Reduction of 1,4-Dien-3-one Steroids with $LiAl^2H_4$ or NaB^2H_4: Stereospecific Deuterium-Labeling at the C-1α Position of a 4-En-3-one Steroid

Mitsuteru NUMAZAWA* and Wakako HANDA

Tohoku Pharmaceutical University; 4–4–1 Komatsushima, Aoba-ku, Sendai 981–8558, Japan.
Received October 6, 2005; accepted December 5, 2005

Reduction of a double bond at C-1 of 1,4-dien-3-one steroids 7 and 8 with $LiAl^2H_4$ in THF or NaB^2H_4 in MeOH and H_2O gave stereospecifically [1α-^{2}H]-labeled 4-en-3-one steroids 9 and 10, respectively. When the deuterated solvents, MeO^2H and ^2H_2O, were used for the reaction of steroid 8 with NaB^2H_4, [1α,2ξ-$^2H_2$]-labeled compound 10 was produced. This indicates that the reaction proceeds through the initial hydride attack at the C-1α position, followed by ketonization of the 2-en-3-ol intermediate.

Key words lithium aluminum deuteride; sodium borodeuteride; reduction; 1,4-dien-3-one steroid; deuterium labeling; [1α-^{2}H]testosterone

Aromatase is a cytochrome P-450 enzyme responsible for catalyzing the conversion of the androgens androstenedione (AD, **1**) and testosterone (**10**) to the estrogens estrone (**3**) and estradiol, respectively.[1—3] This process appears to proceed with three oxygenations of the androgens, each of which requires 1 mol of O_2 and 1 mol of NADPH (Fig. 1). The 19-methyl group, as well as 1β- and 2β-hydrogens, are eliminated in the third oxygenative step, resulting in aromatization of the A-ring of the androgens.[4—10] The exact nature of the final step remains uncertain, however, the A-ring conformation is thought to play a critical role in the stereospecific removal of the two hydrogens. Inhibitors of aromatase are useful in treating estrogen-dependent breast cancer.[11]

We have previously studied the structure–activity relationships of steroidal aromatase inhibitors to gain insights into the spatial nature of the AD binding (active) site of aromatase in relation to the catalytic function of aromatase.[12—18] It was recently found that the 2-methylene derivative of AD, compound **2**, a good competitive inhibitor of aromatase, acts as a substrate of aromatase to be converted into 2-methylestrone (**4**), although the conversion rate is low.[19] To explore the uncertain last step of the aromatase reaction, we focused on the stereochemistry of the hydrogen loss at the C-1 position of the 2-methylene steroid **2**, which has an unusual A-ring conformation distorted by the introduced 2-methylene moiety, during the aromatization sequence. Then, we needed the 1α- or 1β-deuterium-labeled 17β-*tert*-butyldimethylsiloxy-4-en-3-one steroid **9**, a key intermediate for the synthesis of the labeled steroid **2**.[19] In this paper, we report stereospecific ^{2}H-labeling at the C-1α position of steroid **9** and its 17β-hydroxy analog **10**, by reduction of the corresponding 1,4-dien-3-one derivatives **7** and **8** with $LiAl^2H_4$ or NaB^2H_4.

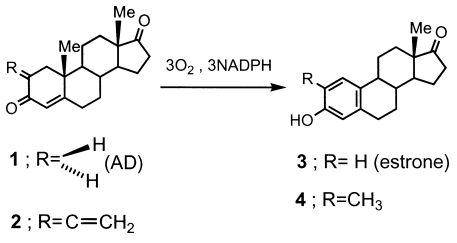

Fig. 1. Biochemical Aromatization of AD (**1**) and Its 2-Methylene Analog (**2**)

