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X-ray and deuterium labeling studies on the abnormal ring cleavages of a 5β-epoxide precursor of formestane

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Abstract

A new convergent synthesis of the antitumor steroid formestane (4-OHA) **5** has been performed from the easily available epimeric mixture of 5α- and 5β-androst-3-en-17-one **1a** and **1b** in order to attempt a yield improvement. A two-step oxidative route followed by base-catalyzed isomerization was applied to the 5α- and 5β-epimers **1a** and **1b**, either as a mixture or separately, leading to the title compound **5**. From epimer **1a** an efficient process was attained to prepare the desired aromatase inhibitor formestane. Epimer **1b** led to the formation of the same compound **5**. Additionally, **1b** have also been converted in 5β-hydroxyandrostane-3,17-dione **12** and androst-4-ene-3,17-dione **13**, revealing an unexpected reactivity of the 3β,4β-epoxy-5β-androstan-17-one intermediate **6** formed from **1b** during the first oxidative step with performic acid. Cleavage of the epoxide **6** led to the *trans*-diaxial and the *trans*-diequatorial *vic*-diols **7** and **8** and to the 1,3-diol **9**. The formation of the abnormal products **8** and **9** were investigated through X-ray and deuterium labeling studies. Diol **8** was formed through a *trans*-diequatorial epoxide ring opening and the 1,3-diol **9** was formed through an intramolecular rearrangement involving a 1,2-hydride shift. All the *vic*-diols **3**, **7** and **8** formed, proved to be good precursors for the synthesis of the target compound **5**.

Keywords: Formestane; Aromatase inhibitors synthesis; Antitumor steroid; Epoxide cleavages; X-ray; Deuterium labeling

1. Introduction

Since aromatase catalyses the final step in the biosynthesis of estrogens, the potent aromatase inhibitor formestane **5** (4-hydroxyandrost-4-ene-3,17-dione, 4-OHA) (Scheme 1) has been proved very effective in the treatment of advanced estrogen-dependent breast cancer [1,2] and, quite recently has found clinical use [3]. A number of synthetic strategies have been developed previously for the preparation of 4-OHA, but the overall yields found were generally low [4–8]. In order to overcome this problem we recently developed alternative synthetic strategies to generate efficiently the desired diosphenol ring A of formestane [9,10]. One of the studied strategies is a two-step oxidative route and uses the 5α-androst-3-en-17-one **1a** as starting material [10]. Due to the lack of chirality at C-5 in the target steroid **5** and to the easy access to 5α- and 5β-androst-3-en-17-one (**1a** and **1b**) as a mixture through the reduction of androstenedione with zinc in acetic acid [11] we embarked to attempt the improvement of the previously developed synthesis of 4-OHA using the whole epimeric mixture of 5α- and 5β-olefins **1a** and **1b** as starting material.

In this paper we wish to report the results of a comparative study on the use of the 5β-olefin **1b** as starting material for the synthesis of 4-OHA, either alone or as a mixture with the 5α-epimer **1a**. We also report the results on the evaluation of the unusual reactivity detected for the 5β-epoxide precursor **6** of 4-OHA, through the preparation of deuterium labeled compounds and X-ray studies.

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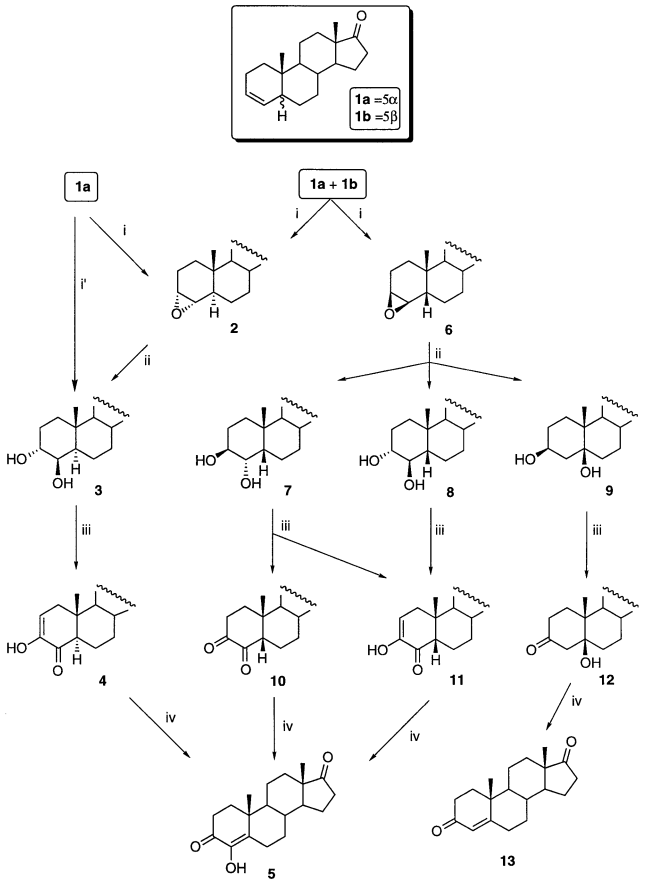


Scheme 1. The synthesis of formestane **5** from the 5α- and 5β-olefins **1a** and **1b.** *Reagents and conditions*: i) H_2O_2, HCO_2H, CH_2Cl_2, rt, 6 h; i') HCO_2H, H_2O_2, rt, 1 h; ii) HCO_2H, rt, 30′; iii) DMSO, TFAA, −60°C, 3 h then Et_3N, −60°C, 15′; iv) Na, MeOH, rt, 1 h.

2. Experimental

2.1. General

Mps were determined on a Reichert Thermopan hot block apparatus and were not corrected. IR spectra were recorded on a Perkin Elmer 257-IR Grating Infrared spectrophotometer. The ^{1}H NMR spectra were recorded at 500 MHz, on a Varian Unity 500 and at 300 MHz on a Bruker-AC 300 spectrometers. The ^{13}C NMR spectra were recorded at 125 MHz on a Varian Unity 500, at 75.6 MHz on a Bruker-AC 300 and at 50.3 MHz on a Bruker-AC 200 spectrometers. Chemical shifts were recorded in δ values in parts per million (ppm) downfield from tetramethylsilane as an internal standard. All J -values are given in Hz. Mass spectra EIMS and HRMS were obtained with VG AutoSpecQ EI and AutoSpecEQ EI mass spectrometers. Deuterated acetic acid ($CH_3CO_2{}^2H$) was purchased from Aldrich. Solvents were dried, when refered, according to described procedures. All commercially available chemicals were used as supplied by the manufacturers. The deuterium labeled compounds described here exhibited the spectra properties expected from comparison with their unlabeled counterparts.

2.2. General procedure for the preparation of 3-olefins

2.2.1. 5α and 5β-androst-3-en-17-one (*1a* and *1b*)

To a boiling solution of androstenedione (500 mg, 1.75 mmol) in glacial acetic acid (30 ml), zinc dust (3.0 g, 325 mesh Aldrich) was added in several portions during 10 min after which the reaction was complete (TLC control). The zinc suspension was filtered, the zinc was washed with glacial acetic acid and the filtrate was evaporated to dryness. The residue was diluted with water (100 ml) and extracted with diethyl ether (3 × 100 ml). The organic layers were washed with 10% aq. $NaHCO_3$ (3 × 100 ml) and water (3 × 100 ml), dried ($MgSO_4$) and evaporated to dryness to give a white crystalline solid (476 mg) composed by an isomeric mixture (2.3:1 by NMR) of 5α-olefin **1a** and 5β-olefin **1b**. Crystallization of the mixture from *n*-hexane gave the pure **1a** (285 mg, 60%).

