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Ergosteroids III. Syntheses and biological activity of seco-steroids related to dehydroepiandrosterone

Ieva L. Reich,*† Henry Lardy,* Yong Wei,* Padma Marwah,* Nancy Kneer,* Douglas R. Powell,† and Hans J. Reich†

**Institute for Enzyme Research and †Department of Chemistry, University of Wisconsin-Madison, Madison, Wisconsin 53705*

*The unusual activity of some D-ring-seco estrogens led us to prepare several seco steroids related to dehydroepiandrosterone (DHEA) and to test for their ability to mimic thyroid hormone and 7-oxo-DHEA (***1***) as inducers of thermogenic enzymes in rats' livers. Only one, 3β-acetoxy-17a-oxa-androst-5-ene-7,17-dione (***17***), was capable of inducing both mitochondrial glycerophosphate dehydrogenase and malic enzyme. The closely related 3β-hydroxy-17a-oxa-androsta-5,15-diene-7,17-diones (both 14α and 14β,* **14** *and* **15***) induce the formation of malic enzyme but not of glycerophosphate dehydrogenase. The 3β-propionyl ester of the above 14α steroid was not active, presumably because it was not deacylated in vivo. The 16,17 dicarboxylic acid (***9***) produced by opening the D-ring also induced the formation of malic enzyme but not of glycerophosphate dehydrogenase. 3β-Acetoxyandrost-5-ene-7,16,17-trione, an intermediate in the synthesis of D-ring seco compounds enhanced the formation of both enzymes. Twelve other D-ring seco compounds were not active. Seco androstanes oxygenated at position 7 and with expanded A or B rings were not active.* (*Steroids* **63**:542–553, 1998) *© 1998 by Elsevier Science Inc.*

Keywords: ergosteroids; 7-oxo-DHEA; DHEA; seco-steroids; thermogenic enzymes

Introduction

In 1932 Marrian and Haselwood,[1] and independently, Doisy and coworkers[2] reported that alkali fusion of theelol (estriol) yielded a dicarboxylic acid. Soon thereafter the Doisy group[3] demonstrated that similar treatment of theelin (estrone) yielded a monocarboxylic acid and that both of these products possessed several times the estrogen potency of estrone. The structures of these fusion products were established by Miescher[4,5] who named them marrianolic acid and doisynolic acid, respectively. The latter is 10 to 20 times as active as estrone and a levorotatory bisdehydrodoisynolic acid is more than 100 times as active when given orally.[5]

Heer and Miescher[6] prepared a series of D-ring seco compounds beginning with dehydroepiandrosterone (hereafter DHEA) but found them devoid of androgen or estrogen activity.[5] In our search for possible metabolites of DHEA with greater ability than the parent steroid to induce the formation of thermogenic enzymes when administered to rats, we have found that activity is retained in compounds with wide variations of substituents on the D-ring[7] (and manuscript in preparation). This was an inducement to examine the seco compounds as well. We report here the synthesis of several known and new A-, B- and D-ring seco derivatives of DHEA. The ability of these steroids, and of intermediates in their chemical synthesis, to induce thermogenic enzymes in rats has been tested. Some expanded D-ring steroids retain the ability to induce one or both of the thermogenic enzymes whereas androstanes with expanded A or B rings were not effective.

Experimental

Nuclear magnetic resonance (NMR) spectra were obtained on Bruker WP-200, WP-270, AM-300 or AM-500 spectrometers. Unless otherwise indicated, the spectra were measured in $CDCl_3$ using tetramethylsilane (δ 0) as reference for the ^1H NMR spectra, and the $CDCl_3$ triplet (δ 77.0) for the ^{13}C spectra. Infrared (IR) spectra in $CHCl_3$ were recorded on a Mattson Polaris spectrophotometer. A Kratos MS-80RFA spectrometer was employed to obtain mass spectra (MS). Elemental analyses were done by Galbraith Laboratories. Other conditions were as described.[8]

Diethyl ether and tetrahydrofuran (THF) were freshly distilled from sodium benzophenone ketyl. Dry CH_2Cl_2 was distilled from CaH_2 as were diisopropylamine and triethylamine. Pyridine was distilled from NaOH. n-Butyllithium in hexane was obtained from Foote Mineral Co. and titrated using n-propanol with 1,10-phenanthroline as indicator.[9] Column chromatography was carried

Address reprint requests to Ieva L Reich, Department of Chemistry, 1101 University Avenue, Madison, WI 53706 or Henry Lardy, Institute for Enzyme Research, 1710 University Avenue, Madison, WI 3705, USA.
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655 Avenue of the Americas, New York, NY 10010

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out with Baker silica gel (60–200 mesh). Preparative thin-layer chromatography was done on Brinkmann silica gel MN PUV254.

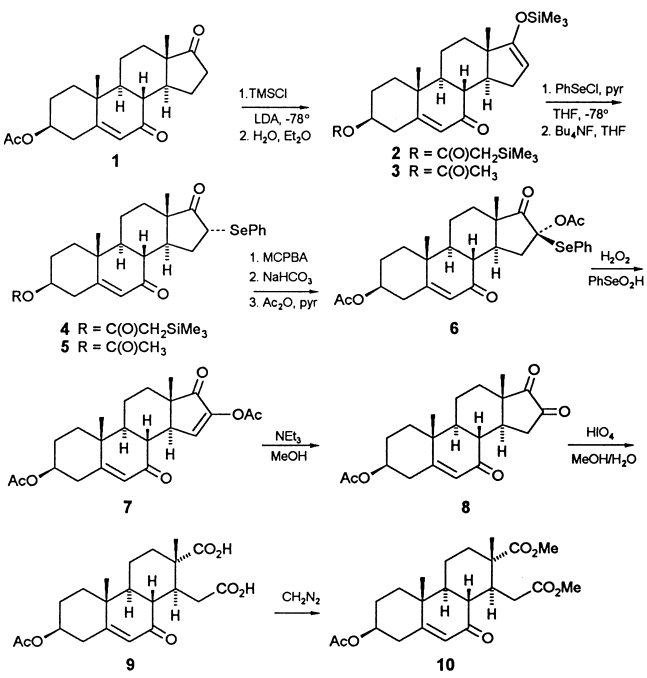
Assays of compounds for their ability to induce mitochondrial glycerophosphate dehydrogenase and cytosolic malic enzyme in rat livers have been described.[8,10] Again, we emphasize that the assays are not quantitative, but serve as a guide to further work. Compounds are considered active only if they enhance the assayed activity of these thermogenic enzymes to greater than 150% of that in livers of control rats.

Syntheses

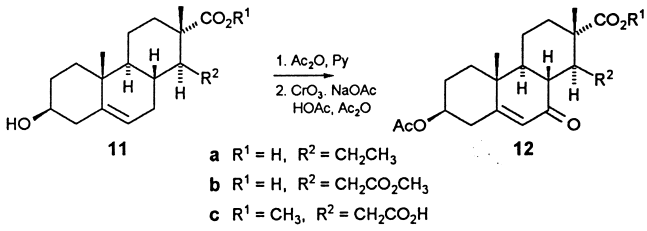
Synthesis of 16,17-dione and D-ring seco androsterones

16,17-Diones in the 7-oxo-dehydroepiandrosterone or 3β-hydroxyandrost-5-ene-7,17-dione (7-oxo-DHEA) series have not been reported, although structures without the 7-oxo group have been prepared in poor yield.[11] Scheme 1 presents a new approach in which a seleno–Pummerer reaction[12] is used to oxidize the 16-position. This route is more efficient than the previously published procedure.

The starting material for the synthesis of **8** was **1**, 3β-acetoxyandrost-5-ene-7,17-dione (7-oxo-DHEA acetate), which was prepared from dehydroepiandrosterone acetate as described.[8] Treatment of **1** with 2.4 equivalents of lithium diisopropylamide in the presence of trimethylchlorosilane (TMSCl) gave the 17-silyl enol ether (70% of the material was also C-silylated on the 3β-acetoxy group). This mixture was selenenylated with benzeneselenenyl chloride and desilylated with tetra-n-butyl ammonium fluoride (nBu_4NF) to give the 16α-selenide **5** in ~90% yield from **1**. Oxidation of the selenide **5** to the selenoxide and treatement with acetic anhydride (the seleno-Pummerer reaction) gave the 16-acetoxyselenide **6** as a mixture of diastereomers. Oxidative elimination formed the enol acetate **7** in 60% yield from **5**. A small amount (7%) of the enol *m*-chlorobenzoate was also formed and separated in the chromatography. The enol acetate and enol benzoate were hydrolyzed in methanol-triethylamine to form 58% of the yellow solid 16, 17-dione **8**. Oxidative cleavage with sodium metaperiodate gave the crystalline diacid **9** in 49% yield.



Scheme 1 Preparation of 3β-acetoxy-7-oxo-16,17-secoandrost-5-ene-16,17-dioic acid (**9**).



Scheme 2

The related secoandroectenenoic acid **12a** was prepared from the doisynolic acid analog **11a**[6] as shown in Scheme 2. The half-esters of 3β-hydroxy-16,17-secoandrost-5-ene-16,17-dioic acid **11b** and **11c**, prepared according to literature procedures,[13] were converted to the corresponding 7-oxo compounds **12b** and **12c** by chromic acid oxidation as described for **11a**.

*3β-(trimethylsilyl)acetoxy-17-hydroxyandrosta-5,16-dien-7-one trimethylsilyl ether (**2**)*

To a solution of 1.00 g (2.90 mmol) of 3β-acetoxyandrost-5-ene-7,17-dione (**1**) in 20 mL of dry THF under N_2, was added 1.07 mL (8.43 mmol) of TMSCl. The reaction mixture was cooled to −78°C. Lithium diisopropylamide (LDA) was prepared at −78°C under N_2 from 1.07 mL (7.66 mmol) of diisopropylamine and 3.60 mL of a 1.94 M solution of n-butyllithium in hexane (6.96 mmol) in 4 mL of THF. The LDA solution was added under N_2 via cannula to the cold (−78°C) THF solution of **1**. The reaction mixture was allowed to warm to room temperature, poured into a separatory funnel with 40 mL of hexane and 40 mL of saturated $NaHCO_3$ solution, and extracted. The organic extract was washed with saturated NaCl solution, dried (Na_2SO_4) and solvent was removed under reduced pressure to yield 1.63 g of a solid which NMR showed to be a 70:30 mixture of the 17-enol silyl ether with C- (**2**) and O-silylation (ketene acetal) on the 3-acetate. This mixture was stirred rapidly in 60 mL of ether and 60 mL of H_2O for 15 min to hydrolyze the ketene acetal, yielding a mixture of **2** and **3**. ^{1}H NMR of **2** in mixture (300 MHz) δ 5.76 (d, J = 2 Hz, 6-H), 4.73 (tt, J = 11, 5 Hz, 3α-H), 4.53 (dd, J = 3, 1.5 Hz, 16-H), 2.73 (ddd, J = 14.5, 6, 3 Hz, 15α-H), 2.15 (ddd, J = 14.5, 10.5, 1.5 Hz, 15β-H), 1.90 (s, CH_2-Si), 1.22 (s, 19-CH_3), 0.86 (s, 18-CH_3), 0.20 (s, OSiMe_3), 0.14 (s, C-SiMe_3). NMR of **3** in the mixture is essentially the same as **2** except that the peak at δ 1.90 is replaced by a peak at δ 2.06 (s, CH_3COO) and the peak at δ 0.14 is absent.

