Expedient synthesis of lactone analogues of formestane as new potential aromatase inhibitors

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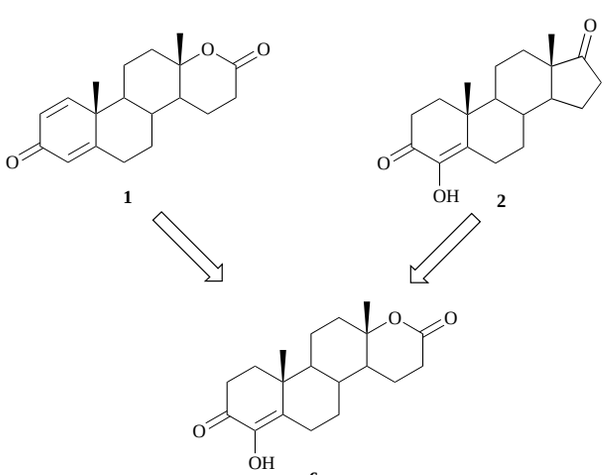
A convenient synthetic strategy for the preparation of ring-D lactones of the androstane derivatives 4, 5 and 6 has been achieved through a high yielding, three-step sequence. Baeyer–Villiger oxidation of 3α,4β-dihydroxy-5α-androstan-17-one 3, previously prepared from a 3-olefin, followed by a TFAA mediated Swern oxidation and subsequent isomerization allowed the preparation of the ring-D lactone analogue of formestane 6.

Inhibition of aromatase, an enzyme which catalyses the final step in the biosynthesis of estrogens, is of importance, since it has been identified as a good endocrine therapy for the treatment of estrogen-dependent breast cancer and may be important in the treatment of other malignant diseases in ageing patients.[1]

Of the various types of aromatase inhibitors, steroids which closely resemble the enzyme natural substrates androstenedione and testosterone have been shown to be useful for the treatment of breast cancer. Ring-D lactones related to testosterone proved to be effective aromatase inhibitors[2] and testolactone **1** (Scheme 1) was one of the first steroids used in the clinical

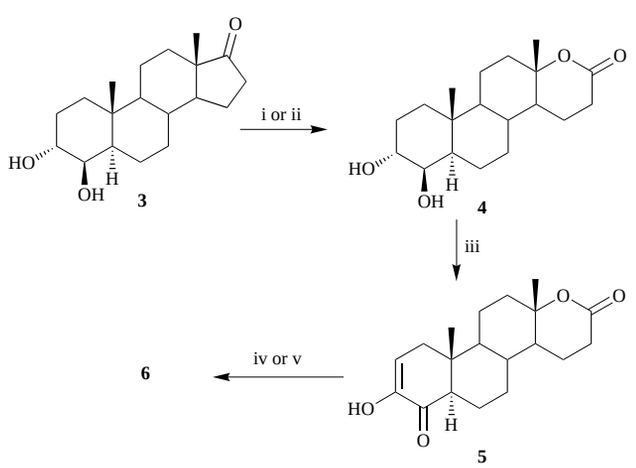


Scheme 1

treatment of breast cancer,[3] although it has been withdrawn before its activity as an aromatase inhibitor had been established.[4] Moreover, the 4-hydroxyandrost-4-ene-3,17-dione (4-OHA, Formestane) **2** (Scheme 1), has proved very effective in the treatment of advanced breast cancer[5] and quite recently has found clinical use.[6]

In order to enhance the anti-cancer activity and to prepare affinity labels for the elucidation of the active site of aromatase, the synthesis of new drugs related to 4-OHA, is a very active field of research.[7] Following this idea, the synthesis of a new target molecule, with the A-ring moiety of 4-OHA and the D-ring moiety of testolactone, steroid **6**, (Scheme 1), has been our goal. Androstenedione derivatives which often serve as synthetic precursors for the ring-A diosphenol moiety in 4-OHA[8] were not suitable for our target molecule, for which only D-lactone formation is required.

