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Ergosteroids IV: synthesis and biological activity of steroid glucuronosides, ethers, and alkylcarbonates

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Abstract

The 7-oxo derivative of dehydroepiandrosterone is more active than the parent steroid and is devoid of adverse side effects in rats, monkeys and humans. In anticipation of possible therapeutic use we have sought more active, longer lasting forms of 7-oxo- and 7β-hydroxydehydroepiandrosterones. The 7-oxo- and 7-hydroxy steroids have been converted to glucuronides, ethers and carbonate esters. The syntheses of these compounds are described and their ability to induce the formation of liver thermogenic enzymes when fed to rats is reported. Some of the new derivatives were found to be somewhat more effective than the equimolar amounts of 7-oxo-DHEA with which they were compared in each experiment.

Keywords: 7-Oxo-dehydroepiandrosterone; 7β-Hydroxydehydroepiandrosterone; Glucuronosides; Ether and alkyl carbonate derivatives of 7-oxygenated steroids

1. Introduction

In addition to serving as a precursor of both androgens and estrogens, dehydroepiandrosterone (DHEA) exerts a variety of other beneficial biologic effects. In experimental animals it decreases body fat without altering food intake, enhances the immune system, suppresses spontaneous and carcinogen-induced tumors, decreases blood glucose in diabetic mice and enhances the memory of old mice [1,2]. The quantities of DHEA required to elicit these responses are in excess of the amounts of steroid *hormones* needed to demonstrate their physiological function. This, together with the lack of a demonstrated receptor for DHEA, indicates that DHEA may not be a hormone in somatic cells but may act as a precursor of one or more steroids displaying higher, and possibly more selective, activity. It has been known since 1962 that DHEA is converted by tissue preparations in vitro to both 7α- and 7β-hydroxy-DHEA [3–5] and also to 7-oxo-DHEA, but until 1988 no biologic activities for these metabolites had been reported [6,7]. Elsewhere we shall present evidence that, in the rat, the formation of these derivatives is by the sequence: DHEA $\rightarrow$ 7α-hydroxy-DHEA $\rightarrow$ 7-oxo-DHEA $\rightarrow$ 7β-hydroxy-DHEA. The ability to induce the formation of liver thermogenic enzymes increases as the steroid is converted toward 7β-hydroxy-DHEA.

In human therapy there are some undesired responses to administered DHEA because it elevates blood testosterone and dihydrotestosterone concentrations in women [8]. Its potentially hazardous effects in persons at risk of breast cancer and prostatic cancer have been cited by the National Institute on Aging [9]. In previous publications we have demonstrated that 7-oxo-DHEA has no androgenic activity [10], is more active than DHEA [11], is non-toxic to rats [12] and monkeys [13] even at doses of a g/kg body weight, and is well tolerated by men given 200 mg daily for four weeks [14]. Administered orally as the acetyl ester, this steroid is rapidly absorbed and converted to the 3-sulfate ester [15]. Its half life in blood plasma of men is 2.17 h [14]. In preparation for clinical efficacy trials we have sought new derivatives of 7-oxo-DHEA that might have a longer half life. We have prepared a variety of 3-, 7- or 17-substituted glucuronides, ethers, and alkylcarbonates of 7-oxygenated steroids that might more slowly yield active steroids. Methyl ethers are of special interest because of the occurrence of such steroids in brain [16,17]. The present report describes the synthesis and properties of these steroids, and their ability to induce the formation in rat livers

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of two enzymes that participate in thermogenesis [18]. The ability of some of the steroids used as starting material to induce these enzymes has been reported [11,19].

2. Experimental

All melting points were determined in open capillaries in an electrically heated and stirred Thiel-type bath and are uncorrected. Solvents were removed on a rotary evaporator under water pump vacuum at 30°C or less. Nuclear magnetic resonance (NMR) spectra were taken on Bruker WP-200SY and AM-300 MHz spectrometers. Spectra were usually measured in deuterated chloroform ($CDCl_3$) using tetramethylsilane (δ 0) as reference for ^{1}H NMR spectra and the $CDCl_3$ triplet (δ 77.0) for ^{13}C spectra. Abbreviations are s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet). A Kratos MS-80RFA mass spectrometer was used for high resolution mass spectra and Hewlett-Packard LC-MS, 1100 series, with diode array (DAD) UV detector and AP-CI and API-ES mass detectors, were employed for simultaneous recording of the UV and the mass spectra of the compounds. Spectral data were included for some known compounds where such data were not previously reported. Reagents and solvents used were purchased from Aldrich Chemical Co., Milwaukee, Wisconsin. For column chromatography, Aldrich brand silica gel of 70–230 and 200–400 mesh size was used.