*3β-acetoxy-16α-phenyl selenoandrost-5-ene-7,17-dione (**5**)*

To a solution of 2.90 mmol of enol silyl ether (**2** + **3**) in 20 mL of dry THF and 0.320 mL (4.00 mmol) of dry pyridine at −78°C under N_2 was added via cannula a THF solution of 0.710 g (3.71 mmol) of PhSeCl in 4 mL of THF. The reaction mixture was immediately poured into 60 mL of CH_2Cl_2 and 60 mL of 0.5 N HCl solution and extracted. The organic extract was washed with water and saturated $NaHCO_3$ solution. After drying (Na_2SO_4), solvent was removed under reduced pressure yielding crude selenide (**4** + **5**), 1.72 g. Chromatography on 100 g of silica eluting with hexane-ethyl acetate (90:10 to 50:50, 50 mL fractions) separated **4** and **5** from diphenyl diselenide and small amounts of unreacted **1**. Fractions 14–17 were combined to give 1.44 g of **4** + **5**. ^{1}H NMR (300 MHz) of **4**: δ 7.68 (m, *o*-SePh), 7.32 (m, SePh), 5.73 (d, J = 1.5 Hz, 6-H), 4.72 (tt, J = 10.5, 5 Hz, 3α-H), 4.10 (d, J = 7.5 Hz, J_{SeH} = 5 Hz, 16β-H), 2.98 (ddd, J = 14, 5.5, 0.5 Hz, 15α-H), 1.90 (s, CH_2Si), 1.20 (s, 19-CH_3), 0.91 (s, 18-CH_3), 0.14 (s, C-$SiMe_3$).

To a solution of 1.19 mmol of the C-silylated acetate **4** (containing some **5**) in 10 mL of CH_2Cl_2 and 15 mL of H_2O was added 4 mL of a 1 M solution of nBu_4NF in THF. The mixture was stirred vigorously for 45 min and then poured into 20 mL of ether-20 mL of hexane-40 mL of H_2O and extracted. The organic extract was washed with H_2O two times, saturated NaCl, dried (Na_2SO_4) and the solvent was removed under reduced pressure to yield a white solid, 95% pure by ^{1}H NMR, 0.594 g, ~95% yield; m.p. 123–124°C. $_1$H NMR (300 MHz) of **5**: δ 7.68 (m, *o*-SePh), 7.31 (m, SePh), 5.73 (d, J = 1.5 Hz, 6-H), 4.72 (tt, J = 11, 5 Hz, 3α-H), 4.10 (d, J = 7.5 Hz, J_{SeH} = 5 Hz, 16β-H), 2.98 (ddd, J = 14, 5.5, 0.5 Hz, 15α-H), 2.59 (ddd, J = 14.5, 5, 2 Hz, 4α-H), 2.47 (ddd, J = 14.5, 11.5, 2 Hz, 4β-H), 2.06 (s, CH_3COO), 1.21 (s, 19-CH_3), 0.91 (s, 18-CH_3). ^{13}C NMR (62.9 MHz) δ 215.8 (17-CO), 199.8 (7-CO), 170.1 (CH_3COO), 164.7 (5-C), 135.6 (*o*-CH), 129.0 (*m*-CH), 128.8 (*i*-C), 128.3 (*p*-CH), 126.3 (6-CH), 71.8 (3-CH), 49.8 (CH), 48.0 (13-C), 44.5 (CH), 43.9 (CH), 43.7 (CH), 38.2 (10-C), 37.7 (CH_2), 35.8 (CH_2), 33.1 (CH_2), 31.1 (CH_2), 27.1 (CH_2), 21.1 (CH_3COO), 20.4 (11-CH_2), 17.3 (19-CH_3), 14.2 (18-CH_3). HRMS: Calcd. for $C_{27}H_{32}O_4Se$: 500.1470; Found: 500.1476.

*3β,16-diacetoxy-16-phenylselenoandrost-5-ene-7,17-dione (**6**)*

A solution of 1.1 mmol of selenide **5** in 25 mL of CH_2Cl_2 was cooled to 0°C. To the well-stirred solution was added 0.296 g (1.2 mmol) of *m*-chloroperbenzoic acid, 70% (MCPBA). After 5 min, 0.40 mL of dimethyl sulfide was added to quench the oxidant. The CH_2Cl_2 solution was stirred with 50 mL of cold saturated $NaHCO_3$ solution and filtered through Na_2SO_4 at 0°C. Acetic anhydride (1.2 mL) and pyridine (1.2 mL) were added and the solution was stirred at room temperature for 1 h. A solution of saturated $NaHCO_3$ was added and the mixture was stirred until bubbling ceased. Layers were separated and the organic layer was washed again with saturated $NaHCO_3$, dried (Na_2SO_4) and evaporated under reduced pressure to yield 0.713 g of selenoacetate (85:15 mixture of diastereomers). NMR showed some incorporation of *m*-chlorobenzoate, ~7%. ^{1}H NMR of chromatographed material, major isomer in mixture; (300 MHz) δ 7.65 (dm, J = 7.5 Hz, *o*-SePh), 7.35 (m, SePh, 3H), 5.72 (d, J = 1.5 Hz, 6-H), 4.71 (tt, J = 11, 5 Hz, 3α-H), 3.15 (dd, J = 14.5, 6.5 Hz, 15α-H), 2.06 (s, 3-CH_3COO), 2.03 (s, 16-CH_3COO), 1.35 (s, 19-CH_3), 1.23 (s, 18-CH_3); minor isomer in mixture δ 7.61 (dm, J = 7.5 Hz, *o*-SePh), 7.35 (m, SePh), 5.76 (d, J = 1.5 Hz, 6-H), 4.71 (tt, J = 11, 5 Hz, 3α-H), 3.29 (dd, J = 13.5, 4.5 Hz, 15α-H), 2.10 (s, 16-CH_3COO), 2.07 (s, 3-CH_3COO), 1.35 (s, 19-CH_3), 1.24 (s, 18-CH_3).

*3β,16-diacetoxyandrosta-5,15-diene-7,17-dione (**7**)*

To a solution of 1.1 mmol selenoacetate **6** in 25 mL of CCl_4 was added 0.12 mL of pyridine and 10 mg of benzeneseleninic acid. The mixture was stirred vigorously and 3.4 mL of 15% aqueous H_2O_2 was added. After stirring for 45 min (mild exotherm), the yellow color faded. The CCl_4 solution was washed twice with saturated $NaHCO_3$, dried and evaporated under reduced pressure to yield 0.390 g of a crude solid containing 7% of the enol *m*-chlorobenzoate. Chromatography on 50 g of silica gel with 50% ethyl acetate/hexane (25 mL fractions), gave 0.236 g (54% yield) of pure enol acetate (fractions 9 and 10), m.p. 208–210°C. ^{1}H NMR (300 MHz) δ 7.81 (broad s, 15-H), 5.81 (d, J = 1.5 Hz, 6-H), 4.74 (tt, J = 11.5, 4.5 Hz, 3α-H), 2.25 (s, 16-CH_3COO), 2.07 (s, 3-CH_3COO), 1.29 (s, 19-CH_3), 1.23 (s, 18-CH_3). HRMS Calcd. for $C_{23}H_{28}O_6$: 400.1886; Found: 400.1889.

*3β-acetoxyandrost-5-ene-7,16,17-trione (**8**)*

Crude enol acetate **7** (2.90 mmol) was dissolved in 17 mL of THF and 43 mL of methanol. Triethylamine (1.8 mL, 12.9 mmol) was added and the solution was kept under N_2 for 16 h. The mixture was then extracted between $CHCl_3$ and 0.5 N HCl solution. The organic layer was washed with 50% saturated NaCl solution, dried (Na_2SO_4) and the solvent was evaporated to yield a yellow solid. The solid was crystallized from $CHCl_3$-CCl_4-ether mixture to yield yellow crystals, 0.534 g, m.p. 221–222°C. The mother liquor was chromatographed on preparative TLC plates to yield 0.076 g. The total yield was 0.61 g, 58% from **1** (6 steps). ^{1}H NMR (300 MHz) δ 5.78 (d, J = 1.5 Hz, 6-H), 4.73 (tt, J = 11.5, 4.5 Hz, 3α-H), 3.42 (dd, J = 19.5, 6.5 Hz, 15α-H), 2.43 (dd, J = 19.5, 13.5 Hz, 15β-H), 2.07 (s, 3-CH_3COO), 1.29 (s, 19-CH_3), 1.06 (s, 18-CH_3). ^{13}C NMR (75.4 MHz) δ 204.5 (CO), 203.1 (CO), 199.4 (7-CO), 170.1 (CH_3COO), 165.4 (5-C), 126.0 (6-CH), 71.7 (3-CH), 49.4 (CH), 47.8 (13-C), 43.5 (CH), 38.9 (CH_2), 38.6 (CH), 38.5 (10-C), 37.8 (CH_2), 35.6 (CH_2), 29.9 (CH_2), 27.1 (CH_2), 21.1 (CH_3COO), 20.1 (11-CH_2), 17.3 (19-CH_3), 13.6 (18-CH_3). IR (cm^{-1}) 3029, 2953, 1750, 1731, 1671, 1250, 1036. HRMS Calcd. for $C_{21}H_{26}O_5$: 358.1780; Found: 358.1778.

*3β-acetoxy-7-oxo-16,17-secoandrost-5-ene-16,17-dioic acid (**9**)*

To a solution of the pure triketone **8** (0.400 g, 1.12 mmol) in 16 mL of MeOH was added 0.263 g (1.23 mmol) of

sodium metaperiodate, 4 mL of H_2O and 2 mL of 1 N HCl solution. The solution was stirred for 2 h at which point a white solid had formed and the yellow color of **8** was absent. The mixture was partitioned between 200 mL of H_2O and 100 mL of $CHCl_3$. The aqueous layer was extracted two more times with $CHCl_3$. The organic layers were combined and washed with 50% saturated NaCl solution, dried (Na_2SO_4) and evaporated under reduced pressure to yield a white solid (0.390 g) which was crystallized from acetone producing 0.215 g of pure crystals (49%), mp 228–238°C d. ^{1}H NMR (300 MHz) δ 5.77 (d, J = 1.5 Hz, 6-H), 4.72 (tt, J = 11.5, 5 Hz, 3α-H), 2.91 (m, 2H), 2.57 (ddd, J = 13.5, 5, 1.5 Hz, 4α-H), 2.45 (ddd, J = 13, 11.5, 1.5 Hz, 4β-H), 2.06 (s, CH_3COO), 1.24 (s, CH_3), 1.21 (s, CH_3). ^{13}C NMR (75.4 MHz) δ 199.8 (7-CO), 184.0 (17-COOH), 179.5 (16-COOH), 170.2 (CH_3COO), 163.1 (5-C), 126.2 (6-CH), 72.1 (3-CH), 48.7 (CH), 44.9 (13-C), 44.5 (CH), 39.0 (10-C), 37.5 (CH_2), 37.2 (CH), 36.4 (CH_2), 35.8 (CH_2), 34.7 (CH_2), 27.1 (CH_2), 21.2 (CH_3COO), 19.2 (11-CH_2), 16.6 (19-CH_3), 15.4 (18-CH_3). IR (cm^{-1}) 3600, 3300–3700 (br), 3013, 2954, 1715, 1675, 1249, 1205.