Olefin **1a**: White solid from methanol, mp 125–126°C (lit [11] 124–126°C); ν_{max} (KBr)/cm^{-1} 3014 (C-H), 1737 (C = O); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.80 (3H, s, 19-H_3), 0.88 (3H, s, 18-H_3), 5.29 (1H, ddd, $J_{4,3}$ 9.5, $J_{4,5\alpha}$ 3.8, $J_{4,2\alpha}$ 2.0, 4-H), 5.57 (1H, ddd, $J_{3,4}$ 9.5, $J_{3,2\beta}$ 6.8, $J_{3,2\alpha}$ 3.2, 3-H); ^{13}C NMR (75.6 MHz, $CDCl_3$) δ: 11.9 (C-19), 14.0 (C-18), 46.1 (C-5), 125.6 (C-3), 131.0 (C-4), 220.3 (C-17).

The mother liquor of crystallization of **1a** was evaporated to dryness giving 167 mg of a thick and colourless oil constituted of an isomeric mixture of 5α-olefin **1a** and 5β-olefin **1b**, now enriched in the **1b** isomer (1:3 by NMR).

Olefin **1b**: The *5β*-epimer **1b** couldn't be isolated and has been identified in the mixture with the 5α-epimer only by ^{1}H and ^{13}C NMR. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.87 (3H, s, 18-H_3), 0.98 (3H, s, 19-H_3), 5.34 (1H, ddd, $J_{4,3}$ 10.0, $J_{4,5}$ 3.8, $J_{4,2\alpha}$ 1.9, 4-H), 5.67 (1H, ddd, $J_{3,4}$ 10.0, $J_{3,2\beta}$ 6.5, $J_{3,2\alpha}$ 3.5, 3-H); ^{13}C NMR (75.6 MHz, $CDCl_3$) δ: 13.7 (C-18), 22.9 (C-19), 43.4 (C-5), 127.2 (C-3), 132.0 (C-4), 221.6 (C-17).

2.2.2. [3,5α-$^2H_2$]-5α- and [3,5β-$^2H_2$]-5β-androst-3-en-17-one (*14a* and *14b*)

Prepared from androstenedione as **1a** and **1b**, but using the deuterated acetic acid, $CH_3CO_2{}^2H$, instead of glacial acetic acid as reactant. The products obtained in these conditions were the 5α- and 5β-deuterium labeled olefins **14a** and **14b** in a mixture (2.3:1 by NMR) from where the pure 5α-epimer **14a** was isolated by crystallization from *n*-hexane, leaving the liquor mother of the crystallization enriched in **14b**. The position where the deuterium labels were introduced has been easily established by ^{1}H and ^{13}C NMR studies.

Labeled olefin **14a**: ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.80 (3H, s, 19-H_3), 0.88 (3H, s, 18-H_3), 5.29 (1H, d, J 1.5, 4-H), (3-^{2}H, not visible); ^{13}C NMR (75.6 MHz, $CDCl_3$) δ: 11.8 (C-19), 13.9 (C-18), (C-5, not visible), (C-3, not visible), 130.9 (C-4), 221.4 (C-17).

Labeled olefin **14b**: The 5β-epimer **14b** couldn't be

isolated and has been studied in the mixture with the 5α-epimer **14a** by ^{1}H and ^{13}C NMR; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.87 (3H, s, 18-H_3), 0.98 (3H, s, 19-H_3), 5.33 (1H, s, 4-H), (3-^{2}H, not visible); ^{13}C NMR (75.6 MHz, $CDCl_3$) δ: 13.7 (C-18), 22.8 (C-19), (C-5, not visible), (C-3, not visible), 131.8 (C-4), 221.6 (C-17).

2.3. General procedure for the preparation of 3,4-epoxides

2.3.1. 3α,4α-Epoxy-5α-androstan-17-one **2** and 3β,4β-epoxy-5β-androstan-17-one **6**

A stirred solution of the olefin **1a** (67.5 mg, 0.25 mmol) in dichloromethane (1 ml) was treated with 30% hydrogen peroxide (0.05 ml, 0.44 mmol) and 90% formic acid (0.05 ml, 1.18 mmol) at room temperature for 6 h (TLC). After dilution with methanol (10 ml) and basification with 10% aq. NaOH, the solution was neutralized with a 10% aq. HCl and extracted with dichloromethane. The extract was washed with 10% aq. $NaHCO_3$ and water, dried ($MgSO_4$) and evaporated to dryness to give 75 mg of epoxide **2** (96%) as the only detected and isolated product.

Epoxide **2**: White solid from diethyl ether, mp 158–159°C (lit [12] 156–158°C); ν_{max} (KBr)/cm^{-1} 1740 (C = O); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.79 (3H, s, 19-H_3), 0.86 (3H, s, 18-H_3), 2.08 (1H, ddd, $J_{16\alpha,16\beta}$ 19.0, $J_{16\alpha,15\alpha/15\beta}$ 9.5, 16α-H), 2.44 (1H, ddd, $J_{16\beta,16\alpha}$ 19.0, $J_{16\beta,15\beta}$ 8.5, $J_{16\beta,15\alpha}$ 0.0–1.0, 16β-H), 2.71 (1H, d, $J_{4\beta,5\alpha}$ 4.0, 4β-H), 3.17 (1H, dd, $J_{3\beta,2\alpha}$ 3.0, $J_{3\beta,2\beta}$ 3.0, 3β-H); ^{13}C NMR (50.3 MHz, $CDCl_3$) δ: 13.4 (C-19), 13.9 (C-18), 52.1 (C-4), 55.6 (C-3), 220.9 (C-17); EIMS *m/z* 288 (M^+, 100%).

Epoxide **6**: Prepared as described above but using a (1:3) mixture of **1a** and **1b** as starting material. Under these conditions a (1:3) mixture of the epoxides **2** and **6** was obtained from where **6** has been isolated by crystallization; White solid from diethyl ether, mp 135–137°C; ν_{max} (KBr)/cm^{-1} 1737 (C = O); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.87 (3H, s, 18-H_3), 0.90 (3H, s, 19-H_3), 2.08 (1H, ddd, $J_{16\alpha,16\beta}$ 19.0, $J_{16\alpha,15\alpha/15\beta}$ 9.0, 16α-H), 2.46 (1H, ddd, $J_{16\beta,16\alpha}$ 19.0, $J_{16\beta,15\beta}$ 9.0, $J_{16\beta,15\alpha}$ 1.0, 16β-H), 2.86 (1H, d, $J_{4\alpha,3\alpha}$ 3.8, 4α-H), 3.23 (1H, ddd, $J_{3\alpha,4\alpha}$ 3.8, $J_{3\alpha,2\alpha}$ 1.8, $J_{3\alpha,2\beta}$ 1.8, 3α-H); ^{13}C NMR (75.6 MHz, $CDCl_3$) δ: 13.6 (C-18), 22.2 (C-19), 42.9 (C-5), 53.1 (C-3), 56.2 (C-4), 221.0 (C-17); EIMS *m/z* 288 (M^+, 43.2%); HRMS calculated for $C_{19}H_{28}O_2$ (M^+): 288.2089; Found: 288.2080.