Assays of compounds for their ability to induce liver mitochondrial glycerophosphate dehydrogenase and cytosolic malic enzyme when fed to rats have been described [11,19]. In addition to the control rats that received no steroid supplement, each experiment included a group that received DHEA or 7-oxo-DHEA-3β-acetate (usually at 0.04%–0.06% of the diet). Because of variation in enzyme activity of both control and treated groups from one experiment to another the activities are reported relative to the enzyme activities of the control group. Compounds are considered active only if they enhance the activity of these thermogenic enzymes to greater than 150% of that in livers of control rats.

3. Syntheses

*3.1. General procedure: preparation of β-pyranoside forms of steroid glucopyranosides (as triacetylated methyl esters, **7, 10, 11, 14, 15, 17,** Scheme I)*

Steroid (10.0 mmol), methyl 2,3,4-tri-O-acetyl-1-bromo-1-deoxy-α-D-glucopyranosiduronate (10.0 mmol) and freshly pulverized dry calcium sulfate (2.0 g) were taken up in dry benzene (150 ml). Dry silver carbonate (freshly prepared, 20.0 mmol) was added to the reaction mixture which was subsequently stirred at room temperature in the dark. Additional quantities of bromo glucuronate (5 mmol) and silver carbonate (10 mmol) were added after 12 and 24 h. The reaction mixture was stirred for 48–72 h depending upon the nature of the steroid, and then the reaction mixture was filtered on a bed of celite and the clear filtrate was evaporated to dryness. The product was isolated from the crude solid either by crystallization or by chromatography on alumina or silica gel.

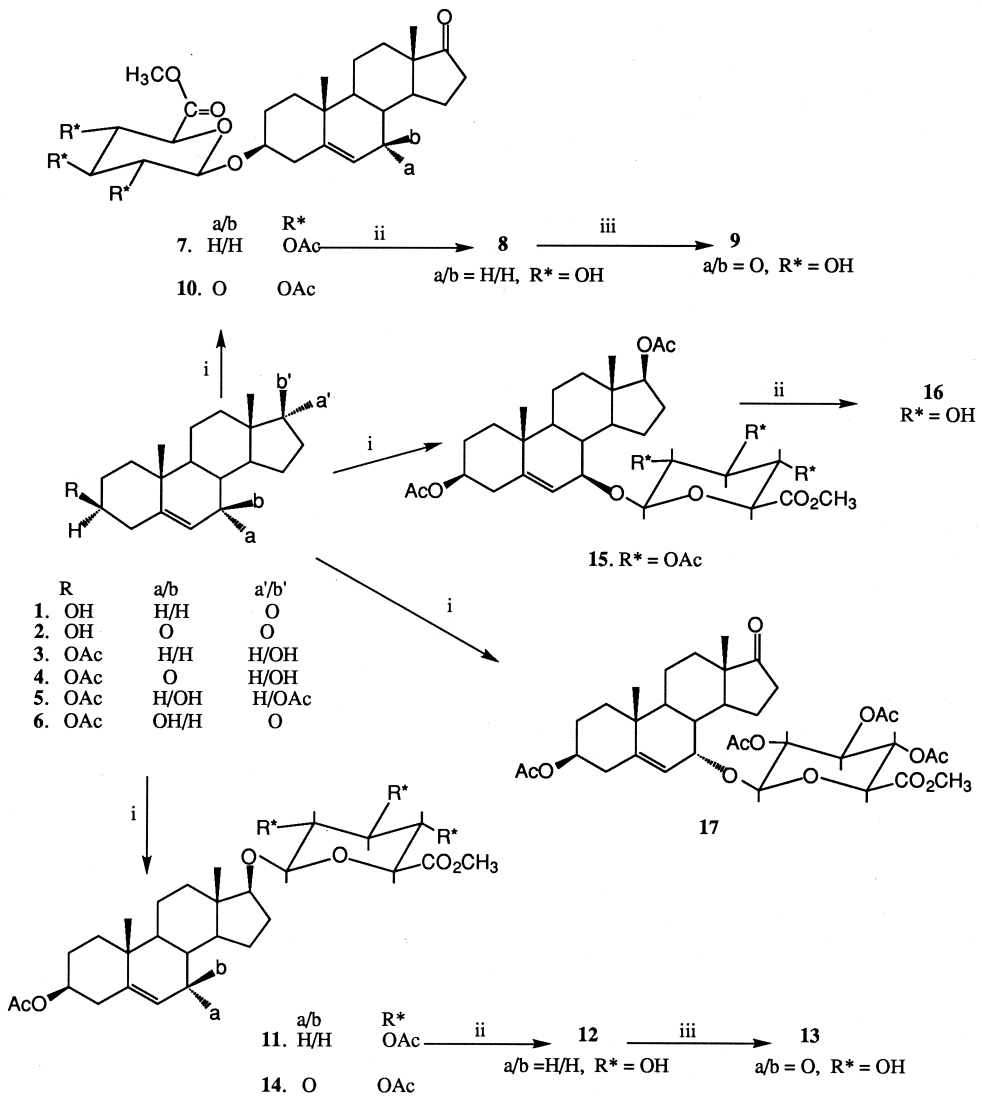
3.1.1. Methyl 2,3,4-tri-O-acetyl -1-O-(17-oxoandrost-5-en-3β-yl)-β-D-glucopyranosiduronate (7)