*Dimethyl 3β-acetoxy-7-oxo-16,17-secoandrost-5-ene-16,17-dioate (**10**)*

The mother liquor from the crystallization of diacid **9** was evaporated under reduced pressure and the solid (0.158 g, 50% diacid) was dissolved in 3 mL of THF and treated with 1 mmol of diazomethane. Preparative TLC (6% MeOH/$CHCl_3$) yielded 75 mg of a white solid; Rf = 0.36, 16% yield from the triketone **8**. Crystallization from ether-hexane gave colorless crystals, 64 mg, m.p. 155–156°C. ^{1}H NMR (300 MHz) δ 5.73 (d, J = 1.5 Hz, 6-H), 4.71 (tt, J = 11, 5 Hz, 3α-H), 3.693 (s, OCH_3), 3.685 (s, OCH_3), 2.73 (dt, J = 11, 4.5 Hz, 14α-H), 2.56 (dd, J = 13.5, 5.5, 2 Hz, 4α-H), 2.46 (ddd, J = 13.5, 11.5, 2 Hz, 4β-H), 2.41 (t, J = 11 Hz, 8-H), 2.36 (d, J = 5 Hz, 15α,β-H), 2.05 (s, CH_3COO), 1.22 (s, 19-CH_3), 1.14 (s, 18-CH_3). ^{13}C NMR (75.4 MHz) δ 199.9 (7-CO), 177.3 (17-*C*OOMe), 173.1 (16-*C*OOMe), 170.1 (CH_3*C*OO), 163.4 (5-C), 126.2 (6-CH), 72.0 (3-CH), 52.0 (OCH_3), 51.4 (OCH_3), 48.8 (CH), 46.5 (13-C), 45.4 (CH), 38.6 (10-C), 37.53 (CH), 37.48 (CH_2), 37.3 (CH_2), 35.7 (CH_2), 34.7 (CH_2), 27.1 (CH_2), 21.1 (CH_3COO), 19.6 (11-CH_2), 16.9 (19-CH_3), 15.6 (18-CH_3). IR (cm^{-1}) 3025, 2953, 1731, 1672, 1437, 1367, 1248, 1175, 1035. HRMS calculated for $C_{23}H_{32}O_7$: 420.2148; Found: 420.2141. Analysis calculated for $C_{23}H_{32}O_7$: C, 65.69; H, 7.67. Found: C, 65.55; H, 7.89.

*3β-acetoxy-7-oxo-16,17-secoandrost-5-en-17-oic acid (**12a**)*

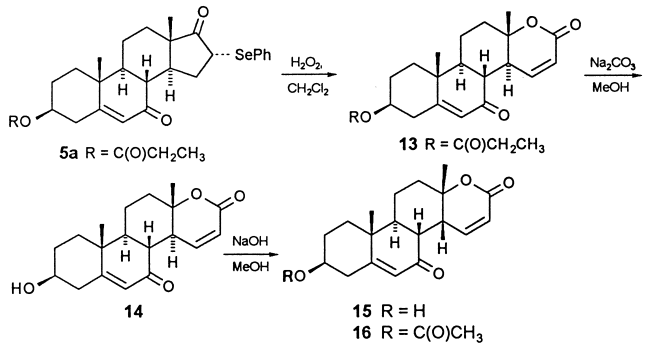
The doisynolic acid analog **11a** (prepared by the method of Heer and Miescher, m.p. 203–205°C; lit.[6] 204–206°C) (700 mg, 2.28 mmol) was stirred with acetic anhydride (4.5 mL), triethylamine (3 mL), and 4-dimethylaminopyridine (42 mg) in CH_2Cl_2 for 2.5 h at room temperature. After removal of the solvent the residue was dissolved in ethyl acetate and the solution was washed with 0.1 N HCl and then with brine, dried ($MgSO_4$) and concentrated to dryness. To the crude product (850 mg) and NaOAc (550 mg) in acetic acid (7 mL), a solution of CrO_3 (690 mg) in acetic anhydride (3.8 mL) was added dropwise during a period of 15 min at room temperature. The reaction mixture was stirred at 45°C under N_2 for 2.5 h and poured into ice-water (250 mL). The product was extracted from the ice-water with ethyl acetate (60 mL × 6). The extracts were combined, washed with brine (60 mL), dried ($MgSO_4$), and concentrated to dryness. The crude product was purified over silica gel ($CHCl_3$:acetone = 20:1) to afford compound **12a** (400 mg, 48%); m.p. 175–179°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.76 (s, 1H, 6-H), 4.71 (m, 1H, 3-H), 2.07 (s, -CO_2CH_3), 1.21 (s, CH_3), 1.08 (s, CH_3), 0.89 (t, J = 7 Hz, CH_3). HRMS calculated for $C_{21}H_{30}O_5$: 362.2093; Found: 362.2089.

*3β-acetoxy- 7-oxo-16,17-secoandrost-5-ene-16,17-dioic acid, 16 and 17-methyl half-esters (**12b** and **12c**)*

The half-esters **11b** and **11c** (1.0 g samples of each, prepared by the procedure of von Seeman and Grant[13]) were oxidized as decribed for **11a**. Chromatography yielded 0.460 g (44%) of **12b** and 0.200 g (19%) of **12c**. The compounds were very hygroscopic and resisted crystallization. **12b**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.72 (s, 1H), 4.72 (m, 1H), 3.68 (s, 3H), 2.05 (s, 3H), 1.20 (s, 3H), 1.12 (s, 3H). **12c**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.78 (s, 1H), 4.72 (m, 1H), 3.69 (s, 1H), 2.05 (s, 3H), 1.23 (s, 3H), 1.10 (s, 3H).

Preparation of D-ring lactones

When the 16-α selenide **5a** (prepared from 3β-propionoxy-7-oxo-DHEA,[8] by the reactions in Scheme 1) was oxidized under the normal conditions for selenoxide elimination,[14,15] the lactone **13** was formed instead of the expected 15,16-enone (Scheme 3). Similar observations have been reported by Williams & Leber[16] in the case of the selenide derived from DHEA acetate, and by Fetizon et al.[17] in the 17-oxo-androstane series. This occurs because the selenoxide elimination, which would place two more sp^2 centers in the already strained trans-fused five-membered ring, is slower than the Baeyer-Villiger reaction to form the six-membered ring lactone. After the ring expansion, the selenoxide eliminates rapidly to form the double bond in the six-membered



Scheme 3 Preparation of 3β-hydroxy-17a-oxa-androsta-5,15-diene-7,17-dione (**15**).

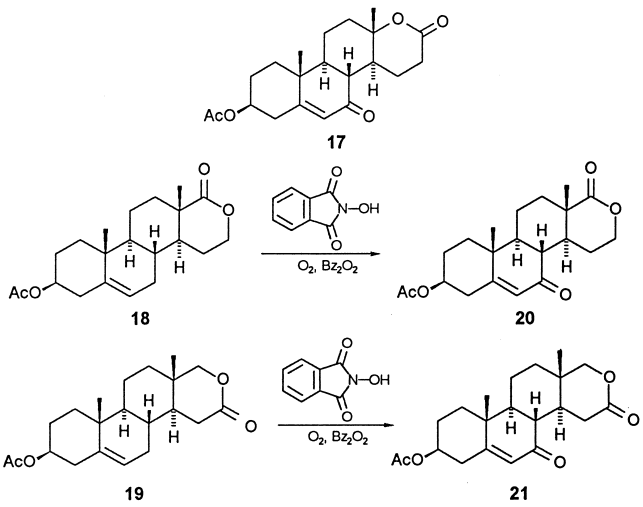
ring. We have been able to form the five-membered ring enone by doing the oxidation with MCPBA at 0°C, removing the acid and pyrolyzing rapidly in refluxing carbon tetrachloride.[15]

The 3-propionoxy group can be hydrolyzed to the alcohol by careful treatment with sodium carbonate in methanol. Under more strongly basic conditions (sodium hydroxide in methanol), the lactone isomerizes at the 14 position to give the cis ring juncture. Ring opening does not occur. Minor products include isomers at the 8 position and methoxide addition to the enone.

It is clear that **14** (14α-H) is the kinetic product which isomerizes under basic conditions to **15** (14β-H). This is confirmed by both the ^{1}H NMR and ^{13}C NMR data. In **14** the 14α-H is axial and upfield, δ 2.80 (shielding by 7 carbonyl) with axial-axial coupling (J = 11.5 Hz). The 15-H is downfield, δ 7.27 (deshielding by 7 carbonyl). In **15** the 14β-H is equatorial with a small coupling and shifted downfield since it is in the deshielding cone of the 7 carbonyl (δ 3.51). The 15-H is upfield (δ 6.38) as expected. In the ^{13}C NMR the major chemical shift changes occur in the C- and D-rings. Most notably the C-18 methyl is significantly upfield (8 ppm) for **14** due to the greater number of γ-gauche interactions present in the trans-fused rings. The C-15 vinyl carbon is upfield in **15** (2 ppm) for the same reason since it occupies an axial position. The C-14 tertiary carbon having an axial substituent is shifted upfield (4 ppm).

A precedent for the formation of the lactone **17** (Scheme 4) was the demonstration by Westerfeld[18] that treating estrone with H_2O_2 converted it to the corresponding 17 → 13 lactone. In the commercial synthesis of 7-oxo-DHEA-acetate by the procedure described earlier,[8] prolonged reaction time results in the formation of by-products. Compound **17** was separated by chromatography from the 7-oxo-DHEA-acetate in such a reaction mixture.

The air oxidation of **18** and **19** to their respective 7-oxo-derivatives (Scheme 4) was based on a patent by Foricher et al.[19] and involved the use of *N*-hydroxyphthalimide as a catalyst.



Scheme 4 Preparation of additional D-ring lactones related to 3β-Acetoxyandrost-5-ene-7,17-dione.