Results and Discussion

We initially tried to obtain the [1β-^{2}H]-labeled 17β-silyl steroid **9** through the mesylation of 1α-hydroxyandrost-4-en-3-one steroid **5**, followed by reductive deoxygenation with a deuterium-labeled reagent. Treatment of the 1α-ol **5** with methanesulfonyl chloride gave mesylate **6**, which was, without isolation, subjected to the deoxygenation reaction with a Zn–NaI–^2H_2O system according to the method[20] previously reported, yielding the elimination product, 1,4-diene compound **7**, without the production of the deoxygenation product [1β-^{2}H]4-en-3-one **9**. The crude mesylate **6** was next treated with $LiAl^2H_4$ in THF under reflux, followed by treatment with diluted H_2SO_4, which gave the 4-en-3-one **9** (8%) along with the A-ring aromatized product **12** (28%). Mass spectrometric analysis of these products showed that compounds **9** and **12** principally consisted of a d_1 molecular species (d_0, 4%; d_1, 96% in each). The aromatic compound was presumed to be an acid-catalyzed dienol-benzene rearrangement product of 3ξ-hydroxy-1,4-dien-3-one steroid.[21] To determine this, compound **7** was then treated with the metal deuteride under the same condition as above, yielding deuterated compounds **9** and **12**, of which the yields and deuterium content were almost the same as above. In these reactions, the production of a 3-hydroxy derivative of the 4-en-3-one **9** was detected by TLC analysis of the reaction mixture; however, this was not isolated because of its instability during the isolation procedure. These results indicated that the aluminum deuteride reduction of the mesylate **6** proceeded through the 1,4-diene steroid **7**. The position, as well as the stereochemistry of deuterium incorporated into compounds **9** and **12** was determined by ^{1}H-NMR spectrometry. Signals of the 1α- and 1β-hydrogens of non-labeled compound **9** appeared at 1.69 ppm for 1α-H and at 2.03 ppm for 1β-H [*cf.* 1.70 ppm for 1α-H and 2.03 ppm for 1β-H of testosterone (**9**)[20]] without interference from the other hydrogen signals. The signals were further assigned by comparison with the ^{1}H-NMR spectrum of [1β-^{2}H]-labeled 4-en-3-one **9**, previously synthesized through catalytic reduction of the 1,4-

* To whom correspondence should be addressed. e-mail: numazawa@tohoku-pharm.ac.jp

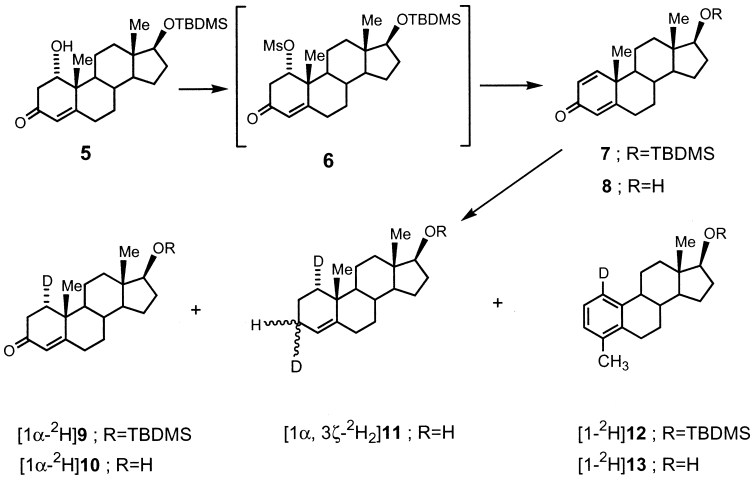

Fig. 2. Reactions of 1α-Mesylate **6** and 1,4-Dien-3-ones **7** and **8** with $LiAL^2H_4$ or NaB^2H_4

diene **7** with $^2H_2$-Pd/charcoal as a key reaction according to the previous method,[23] along with the NOE experiments. Based on NMR analysis, it was found that deuterium was incorporated at the C-1α position in a stereospecific manner (100 : 0 for C-1α : C-1β).

On the other hand, in the ^{1}H-NMR spectrum of the aromatized product **12**, signals of two aromatic hydrogens resonated at 7.00 and 7.08 ppm as a doublet (J=7.6 Hz), respectively. Based on this, as well as the rearrangement mechanism, it was determined that a deuterium was labeled at the C-1 position.

The reaction of the 17β-hydroxy analog **8** with $LiAl^2H_4$ gave [1α,3ξ-$^2H_2$]3ξ-hydroxy-4-ene steroid **11** (28%), along with [1α-^{2}H]4-en-3-one **10** (18%) and a [1-^{2}H]aromatized product **13** (31%). The 3ξ,17β-diol **11** was stable during the isolation procedure, and the deuterium contents of the other products were almost the same as above.