We report herein a convenient synthetic strategy in three-steps to prepare the target compound **6** using the 3α,4β-dihydroxy-5α-androstan-17-one **3**, a key intermediate in a recently reported novel approach to the synthesis of 4-OHA,[9] as starting material. The reaction conditions are summarized in Scheme 2.



Scheme 2 *Reagents and conditions:* i, MCPBA, $NaHCO_3$, CH_2Cl_2, RT, 6 days, 64%; ii, MMPP, MeOH, H_2O, RT, 3 days, 90–94%; iii, DMSO, TFAA, −60 °C, 3 h then Et_3N, −60 °C, 15 min, 98%; iv, Na, MeOH, RT, 1 h, 60%; v, HCl, CH_3CO_2H, RT, 24 h, 99%

Baeyer–Villiger oxidation of the 17-keto group of **3** was first performed with the classical reagent *m*-chloroperoxybenzoic acid (MCPBA), but a long reaction time was required for complete reaction (6 days) and only a limited yield of the required lactone product (64%) was obtained. Besides, the work-up of the reaction requires the chromatographic separation of the formed *m*-chlorobenzoic acid in order to purify the lactone **4** (Scheme 2). Better reaction and work-up performances were achieved with the inexpensive and safe water-soluble monoperoxyphthalic acid magnesium salt hexahydrate (MMPP)[10] to afford the 3α,4β-dihydroxy-D-homo-17a-oxa-5α-androstan-17-one **4** (90–94%). Further oxidation of **4** with dimethyl sulfoxide

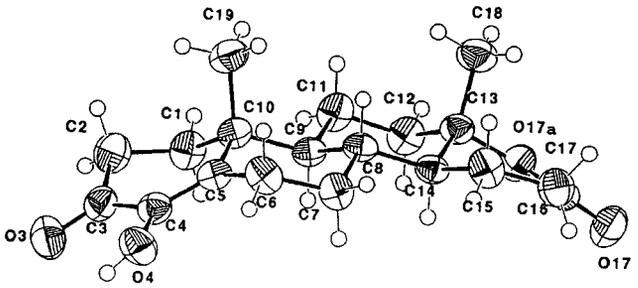


Fig. 1 An ORTEP drawing of **6**

activated with trifluoroacetic anhydride[9] gave the kinetic diosphenol **5** in near quantitative yield (98%). Base-catalyzed isomerization[9] of **5** led to the desired thermodynamic diosphenol, the 4-hydroxy-D-homo-17a-oxaandrost-4-ene-3,17-dione **6**, (60%), revealing that some hydrolysis of the D-ring lactone may also have occurred. The development of acid-catalyzed conditions for the required isomerization allowed us to prepare compound **6** in excellent yield (99%).

Single-crystal X-ray diffraction analysis of the latter unambiguously established the expected structure (Fig. 1).[11]

The generation of the ring-A diosphenol after the Baeyer–Villiger oxidation, proved to be an appropriate synthetic strategy for the synthesis of the lactone analogue of 4-OHA **6** and the precursor **5**. Furthermore, the ring-D lactone moiety remained intact during subsequent TFAA mediated Swern oxidation, and isomerization.

The convenient synthesis described uses moderately cheap reagents and gives a high overall yield (91%) of compound **6**. The three hitherto undescribed lactones **4**, **5** and **6** were fully characterized and are being evaluated as anticancer drugs. Detailed biochemical results will be reported elsewhere.

Experimental

3α,4β-Dihydroxy-5α-androstan-17-one 3

This compound has been prepared as previously described[9] and an analytical sample of **3** for elemental analysis was obtained by crystallization from acetone–hexane (Found: C, 74.33; H, 9.92. $C_{19}H_{30}O_3$ requires C, 74.47; H, 9.87%).