*3β-propionoxy-17a-oxa-14α-androsta-5,15-diene-7,17-dione (***13***)*

The crude selenide **5a** (3.29 mmol), dissolved in 40 mL of CH_2Cl_2, was stirred rapidly and treated with 10 mL of 15% H_2O_2. After about 15 min, reaction began and the mixture warmed slightly. After 45 min the yellow color of diphenyl diselenide disappeared and the reaction mixture was poured into ethyl acetate (200 mL) and H_2O (60 mL) and extracted. The organic layer was washed with 100 mL of saturated $NaHCO_3$ solution and 30 mL of saturated NaCl solution. The crude solid (~80% pure) was crystallized from MeOH to yield 0.518 g, m.p. 233–235°C, 42%. ^{1}H NMR (300 MHz) δ 7.26 (dd, J = 9.5, 2.5 Hz, 15-H), 6.02 (dd, J = 9.5, 3 Hz, 16-H), 5.81 (d, J = 1.5 Hz, 6-H), 4.74 (tt, J = 11.5, 5 Hz, 3α-H), 2.81 (dt, J = 11.5, 2 Hz, 14α-H), 2.62 (ddd, J = 13.5, 5, 2 Hz, 4α-H), 2.49 (ddd, J = 13.5, 11.5, 2 Hz, 4β-H), 2.39 (t, J = 12 Hz, 8β-H), 2.33 (q, J = 7.5 Hz, CH_3CH_2), 1.35 (s, 18-CH_3), 1.25 (s, 19-CH_3), 1.15 (t, J = 7.5 Hz, CH_3CH_2). ^{13}C NMR (67.9 MHz) δ 198.9 (7-CO), 173.5 (CH_3CH_2CO), 164.9 (5-C), 163.6 (17-CO), 148.8 (15-CH), 125.7 (6-CH), 120.2 (16-CH), 82.4 (13-C), 71.5 (3-CH), 48.6 (CH), 43.0 (CH), 42.3 (CH), 38.6 (10-C), 37.6 (CH_2), 37.0 (CH_2), 35.6 (CH_2), 27.6 (CH_2), 27.0 (CH_2), 21.5 (11-CH_2), 18.3 (18-CH_3), 16.9 (19-CH_3), 8.9 (CH_3CH_2). IR (cm^{-1}) 2950, 1674, 1722. HRMS calculated for $C_{22}H_{26}O_5$: 372.1937. Found: 372.1934.

*3β-hydroxy-17a-oxa-14α-androsta-5,15-diene-7,17-dione (***14***)*

To 0.240 g (0.64 mmol) of propionate **13** dissolved in 24 mL of MeOH was added with stirring 12 mL of 5% Na_2CO_3 solution. Stirring was continued for 1 h and a sample was removed to check the extent of hydrolysis. This was necessary because in the heterogeneous mixture the reaction rate depends on the effectiveness of stirring and can't always be reproduced. After workup (*vide infra*), NMR showed 10% propionate **13**, 80% hydrolyzed 3β-OH, 14α lactone **14**, and 10% 14β lactone **15**. The reaction mixture was neutralized with acetic acid, diluted with 100 mL of H_2O and extracted with $CHCl_3$ two times. The organic layers were combined, washed with 50% saturated NaCl, dried (Na_2SO_4) and solvent evaporated. The residue could be used directly or purified by preparative TLC (6% MeOH/$CHCl_3$, Rf = 0.20). ^{1}H NMR (270 MHz) δ 7.27 (d, J = 9, 2.5 Hz, 15-H), 6.03 (d, J = 9, 3 Hz, 16-H), 5.80 (d, J = 1.5 Hz, 6-H), 3.72 (tt, J = 11, 5 Hz, 3α-H), 2.80 (dt, J = 11.5, 2.5 Hz, 14α-H), 2.60 (ddd, J = 13.5, 5, 2 Hz, 4α-H), 2.45 (ddd, J = 13.5, 11, 2 Hz, 4β-H), 2.41 (t, J = 12 Hz, 8β-H), 1.36 (s, 18-CH_3), 1.23 (s, 19-CH_3). ^{13}C NMR (90.6 MHz) δ 199.4 (7-CO), 166.6 (5-C), 163.9 (17-CO), 149.1 (15-CH), 125.1 (6-CH), 120.2 (16-CH), 82.7 (13-C), 70.0 (3-CH), 48.8 (CH), 43.0 (CH), 42.3 (CH), 41.6 (CH_2), 38.6 (10-C), 37.0 (CH_2), 36.0 (CH_2), 30.7 (CH_2), 21.5 (11-CH_2), 18.2 (18-CH_3), 16.9 (19-CH_3).

*3β-hydroxy-17a-oxa-14β-androsta-5,15-diene-7,17-dione (**15**)*

The crude 14α lactone **14** (0.896 g, 2.50 mmol) was dissolved in 30 mL of MeOH and treated under N_2 with 5 mL of 2 M NaOH solution. After 1 h 1.4 mL of AcOH was added and most of the MeOH was removed under vacuum. Water (60 mL) and $CHCl_3$ (80 mL) were added and the mixture was extracted. The aqueous layer was washed two more times with $CHCl_3$. The organic layers were combined, washed with 50% saturated NaCl, dried (Na_2SO_4) and solvent was evaporated to yield 0.610 g of crude 14β lactone **15**. The lactone can be purified by preparative TLC (8% MeOH/$CHCl_3$, Rf = 0.45). To obtain >95% purity, three consecutive chromatographies were necessary. The enzyme induction assays were performed on ~80% pure material after one chromatography. ^{1}H NMR (300 MHz) δ 6.38 (dd, *J* = 10, 2 Hz, 15-H), 6.02 (dd, *J* = 10, 3 Hz, 16-H), 5.91 (d, *J* = 2 Hz, 6-H), 3.74 (tt, *J* = 11.5, 4.5 Hz, 3α-H), 3.51 (broad s, 14β-H), 2.62 (ddd, *J* = 14.5, 5, 2 Hz, 4α-H), 2.54 (dd, *J* = 13, 4.5 Hz, 8β-H), 2.49 (ddd, *J* = 14.5, 11.5, 2 Hz, 4β-H), 1.56 (s, 18-CH_3), 1.16 (s, 19-CH_3). ^{13}C NMR (90.6 MHz) δ 197.4 (7-CO), 168.9 (5-C), 163.2 (17-CO), 147.3 (15-CH), 126.0 (6-CH), 121.7 (16-CH), 82.9 (13-C), 69.7 (3-CH), 45.2 (CH), 44.4 (CH), 41.8 (CH_2), 39.9 (CH), 37.6 (10-C), 35.7 (CH_2), 31.4 (CH_2), 30.6 (CH_2), 25.0 (18-CH_3), 23.0 (11-CH_2), 18.0 (19-CH_3). HRMS calculated for $C_{19}H_{24}O_4$: 316.1674; Found: 316.1615.

*3β-acetoxy-17a-oxa-14β-androsta-5,15-diene-7,17-dione (**16**)*

To 5 mg of alcohol **15** was added 0.1 mL of acetic anhydride, 0.1 mL of dry pyridine and 1 mg of dimethylaminopyridine (DMAP). The solution was allowed to stand for 16 h and was then extracted between 0.5 N HCl solution and CH_2Cl_2. The organic layer was washed with 50% saturated NaCl solution, dried (Na_2SO_4) and solvent was removed to yield 5 mg of the acetate **16.** TLC (6% MeOH/$CHCl_3$, Rf = 0.50) gave 4 mg of pure acetate. ^{1}H NMR (300 MHz) δ 6.37 (dd, *J* = 10, 2 Hz, 15-H), 6.03 (dd, *J* = 10, 3 Hz, 16-H), 5.92 (d, *J* = 2 Hz, 6-H), 4.76 (tt, *J* = 11.5, 4.5 Hz, 3α-H), 3.51 (broad s, 14β-H), 2.67 (ddd, *J* = 14.5, 5, 2 Hz, 4α-H), 2.54 (ddd, *J* = 14.5, 9, 2 Hz, 4β-H), 2.48, 2.51(d,d, *J* = 13, 4.5 Hz, 8β-H), 2.07 (s, CH_3COO), 1.56 (s, 18-CH_3), 1.17 (s, 19-CH_3).

*3β-acetoxy-17a-oxa-androst-5-ene-7,17-dione (**17**)*

Compound **17** was isolated as a by-product in the commercial synthesis of 7-oxo-DHEA-acetate; m.p. 262–263°C; $[\alpha]_D$24°C = 206.3° (c = 0.52, $CHCl_3$). ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.75 (d, *J* = 1.5 Hz, 6-H), 4.71 (m, 3α-H), 2.06 (s, $OCOCH_3$), 1.33 (s, 18-CH_3), 1.22 (s, 19-CH_3). Calculated for $C_{21}H_{28}O_5$: C = 69.98; H = 7.83. Found: C = 69.77; H = 7.70.

*3β-acetoxy-17-oxa-androst-5-en-17a-one (**18**) and 3β-acetoxy-17a-homo-17-oxa-androst-5-en-16-one (**19**)*

Compounds **18** and **19** were prepared according to von Seeman and Grant.[13] Their structures were confirmed by NMR, IR and MS; these data were not previously available. **18** ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.38 (d, *J* = 4 Hz, 6-H), 4.62 (m, 1H, 3α-H), 4.49 (m, 1H, 16-H), 4.28 (m, 1H, 16-H), 2.04 (s, 3H, CH_3COO), 1.23 (s, 3H, 19-CH_3), 1.04 (s, 3H, 18-CH_3). IR (cm^{-1}): 2941, 1750, 1735, 1430, 1376, 1255, 1115, 903, 737. MS m/z: 347 (M +1), 331 (M -CH_3), 304 (M -CH_2CO) 302 (M -CO_2), 286 (M -AcOH), 271 (286 -CH_3). **19** ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.38 (d, *J* = 4 Hz, 6-H), 4.62 (m, 3α-H), 3.99 (d, *J* = 11 Hz, 17-H), 3.91 (d, *J* = 11 Hz, 17-H), 2.72 (dd, *J* = 19, 5 Hz, 15-H), 2.13 (dd, *J* = 19, 13 Hz, 15-H), 2.05 (s, CH_3COO), 1.04 (s, CH_3), 1.03 (s, CH_3). IR (cm^{-1}): 2949, 1730, 1467, 1376, 1249, 1199, 1034, 912, 733. MS m/e: 346 (M^+), 330 (M -CH_3, -H), 304 (M -CH_2CO), 302 (M -CO_2), 286 (M -AcOH), 271 (286 -CH_3), 242 (286 -CO_2), 227 (271 -CO_2).