2.3.2. 3β,4β-Epoxy-[3α,5β-$^{2}H_2$]-5β-androstan-17-one **15**

Prepared as the unlabeled **6**, but using now a (1:3) mixture of deuterium labeled olefins **14a** and **14b**, as starting material. As before, the labeled epoxide **15** was isolated by crystallization and the position of the deuterium label was confirmed by ^{1}H and ^{13}C NMR: ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.87 (3H, s, 18-H_3), 0.90 (3H, s, 19-H_3), 2.84 (1H, s, 4α-H), (3α-^{2}H, not visible); ^{13}C NMR (75.6 MHz, $CDCl_3$) δ: 13.6 (C-18), 22.1 (C-19), (C-5, not visible), (C-3, not visible), 56.1 (C-4), 221.0 (C-17).

2.4. General procedure for the preparation of diols

2.4.1. 3α,4β-Dihydroxy-5α-androstan-17-one **3**

A stirred solution of the olefin **1a** (67.5 mg, 0.25 mmol) in 90% formic acid (2 ml) was treated with 30% hydrogen peroxide (0.05 ml, 0.5 mmol) at room temperature for 1 h (TLC). After methanol dilution (10 ml) and 10% aq. NaOH basification, the solution was neutralized with a 10% aq. HCl and extracted with dichloromethane. The extract was washed with 10% aq. $NaHCO_3$ and water, dried over $MgSO_4$ and evaporated to dryness to give compound **3** (75 mg, 96%).

Vic-diol **3**: White solid from acetone-hexane, mp 232–234°C (lit [12] 235–237°C); ν_{max} (KBr)/cm^{-1} 3440 (OH), 1740 (C = O); ^{1}H NMR (500 MHz, CD_3OD) δ: 0.86 (3H, s, 18-H_3), 1.06 (3H, s, 19-H_3), 3.51 (1H, ddd, $J_{3\beta,2\alpha}$ 3.0, $J_{3\beta,4\alpha}$ 3.0, $J_{3\beta,2\beta}$ 1.5, 3β-H), 3.76 (1H, dd, $J_{4\alpha,5\alpha}$ 6.0, $J_{4\alpha,3\beta}$ 3.0, 4α-H); ^{13}C NMR (75.6 MHz, $CDCl_3$) δ: 13.8 (C-18), 14.2 (C-19), 70.5 (C-3), 76.1 (C-4), 220.4 (C-17); EIMS *m/z* 306 (M^+, 100%).

2.4.2. 3β,4α-Dihydroxy-5β-androstan-17-one **7**, 3α,4β-dihydroxy-5β-androstan-17-one **8** and 3β,5β-dihydroxyandrostan-17-one **9**

A solution of epoxide **6** (200 mg, 0.69 mmol) in 90% formic acid (6.0 ml) was stirred at room temperature during 30 min. After this time, the solution was diluted with methanol (30 ml) and basified with 10% aq. NaOH (8.0 ml). The resulting mixture was stirred for 5 min, neutralized with 10% aq. HCl and 10% aq. $NaHCO_3$ and extracted with dichloromethane (3 × 100 ml). The extract was washed with 10% aq. $NaHCO_3$ (100 ml) and water (3 × 100 ml), dried ($MgSO_4$) and evaporated to dryness giving 208 mg of a crystalline crude composed by a mixture of the title diols **7**, **8** and **9**. The three diols were separated by column chromatography [silica gel; light petroleum (bp 40–60°C)-ethyl acetate (from 9:1 to 2:8)] giving the *trans*-diaxial *vic*-diol **7** (102 mg, 48%), the *trans*-diequatorial *vic*-diol **8** (23 mg, 11% - smaller Rf) and the non-vicinal diol **9** (53 mg, 25% - greater Rf).

Vic-diol **7**: White solid from ethyl acetate-*n*-hexane mp 231–232°C; ν_{max} (KBr)/cm^{-1} 3400 (OH), 1725 (C = O); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.86 (3H, s, 18-H_3), 0.98 (3H, s, 19-H_3), 2.06 (1H, ddd, $J_{16\alpha,16\beta}$ 19.0, $J_{16\alpha,15\alpha/15\beta}$ 9.5, 16α-H), 2.44 (1H, dd, $J_{16\beta,16\alpha}$ 19.0, $J_{16\beta,15\beta}$ 9.0, 16β-H), 3.65 (1H, dddd, $J_{3\alpha,4\beta}$ 5.0–6.0, $J_{3\alpha,2\beta}$ 5.0–6.0, $J_{3\alpha,2\alpha}$ 3.0, $J_{3\alpha,3\beta OH}$ 3.0, 3α-H), 3.79 (1H, ddd, $J_{4\beta,5\beta}$ 5.0–6.0, $J_{4\beta,3\alpha}$ 5.0–6.0, $J_{4\beta,4\alpha OH}$ 5.0, 4β-H); ^{13}C NMR (75.6 MHz, $CDCl_3$) δ: 13.7 (C-18), 22.6 (C-19), 42.5 (C-5), 72.0 (C-3), 76.5 (C-4), 220.5 (C-17); EIMS *m/z* 306 (M^+, 100%); HRMS calculated for $C_{19}H_{30}O_3$ (M^+): 306.2195; Found: 306.2190.

Vic-diol **8**: Amorphous solid, ν_{max} (KBr)/cm^{-1} 3400 (OH), 1730 (C = O); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.86 (3H, s, 18-H_3), 1.00 (3H, s, 19-H_3), 2.09 (1H, ddd, $J_{16\alpha,16\beta}$ 19.0, $J_{16\alpha,15\alpha/15\beta}$ 9.5, 16α-H), 2.45 (1H, dd, $J_{16\beta,16\alpha}$ 19.0, $J_{16\beta,15\beta}$ 8.5, 16β-H), 3.42 (1H, ddd, $J_{3\beta,2\alpha}$ 11.0–12.0, $J_{3\beta,4\alpha}$

9.0, $J_{3\beta,2\beta}$ 5.0, 3β-H), 3.71 (1H, dd, $J_{4\alpha,5\beta}$ 11.0–12.0, $J_{4\alpha,3\beta}$ 9.0, 4α-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 13.9 (C-18), 23.6 (C-19), 48.6 (C-5), 72.7 (C-4), 76.7 (C-3), 220.4 (C-17); EIMS *m/z* 306 (M^+, 83.5%); HRMS calculated for $C_{19}H_{30}O_3$ (M^+): 306.2195; Found: 306.2210.