The reduction of 17-ol **8** with NaB^2H_4 in MeOH and H_2O or in MeO^2H and ^2H_2O was studied next. The reaction in the non-labeled solvents yielded [1α-^{2}H]4-en-3-one **10** (15%) and [1α,3ζ-$^2H_2$]3ζ-ol **11** (30%), along with the recovered substrate (20%), where neither 3ξ-hydoxy-1,4-diene or the aromatized product **13** was produced on TLC analysis. When the deuterated solvents were employed for the reduction, one other deuterium atom was incorporated at the C-2 position of the 4-en-3-one **10** in addition to the C-1α position. The deuterium labeling at C-2 was not stereoselective on the basis of the reaction sequences, or from the ^{1}H-NMR analysis (δ; 2.28 for 2α-H and 2.39 for 2β-H). The 3ξ-ol **11** obtained had three deuterium atoms at the positions of C-1α, C-2ξ, and C-3ξ, and oxidation of the labeled steroid **11** with MnO_2 gave the [1α-^{2}H]3-ketone **10** in good yield.

It has previously been reported that the treatment of 1,4-dien-3-one steroids and their 6-ene analogs with $LiAlH_4$ or $NaBH_4$ yields corresponding 3ζ-hydroxy-4-ene products.[24] On the basis of the present findings, the metal hydride reductions of the 1,4-dien-3-one **7** are thought to proceed as follows (Fig. 3). The attack of a hydride of the metal hydride at the C-1 position of steroid **7** from the sterically less hindered α-side, yielding enolate **14**, followed by ketonization produces the enone **9** of which the C-3 carbonyl group is next reduced to produce the 3-ol **11** (path A); in contrast, the initial attack of the hydride at the C-3 position, followed by the acid-catalyzed dienol-benzene rearrangement, produces the aromatic compound **13** (path B). In the case of the $LiAlH_4$ reduction, both paths are involved, whereas the reaction with NaB^2H_4 principally proceeds through path A.

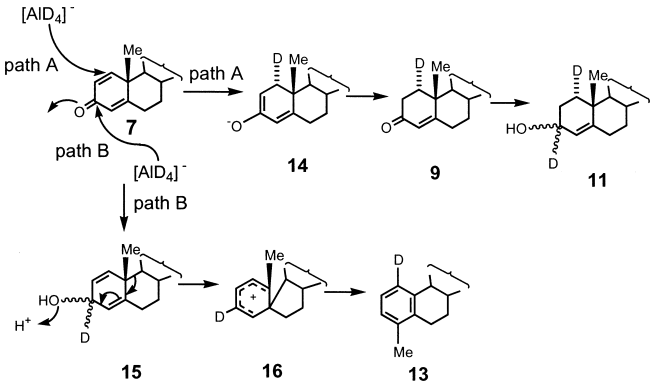

Fig. 3. Reaction Sequences for the Reduction of a 1,4-Dien-3-one Steroid with $LiAl^2H_4$

The deuterium atom ^{2}H is expressed as D for convenience.

Catalytic hydrogenation of a 1,4-dien-3-one steroid using tris(triphenylphosphine)rhodium chloride and $^2H_2$ or $^3H_2$ has been used for the synthesis of [1α-^{2}H]- or [1α-^{3}H]-labeled AD (**1**) and testosterone (**10**).[23,25] Osawa's group has previously reported that this reaction incorporates two deuterium atoms at not only the 1α,2α-positions, but also at the 1β,2β-positions (α : β=86 : 14).[25] Thus, the reduction of a 1,4-dien-3-one steroid with the deuterated metal hydride reported here is found to be the first stereospecific synthesis of a [1α-^{2}H]4-en-3-one steroid. This synthesis has advantages over catalytic hydrogenation with rhodium chloride in terms both regio- and stereospecificities.