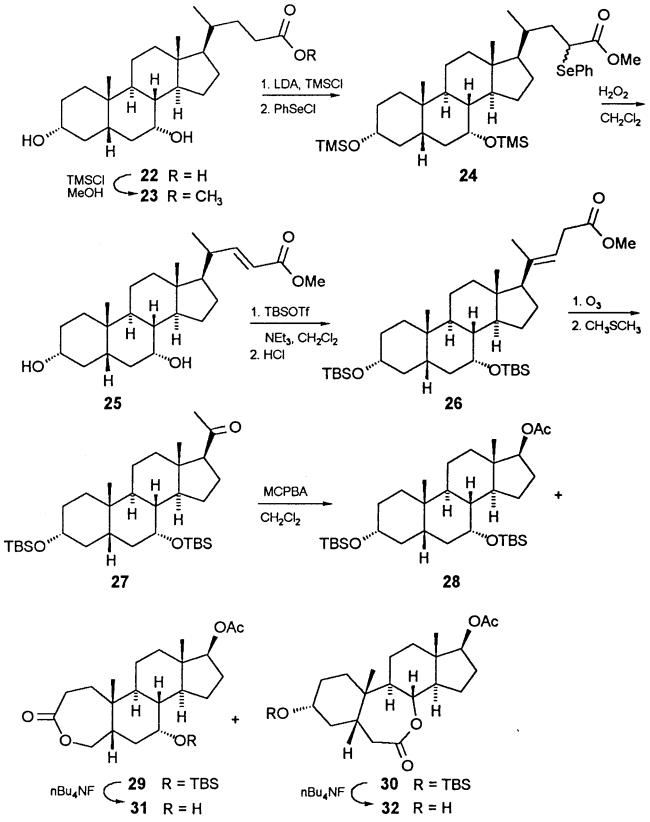
*3β-acetoxy-17-oxa-androst-5-ene-7,17a-dione (**20**) and 3β-acetoxy-17a-homo-17-oxa-androst-5-ene-7,16-dione (**21**)*

The oxidation of **18** and **19** to their respective products (Scheme 4) was based on a patent of Foricher et al.[19] (*cf* ref. 8). To a refluxing solution of lactone **18** or **19** (1.0 g, 2.9 mmol) and *N*-hydroxyphthalimide (0.5 g, 2.9 mmol) in acetone, dibenzoyl peroxide (30 mg) was added and a slow stream of compressed air was passed into the solution for 8–9 h. Acetone was removed under vacuum and the resultant mass was taken up in CCl_4. After stirring for some time the precipitated *N*-hydroxyphthalimide was filtered and washed with two 5 mL portions of CCl_4. The combined solutions were washed thoroughly with 1% $NaHCO_3$, water and brine and dried over $MgSO_4$. After evaporating the solvent under vacuum, the product was crystallized from acetone-hexane or may be further purified by chromatography on silica gel using 25% ethyl acetate in hexane. The white crystalline compounds were obtained in 58–60% yields.

20. Mp 154–155°. ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.74 (s, 6-H), 4.72 (m, 3α-H), 4.45 (m, 2H, 16-H), 2.82 (br d, *J* = 15 Hz 15α-H), 2.59 (dd, *J* = 15, 4 Hz, 4α-H), 2.47 (t, *J* = 13 Hz, 4β-H), 2.33 (t, *J* = 11 Hz, 8-H), 2.22 (br d, *J* = 13 Hz, 15β-H), 2.08 (s, CH_3COO), 1.25 (s, CH_3), 1.24 (s, CH_3). IR (cm^{-1}): 2953, 1734, 1673, 1630, 1456, 1377, 1246, 1053, 736. MS m/z: 360 (M^+), 344 (M -CH_3, -H), 316 (M -CH_2CH_2O or CO_2), 300 (M-AcOH), 299 (M -H, -AcOH), 284 (300 -CH_3, -H), 255 (299 -CH_2CH_2O or CO_2). **21** m.p. 213–214°C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.76 (s, 6-H), 4.72 (m, 3α-H), 3.98 (s, 2H, 17-H), 3.75 (dd, *J* = 20, 6 Hz, 15α-H), 2.59 (dd, *J* = 14, 5 Hz, 4α-H), 2.49 (t, *J* = 13 Hz, 4β-H), 2.17 (dd, *J* = 20, 13 Hz, 15β-H), 2.16 (t, *J* = 9 Hz, 8-H), 2.08 (s, CH_3COO), 1.27 (s, 19-CH_3), 1.04 (s, 18-CH_3). IR (cm^{-1}): 2960, 1725, 1670, 1650, 1372, 1237, 1031. MS m/z: 360 (M^+), 344 (M -CH_3, -H), 318 (M -CH_2CO), 316 (M -CO_2), 300 (M -AcOH), 285 (300 -CH_3), 272 (300 -CO), 270 (300 -CH_2O), 256 (300 -CO_2).

Degradation of the chenodeoxycholic acid side chain

Scheme 5 presents a new sequence for removal of the chenodeoxycholic acid side chain, related to a procedure



Scheme 5 Preparation of 4-oxa-4a-homo-7α-hydroxy-17β-acetoxy-5β-androstan-3-one and 3α-hydroxy-7a-oxa-17β-acetoxy-5β-androstan-7-one.

reported by Barton et al. with 11-oxolithocholic acid.[20] The methyl ester **23** was selenenylated via the silyl enol ether formed in situ. Oxidation with hydrogen peroxide resulted in selenoxide elimination to form the α,β-unsaturated side chain compound **25** in nearly quantitative yield. Attempted deconjugation of the 22, 23 double bond by base-catalyzed enolization-reprotonation of **25** with amide bases such as lithium diisopropylamide and hexamethylphosphoridriamide (HMPA) was unsuccessful. A successful procedure involved conversion of **25** to the β,γ-unsaturated ketene acetal with *t*-butyldimethylsilyl trifluoromethanesulfonate (TBSOTf), followed by hydrolysis to **26**. Ozonolysis and reductive workup gave the 17-acetyl compound **27**. Baeyer–Villiger oxidation produced the 17-acetoxy compound **28** in 69% yield and the two lactones **29** and **30** in 7% and 9% yield, respectively, after chromatographic separation.

The two minor products **29** and **30** are formed by Baeyer–Villiger oxidation of ketone precursors. One can envision formation of these ketones by a direct oxidation of the silyl ether or cleavage of one of the silyl groups by the peracid yielding the alcohol which is subsequently oxidized.

*Methyl 3α,7α-dihydroxy-5β-cholan-24-oate (***23***)*

To 4.0 g (10.2 mmol) of 3α,7α-dihydroxy-5β-cholan-24-oic acid (**22**) in 15 mL of dry MeOH was added 0.80 mL of TMSCl (6.31 mmol). The solution was heated under reflux for 20 min. After cooling, the organic product was extracted into CH_2Cl_2. The organic layer was washed with 5% saturated $NaHCO_3$ solution. NMR showed quantitative conversion to the ester **23**. ^{1}H NMR (270 MHz) δ 3.86 (q, J = 3 Hz, 7β-H), 3.68 (s, OCH_3), 3.47 (tt, J = 10.5, 4.5 Hz, 3β-H), 0.94 (d, J = 6.5 Hz, 21-CH_3), 0.91 (s, 19-CH_3), 0.66 (s, 18-CH_3).

*Methyl 3α,7α-dihydroxy-5β-chol-22-en-24-oate (***25***)*

To 4.9 mL of diisopropylamine (35 mmol) in 18 mL of dry THF at −78°C under N_2 was added 14.6 mL of nBuLi (2.38 M in hexane, 34.5 mmol) followed by 5.1 mL of TMSCl (40 mmol). The ester **23** (10.2 mmol) in 35 mL of THF was added slowly via cannula to the LDA-TMSCl solution at −78°C. The mixture was warmed to 0°C for 15 min and then cooled again to −78°C. To the cooled solution PhSeCl (2.35 g, 12.2 mmol) was added in 25 mL of THF rapidly via cannula. Triethylamine (5.0 mL) was added and the mixture was extracted between CH_2Cl_2 and 5% $NaHCO_3$ solution. The organic layer was dried (Na_2SO_4) and evaporated to yield the crude **24** as a mixture of epimers at C-23, but otherwise pure. ^{1}H NMR (200 MHz) δ 7.61 (m, *o*-SePh), 7.29 (m, SePh), 3.87 (m, 7β-H), 3.80 (dd, J = 12, 3.5 Hz, 23-H), 3.70 (dd, J = 9.5, 5 Hz, 23-H), 3.62, 3.60 (s, OMe), 3.42 (m, 3β-H), 0.92 (d, J = 6.5 Hz, 21-CH_3), 0.89, 0.87 (s, 19-CH_3), 0.63, 0.59, (s, 18-CH_3), 0.12 (s, 3-$OSiMe_3$), 0.07, 0.06, (s, 7-$OSiMe_3$).

To 10.2 mmol of the selenide mixture in 65 mL of CH_2Cl_2 was added 1.6 mL of pyridine and 7 mL of 15% H_2O_2 (40 mmol). The mixture was stirred vigorously until the yellow color was gone (15 min). The mixture was partitioned between CH_2Cl_2 and 5% $NaHCO_3$ solution. The solvent was evaporated and the residue dissolved in 70 mL of THF and 70 mL of 1 N HCl solution. The mixture was stirred vigorously for 1 h then extracted between H_2O and EtOAc. The aqueous layer was washed two more times with EtOAc. The organic layers were combined, dried (Na_2SO_4) and evaporated. A small sample of **25** was purified by preparative TLC (6% MeOH/$CHCl_3$, Rf = 0.18). ^{1}H NMR (300 MHz) δ 6.84 (dd, J = 15.5, 9 Hz, 22-H), 5.74 (dd, J = 15.5, 0.5 Hz, 23-H), 3.84 (q, J = 2.5 Hz, 7β-H), 3.72 (s, OMe), 3.46 (tt, J = 11, 4.5 Hz, 3β-H), 1.09 (d, J = 6.5 Hz, 21-CH_3), 0.91 (s, 19-CH_3), 0.70 (s, 18-CH_3). ^{13}C NMR (75.4 MHz) δ 167.4 (24-CO), 154.8 (22-CH), 118.6 (23-CH), 71.6 (3-CH), 68.3 (7-CH), 54.9 (CH), 51.3 (OCH_3), 50.2 (CH), 42.9 (13-C), 41.5 (CH), 39.8 (CH_2), 39.7 (CH_2), 39.5 (CH), 39.4 (CH), 35.3 (CH_2), 35.0 (CH_2), 35.0 (10-C), 34.7 (CH_2), 32.8 (CH), 30.6 (CH_2), 28.1 (CH_2), 23.7 (CH_2), 22.8 (21-CH_3), 20.5 (11-CH_2), 19.2 (19-CH_3), 12.0 (18-CH_3). IR (cm^{-1}) 3613, 3410, 2934, 2869, 1711, 1437, 1283, 1238, 1075, 908. HRMS calculated for $C_{25}H_{40}O_4$: 404.2927; Found: 404.2934.

*Methyl 3α,7α-bis(tert-butyldimethylsilyloxy)-5β-chol-20-en-24-oate (***26***)*

To 6.38 g (15.8 mmol) of diol **25** in 50 mL of freshly distilled CH_2Cl_2 at 0°C was added, under N_2, 12 mL of triethylamine (86 mmol) and 13 mL of tert-butyldimethylsilyl triflate (56.8 mmol). The mixture was allowed

to stand at room temperature for 48 h after which NMR showed the reaction to be 92% complete. The reaction mixture was poured into 200 mL of 1 N HCl solution and extracted with CH_2Cl_2. The CH_2Cl_2 layer was washed with 5% $NaHCO_3$ solution. The solvent was dried (Na_2SO_4), and evaporated to yield 14.3 g. A small sample of **26** was purified by preparative TLC (5% EtOAc-hexane, Rf = 0.30). ^{1}H NMR (270 MHz) δ 5.37 (tm, J = 6.5 Hz, 23-H), 3.82 (q, J = 2.5 Hz, 7β-H), 3.68 (s, OMe), 3.41 (tt, J = 11.5, 4.5 Hz, 3β-H), 3.10 (d, J = 6.5 Hz, 24-H, 2H), 2.28 (td, J = 13, 11 Hz, 4α-H), 2.04 (t J = 10 Hz, 17β-H), 1.64 (s, 21-H), 0.89 (s, t-BuSiO), 0.87 (s, t-BuSiO), 0.87 (s, 19-CH_3), 0.50 (s, 18-CH_3), 0.10 (s, CH_3SiO, 6H), 0.04 (s, CH_3SiO, 3H), 0.01 (s, CH_3SiO, 3H).