A mixture of 3β-hydroxyandrost-5-en-17-one (DHEA, **1,** Scheme I) and methyl 2,3,4-tri-O-acetyl-1-bromo-1-deoxy-α-D-glucopyranosiduronate, dry calcium sulfate and silver carbonate in benzene was stirred at room temperature and worked up after 48 h. The ^{1}H NMR of the reaction mixture showed 60% conversion of the starting material to the product. After the usual work-up and subsequent crystallization of the crude product, twice, from acetone-hexane, product **7** (Scheme I) was obtained as a pure white solid (61%, based on 60% conversion), m.p. 191–92°C. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 5.39 (1H, d, J = 5.2 Hz, 6-H), 5.24 (2H, m, 3,4-H (glu)), 4.98 (1H, dd, J = 8.0, 10.0 Hz, 2-H (glu)), 4.66 (1H, d, J = 8.0 Hz, 1-H (glu)), 4.02 (1H, d, J = 10.0 Hz, 5-H (glu)), 3.75 (3H, s, OCH_3), 3.52 (1H, m, 3α-H), 2.10 (3H, s, $OCOCH_3$), 2.05 (6H, s, 2$\times$ $OCOCH_3$), 1.02 (3H, s, 19-CH_3), 0.89 (3H, s, 18-CH_3). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 220.7 (17-CO), 170.0 (CO, acetate), 169.2 (CO, acetate), 169.0 (CO, acetate), 167.5 (CO, ester), 140.3 (5-C), 121.1 (6-C), 99.2 (1-C (glu)), 79.6 (3-C), 72.9 (CH (glu)), 72.0 (CH (glu)), 70.2 (CH (glu)), 69.3 (CH (glu)), 52.8, 51.5, 50.0, 47.4, 38.4, 37.2, 37.0, 35.6, 31.6, 31.1, 29.6, 22.2 (CH_2(s), CH(s) and OCH_3), 21.0 (Ac-CH_3), 20.9 (Ac-CH_3), 20.8 (Ac-CH_3), 19.5 (19-CH_3), 14.4 (18-CH_3).

3.1.2. Methyl 2,3,4-tri-O-acetyl-1-O-(7,17-dioxoandrost -5-en-3β-yl)-β-D-glucopyranosiduronate (10)

The product **10** was prepared by the glucuronidation of 3β-hydroxyandrost-5-ene-7,17-dione (**2,** Scheme I). The reaction mixture was stirred at room temperature in the dark for 72 h. The crude product was chromatographed on alumina and eluted with ethyl acetate-hexane (15:85, v/v). The first fraction eluted from the column was crystallized from methanol and identified as androsta-3,5-diene-7,17-dione (15%, based on 69% conversion), m.p. 167–68°C. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 6.19 (2H, m, 3,4-H), 5.65 (1H, s, 6-H), 1.15 (3H, s, 19-CH_3), 0.90 (3H, s, 18-CH_3).