Diol **9**: White solid from acetone-hexane mp 202–204°C (lit [13] 201–203°C); ν_{max} (KBr)/cm^{-1} 3460 (OH), 1735 (C = O); ^{1}H NMR (500 MHz, CD_3OD) δ: 0.86 (3H, s, 18-H_3), 0.97 (3H, s, 19-H_3), 2.07 (1H, ddd, $J_{16\alpha,16\beta}$ 19.0, $J_{16\alpha,15\alpha/15\beta}$ 9.5, 16α-H), 2.26 (1H, dd, $J_{4\alpha,4\beta}$ 15.0, $J_{4,3\alpha}$ 3.0, 4-H), 2.44 (1H, dd, $J_{16\beta,16\alpha}$ 19.0, $J_{16\beta,15\beta}$ 9.0, 16β-H), 4.11 (1H, dddd, $J_{3\alpha,2\alpha}$ 3.0, $J_{3\alpha,2\beta}$ 3.0, $J_{3\alpha,4\alpha}$ 3.0, $J_{3\alpha,4\beta}$ 3.0, 3α-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 13.8 (C-18), 17.0 (C-19), 37.1 (C-4), 68.1 (C-3), 75.1 (C-5), 220.5 (C-17); EIMS *m/z* 306 (M^+, 14.6%); HRMS calculated for $C_{19}H_{30}O_3$: 306.2195; Found: 306.2184.

2.4.3. 3β,4α-Dihydroxy-[3α,5β-$^2H_2$]-5β-androstan-17-one ***16****, 3α,4β-dihydroxy-[3β,5β-$^2H_2$]-5β-androstan-17-one* ***17*** *and 3β,5β-dihydroxy-[3α,4ξ-$^2H_2$]-androstan-17-one* ***18***

Prepared as **7**, **8** and **9**, but using the deuterium labeled epoxide **15**, as starting material. In these conditions the isolated products were the labeled *trans*-diaxial and *trans*-diequatorial *vic*-diols **16** and **17** and the labeled non-vicinal diol **18** with identical yields. The position of the deuterium label was unambiguously confirmed by ^{1}H and ^{13}C NMR.

Labeled *vic*-diol **16**: ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.87 (3H, s, 18-H_3), 0.99 (3H, s, 19-H_3), (3α-^{2}H, not visible), 3.79 (1H, s, 4β-H); ^{13}C NMR (75.6 MHz, $CDCl_3$) δ: 13.6 (C-18), 22.5 (C-19), (C-5, not visible), (C-3, not visible), 76.2 (C-4), 221.6 (C-17).

Labeled *vic*-diol **17**: ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.87 (3H, s, 18-H_3), 1.01 (3H, s, 19-H_3), (3β-^{2}H, not visible), 3.71 (1H, s, 4α-H); ^{13}C NMR (75.6 MHz, $CDCl_3$) δ: 13.8 (C-18), 23.4 (C-19), (C-5, not visible), 72.5 (C-4), 76.7 (C-3, not visible), 221.3 (C-17).

Labeled diol **18**: ^{1}H NMR (300 MHz, CD_3OD) δ: 0.87 (3H, s, 18-H_3), 0.98 (3H, s, 19-H_3), 2.23 (1H, s, 4-H), (3α-^{2}H, not visible); ^{13}C NMR (75.6 MHz, $CDCl_3$) δ: 13.7 (C-18), 17.0 (C-19), (C-4, not visible), (C-3 not visible), 75.0 (C-5), 221.1 (C-17).

2.5. General procedure for the DMSO oxidation of diols ***3****,* ***7****,* ***8****,* ***9***

To a stirred and cooled (−60°C) mixture of dimethylsulfoxide (DMSO) (0.15 ml, 2.11 mmol) in dichloromethane (9 ml) under nitrogen, trifluoroacetic anhydride (TFAA) (0.27 ml, 1.91 mmol) was added dropwise. After 10 min a solution of diol **3** (203 mg, 0.66 mmol) in a mixture of dichloromethane and dimethylsulfoxide (1 ml) was added and stirred until the steroid was consumed (3 h, TLC control). Triethylamine (Et_3N) (0.62 ml, 4.41 mmol) was then added and after 15 min at −60°C the temperature was raised at 5°C. The solution was poured into 2N HCl (20 ml) and extracted with dichloromethane (3 × 20 ml). The extract was washed with 10% aq. $NaHCO_3$ (2 × 40 ml), water (4 × 40 ml), dried ($MgSO_4$) and evaporated to dryness yielding the crude diosphenol *3-hydroxy-5α-androst-2-ene-4,17-dione* **4** (199 mg, 98%) as the only detected product. This pale yellow solid could not be crystallized, and an analytical sample was purified by column chromatography [silica gel; ethyl acetate-light petroleum (b.p. 60–80°C) (1:1)].

Diosphenol **4**: Amorphous solid, ν_{max} (KBr)/cm^{-1} 3400 (OH), 1740 (C = O), 1675 (C = O), 1650 (C = C); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.88 (3H, s, 18-H_3), 0.93 (3H, s, 19-H_3), 5.85 (1H, s, disappeared with D_2O, 2-OH), 5.96 (1H, dd, $J_{2,1}$ 3.0, $J_{2,1}$ 7.0, 2-H); ^{13}C NMR (50.3 MHz, $CDCl_3$) δ: 13.2 (C-18), 13.7 (C-19), 113.9 (C-2), 145.9 (C-3), 196.8 (C-4), 220.7 (C-17); EIMS *m/z* 302 (M^+, 100%); Elemental analysis calculated for $C_{19}H_{26}O_3$: C, 75.46; H, 8.67. Found: C, 75.46; H, 8.97.

The same oxidation reaction conditions used for the *vic*-diol **3** were applied to diol **7** giving a mixture with a variable composition (1:2 to 2:1 by NMR) of the triketone *5β-androstane-3,4,17-trione* **10** and the diosphenol *3-hydroxy-5β-androst-2-ene-4,17-dione* **11**. The unstable compounds **10** and **11** couldn't be separated because of their rapid conversion into diosphenol **5** and were identified in the mixture only by ^{1}H NMR.

Triketone **10**: ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.86 (3H, s, 18-H_3), 1.17 (3H, s, 19-H_3), 2.65 (1H, ddd, $J_{2\alpha,2\beta}$ 18.0, $J_{2\alpha,1\beta}$ 15.0, $J_{2\alpha,1\alpha}$ 6.0, 2α-H), 2.77 (1H, ddd, $J_{2\beta,2\alpha}$ 18.0, $J_{2\beta,1\beta}$ 5.0, $J_{2\beta,1\alpha}$ 2.5, 2β-H).

Diosphenol **11**: ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.86 (3H, s, 18-H_3), 1.17 (3H, s, 19-H_3), 5.91 (1H, dd, $J_{2,1\alpha}$ 7.0, $J_{2,1\beta}$ 3.0, 2-H), 6.02 (1H, broad s, disappeared with D_2O, 3-OH).

Applying the same oxidation reaction conditions to the *vic*-diol **8**, the diosphenol **11** was the only product detected in the crude obtained from the reaction. Attempts to recrystallize this compound where unsuccessful also due to its rapid conversion into diosphenol **5** and it was identified only by IR, ^{1}H and ^{13}C NMR.

Diosphenol **11**: Amorphous slightly yellow solid, ν_{max} (KBr)/cm^{-1} 3340 (OH), 1735 (C = O), 1670 (C = O), 1655 (C = C); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.86 (3H, s, 18-H_3), 1.17 (3H, s, 19-H_3), 5.91 (1H, dd, $J_{2,1\alpha}$ 7.0, $J_{2,1\beta}$ 3.0, 2-H), 6.02 (1H, broad s, disappeared with D_2O, 3-OH); ^{13}C NMR (50.3 MHz, $CDCl_3$) δ: 13.8 (C-18), 23.0 (C-19), 113.2 (C-2), 146.4 (C-3), 196.8 (C-4), 220.5 (C-17).