Experimental

Melting points were measured on a Yanagimoto melting point apparatus and are uncorrected. ^{1}H-NMR spectra were obtained in $CDCl_3$ solution with JEOL EX270 (270 MHz) and JEOL JNM-LA 600 (600 MHz) spectrometers (Tokyo, Japan) using tetramethylsilane as an internal standard. Mass spectra (MS) were obtained with a JEOL JMS-DX303 spectrometer. Column chromatography was conducted with silica gel (E. Merck, Darmstadt, Germany, 70—230 mesh) (solvent: hexane–EtOAc). 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 preparative use, respectively; solvent: hexane–

EtOAc). $LiAl^2H_4$ (98 atom%) and NaB^2H_4 (98 atom%) were obtained from Aldrich Chem. Co. (Milw., WI, U.S.A.). The 17β-siloxy-1α-hydroxy steroid **5** was synthesized according to the previous method.[24]

Mesylation of 17β-*tert*-Butyldimethylsilyl-1α-hydroxyandrosta-1,4-dien-3-one (5) with Methanesulfonyl Chloride Methanesulfonyl chloride (0.6 ml, 0.01 mmol) was added slowly to a solution of the 1α-ol **5** (200 mg, 0.48 mmol) in pyridine (2 ml) at 0 °C with stirring, and the mixture was stirred for 22 h at 0 °C. After this time, water was added to the mixture to decompose the reagent, and the product was extracted with EtOAc. The organic layer was washed with saturated $NaHCO_3$ solution and water, then dried with Na_2SO_4. Evaporation of the solvent gave an oily crude product **6** (203 mg) which was used without further purification in the next reaction.

Reaction of the Mesylate 6, 17β-*tert*-Butyldimethylsiloxyandrosta-1,4-dien-3-one (7) or 17β-Hydroxyandrosta-1,4-dien-3-one (8) with $LiAl^2H_4$ A solution of the crude mesylate **6** (370 mg, about 0.75 mmol) or the 1,4-dien-3-one **7** or **8** (300 mg or 215 mg, 0.75 mmol) was added dropwise to a suspension of $LiAl^2H_4$ (380 mg, 9.1 mmol) in dry THF (45 ml) under reflux with stirring in a stream of nitrogen gas for 2 h for the experiment with **6** or **7**, and for 15 min for that with **8**. After cooling the reaction mixture, water was added to stop the reaction, then 10% H_2SO_4 was added to clarify the mixture. Products were extracted with EtOAc, and the organic layer was washed with saturated $NaHCO_3$ solution and water and dried with Na_2SO_4. Evaporation of the solvent afforded an oil which was purified by column chromatography (hexane–EtOAc, 10 : 1 for the experiments with the silyl steroids or 3 : 1 for that with **8**) followed by preparative TLC (hexane–EtOAc, 3 : 1 or 1 : 1).

[1α-^{2}H]17β-*tert*-Butyldimethylsiloxyandrost-4-en-3-one (**9**): Yield 8 or 10% from **6** or **7**; mp 131—133 °C (from acetone) (lit.[26] mp 139—140.5 °C for non-labeled **9**). ^{1}H-NMR (600 MHz) δ: 0.01 and 0.08 (3H each, s, Si–Me_2), 0.75 (3H, s, 18-Me), 0.88 (9H, s, Si–Me_3), 1.18 (3H, s, 19-Me), 2.03 (1H, s, 1β-H), 3.55 (1H, t, J=8.2 Hz, 17α-H), and 5.72 (1H, s, 4-H). MS *m*/*z* (relative intensity): 403 (M^+, 3), 346 (100), 270 (10), 75 (63), d_0 4%, d_1 96%.

[1α-^{2}H]Testosterone (**10**): Yield 18% ($LiAl^2H_4$) or 15% (NaB^2H_4) from **8**; mp 133—136 °C (from acetone) (lit.[27] mp 155 °C for non-labeled **10**). ^{1}H-NMR (600 MHz) δ: 0.80 (3H, 18-Me), 1.20 (3H, s, 19-Me), 2.05 (1H, s, 1β-H), 3.65 (3H, t, J=8.5 Hz, 17α-H), 5.73 (1H, s, 4-H). MS *m*/*z* (relative intensity): 289 (M^+, 62), 247 (18), 229 (14), 125 (100), d_0 4%, d_1 96%.