Further elution with a 30:70 mixture of ethyl acetate-hexane afforded a thick syrupy material which was triturated with diethyl ether and cooled in the refrigerator for 2 h, to afford white crystalline compound **10** (Scheme I) m.p. 206–8°C (25%, based on 69% conversion). ^{1}H NMR ($CDCl_3$, 200 MHz): δ 5.75 (1H, s, 6-H), 5.24 (2H, m, 3,4-H (glu)), 4.98 (1H, t, J = 8.3 Hz, 2-H (glu)), 4.65 (1H, d, J = 8.3 Hz, 1-H (glu)), 4.03 (1H, d, J = 9.9 Hz, 5-H (glu)), 3.75 (3H, s, OCH_3), 3.65 (1H, m, 3α-H), 2.04 (3H, s, $OCOCH_3$),



Scheme 1.

2.0 (6H, s, 2× $OCOCH_3$), 1.20 (3H, s, 19-CH_3), 0.91 (3H, s, 18-CH_3). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 220.4 (17-CO), 201.1 (7-CO), 170 (CO, acetate), 169.4 (CO, acetate), 169.2 (CO, acetate), 167.1 (CO, ester), 165.1 (5-C), 126.2 (6-C), 100.0 (1-C (glu)), 78.4 (3-C), 72.9 (CH (glu)), 72.1 (CH (glu)), 71.4 (CH (glu)), 69.4 (CH (glu)), 53.2, 48.2, 45.8, 44.6, 39.6, 39.0, 39.2, 36.2, 35.6, 31.0, 29.0, (CH_2(s), CH(s) and OCH_3), 24.3 (Ac-CH_3), 21.0 (Ac-CH_3), 21.0 (Ac-CH_3), 17.6 (19-CH_3), 14.1 (18-CH_3). FAB m/z: 641.1 (M+Na, 100%), 498 (M, -AcOH, -AcOH, 1%), 285 (M+1, -334 (methyl triactylglucuronate, -H), 4%).

A final fraction eluted with the same eluent afforded the starting 3β-hydroxyandrost-5-ene-7,17-dione (**2,** 31**%**).

3.1.3. Methyl 2,3,4-tri-O-acetyl-1-O-(3β-acetoxyandrost-5-en-17β-yl)-β-D-glucopyranosiduronate (11)

3β-Acetoxy-17β-hydroxyandrost-5-ene (**3,** Scheme I) in dry benzene was stirred with acetobromoglucuronate methyl ester and silver carbonate for 48 h at room temperature. The ^1H NMR spectrum of the reaction mixture showed the presence of equal amounts of starting 3β-acetoxy-17β-hydroxyandrost-5-ene (**3**) and the product steroid glucopyranoside **11.** Prolonged stirring beyond 48 h did not alter the product ratio. The reaction mixture was processed as described before and the resultant thick mass was heated to dissolve in a mixture of acetone-hexane. The solution was allowed to stand at room temperature for 4 h and the white crystalline compound **11** was collected and dried. ^1H NMR spectrum of the crystalline solid confirmed the purity and structure of product **11** (Scheme I) yield 94.8% (based on 50% conversion of starting steroid), m.p. 187–9°C. ^1H NMR ($CDCl_3$, 200 MHz): δ 5.36 (1H, d, J = 5.2 Hz, 6-H), 5.23 (2H, m, 3,4-H (glu)), 5.02 (1H, t, J = 8.4 Hz, 2-H (glu)), 4.60 (1H, d, J = 8.4 Hz, 1-H (glu)), 4.60 (1H, m, 3α-H), 3.99 (1H, d, J = 10 Hz, 5-H (glu)), 3.75 (3H, s, OCH_3), 3.59 (1H, t, J = 8.7 Hz, 17α-H), 2.07 (3H, s,

$OCOCH_3$), 2.05 (3H, s, $OCOCH_3$), 2.04 (3H, s, $OCOCH_3$), 2.03 (3H, s, $OCOCH_3$), 1.03 (3H, s, 19-CH_3), 0.74 (3H, s, 18-CH_3). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 170.5 (CO acetate), 170.2 (CO acetate), 169.4 (CO acetate), 169.1, 167.3 (CO ester), 139.7 (5-C), 122.2 (6-C), 101.4 (1-C (glu)), 90.0 (3-C), 73.8, 72.5, 72.2, 71.4, 69.5 (17-C and CH(s) (glu)), 52.8, 50.95, 50.1, 42.8, 38.7, 37.4, 37.0, 36.6, 31.6, 31.4, 28.6, 27.7, 23.4 (CH_2(s), CH(s), quaternary-C, and OCH_3), 21.4 (Ac-CH_3), 20.7 (Ac-CH_3), 20.6 (Ac-CH_3), 20.5 (Ac-CH_3), 19.3 (19-CH_3), 11.5 (18-CH_3).