Oxidation of the diol **9** with the same reaction conditions above described gives the hydroxyketone *5β-hydroxyandrostane-3,17-dione* **12** which is converted into the enone *androst-4-ene-3,17-dione* **13** during the work-up procedure. Analytical samples of **12** and **13** were isolated by preparative TLC [silica gel; ethyl acetate-*n*-hexane (1:1)]. Attempts to recrystallize compound **12** where unsuccessful due to its conversion into enone **13** and it was identified only by IR, ^{1}H and ^{13}C NMR.

Hydroxyketone **12**: Amorphous white solid, ν_{max} (KBr)/cm^{-1} 3480 (OH), 1700–1730 (C = O); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.90 (3H, s, 18-H_3), 1.03 (3H, s, 19-H_3),

2.11 (1H, ddd, $J_{16\alpha,16\beta}$ 19.0, $J_{16\alpha,15\alpha/15\beta}$ 9.5, 16α-H), 2.30 (1H, ddd, $J_{2\alpha,2\beta}$ 14.5, $J_{2\alpha,1\beta}$ 14.5, $J_{2\alpha,1\alpha}$ 6.0, 2α-H), 2.48 (1H, dd, $J_{16\beta,16\alpha}$ 19.0, $J_{16\beta,15\beta}$ 9.0, 16β-H), 3.04 (1H, d, $J_{4\alpha,4\beta}$ 15.0, 4α-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 13.8 (C-18), 16.2 (C-19), 37.3 (C-2), 49.3 (C-4), 78.4 (C-5), 210.8 (C-3), 220.4 (C-17).

Enone **13**: White solid from hexane mp 172–174°C (lit [14] 173–174°C); ν_{max} (KBr)/cm^{-1} 1740 (C = O), 1660 (C = O), 1620 (C = C); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.92 (3H, s, 18-H_3), 1.22 (3H, s, 19-H_3), 5.76 (1H, s, 4-H). Spectroscopic data were in agreement with those reported in the literature [15].

*2.6. General procedure for the preparation of the diosphenol 4-hydroxyandrost-4-ene-3,17-dione **5***

*2.6.1. From isomerization of diosphenol **4***

To a stirred and cooled (0°C) solution of **4** (60 mg, 0.2 mmol) in methanol (3 ml) under nitrogen, sodium metal (50 mg, 2.2 mmol) was added. After 1 h at room temperature the suspension was neutralized with 10% aq. HCl (2 ml), diluted with water (30 ml) and extracted with dichloromethane (3 × 50 ml). The extract was washed with 10% aq. $NaHCO_3$ (50 ml), water (3 × 50 ml), dried ($MgSO_4$) and evaporated to dryness giving a crystalline crude. The crude was purified by flash chromatography [silica gel; diethyl ether-carbon tetrachloride (1:1)] to give 48 mg of **5** (80%).

Diosphenol **5**: White solid from ethyl acetate, mp 202–203°C (lit [6] 201–203°C); ν_{max} (KBr)/cm^{-1} 3415, 3390 (OH), 1740 (C = O), 1670 (C = O), 1630 (C = C); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.92 (3H, s, 18-H_3), 1.20 (3H, s, 19-H_3), 3.07 (1H, m, 6α-H), 6.16 (1H, broad s, disappeared with D_2O, 4-OH); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 13.7 (C-18), 17.1 (C-19), 139.2 (C-4), 141.3 (C-5), 193.5 (C-3), 220.7 (C-17). Spectroscopic data were in agreement with those reported in the literature [6].

*2.6.2. From isomerization of diosphenol **11***

The same reaction conditions used for the diosphenol **4** were applied to **11** giving, as before, a similar yield of the desired diosphenol ***5***.

*2.6.3. From isomerization of triketone **10***

Applying the same reaction conditions to a (2:1) mixture of triketone **10** and diosphenol **11,** due to the instability of the pure **10**, the desired diosphenol **5** was also obtained in similar yield.

*2.7. Using the epimeric mixture of olefins **1a** and **1b** as starting material*

A stirred solution of the (2.3:1) mixture of **1a** and **1b** (200 mg, 0.73 mmol) was treated with 30% hydrogen peroxide (0.02 ml, 2.0 mmol) at room temperature for 12 h (TLC). After the usual work-up a crude (200 mg) of complex composition (TLC) was obtained. A solution of this crude in a mixture of DMSO and dichloromethane (3 ml) was added dropwise to a stirred and cooled (60°C) mixture of DMSO (0.22 ml, 3.1 mmol) and TFAA (0.27 ml, 1.91 mmol) in dichloromethane (10 ml) and Et_3N (0.62 ml, 6.45 mmol) was then added according to the usual procedure. After the usual work-up a crude oil (214 mg) of complex composition was obtained. A solution of this crude in methanol (20 ml) was finally treated with sodium metal (50 mg, 13.6 mmol) at room temperature for 2 h giving after the usual work-up a new crude (191 mg) containing a mixture of the desired diosphenol **5**, the hydroxyketone **12** and the enone **13**. Column chromatography of this crude with diethyl ether-carbon tetrachloride (1:1) yielded 119 mg (54%) of diosphenol **5** still contaminated with traces of by-products.

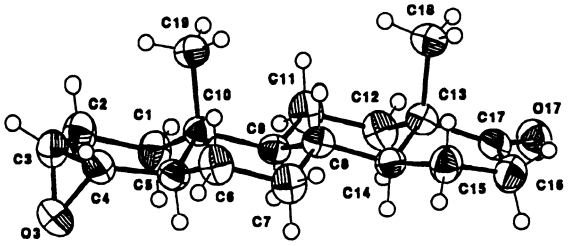


Fig. 1. ORTEP plot of the epoxide intermediate **2**.

3. Results and discussion

An optimized synthesis of formestane previously developed in our laboratory uses the available 5α-androst-3-en-17-one **1a** as starting material [10]. Treatment of **1a** (Scheme 1) with performic acid generated in situ led after work-up to the *trans*-diaxial diol, 3α,4β-dihydroxy-5α-androstan-17-one **3**, in 96% yield. Using dichloromethane instead of formic acid as a solvent, due to the low solubility of the formic acid in this solvent further cleavage of the epoxide was prevented and the 3α,4α-epoxide **2** was the only product formed. The stereochemistry of the 3α,4α-oxirane ring and the *trans*-A/B fusion were previously established by ^{1}H and ^{13}C NMR spectroscopic analysis [10] and afterwards confirmed by X-ray diffraction analysis (Fig. 1) [16]. Further oxidation of the *trans*-diaxial diol **3**, with dimethyl sulfoxide activated with trifluoroacetic anhydride, gave quantitatively the kinetic diosphenol, 3-hydroxy-5α-androst-2-ene-4,17-dione **4**, which by base-catalyzed isomerization with NaOMe/MeOH led to the desired thermodynamic diosphenol, 4-OHA **5**, as the sole product in high yield (80%).