[1α,3ξ-^{2}H$_2$]Androst-4-ene-3ζ,17β-diol (**11**): Yield 28% ($LiAl^2H_4$) or 30% (NaB^2H_4) from **8**; mp 156—159 °C (from acetone). ^{1}H-NMR (270 MHz) δ: 0.76 (3H, s, 18-Me), 1.06 (3H, s, 19-Me), 3.64 (1H, dd, J=16.6, 4.1 Hz, 17α-H), 5.27 (1H, s, 4-H). MS *m*/*z* (relative intensity): 292 (M^+, 24), 247 (15), 220 (17), 57 (100), d_0 2%, d_1 7%, d_2 91%.

[1-^{2}H]4-Methylestra-1,3,5(10)-trien-17β-yl *tert*-Butyldimethylsilyl Ether (**12**): Yield 28% from **6**, 25% from **7**; mp 99—100 °C (from acetone) (mp 100—102 °C for non-labeled **7**). ^{1}H-NMR (270 MHz) δ: 0.025 and 0.037 (3H each, s, 17-OSiMe_2), 0.73 (3H, s, 18-Me), 0.89 (9H, s, 17-SiCMe_3), 2.21 (3H, s, 4-Me), 3.64 (1H, dd, J=7.6, 8.7 Hz, 17α-H), 7.00 and 7.08 (1H each, d, J=7.6 Hz, 2-H, 3-H). MS *m*/*z* (relative intensity): 385 (M^+, 7); 328 (69), 252 (100), d_0 5%, d_1 95%.

[1-^{2}H]4-Methylestra-1,3,5(10)-trien-17β-ol (**13**): Yield 31% from **8**; mp 107—110 °C (from EtOAc) (lit.[28] mp 113—114 °C for non-labeled **13**). ^{1}H-NMR (270 MHz) δ: 0.77 (3H, s, 18-Me), 2.22 (3H, s, 4-Me), 3.74 (1H, t, J=8.4 Hz, 17α-H), 7.01 and 7.08 (1H each, d, J=7.3 Hz, 2-H, 3-H). MS *m*/*z* (relative intensity): 271 (M^+, 100), 238 (8), 212 (48), 159 (39), d_0 3%, d_1 97%.

Reaction of the 1,4-Diene 8 with NaB^2H_4 in MeOH and H_2O or in MeO^2H and ^2H_2O NaB^2H_4 (120 mg, 0.28 mmol) was added to a solution of compound **8** (100 mg, 0.35 mol) in MeOH (6.6 ml) and H_2O (0.75 ml), or in MeO^2H (6.6 ml) and ^2H_2O (0.75 ml), and the mixture was stirred at room temperature for 5 h, to which was added a few drops of AcOH, then the mixture was diluted with EtOAc, washed with saturated $NaHCO_3$ solution and water, and dried with Na_2SO_4. Evaporation of the solvent gave a solid which was purified by column chromatography (hexane–EtOAc, 4 : 1) and preparative TLC (hexane–EtOAc, 1 : 1, three developments).

[1α,2ζ,3ζ-^{2}H$_3$]Compound **11** was produced in the experiment with the deuterated solvents: yield 30%; MS *m*/*z* (relative intensity): 293 (M^+, 20), 257 (10), 57 (100), d_1 6%, d_2 15%, d_3 79%.

[1α,2ζ-^{2}H$_2$]Compound **10** was produced in the experiment with the deuterated solvents: yield 15%; MS *m*/*z* (relative intensity): 290 (M^+, 13), 248 (8), 57 (100), d_0 3%, d_1 18%, d_2 79%.

Oxidation of the 3ζ-Hydroxy Steroid 10 with MnO_2 A mixture of [1α,3ζ-^{2}H$_2$]compound **10** (234 mg, 0.8 mmol), MnO_2 (1.2 g, 13.3 mmol) and $CHCl_3$ (15 ml) was stirred overnight at room temperature. The solid material was removed by filtration, and the filtrate was evaporated to yield a solid which was purified by column chromatography (hexane–EtOAc). Subsequent recrystallization from acetone yielded the 3-one product **9** (162 mg, 70%). MS *m*/*z* (relative intensity): 403 (M^+, 1.5), 346 (100), 270 (8), d_0 4%, d_1 96%.