3α,4β-Dihydroxy-D-homo-17a-oxa-5α-androstan-17-one 4

To a stirred solution of the diol **3** (50 mg, 0.16 mmol) in a mixture of methanol (4 cm^3) and water (1 cm^3) at room temperature, magnesium monoperphthalate 80% (7–10 mole/mole of substrate) was added sequentially in 3–4 equal proportions (2.5 mol/portion) during 3–4 days (1 portion/day) until the reaction was complete (TLC control). After methanol evaporation and dichloromethane dilution, the organic solution was washed with aq. $NaHCO_3$ and water, dried and evaporated to dryness to give compound **4** (47.5–49.5 mg, 90–94%) as a pure white crystalline solid. An analytical sample of the dihydroxy-lactone **4** was obtained by crystallization or alternatively by preparative TLC, mp 225–227 °C (from ethyl acetate); ν_{max}(KBr)/cm^{-1} 3360–3320 (OH), 1710–1680 (C=O), 1230, 1170, 1110 and 1075 (C–O); δ_H(500 MHz; $CDCl_3$; Me_4Si) † 0.99 (3H, s, 18-H_3), 1.30 (3H, s, 19-H_3), 2.57 (1H, ddd, $J_{16\alpha,16\beta}$ 19.0, $J_{16\alpha,15\beta}$ 9.5, $J_{16\alpha,15\alpha}$ 8.5, 16α-H), 2.67 (1H, ddd, $J_{16\beta,16\alpha}$ 19.0, $J_{16\beta,15\beta}$ 9.0, $J_{16\beta,15\alpha}$ 2.5, 16β-H), 3.64 (1H, dddd, $J_{3\beta,4\alpha}$ 3.0, $J_{3\beta,3\alpha OH}$ 3.0, $J_{3\beta,2H}$ 3.0, $J_{3\beta,2H}$ 1.5, 3β-H) and 3.90 (1H, ddd, $J_{4\alpha,5\alpha}$ 6.0, $J_{4\alpha,3\beta}$ 3.0, $J_{4\alpha,4\beta OH}$ 3.0, 4α-H); δ_C(50.3 MHz, [$^2H_4$]-MeOH; Me_4Si) 14.6 (CH_3, C-19), 20.3 (CH_3, C-18), 20.6 (CH_2), 22.2 (CH_2), 25.0 (CH_2), 26.3 (CH_2), 29.4 (CH_2), 32.1 (CH_2), 32.9 (CH_2), 36.9 (Cq), 39.2 (CH), 40.5 (CH_2), 44.5 (CH), 47.5 (CH), 55.4 (CH), 71.2 (CH, C-3), 76.2 (CH, C-4), 85.4 (Cq, C-13) and 174.9 (Cq, C-17); *m*/*z* (EI) 322 (M^+, 7.9%).

† *J* Values recorded in Hz.

3-Hydroxy-D-homo-17a-oxa-5α-androst-2-ene-4,17-dione 5

To a stirred and cooled (−60 °C) mixture of dried dimethylsulfoxide (0.3 cm^3, 4.22 mmol) in dried dichloromethane (17 cm^3) under a nitrogen atmosphere, trifluoroacetic anhydride (0.54 cm^3, 3.82 mmol) was added dropwise. After 10 min, a solution of the dihydroxy-lactone **4** (400 mg, 1.24 mmol) in dichloromethane–dimethyl sulfoxide (2 cm^3) was added to the reaction mixture which was then stirred until the steroid had been consumed (4 h 20 min, TLC control). Triethylamine (1.7 cm^3, 12.3 mmol) was then added to the mixture and after 15 min at −60 °C, the temperature was raised to 5 °C. The solution was poured into 2 M hydrochloric acid (50 cm^3), extracted with dichloromethane (3 × 50 cm^3) and worked-up in the usual manner to yield the crude *kinetic diosphenol lactone* **5** (387 mg, 98%) as the only detected product by TLC and NMR. This white solid could not be crystallized, and the analyses were performed on the crude material; δ_H(500 MHz; $CDCl_3$; Me_4Si) 0.88 (3H, s, 18-H_3), 1.32 (3H, s, 19-H_3), 2.42 (1H, dd, $J_{1\alpha,1\beta}$ 17.0, $J_{1H,2H}$ 7.0, 1-H), 2.58 (1H, ddd, $J_{16\alpha,16\beta}$ 19.0, $J_{16\alpha,15\beta}$ 9.5, $J_{16\alpha,15\alpha}$ 8.5, 16α-H), 2.70 (1H, ddd, $J_{16\beta,16\alpha}$ 19.0, $J_{16\beta,15\beta}$ 9.25, $J_{16\beta,15\alpha}$ 2.25, 16β-H), 5.83 (1H, s, sharp, 3-OH) and 5.97 (1H, dd, $J_{2H,1H}$ 7.0, $J_{2H,1H}$ 2.5, 2-H); δ_C(50.3 MHz, $CDCl_3$; Me_4Si) 13.2 (CH_3, C-19), 19.7 (CH_2), 20.0 (CH_2), 20.1 (CH_3, C-18), 21.6 (CH_2), 28.5 (CH_2), 29.1 (CH_2), 37.1 (CH), 37.6 (CH_2), 38.9 (CH_2), 40.8 (Cq, C-10), 45.9 (CH), 52.5 (CH), 54.0 (CH), 83.1 (Cq, C-13), 114.0 (CH, C-2), 146.0 (Cq, C-3), 171.6 (Cq, C-17) and 196.7 (Cq, C-4); *m*/*z* (EI) 318 (M^+, 90%).