Since the chiral center at C-5 in 5α-olefin **1a** is lost during the synthetic process described for the preparation of 4-OHA, the epimeric mixture of 5α- and 5β-olefins, **1a** and **1b**, easily available from the reduction of androstenedione with zinc in acetic acid [11], was treated in a sequential way with performic acid, dimethyl sulfoxide activated with tri-

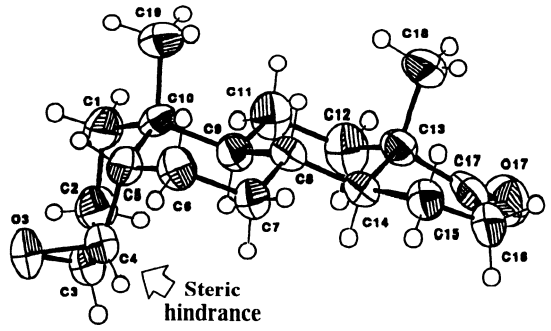


Fig. 2. ORTEP plot of the epoxide intermediate **6**.

fluoroacetic anhydride, and finally with sodium metal in methanolic solution under the conditions depicted in Scheme 1. After the work-up, the diosphenol **5** was obtained as a mixture with two by-products, the hydroxyketone **12** and the enone **13** thus making the isolation of the desired compound difficult and not affording the improvement in yield.

The formation of the by-products **12** and **13** from the 5β-olefin **1b** was studied through the reaction of the 3β,4β-epoxy-5β-androstan-17-one intermediate **6**, formed during the first oxidative step, with formic acid (Scheme 1). Preparation of **6** was carried out by reaction in dichloromethane of a mixture of **1b**+ **1a,** enriched in **1b**, with performic acid generated in situ. As in the preparation of **2**, the low solubility of the resulting formic acid in dichloromethane prevents further cleavage of the epoxide. The β configuration of the oxirane ring and the *cis*-A/B fusion were established by the chemical shift, 22.2 ppm, of the C-19 carbon in ^{13}C NMR spectroscopic analysis and by X-ray diffraction analysis [17] (Fig. 2).

Reaction of the intermediate epoxide **6** with formic acid at room temperature affords a mixture of diols, the expected *trans*-diaxial diol, 3β,4α-dihydroxy-5β-androstan-17-one **7** (48%) and the *trans*-diequatorial diol, 3α,4β-dihydroxy-5β-androstan-17-one **8** (11%) as well as the non-vicinal diol, 3β,5β-dihydroxyandrostan-17-one **9** (25%).

The stereochemistry of the hydroxyl groups was unambiguously established by ^{1}H NMR spectroscopic and X-ray diffraction analyses [18] for compound **7** (Fig. 3) and by ^{1}H NMR spectroscopic analysis for compounds **8** and **9**. The

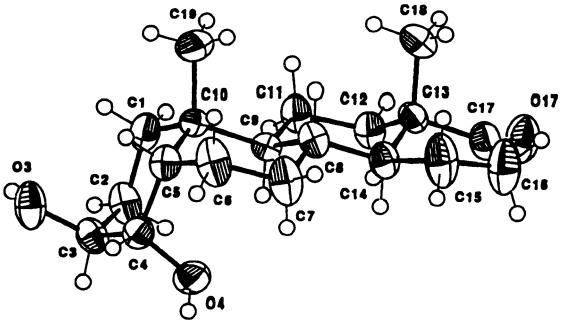


Fig. 3. ORTEP plot of the *vic*-diol **7**.

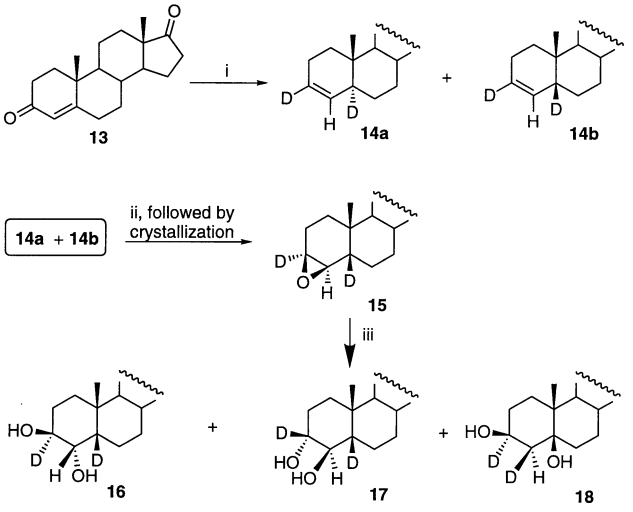
magnitude of the coupling constant, $J_{3:4}$ 5–6 Hz, measured in the ^{1}H NMR spectrum of **7**, revealed a *trans*-diequatorial interaction between these protons which was confirmed by X-ray analysis (Fig. 3), while the magnitude of J = 9 Hz in **8** revealed a *trans*-diaxial relationship for the protons 3β,4α-H. Coupling constants of 5–6 Hz for the 4β-H, 5β-H interaction in **7** and 11–12 Hz for the 4α-H, 5β-H interaction in **8** also revealed the corresponding diequatorial and diaxial proton relationships in the aforementioned compounds. The two 4-H protons of **9** interact geminally, with a coupling constant of 15 Hz and one of them interacts with the 3α-equatorial proton with a coupling constant of 3 Hz.

Further oxidation of the *trans*-diaxial *vic*-diol **7** with dimethyl sulfoxide (DMSO) activated with trifluoroacetic anhydride (TFAA) yields the triketone, *5β-androstane-3,4,17-trione* **10**, and the kinetic diosphenol, 3-hydroxy-5β-androst-2-ene-4,17-dione **11**, in a mixture of variable proportions (from 1:2 to 2:1 by NMR). When the same oxidative conditions were applied to the *trans*-diequatorial *vic*-diol **8** the kinetic diosphenol **11** was quantitatively afforded.

The base-catalyzed isomerization of the diosphenol **11** either alone or as a mixture with the triketonic form **10** led to the 4-OHA **5** in high yield (80%).

Oxidation of diol **9** with dimethyl sulfoxide activated with trifluoroacetic anhydride gave 5β-hydroxyandrostane-3,17-dione **12** and androst-4-ene-3,17-dione **13** in a 2:1 mixture. Compound **13** was also obtained after base-catalyzed dehydration of **12**.

In order to understand the mechanism for the formation of the abnormal diols **8** and **9**, the 3,5-deuterium labeled analog **15** of epoxide **6** was prepared through selective introduction of the isotope in positions 3 and 5 of the steroid nucleus and its cleavage with formic acid was further studied (Scheme 2). Preparation of the desired labeled steroid **15**



Scheme 2. The strategy for C-3 and C-5 deuterium labeling of the epoxide intermediate **15** and study of the normal and abnormal cleavages. *Reagents and conditions*: i) Zn, $CH_3CO_2{}^2H$, reflux, 10′; ii) H_2O_2, HCO_2H, CH_2Cl_2, rt, 6 h; iii) HCO_2H, rt, 30′.