*3α,7α-bis(tert-butyldimethylsilyloxy)-5β-pregnan-20-one (***27***)*

A solution of the silyl-protected diol **26** (15.8 mmol) in 60 ml of CH_2Cl_2 and 25 mL of MeOH was divided in half for ozonolysis. Each half was cooled to −78°C and ozone bubbled in for about 0.5 h until the solution was blue. The solution was purged of ozone with N_2 at −78°C, dimethyl sulfide (3.3 mL, 45 mmol) was added and the solution was warmed to room temperature for 1 h. The mixture was poured into CH_2Cl_2 and extracted with H_2O two times. The organic layer was washed with 5% $NaHCO_3$ solution. A small sample was purified by preparative TLC (5% EtOAc-hexane, Rf = 0.16). ^{1}H NMR (300 MHz) δ 3.83 (q, J = 2.8 Hz, 7β-H), 3.42 (tt, J = 11.5, 4.5 Hz, 3β-H), 2.54 (t, J = 9 Hz, 17α-H), 2.28 (td, J = 13, 11Hz, 4α-H), 2.13 (s, 21-CH_3), 0.92 (s, 7-t-Bu), 0.88 (s, 19-CH_3 + 3-t-Bu), 0.58 (s, 18-CH_3), 0.106 (s, 7-OSiCH_3), 0.050 (s, 7-OSiCH_3), 0.038 (s, 3-OSiCH_3), 0.036 (s, 3-OSiCH_3). ^{13}C NMR (75.4 MHz) δ 72.5 (3-CH), 69.5 (7-CH), 63.8 (17-CH), 50.5 (CH), 44.1 (13-C), 41.8 (CH), 40.6 (CH), 40.5 (CH_2), 38.8 (CH_2), 35.6 (CH_2), 35.1 (10-C), 34.5 (CH_2), 32.4 (CH), 31.6 (21-CH_3), 31.0 (CH_2), 26.1 (t-Bu-CH_3), 25.9 (t-Bu-CH_3), 24.1 (CH_2), 22.8 (19-CH_3), 22.7 (CH_2), 20.5 (11-CH_2), 18.4 (C-tBu), 18.1 (C-tBu), 13.3 (18-CH_3), −2.37 (7-OSiCH_3), −4.62 (3-OSiCH_3), −5.56 (7-OSiCH_3). IR (cm^{-1}) 2952, 2931, 2857, 1698, 1472, 1462, 1254, 1092, 1027. HRMS calculated for $C_{33}H_{61}O_3Si_2$: 561.4159; Found: 561.4159.

*3α,7α-bis(tert-butyldimethylsilyloxy)-17β-acetoxy-5β-androstane (***28***), 4-oxa-4a-homo-7α-tert-butyldimethylsilyloxy-17β-acetoxy-5β-androstan-3-one (***29***) and 3α-tert-butyldimethylsilyloxy-7a-oxa-17β-acetoxy-5β-androstan-7-one (***30***)*

A solution of 15.8 mmol of the methyl ketone **27** in 80 mL of CH_2Cl_2 was treated with 10 g (~85%, 49.2 mmol) of *m*-chloroperoxybenzoic acid and stirred at room temperature for 5 days. The mixture was diluted with more CH_2Cl_2 and extracted two times with 5% $NaHCO_3$ solution. The organic layer was dried (Na_2SO_4) and evaporated to yield 11.7 g of crude product, which was chromatographed on 250 g of silica gel packed with hexane. The column was eluted with 1.2 L of 20% EtOAc-hexane, 200 mL of 50% EtOAc-hexane and then EtOAc (100 mL fractions).

Fractions 7–9 yielded 6.21 g (69%) of the 17-acetate **28**. ^{1}H NMR (300 MHz) δ 4.57 (dd, J = 9, 7.5 Hz, 17α-H), 3.80 (q, J = 2.5 Hz, 7β-H), 3.40 (tt, J = 11, 4.5 Hz, 3β-H), 2.26 (td, J = 13, 11 Hz, 4α-H), 0.90 (s, t-BuSiO), 0.88 (s, 19-CH_3), 0.87 (s, t-BuSiO), 0.75 (s, 18-CH_3), 0.088 (s, CH_3SiO), 0.026 (s, CH_3SiO), 0.022 (s, CH_3SiO), 0.018 (s, CH_3SiO). ^{13}C NMR (75.4 MHz) δ 171.2 (CH_3COO), 82.9 (17-CH), 72.6 (3-CH), 69.0 (7-CH), 44.5 (CH), 42.5 (13-C), 41.9 (CH), 40.5 (CH_2), 36.6 (CH_2), 35.7 (CH_2), 35.1 (10-C), 34.5 (CH_2), 32.5 (CH), 30.9 (CH_2), 27.6 (CH_2), 25.9 (t-Bu-CH_3), 23.4 (CH_2), 22.8 (19-CH_3), 21.2 (CH_3COO), 20.0 (11-CH_2), 18.3 (t-Bu-C), 18.1 (t-Bu-C), 12.0 (18-CH_3), −2.38 (7-OSiCH_3), -4.61 (3-OSiCH_3), -5.51 (7-OSiCH_3). IR (cm^{-1}) 2958, 2935, 2857, 1724, 1256, 1093, 1028, 837. HRMS calculated for $C_{33}H_{62}O_4Si_2$: 578.4200; Found: 578.4198.

Fraction-11 yielded solid lactone **29**, 0.55 g (7%). A portion was crystallized from hexane-CH_2Cl_2, m.p. 222–223°C. ^{1}H NMR (300 MHz) δ 4.73 (dd, J = 13.5, 10 Hz, 4a-H), 4.59 (dd, J = 9, 7.5 Hz, 17α-H), 3.93 (dd, J = 13.5, 1.5 Hz, 4a-H), 3.79 (q, J = 2.5 Hz, 7β-H), 2.76 (dd, J = 14.5, 12.5 Hz, 2-H), 2.35 (dd, J = 14.5, 8 Hz, 2-H), 2.04 (s, 17-CH_3COO), 1.01 (s, 19-CH_3), 0.90 (s, t-BuSiO), 0.76 (s, 18-CH_3), 0.09 (s, CH_3SiO), 0.06 (s, CH_3SiO). ^{13}C NMR (75.4 MHz) δ 176.4 (3-COO), 171.1 (CH_3COO), 82.4 (17-CH), 71.7 (4a-CH_2), 68.0 (7-CH), 45.3 (CH), 44.2 (CH), 42.4 (13-C), 40.5 (CH), 37.0 (10-C), 36.4 (CH_2), 34.3 (CH_2), 33.3 (CH_2), 32.8 (CH), 28.0 (CH_2), 27.4 (CH_2), 26.0 (t-Bu-CH_3), 23.3 (CH_2), 23.2 (CH_3COO), 21.1 (19-CH_3), 20.2 (11-CH_2), 18.2 (t-Bu-C), 11.9 (18-CH_3), -2.19 (7-OSiCH_3), -5.48 (7-OSiCH_3). IR (cm^{-1}) 2956, 2935, 2881, 2859, 1728, 1256, 1056, 1048, 1024. HRMS calculated for $C_{27}H_{46}O_5Si$: 478.3115. Found: 478.3096.

Fractions 14–16 yielded semi-solid lactone **30**, 0.70 g (9%). ^{1}H NMR (300 MHz) δ 4.64 (dd, J = 9, 7.5 Hz, 17α-H), 4.21 (dd, J = 10, 9.5 Hz, 8β-H), 3.59 (tt, J = 10.5, 5 Hz, 3β-H), 2.98 (dd, J = 14.5, 0.5 Hz, 6-H), 2.35 (dd, J = 14.5, 6 Hz, 6-H), 2.02 (CH_3COO), 1.04 (19-CH_3), 0.84 (t-BuSiO), 0.74 (18-CH_3), 0.007 (CH_3SiO), 0.004 (CH_3SiO). ^{13}C NMR (75.4 MHz) δ 174.0 (7-COO), 170.8 (CH_3COO), 81.8 (17-CH), 79.2 (8-CH), 71.3 (3-CH), 49.1 (CH), 43.1 (13-C), 41.7 (CH), 40.0 (CH), 37.5 (10-C), 36.0 (CH_2), 35.8 (CH_2), 35.60 (CH_2), 35.55 (CH_2), 30.8 (CH_2), 27.2 (CH_2), 25.8 (t-Bu-CH_3), 24.2 (CH_2), 23.3 (19-CH_3), 22.1 (11-CH_2), 21.0 (CH_3COO), 18.1 (t-BuC), 11.5 (18-CH_3), -4.70 (CH_3SiO), -4.77 (CH_3SiO). IR (cm^{-1}) 2962, 2956, 2947, 2940, 1728, 1472, 1361, 1099, 1015, 909, 868, 837. HRMS calculated for $C_{27}H_{46}O_5Si$: 478.3115. Found: 478.3118.

*4-oxa-4a-homo-7α-hydroxy-17β-acetoxy-5β-androstan-3-one (***31***)*

To a solution of 450 mg (0.94 mmol) of the lactone **29** in 15 mL of dry THF was added 5 mL of a 1 M solution of nBu_4NF in THF. The solution was kept at room temperature for 17 h. The mixture was partitioned between $CHCl_3$ and water. The organic layer was washed three times with water, 50% saturated NaCl solution, dried (Na_2SO_4) and evaporated. Preparative TLC yielded 149 mg (44%) of the alcohol **31**, Rf = 0.47 (5% MeOH/$CHCl_3$). ^{1}H NMR (300 MHz) δ 4.82 (dd, J = 13.5, 10 Hz, 4a-H), 4.63 (dd, J = 9, 7.5 Hz,

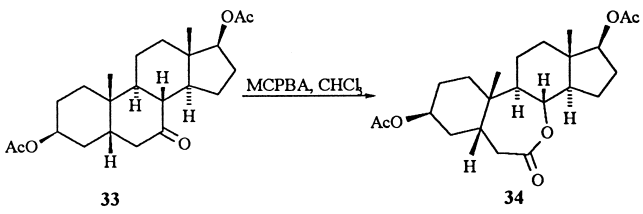
17α-H), 3.96 (dd, J = 14, 1.5 Hz, 4a-H), 3.82 (q, J = 2.5 Hz, 7β-H), 2.79 (t, J = 14 Hz, 2-H), 2.35 (dd, J = 14.5, 8 Hz, 2-H), 2.04 (s, 17-CH_3COO), 1.00 (s, 19-CH_3), 0.78 (s, 18-CH_3). ^{13}C NMR (75.4 MHz) δ 176.7 (3-COO), 171.2 (CH_3COO), 82.5 (17-CH), 71.5 (4a-CH_2), 66.6 (7-CH), 45.2 (CH), 44.7 (CH), 42.5 (13-C), 39.3 (CH), 37.0 (10-C), 36.4 (CH_2), 34.1 (CH_2), 33.4 (CH), 33.3 (CH_2), 28.2 (CH_2), 27.5 (CH_2), 23.3 (CH_3COO), 22.9 (CH_2), 21.1 (19-CH_3), 20.2 (11-CH_2), 11.8 (18-CH_3). IR (cm^{-1}) 3700 (broad), 2943, 2878, 1727, 1375, 1291, 1258, 1213, 1055, 1030, 908. HRMS calculated for $C_{21}H_{32}O_5$: 364.2250; Found: 364.2250.

