639.

(17) A Varian Model 3700 GC instrument equipped with a glass capillary column (25 m) coated with OV-101 was used. Injection port temperature 270 °C; column temperature 70–280 °C; temperature gradient 3 °C/min. The GC was linked to a Varian-MAT Model 312 mass spectrometer via a direct inlet. Spectra were recorded at 70 eV.

Reaction of Ketene Alkylsilyl Acetals with Bromoform–Diethylzinc. An Unprecedented Cyclopropanation Reaction

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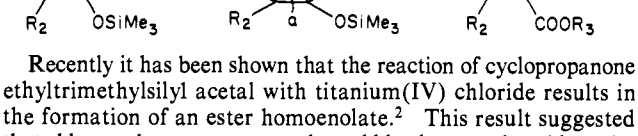
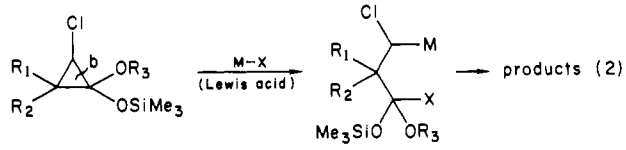
Received July 24, 1984

In conjunction with our program dealing with the reactivity of ketene alkylsilyl acetals and carbenes, we have reported that the reaction of those species with chlorocarbenes provides a convenient route to α-substituted α,β-ethylenic esters via a-bond cleavage of an intermediate cyclopropane (eq 1[1]).



(1)

Recently it has been shown that the reaction of cyclopropanone ethyltrimethylsilyl acetal with titanium(IV) chloride results in the formation of an ester homoenolate.[2] This result suggested that chlorocyclopropanone acetals could lead to a carbenoid species by cyclopropane b-bond ring cleavage (eq 2) if the reaction was carried out in the presence of a Lewis acid.



(2)

(1) Slougui, N.; Rousseau, G.; Conia, J. M. *Synthesis* **1982**, 58. Slougui, N.; Rousseau, G. *Synth. Commun.* **1982**, *12*, 401.

(2) Nakamura, E.; Kuwajima, I. *J. Am. Chem. Soc.* **1983**, *105*, 651.

0002-7863/84/1506-7283$01.50/0