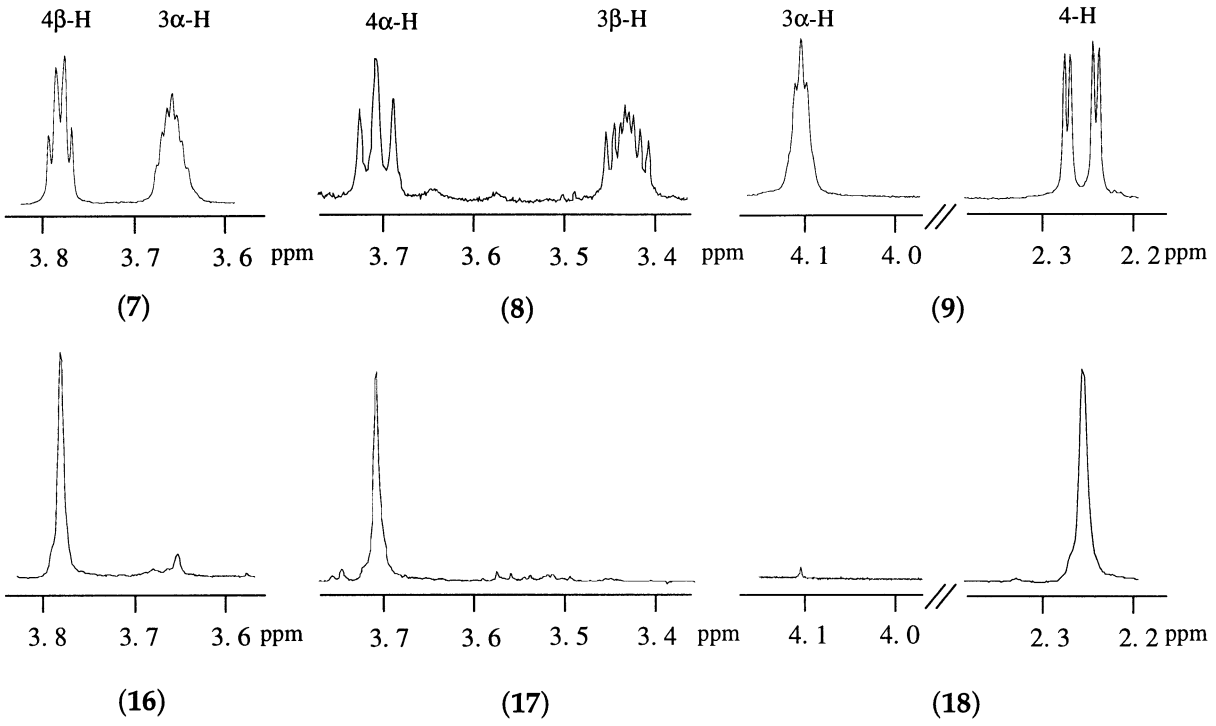
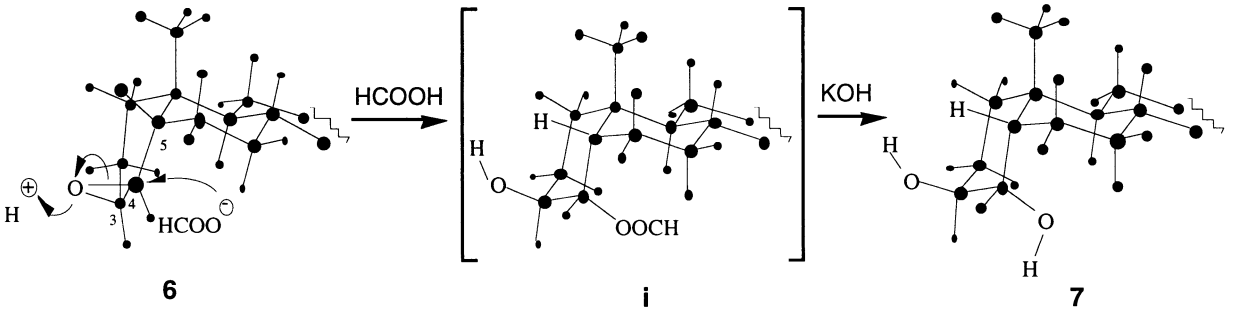


Fig. 4. Portions of ^{1}H NMR spectra with the signals of 3-H and 4-H protons of the unlabeled compounds **7**, **8** and **9** and the counterpart spectra portions of deuterium labeled compounds **16**, **17** and **18**.

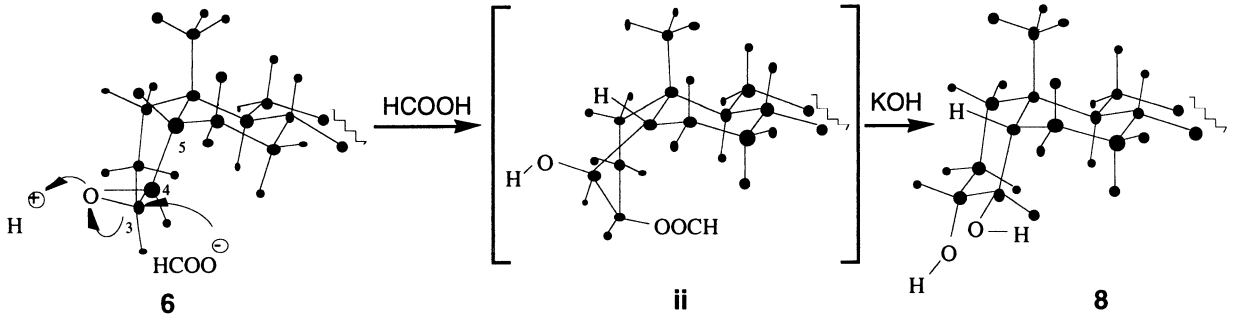
was achieved by epoxidation of the labeled olefin **14b**. This olefin was obtained, in mixture with **14a**, by selective deuteration of enone **13** through reduction with metal zinc in deuterated acetic acid $CH_3CO_2{}^2H$. The presence of deuterium in the desired 3 and 5 positions of the steroid nucleus of **14b** and **15** has been inferred by comparing the ^{1}H and ^{13}C NMR spectra of these compounds with the corresponding spectra of the non-labeled analogues **1b** and **6**. In fact, the multiplet of the 4-H proton, in **1b** and **6**, shows the coupling with the vicinal 3-H and 5-H protons, which is not visible in the case of **14b** and **15**, due to the substitution of these protons by deuterium. Additionally, the 3-H, C-3 and C-5 signals observed in the ^{1}H and ^{13}C NMR spectra, respectively, for **1b** and **6** have disappeared in the spectra of **14b** and **15**, in accordance with the introduction of deuterium in the above-mentioned carbon atoms.

When the 3,5-deuterium substituted compound **15**, prepared as previously described, was treated with formic acid, three new compounds, **16**, **17** and **18**, were obtained after conventional work-up, due to the cleavage of the epoxide ring of **15** (Scheme 2).