4-Hydroxy-D-homo-17a-oxaandrost-4-ene-3,17-dione 6

Method 1. To a stirred and cooled (0 °C) solution of the diosphenol **5** (30.4 mg, 0.095 mmol) in methanol (3.5 cm^3) under a nitrogen atmosphere, sodium metal (48 mg, 2.08 mmol) was added. After 1 h at room temperature, the solution was neutralized with 10% hydrochloric acid and the usual work-up procedure was followed. The crude mixture, a white crystalline powder (23 mg, 76%) was composed of a mixture (4:1; NMR) of 4-hydroxy-D-homo-17a-oxaandrost-4-ene-3,17-dione **6** and starting material **5**.

Method 2. The kinetic diosphenol lactone **5** (50 mg, 0.16 mmol) in a 0.3 M acetic acid solution of hydrochloric acid (2 cm^3) was stirred at room temperature for 24 h. The solution was neutralized with 10% aqueous sodium hydrogencarbonate (25 cm^3) and worked-up in the usual way, to yield compound **6** (49.5 mg, 99%) as the only detected product by TLC and NMR as a pure pale yellow solid; mp 235–237 °C (from ethyl acetate–isopropyl ether) (Found: C, 71.64; H, 8.32. $C_{19}H_{26}O_4$ requires C, 71.67; H, 8.23%) ν_{max}(KBr)/cm^{-1} 3380 (OH), 1720 (17-C=O), 1665 (3-C=O), 1635 (C=C), 1225, 1160 and 1095 (C–O); δ_H(500 MHz; $CDCl_3$; Me_4Si) 1.16 (3H, s, 19-H_3), 1.35 (3H, s, 18-H_3), 2.59 (1H, ddd, $J_{16\alpha,16\beta}$ 19.0, $J_{16\alpha,15\alpha}$ 9.0, $J_{16\alpha,15\beta}$ 9.0, 16α-H), 2.71 (1H, ddd, $J_{16\beta,16\alpha}$ 19.0, $J_{16\beta,15\beta}$ 8.5, $J_{16\beta,15\alpha}$ 2.5, 16β-H), 3.06 (1H, ddd, $J_{6\alpha,6\beta}$ 15.0, $J_{6\alpha,7H}$ 4.0, $J_{6\alpha,7H}$ 2.5, 6α-H) and 6.11 (1H, s, 4-OH); δ_C(50.3 MHz, $CDCl_3$; Me_4Si) 17.1 (CH_3, C-19), 19.9 (CH_2), 20.1 (CH_3, C-18), 21.8 (CH_2), 22.7 (CH_2), 28.5 (CH_2), 29.5 (CH_2), 31.7 (CH_2), 34.4 (CH_2), 37.6 (CH), 37.7 (Cq, C-10), 39.0 (CH_2), 45.8 (CH), 52.9 (CH), 82.8 (Cq, C-13), 138.0 (Cq, C-5), 141.2 (Cq, C-4), 171.3 (Cq, C-17) and 193.3 (Cq, C-3); *m*/*z* (EI) 318 (M^+, 84.6%).