Acknowledgments This work was supported in part by a Grant-in-Aid for Scientific Research and a High Technology Research Center Project, from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

References and Notes

1) Thompson E. A., Jr., Siiteri P. K., *J. Biol. Chem.*, **249**, 5373—5378 (1974).
2) Kellis J. T., Jr., Vickery L. E., *J. Biol. Chem.*, **262**, 4413—4420 (1987).
3) Yoshida N., Osawa Y., *Biochemistry*, **30**, 3003—3010 (1991).
4) Akhtar M., Calder M. R., Corina D. L., Wright J. N., *Biochem. J.*, **201**, 569—580 (1982).
5) Caspi E., Arunachalam T., Nelson P. A., *J. Am. Chem. Soc.*, **108**, 1847—1852 (1986).
6) Townsley J. D., Brodie H. J., *Biochemistry*, **7**, 33—40 (1968).
7) Fishman J., Guzik H., *J. Am. Chem. Soc.*, **91**, 2805—2806 (1969).
8) Cole P. A., Robinson C. H., *J. Med. Chem.*, **33**, 2933—2944 (1990).
9) Covey D. F., "Sterol Biosynthesis Inhibitors," ed. by Berg D., Plempel M., Ellis Horwood Ltd., Chichester, England, 1988, pp. 535—571.
10) Montellano P. R. O., "Cytochrome P-450 Structure, Mechanism, and Biochemistry," ed. by Montellano P. R. O., Pelnum Press, New York, 1995, pp. 280—282.
11) For a review see: Santen R. J., *Steroids*, **68**, 559—567 (2003).
12) Numazawa M., Yoshimura A., Oshibe M., *Biochem. J.*, **329**, 151—156 (1998).
13) Numazawa M., Yoshimura A., *J. Steroid Biochem. Mol. Biol.*, **70**, 189—196 (1999).
14) Numazawa M., Nagaoka M., Morio M., Kamiyama T., *J. Steroid Biochem. Mol. Biol.*, **71**, 173—179 (1999).
15) Numazawa M., Yoshimura A., *Biol. Pharm. Bull.*, **23**, 1059—1065 (2000).
16) Numazawa M., Yamada K., Nagaoka M., *Biol. Pharm. Bull.*, **24**, 332—335 (2001).
17) Numazawa M., Ando M., Zennyoji R., *J. Steroid Biochem. Mol. Biol.*, **82**, 65—73 (2002).
18) Numazawa M., Nagaoka M., Sohtome N., *Biochemistry*, **44**, 10839—10845 (2005).
19) Numazawa M., Watari Y., Yamada K., Uemura N., Handa W., *Steroids*, **68**, 503—513 (2003).
20) Turecek F., Veres K., Kocovsky P., Pouzar V., Fajkos J., *J. Org. Chem.*, **48**, 2233—2237 (1983).
21) Kirk D. N., Hartshorn M. P., "Steroid Reaction Mechanisms," Elsevier Publishing Company, Amsterdam, 1968, pp. 277—313.
22) Kirk D. N., Toms H. T., Douglas C., White K. A., Smith K. E., Latif S., Hubbard R. W. P., *J. Chem. Soc. Perkin Trans. 2*, **1990**, 1567—1594 (1990).
23) Tökés L., Throop L. J., "Organic Reactions in Steroid Chemistry," Vol. 1, ed. by Fried J., Edwards J. A., Van Nostrand Reinhold Company, New York, 1972, pp. 145—188.
24) Mann J., Pietrzak B., *Tetrahedron*, **45**, 1549—1552 (1989).
25) Osawa Y., Spaeth D. G., *Biochemistry*, **10**, 66—71 (1971).
26) Hosoda H., Yamashita K., Sagae H., Nambara T., *Chem. Pharm. Bull.*, **23**, 2118—2122 (1975).
27) "The Merck Index," 13th ed., Merck & Co., Inc., Whitehouse Station, NJ, 2001, p. 1638.
28) Gentles M. J., Moss J. B., Herzog H. L., Hershberg E. B., *J. Am. Chem. Soc.*, **80**, 3702—3705 (1958).