^{1}H and ^{13}C NMR spectroscopic analysis of **16**, **17** and **18** revealed that **16** and **17** are respectively, the 3,5-deuterated analogues of the diols **7** and **8**, while compound **18** is the 3,4-deuterated analog of diol **9** (Fig. 4). In fact, the vicinal 4H-3H and 4H-5H couplings, observed for the 4-H multiplets of the ^{1}H NMR spectra of compounds **7** and **8**, were absent in the case of **16** and **17**. Furthermore, the 3-H, C-3 and C-5 signals have also disappeared from the ^{1}H and ^{13}C NMR spectra of these two diols. In the same way, comparing the ^{1}H NMR spectra of **9** and **18** we could observe a double doublet, for the geminal coupling between the two 4-H protons and for the vicinal coupling between 4H-3αH of **9**, which collapses to a singlet in the spectrum of **18**, due



Scheme 3. Formation of the *vic*-diol **7** through the classic Fürst-Plattner rule.



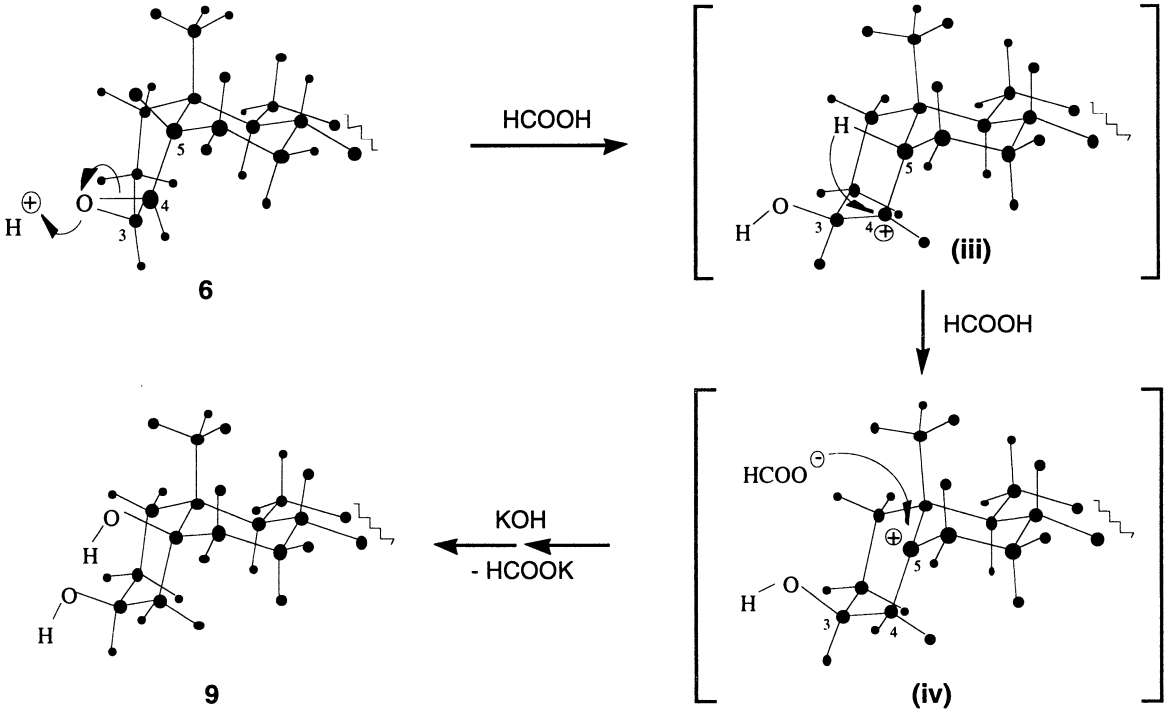
Scheme 4. Proposed mechanism for the formation of *vic*-diol **8**.

to the presence of a deuterium atom at C-3 and C-4 carbons. As observed in the spectra of compounds **16** and **17**, the 3-H signal disappeared from the ^{1}H NMR spectra of **18** as a result of the presence of a deuterium atom at C-3. Additionally, the collapse of the C-4 signal and the reappearance of the C-5 signal in the ^{13}C NMR spectrum of **18** confirms the transfer of the deuterium from C-5 to C-4 during the conversion of **15** into **18**.

Based on these results, we can conclude that epoxide **6** is cleaved normally by the nucleophilic attack of the formic acid anion at C-4 carbon, with the formation of the *trans*-diaxial intermediate **i** as a result of a product like transition state (Scheme 3), which leads to the *vic*-diol **7**, according to the classic Fürst-Plattner rule [19,20].

Moreover, the formic acid anion can also attack the C-3 carbon atom, resulting in the formation of the twist boat anti-periplanar intermediate **ii**, as depicted in Scheme 4, which undergo rotation to the more stable chair conformer, the *trans*-diequatorial diol **8** [21,22].

On the other hand, the study of the reaction with isotopic labeled compounds allowed us to conclude that the epoxide ring cleavage of **6** can also be accompanied by a hydride transfer from C-5 to C-4, leading to the formation of the non-vicinal diol **9** (Scheme 5) [23–27]. As a mechanism for this reaction, we also propose the protonation of the oxygen atom, which is followed by the C_4–O bond cleavage to generate the secondary carbocation **iii** (Scheme 5) in the C-4 carbon atom followed by the vicinal 5-H hydride transfer to C-4 with formation of a more stable tertiary carbocation **iv**, which will be finally attacked by the nucleophilic formic acid anion. The hydride ion must approach the epoxide at the C-4 carbon atom from the β-face of the steroid framework as depicted in **iii**. The formation of the refered carbocations should be favored by the high polarity and strength of the



Scheme 5. Proposed mechanism for the formation of 1,3-diol **9**.

formic acid, used as solvent and reagent, and so the C–O epoxide bond cleavage should precede the hydride shift to C-4, accordingly to a non-concerted type of reaction mechanism [21]. Hydrolysis of the resultant formate derivative led to the diol **9**.

In an attempt to understand why the formic acid does not attack only the C-4, but also the C-3 carbon atom, and why the 5-H hydride undergoes migration to C-4, competing with the predicted nucleophilic attack at this position, epoxides **2** and **6** were studied by X-ray diffraction analyses [16,17] (Figs. 1 and 2). These studies confirm that C-4 carbon atom is stereochemically hindered due to the *cis*-A/B ring fusion in epoxide **6** (Fig. 2). This hindrance should be, most probably, the principal cause for the partial shift of the nucleophilic attack from C-4 to C-3 and for the hydride rearrangement which occurs in the reaction of **6** with formic acid.

In summary, 4-OHA **5** can be prepared either from the olefin **1a** or from the 5β-epimer **1b** through the epoxides **2** and **6**. However, an efficient synthesis is only achieved from **1a**, since by-products **12** and **13** formed during the synthesis from the 5β-olefin **1b**, complicate the final isolation of the desired diosphenol **5**. As epoxide **2** cleaves giving diol **3**, epoxide **6** cleaves also by a normal way giving the diol **7**. Furthermore another two abnormal products, diols **8** and **9**, have been formed from **6** suggesting abnormal epoxide ring cleavages. Isotopic and X-ray diffraction studies allowed us to further conclude that diol **9** will be formed through an 1,2-intramolecular hydride shift from C-5 to C-4 which accompanies the epoxide ring cleavage according, most probably, to a non-concerted type reaction mechanism.

In spite of considering all the mentioned *vic*-diols **3**, **7** and **8** as precursors for the synthesis of the desired anticancer diosphenol **5**, the best synthetic route uses the key intermediate **3**, the only product formed from the olefin **1a**.

Acknowledgments

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