Acknowledgements

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References

1 F. J. Zeelen, *Medicinal Chemistry of Steroids*, Elsevier, Amsterdam, 1990, p. 280; P. A. Cole and C. H. Robinson, *J. Med. Chem.*, 1990,

33, 2933; D. Lesuisse, J. F. Gourvest, C. Hartmann, B. Tric, O. Benslimane, D. Philibert and J. P. Vevert, *J. Med. Chem.*, 1992, **35**, 1588; A. M. H. Brodie and R. J. Santen, *Breast Cancer Res. Tr.*, 1994, **30**, 1.
2 G. Leclercq, *Antitumor Steroids*, ed. R. T. Blickenstaff, Academic Press, New York, 1992, p. 81; I. Barta, K. Albrecht, G. Ambrus, E. Csanyi, J. Gyimesi and E. Kiraly, Hung. Teljes 14, 361/1977 (*Chem. Abstr.*, 1978, **89**, 24624v); P. F. Sherwin, P. C. MacMullan and D. F. Covey, *J. Med. Chem.*, 1989, **32**, 651; L. H. Knox, R. Villoti, F. A. Kincl and H. J. Ringold, *J. Org. Chem.*, 1961, **26**, 501.
3 A. Segaloff, J. B. Weeth, K. K. Meyer, E. L. Rongone and M. E. G. Cunningham, *Cancer*, 1962, **15**, 633.
4 P. K. Siiteri and E. A. Thompson, *J. Steroid Biochem.*, 1975, **6**, 317.
5 K. Hoffken, W. Jonat, K. Possinger, M. Kolbel and T. Kunz, *J. Clin. Oncol.*, 1990, **8**, 875; T. Pickles, L. Perry, V. Murray and P. Plowman, *Br. J. Cancer*, 1990, **62**, 309.
6 A. M. Brodie, *J. Steroid Biochem. Mol. Biol.*, 1994, **49**, 281.
7 X. P. Liu, D. M. Lambert and Y. J. Abul-Hajj, *J. Med. Chem.*, 1995, **38**, 4135; M. B. Martin, A. F. Mateos and R. R. González, *J. Chem. Soc.*, *Perkin Trans. 1*, 1995, **5**, 569; M. Numazawa and M. Tachibana, *J. Chem. Soc.*, *Perkin Trans. 1*, 1993, **23**, 2975; P. F. Sherwin, P. C. MacMullan and D. F. Covey, *J. Med. Chem.*, 1989, **32**, 651.
8 D. A. Marsh, H. J. Brodie, W. Garrett, C. H. Tsai-Morris and A. M. H. Brodie, *J. Med. Chem.*, 1985, **28**, 788; P. J. Bednarski and S. D. Nelson, *J. Med. Chem.*, 1989, **32**, 203; J. Mann and B. Pietrzak, *J. Chem. Soc.*, *Perkin Trans. 1*, 1983, **11**, 2681; M. Numazawa and M. Ogata, *J. Chem. Soc.*, *Chem. Commun.*, 1986, 1092; P. G. Ciattini, E. Morera and G. Ortar, *Synth. Commun.*, 1992, **22**, 1949.
9 E. J. Tavares da Silva, M. L. Sá e Melo and A. S. Campos Neves, *J. Chem. Soc.*, *Perkin Trans. 1*, 1996, 1649, and references cited therein.
10 P. Brougham, M. S. Cooper, D. A. Cummerson, H. Heaney and N. Thompson, *Synthesis*, 1987, 1015.
11 J. A. Paixão, L. C. R. Andrade, M. J. Almeida, M. M. R. Costa, E. J. Tavares da Silva, M. L. Sá e Melo and A. S. Campos Neves, submitted for publication in *Acta Crystallogr.*, *Sect. C.*

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