*3α-hydroxy-7a-oxa-17β-acetoxy-5β-androstan-7-one (***32***)*

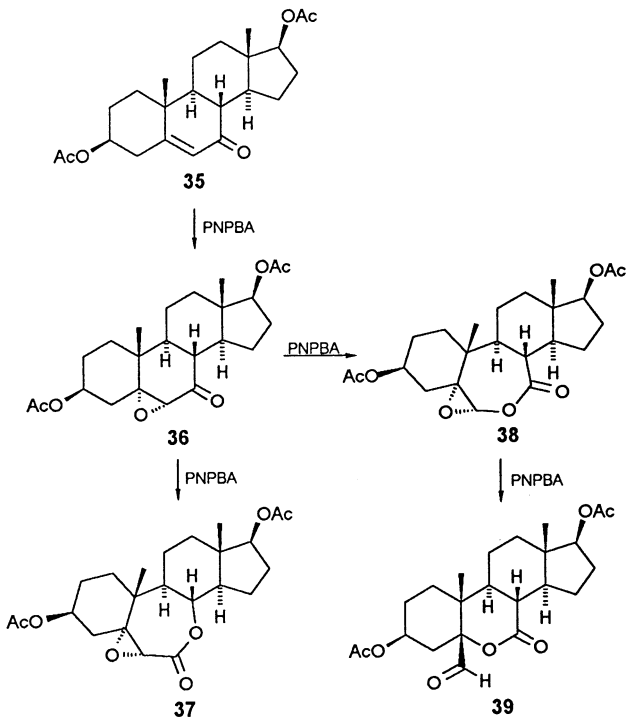
The *t*-butyldimethylsilyl group was cleaved from **30** (30 mg, 0.063 mmol) by treatment with nBu_4NF for 2.5 h as described above. The yield after chromatography (Rf = 0.20, 5% MeOH/$CHCl_3$) was 12 mg (52%). ^{1}H NMR (300 MHz) δ 4.68 (dd, J = 9.5, 7.5 Hz, 17α-H), 4.26 (t, J = 9.5 Hz, 8β-H), 3.67 (tt, J = 10.5, 5 Hz, 3β-H), 3.04 (dd, J = 14.5, 1.0 Hz, 6-H), 2.40 (dd, J = 14.5, 6.5 Hz, 2-H), 2.05 (s, 17-CH_3COO), 1.09 (s, 19-CH_3), 0.78 (s, 18-CH_3). ^{13}C NMR (75.4 MHz) δ 174.0 (7-COO), 170.9 (CH_3COO), 81.8 (17-CH), 79.3 (8-CH), 70.7 (3-CH), 49.2 (CH), 43.1 (13-C), 41.7 (CH), 40.2 (CH), 37.6 (10-C), 35.9 (CH_2), 35.7 (CH_2), 35.59 (CH_2), 35.55 (CH_2), 30.2 (CH_2), 27.3 (CH_2), 24.2 (CH_2), 23.4 (19-CH_3), 22.2 (11-CH_2), 21.1 (CH_3COO), 11.5 (18-CH_3), IR (cm^{-1}) 3600 (broad), 2964, 2879, 1727, 1359, 1250, 1214, 1068, 1005, 909. HRMS calculated for $C_{21}H_{32}O_5$: 364.2250; Found: 364.2243.

Formation of B-ring lactones by peroxidation of 7-keto steroids

Several B-ring lactones were prepared by Baeyer–Villiger oxidation. The 7-keto androstane **33** gave the normal product of migration (**34**) of the secondary carbon in preference to the primary one on treatment with *m*-chloroperbenzoic acid (Scheme 6). The enone 3β,17β-diacetoxyandrost-5-en-7-one (**35**) showed more complex behavior. Treatment with excess *p*-nitroperoxybenzoic acid (PNPBA) in dichloromethane for several days at room temperature gave mixtures of the α-5,6 epoxide (**36**) and the two Baeyer–Villiger products **37** and **38** (Scheme 7). When the solvent was changed to refluxing chloroform, 70% of the aldehyde **39** was formed. When epoxide lactone **38** was submitted to these reaction conditions the aldehyde **39** was the only product. The structures of the epoxide **36**, and the aldehyde



Scheme 6



Scheme 7

39 were determined unambiguously by single crystal X-ray crystallography. The α configuration of the epoxide translates into the β configuration for the formyl group in the rearrangement of **38** to **39** after the Baeyer–Villiger reaction.

*3β,17β-diacetoxy-7a-oxa-5α-androstan-7-one (***34***)*

To a solution of 3β,17β-diacetoxyandrostan-7-one (**33**, 0.45 g; 1.15 mmol) in dry chloroform (5 mL), was added *m*-chloroperbenzoic acid (MCPBA) (0.5 g). After stirring at room temperature for 20 h the reaction mixture was poured into water and the organic layer separated. The $CHCl_3$ extract was washed with 10% $NaHCO_3$ and water, dried over $MgSO_4$ and the solvent was removed in vacuo. The residue was purified by chromatography on a column of silica gel and eluted with acetone-petroleum ether (1.5:8.5). The starting steroid (0.1 g, 22% recovered) eluted first followed by the product which was crystallized from ether-hexane. Yield 0.30 g (82% based on 78% conversion); m.p. 163–165°C. ^{1}H NMR (200 MHz): δ 4.65 (m, 2H, 3α-,17α-H), 4.22 (m, 1H, 8-H), 2.05 (s, 3H, $OCOCH_3$), 2.03 (s, 3H, $OCOCH_3$), 1.03 (s, 3H, 19-CH_3), 0.79 (s, 3H, 18-CH_3). ^{13}C NMR (300 MHz): δ 174.6 (C═O, C-7), 170.8 & 170.3 (acetate C═O), 81.79, 79.1, 72.05 (CH, C-3,8,17), 52.3, 49.1, 40.9 (CH, C-5,9,14), 37.5, 36.7, 35.8, 35.5, 27.2, 26.7, 24.2, 22.5 (CH_2, C-1,2,4,6,11,12,15,16), 21.3, 21.0 (acetate-CH_3), 12.9, 11.6 (CH_3, C-18,19).

*3β,17β-diacetoxy-5α,6-epoxy-androstan-7-one (***36***)*

3β,17β-Diacetoxyandrost-5-en-7-one (0.349 g, 0.90 mmol) in 12 mL of CH_2Cl_2 was treated with 0.474 g (1.56 mmol,

65%) of PNPBA and 4 mg of 3-*tert*-butyl-4-hydroxy-5-methylphenyl sulfide (radical inhibitor) and stirred for 24 h. At this point NMR analysis indicated that 36% starting material remained. More PNPBA (0.256 g, 1.09 mmol, 65%) was added and stirring was continued for 48 h. NMR analysis showed 42% epoxide **36**, 37% aldehyde **39**, and 21% lactone **37**. The crude mixture was chromatographed on silica gel eluting with 50% ethyl acetate-hexane. The first fraction (Rf = 0.70) contained 0.086 g of **36** and some methyl *p*-nitrobenzoate. Pure **36** was crystallized from CH_2Cl_2-hexane to yield 27 mg, m.p. 207–208°C. ^{1}H NMR (300 MHz) δ 4.92 (tt, J = 11.5, 4.5 Hz, 3α-H), 4.60 (dd, J = 9, 7.5 Hz, 17α-H), 3.04 (s, 6β-H), 2.28 (dd, J = 13, 12 Hz, 15α-H), 2.038 (s, CH_3COO), 2.036 (s, CH_3COO), 1.98 (t, J = 11.5, 8β-H), 1.07 (s, 19-CH_3), 0.80 (s, 18-CH_3). ^{13}C NMR (75.4 MHz) δ 206.5 (7-CO), 171.0 (CH_3*C*OO), 170.0 (CH_3*C*OO), 81.9 (17-CH), 70.7 (3-CH), 67.5 (5-C), 62.5 (6-CH), 46.7 (CH), 46.2 (CH), 44.0 (13-C), 43.1 (CH), 36.6 (CH_2), 35.2 (10-C), 35.1 (CH_2), 32.6 (CH_2), 27.6 (CH_2), 27.1 (CH_2), 24.6 (CH_2), 21.2 (*C*H_3COO), 21.1 (*C*H_3COO), 20.6 (11-C), 15.5 (19-CH_3), 12.2 (18-CH_3).

The stereochemistry was determined by X-ray crystallography. A colorless transparent plate-shaped crystal of dimensions 0.58 × 0.24 × 0.04 mm was selected for structural analysis. Intensity data for this compound were collected using a Siemens SMART ccd area detector[21,22] mounted on a Siemens P4 diffractometer equipped with graphite-monochromated Mo Kα radiation (λ = 0.71073 Å). The sample was cooled to 133 ± 2°K. The intensity data, which nominally covered one and a half hemispheres of reciprocal space, were measured as a series of ϕ oscillation frames each of 0.4° for 60 sec/frame. The detector was operated in 512 × 512 mode and was positioned 5.26 cm from the sample. Cell parameters were determined from a non-linear least-squares fit of 5794 peaks in the range $3.0 < \theta < 25.0°$. The first 50 frames were repeated at the end of data collection and yielded a total of 249 peaks showing a variation of 0.05% during the data collection. A total of 10361 data were measured in the range $2.50 < \theta < 29.19°$. The data were corrected for absorption by the empiric method[23] giving minimum and maximum transmissions of 0.9498 and 0.9964. The data were merged to form a set of 5126 independent data with R_{int} = 0.0234. Over 99.8% of the unique data were measured to 25° in θ.