*3.1.4. Methyl 2,3,4-tri-O-acetyl-1-O-(3β-acetoxyandrost-5-en-7-one-17β-yl)-β-D-glucopyranosiduronate (**14**)*

Glucuronidation of 3β-acetoxy-17β-hydroxyandrost-5-en-7-one (**4,** Scheme I) was performed similarly and the reaction mixture was worked up after stirring for 48 h. ^{1}H NMR analysis of the filtrate showed 60% of the starting material and 40% of the product. The crude mixture was treated with hexane and crystallization at room temperature afforded a crystalline white solid that was filtered and air dried. It was found by ^{1}H NMR to be pure compound **14**. M.p. 122–25°C, yield 78.5% (based on 40% conversion). ^{1}H NMR ($CDCl_3$, 200 MHz): δ 5.71 (1H, d, 6-H), 5.23 (2H, m, 3, 4-H (glu)), 5.02 (1H, t, J = 8.0 Hz, 2-H (glu)), 4.72 (1H, m, 3α-H), 4.58 (1H, d, J = 8.0 Hz, 1-H (glu)), 4.0 (1H, d, J = 10.0 Hz, 5-H (glu)), 3.76 (3H, s, OCH_3), 3.6 (1H, t, J = 8.0 Hz, 17α-H), 2.06 (3H, s, $OCOCH_3$), 2.046 (3H, s, $OCOCH_3$), 2.025 (3H, s, $OCOCH_3$), 2.02 (3H, s, $OCOCH_3$), 1.22 (3H, s, 19-CH_3), 0.74 (3H, s, 18-CH_3). FAB m/z: 685.1 (M+Na, 100%), 625.1 (M+Na, -AcOH, 6%), 269.2 (M+1, -334 (methyl triactylglucuronate), -AcOH, 8%).

*3.1.5. Methyl 2,3,4-tri-O-acetyl-1-O-(3β,17β-diacetoxyandrost-5-en-7β-yl)-β-D-glucopyranosiduronate (**15**)*

The reaction mixture containing 3β,17β-diacetoxyandrost-5-en-7β-ol (**5,** Scheme I**),** acetobromoglucuronate methyl ester, freshly dried calcium sulfate and silver carbonate in anhydrous benzene was stirred for 48 h at room temperature during which time 60% conversion of the starting steroid was detected on the basis of the NMR of the reaction mixture. The mixture was filtered through a small bed of celite and the clear filtrate was evaporated to dryness under vacuum at 30°C. The resulting solid was chromatographed on a silica gel column using ethyl acetate-hexane (3:7, v/v) as eluent to afford first the starting steroid followed by the product **15**. Crystallization of steroid glucopyranoside **15** (Scheme I) from methanol afforded a white compound m.p. 230–31°C, yield 54.5% (based on 61% conversion). ^{1}H NMR ($CDCl_3$, 200 MHz): δ 5.55 (1H, m, 6-H), 5.22 (2H, m, 3,4-H (glu)), 5.02 (1H, dd, J = 8.0 Hz, 9.8 Hz, 2-H (glu)), 4.69 (1H, d, J = 8.0 Hz, 1-H (glu)), 4.6 (1H, t, J = 8.3 Hz, 17α-H), 4.6 (1H, m, 3α-H), 3.99 (1H, d, J = 9.8 Hz, 5-H (glu)), 3.9 (1H, dm, J = 8.0 Hz, 7α-H), 3.76 (3H, s, OCH_3), 2.04 (6H, s, 2× $OCOCH_3$), 2.02 (3H, s, $OCOCH_3$), 2.00 (6H, s, 2× $OCOCH_3$), 1.05 (3H, s, 19-CH_3), 0.76 (3H, s, 18-CH_3). FAB m/z: 729.1 (M+Na, 100%), 669.1 (M+Na, -AcOH, 5%), 609 (M+Na, -AcOH, -AcOH, 20%), 313.2 (M+1, -334 (methyl triacetylglucuronate), -AcOH, 15%), 253.2 (M+1, -methyl triacetylglucuronate, -AcOH, -AcOH, 7%).

*3.1.6. Methyl 2,3,4-tri-O-acetyl-1-O-(3β-acetoxy-17-oxoandrost-5-en-7α-yl)-β-D-glucopyranosiduronate (**17**)*

The reaction mixture comprised of 3β-acetoxy-7α-hydroxyandrost-5-en-17-one (**6,** Scheme I), acetobromoglucuronate methyl ester, freshly dried calcium sulfate, silver carbonate and a catalytic amount (0.5 mole %) of silver triflate in anhydrous benzene was stirred for 48 h at room temperature in the dark. A total of 60% conversion of the starting steroid was detected on the basis of the NMR of the reaction mixture. The mixture was filtered over a small bed of celite and the clear filtrate was evaporated to dryness. The product, **17** (Scheme I), was obtained in 34.8% yield (based on 60% conversion) by crystallization from acetone-hexane, m.p. 240–42°C. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 5.64 (1H, d, J = 5.37 Hz, 6-H), 5.24 (2H, m, 3,4-H (glu)), 4.96 (1H, t, J = 7.81 Hz, 2-H (glu)), 4.68 (1H, d, J = 7.81 Hz, 1-H (glu)), 4.58 (1H, m, 3α-H), 4.02 (1H, d, J = 9.77 Hz, 5-H (glu)), 4.02 (1H, m, 7β-H), 3.74 (3H, s, OCH_3), 2.047 (3H, s, $OCOCH_3$), 2.034 (3H, s, $OCOCH_3$), 2.03 (3H, s, $OCOCH_3$), 2.02 (3H, s, $OCOCH_3$), 1.03 (3H, s, 19-CH_3) 0.84 (3H, s, 18-CH_3). FAB m/z: 685.1 (M+Na, 50%), 329 (M+1, -334 (methyl triacetylglucuronate), 16%), 269 (M+1, -AcOH, -methyl triacetylglucuronate, 6.9%).

3.1.7. Methyl 1-O-(17-oxoandrost-5-en-3β-yl)-β-D-glucopyranosiduronate (***8***)

Methyl 2,3,4-tri-O-acetyl-1-O-(17-oxoandrost-5-en-3β-yl)-β-D-glucopyranosiduronate (**7**, Scheme I) (0.5 g, 0.83 mmol) was placed in a flame-dried flask with 50.0 ml of freshly dried and distilled methanol. A freshly prepared solution (0.65 ml) of sodium methoxide (prepared by dissolving 0.14 g sodium in 5 ml of methanol) was added to the solution and the mixture was stirred at room temperature for 3 h. Solvent was evaporated and the residue was taken up in distilled water (25 ml). The excess base was neutralized with solid carbon dioxide or dilute acetic acid and the aqueous layer was saturated with sodium chloride. A white precipitated solid, thus formed, was extracted with ethyl acetate and the organic layer washed with brine, dried over magnesium sulfate and filtered. Solvent was removed and the residue, after trituration with ether, afforded a white solid which was filtered and dried. Recrystallization from acetone-hexane yielded 0.23 g (75.8%) of crystalline product **8** (Scheme I), m.p. 208–10°C. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 5.41 (1H, d, J = 4.6 Hz, 6-H), 4.45 (1H, d, J = 8.3 Hz, 1-H (glu)), 3.92–3.24 (5H, m, 2–5H (glu) and 3α-H), 3.83 (3H, s, OCH_3), 1.04 (3H, s, 19-CH_3), 0.89 (3H, s, 18-CH_3). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 221.2 (17-C), 170.0 (ester CO), 140.9 (5-C), 121.8 (6-C), 101.4 (1-C

(glu)), 79.4 (3-C), 77.6 (CH (glu)), 74.3 (CH (glu)), 72.3 (CH (glu)), 71.8 (CH (glu)), 53.1, 52.2, 50.7, 48.1, 39.2, 37.4, 37.0, 36.2, 31.6, 31.2, 29.6, 22.2, 20.5 (CH_2(s), CH(s), quaternary-C, and OCH_3), 19.4 (19-CH_3), 14.6 (18-CH_3).