The orthorhombic space group $P2_12_12_1$ was determined by systematic absences and statistical tests and verified by subsequent refinement. The structure was solved by direct methods and refined by full-matrix least-squares methods on F^2.[24,25] Hydrogen atom positions were initially determined by geometry and refined by a riding model. Non-hydrogen atoms were refined with anisotropic displacement parameters. A total of 263 parameters were refined against 5126 data to give $wR(F^2)$ = 0.0884 and S = 0.985 for weights of $w = 1/[\sigma^2(F^2) + (0.0500P)^2]$, where $P = [F_o^2 + 2F_c^2]/3$. The final R(F) was 0.0402 for the 4290 observed, $[F > 4\sigma(F)]$, data. The largest shift/s.u. was 0.002 in the final refinement cycle. The final difference map had maxima and minima of 0.284 and −0.165, respectively. The absolute structure was determined by refinement of the Flack parameter.[26]

*3β,17β-diacetoxy-5α,6-epoxy-7a-oxa-androstan-7-one (**37**) and 3β,17β-diacetoxy-5α,6-epoxy-7-oxa-androstan-7a-one (**38**)*

3β,17β-Diacetoxyandrost-5-en-7-one (**35**) (0.212 g, 0.55 mmol) in 7 mL of CH_2Cl_2 was treated with 0.328 g (65%, 1.16 mmol) of PNPBA and the mixture was stirred at room temperature for 70 h. At this point NMR analysis showed the starting material to be consumed and a mixture of **37** (49%) and **38** (36%) was formed along with minor amounts of other oxidation products. The CH_2Cl_2 solution was extracted with 10% aqueous NaOH and washed with saturated NaCl. Solvent was removed under reduced pressure. This mixture, which also contained some methyl *p*-nitrobenzoate, was used in the biological assay. A small sample was chromatographed on silica gel, eluting with 50% ethyl acetate-hexane two times, to yield pure **38**. ^{1}H NMR (300 MHz) δ 4.85 (m, 3α-H), 4.82 (s, 6β-H), 4.62 (dd, J = 9.5, 7.0 Hz, 17α-H), 2.41 (t, J = 10.0 Hz, 8β-H), 2.04, 2.01 (s, 3-CH_3COO, 17-CH_3COO), 1.13 (s, 19-CH_3), 0.82 (s, 18-CH_3). ^{13}C NMR (75.4 MHz) δ 174.1 (7-CO), 170.8 (CH_3*C*OO), 170.1 (CH_3*C*OO), 82.5 (6-C), 81.7 (17-CH), 69.5 (3-CH), 67.5 (5-C), 48.8 (CH), 46.9 (CH), 43.1 (13-C), 40.0 (CH), 38.6 (10-C), 36.3 (CH_2), 34.6 (CH_2), 32.5 (CH_2), 27.1 (CH_2), 26.6 (CH_2), 23.5 (CH_2), 21.2 (*C*H_3COO), 21.1 (*C*H_3COO), 20.3 (11-CH_2), 15.9 (19-CH_3), 11.9 (18-CH_3). ^{1}H NMR of **37** in mixture (300 MHz) δ 4.85 (m, 3α-H), 4.66 (dd, J = 10, 7 Hz, 17α-H), 3.27 (s, 6β-H), 2.05, 2.03 (s, 3-CH_3COO, 17-CH_3COO), 1.17 (s, 19-H), 0.77 (s, 18-H).

*3β,17β-diacetoxy-5α-formyl-6-oxa-androstan-7-one (**39**)*

3β,17β-Diacetoxyandrost-5-en-7-one (0.346 g, 0.89 mmol) was dissolved in 12 mL of $CHCl_3$ and treated with 0.474 g (65%, 1.68 mmol) of PNPBA and 4 mg of 3-*tert*-butyl-4-hydroxy-5-methylphenyl sulfide (radical inhibitor) and refluxed for 2 h. More PNPBA (0.450 g, 1.90 mmol, 65%) was added and the mixture was refluxed for 2 more h. The $CHCl_3$ solution was extracted with 10% aqueous NaOH and washed with saturated NaCl. Solvent was removed under reduced pressure to yield a white solid. A small sample was purified by preparative TLC on silica gel eluting with 5% MeOH-$CHCl_3$ to give 15 mg of pure aldehyde **39** which was crystallized from ether; m.p. 143–145°C. ^{1}H NMR (300 MHz) δ 9.66 (s, HCO), 5.16 (apparent quintet, $J \sim 5$ Hz, 3α-H), 4.65 (dd, J = 9, 7 Hz, 6α-H0, 2.05, 2.04 (s, 3-CH_3COO, 17-CH_3COO), 1.22 (s, 19-CH_3), 0.79 (s, 18-CH_3). ^{13}C NMRδ 197.3 (HCO), 171.0 (CH_3*C*OO), 170.9 (CH_3*C*OO), 86.6 (5-C), 81.4 (17-CH), 68.3 (3-CH), 46.1 (CH), 43.4 (13-C), 43.3 (CH), 41.5 (CH), 37.6 (10-C), 35.9 (CH_2), 33.9 (CH_2), 32.7 (CH_2), 27.3 (CH_2), 25.0 (CH_2), 24.7 (CH_2), 21.22 (11-CH_2), 21.14 (*C*H_3COO), 21.09 (*C*H_3COO), 17.6 (19-CH_3), 12.1 (18-CH_3). IR (cm^{-1}) 3026, 2953, 1736, 1375, 1246, 1030, 908.

The crude mixture containing 65% aldehyde by NMR was used in the biological assay. The stereochemistry was determined by x-ray crystallography of a colorless transparent crystal of dimensions 0.60 × 0.40 × 0.02 mm. Intensity data for this compound were collected as described for

Table 1. Induction of thermogenic enzymes in rats' livers by seco analogs of DHEA and related steroids

Steroid	Number of rats	Concentration in diet %	% of Control GPDH	% of Control ME
3β-Acetoxyandrost-5-en-17-one(DHEA-acetate)[a]	42	0.057	241 ± 55	329 ±75
3β-Acetoxyandrost-5-en-7,17-dione (**1**)[a]	9	0.06	432 ± 114	416 ± 47
3β-Acetoxy-17a-oxa-androst-5-ene-7,17-dione (**17**)	4	0.064	328	485
3β-Hydroxy-17a-oxa-androsta-5,15-diene-7,17-dione (14α) (**14**)	4	0.052	128	396
3β-Propionoxy-17a-oxa-androsta-5,15-diene-7,17-dione(14α) (**13**)	5	0.065	100	120
3β-Hydroxy-17a-oxa-androsta-5,15-diene-7,17-dione (14β) (**15**)	2	0.068	112	207
3β-Hydroxy-7-oxo-16,17-secoandrost-5-ene-16,17-dioic acid (**9**)	2	0.063	80	205
3β-Acetoxyandrost-5-ene-7,16,17-trione (**8**)[b]	3	0.066	253	460

Other inactive compounds tested at 0.05–0.1% of the diet: 3β-Hydroxy-7-oxo-16,17-secoandrost-5-en-17-oic acid (**12a**); 3β-hydroxy-16,17 secoandrost-5-en-17-oic acid (**11**); methyl 3β-hydroxy-7-oxo-17-oic-16,17-secoandrost-5-en-16-oate; methyl 3β-hydroxy-17-oic-16,17-secoandrost-5-en-16-oate; methyl 3β-acetoxy-7-oxo-16-oic-16,17-secoandrost-5-en-17-oate; methyl 3β-acetoxy-16-oic-16,17-secoandrost-5-en-17-oate; dimethyl 3β-hydroxy-7-oxo-16,17-secoandrost-5-ene-16,17-dioate (**10**); dimethyl 3β-hydroxy-16,17-secoandrost-5-ene-16,17-dioate; 3β-acetoxy-16a-oxa-androst-5-ene-7,17-dione; 3β-acetoxy-16a-oxa-androst-5-ene-7,16,-dione (**21**); 3α-hydroxy-7a-oxa-17β-acetoxy-5β-androstan-7-one (**32**); 4-oxa-4a-homo-7α-hydroxy-17β-acetoxy-5β-androstan-3-one (**31**); 3β,17β-diacetoxy-5α,6-epoxy-6a-oxa-androstan-7-one and the corresponding 7a-oxa steroid (**38, 37**); 3β,17β-diacetoxy-5α-formyl-6-oxa-androstan-7-one; 3β,17β-diacetoxy-7a-oxa-androstan-7-one (**34**).
Two to 5 rats were used in each assay of the inactive compounds.
[a]These data from ref. 8 are shown for comparative purposes.
[b]In assays not shown, the 3-propionyl ester of this trione was as active as equimolar 7-oxo-DHEA.

compound **36** except that the temperature was 293 ± 2°K. Cell parameters were determined from a non-linear least-squares fit of 6523 peaks in the range $3.0 < \theta < 25.0°$. The first 50 frames were repeated at the end of data collection and yielded a total of 270 peaks showing a variation of 0.04% during the data collection. A total of 13927 data were measured in the range $5.36 < \theta < 24.99°$. The data were corrected for absorption by the empiric method[23] giving minimum and maximum transmissions of 0.9466 and 0.9982. The data were merged to form a set of 7763 independent data with $R_{int} = 0.0461$. Over 97.0% of the unique data were measured to 25° in θ. A total of 571 anisotropic displacement parameters were refined against 1 restraint and 7763 data to give $wR(F^2) = 0.3113$ and $S = 1.244$ for weights of $w = 1/[\sigma^2 (F^2) + (0.1182P)^2 + 11.8340P]$, where $P = [F_O^2 + 2F_C^2]/3$. The final R(F) was 0.1269 for the 5784 observed, $[F > 4\sigma(F)]$, data. The largest shift/s.u. was 0.028 in the final refinement cycle. The final difference map had maxima and minima of 0.724 and -0.448, respectively. The absolute structure was determined by refinement of the Flack parameter.[26]

Biological activity: Results and discussion

D-Ring seco estrogens have unusual chemical[4,27,28] and biologic properties.[3,4,29] Despite low affinity for estrogen receptors, some of the seco compounds have much greater oral estrogenic activity than estrone or estradiol.[5,29] D-ring seco steroids related to DHEA have been made and were found to have no androgen or estrogen activity.[6] Because DHEA has a wide variety of biologic effects we thought it worthwhile to assay these derivatives for an activity other than those of the sex hormones. Of the open ring structures synthesized only the 16,17 dicarboxylic acid (9) induced the formation of cytosolic malic enzyme but not of glycerophosphate dehydrogenase when fed to rats (Table 1). Two 5,15-diene-17 → 13 lactones, with their 14-hydrogen atom in α- and β- configuration respectively, also induced the formation of malic enzyme. The 3-β propionyl ester of the former was apparently not hydrolyzed in vivo for it was inactive. Compound **17**, the 17 → 13 lactone of 7-oxo-DHEA acetate, induced both enzymes as did 3β-acetoxyandrost-5-ene-7,16,17-trione, an intermediate in the synthesis of the 16, 17 dicarboxylic acid. Some ability to induce liver enzymes is retained despite extensive alterations of the D-ring. Of special interest is the finding that introduction of a double bond at 15, 16 abolishes the ability to induce glycerophosphate dehydrogenase while activity toward malic enzyme is retained (**14** and **15** vs **17**; other examples will be presented: I. L. Reich et al., in preparation).

We presume that DHEA, its two 7-hydroxy derivatives, and 7-oxo-DHEA are not hormones but are precursors of one or more steroid hormones with higher specific activity and for which specific receptors will be found. The enzyme-inducing activities of compounds reported here, in Reference [8,10] and I. L. Reich et al. (in preparation) indicate that the D ring of a hormone they form probably does not participate in binding as specifically to a receptor as do some other parts of the steroid nucleus.

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