*3.1.8. Methyl 1-O-(3β-acetoxyandrost-5-en-17β-yl)-β-D-glucopyranosiduronate (**12**)*

To a solution of compound **11** (2.0 g, 3.08 mmol) in anhydrous methanol (100 ml), 1.3 ml of a freshly prepared solution of sodium methoxide was added under argon. After 1 h stirring at room temperature, the methanol was distilled off at 25–30°C, the residue was taken up in cold water and neutralized with dilute acetic acid. The aqueous layer was saturated with sodium chloride and the solid was extracted with dichloromethane. The organic layer was filtered, washed with saturated bicarbonate, dried over magnesium sulfate, and evaporated. On trituration with ether the product **12** (Scheme I) was obtained as pure white crystals (1.4 g, 87%), m.p. 192–5°C. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 5.37 (1H, d, J = 5.5 Hz, 6-H), 4.6 (1H, m, 3α-H), 4.41 (1H, d, J = 8.2 Hz, 1-H (glu)), 3.83 (3H, s, OCH_3), 3.88–3.38 (5H, m, 17α-H, and 2–5-H (glu)), 2.04 (3H, s, $OCOCH_3$), 1.03 (3H, s, 19-CH_3), 0.82 (3H, s, 18-CH_3). ^{13}C NMR (DMSO, 300 MHz): δ 170.0 (CO, acetate), 169.8 (CO, ester), 140.5 (5-C), 122.3 (6-C), 104.3 (1-C (glu)), 89.1 (3-C), 76.8 (CH), 76.2 (CH), 74.4 (CH), 74.2 (CH), 71.1 (CH), 21.0 (acetate-CH_3), 19.2 (19-CH_3), 11.2 (18-CH_3). FAB m/z: 559.2 (M+Na, 100%), 499.2 (M+Na, -AcOH, 12%), 329 (M+1, -208 (methyl glucuronate), 30%), 269 (M+1, -methyl glucuronate, -AcOH, 5%).

*3.1.9. Methyl 1-O-(3β,17β-diacetoxyandrost-5-en-7β-yl)-β-D-glucopyranosiduronate (**16**)*

To a solution of compound **15** (0.19 g, 0.27 mmol) in dry methanol (10.0 ml), 0.2 ml of a solution of sodium methoxide (as described) was added. The solution was stirred at room temperature for 1 h. Methanol was removed at 25°C and the resultant mass was taken up in 10 ml of cold distilled water. Dilute acetic acid was added to neutralize the excess base. The aqueous layer was extracted with ethyl acetate, which was washed with water and brine, and dried over magnesium sulfate. Ethyl acetate was completely evaporated under vacuum at 25°C and the residue, on trituration with ether, afforded a white solid which was filtered, dried and identified as methyl 1-O-(3β,17β-diacetoxyandrost-5-en-7β-yl)-β-D-glucopyranosiduronate (**16,** Scheme I**)** (0.1 g, 64%), m.p. 140–42°C. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 5.59 (1H, s, 6-H), 4.68–4.50 (1H, t, J = 8.5 Hz, 17α-H and 1H, m, 3α-H), 4.48 (1H, d, J = 7.4 Hz, 1-H (glu)), 4.00–3.30 (5H, m, 2–5-H (glu) + 7α-H), 3.82 (3H, s, OCH_3), 2.05 (6H, s, 2× $OCOCH_3$), 1.06 (3H, s, 19-CH_3), 0.80 (3H, s, 18-CH_3). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 172.2 (CO), 171.2 (CO), 170.0 (CO), 144.8 (5-C), 124.4 (6-C), 101.88 (1-C (glu)), 82.94 (CH), 82.60 (CH), 75.61, 74.14, 73.31, 71.23 (7-C, and CH(s) (glu)), 52.68 (OCH_3), 50.64 (CH), 48.61 (CH), 37.9 (CH), 42.93, 36.19 (quaternary-C), 37.83 (CH_2), 36.62 (CH_2), 36.62 (CH_2), 27.66 (CH_2), 27.66 (CH_2), 26.0 (CH_2), 20.9 (CH_2), 21.38 (acetate-CH_3), 21.19 (acetate-CH_3), 18.83 (19-CH_3), 11.84 (18-CH_3). HRMS m/z: 372.2274 for $C_{23}H_{32}O_4$ requires 372.2300 (M^+, -208 (methyl glucuronate), 4%), 330.2213 for $C_{21}H_{30}O_3$ requires 330.2195 (M^+, -191 (methyl glucuronate, -OH), -59 (AcO), 100%), 312.2132 for $C_{21}H_{28}O_2$ requires 312.2089 (M^+, -208, -AcOH, 20%), 271 (M^+, -191 (methyl glucuronate, -OH), -AcO), -59 (AcOH, -H), 8%), 253.1972 for $C_{19}H_{25}$ requires 253.1956 (M^+, - methyl glucuronate, -AcOH, -AcOH, 16%).

*3.1.10. General procedure for allylic oxidation of steroid glucuronides (**8 and 12**, Scheme I)*

A solution of N-hydroxyphthalimide (5.0 mmol) and dibenzoyl peroxide (0.01 g) in acetone-ethyl acetate (2:1, 75.0 ml) was brought to reflux, and a filtered hot solution of steroid methyl glucuronate (2.5 mmol) in acetone (20.0 ml) and dibenzoyl peroxide (0.01 g) was added [20]. A slow stream of compressed air was passed into the solution and the mixture refluxed for 12–16 h (checked with TLC). Solvent was removed completely and the resultant mass was taken up in toluene and the precipitated N-hydroxyphthalimide was filtered off. The organic layer was washed thoroughly with saturated sodium bicarbonate solution followed by water and brine and then dried over magnesium sulfate. The solvent was evaporated and the residue was chromatographed on silica gel using acetone-hexane (1:1, v/v).

*3.1.11. Methyl 1-O-(7,17-dioxoandrost-5-en-3β-yl)-β-D-glucopyranosiduronate (**9**)*

Compound **9** was obtained from compound **8** (Scheme I) as white crystals, m.p. 203–5°C (decomp.), yield 29%. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 5.76 (1H, s, 6-H), 4.47 (1H, d, J = 7.9 Hz, 1-H (glu)), 3.83 (3H, s, OCH_3), 3.95–3.35 (5H, m, 2–5H (glu) and 3α-H), 1.22 (3H, s, 19-CH_3), 0.90 (3H, s, 18-CH_3). ^{13}C NMR (DMSO, 300 MHz): δ 219.1 (17-C) 200.5 (7-C), 169.5 (CO, ester), 166.3 (5-C), 125.2 (6-C), 101.3 (1-C (glu)), 76.4 (CH), 75.8 (CH), 75.3 (CH), 73.0 (CH), 71.7 (CH), 51.8, 49.3, 47.2, 44.98, 43.7, 38.6, 38.1, 35.8, 35.0, 29.2, 24.0, 20.0 (CH_2(s), CH(s), quaternary-C, and OCH_3), 16.93 (19-CH_3), 13.43 (18-CH_3). FAB m/z: 515.2 (M+Na, 4%), 455.3.2 (M+Na, -$HCOOCH_3$, 9%), 413.2 (M, - $HCOOCH_3$, -H_2O, -H, 100%), 241.8 (M, -208 (methyl glucuronate), -28 (CO), -15 (CH_3), 2%).

*3.1.12. Methyl 1-O-(3β-acetoxy-7-oxoandrost-5-en-17β-yl)-β-D-glucopyranosiduronate (**13**)*

The compound **12** on air oxidation afforded 7-oxygenated glucuronide **13** (Scheme I) as a white solid; yield 38%, m.p. 118–20°C (acetone-hexane). ^{1}H NMR ($CDCl_3$, 200 MHz): δ 5.71 (1H, s, 6-H), 4.72 (1H, m, 3α-H), 4.39 (1H, d, J = 8.2 Hz, 1-H (glu)), 3.84 (3H, s, OCH_3), 3.88–3.27 (5H, m, 17α-H, and 2–5-H (glu)), 2.06 (3H, s, $OCOCH_3$), 1.23 (3H, s, 19-CH_3), 0.83 (3H, s, 18-CH_3). ^{13}C NMR (DMSO, 300 MHz): δ 200.5 (7-C), 169.0, 169.2 (CO ace-

tate and ester), 165.0 (5-C), 125.8 (6-C), 104.1 (1-C (glu)), 87.5 (3-C), 76.0, 75.37, 73.6, 71.54 (17-C and CH(s), (glu)), 51.8, 48.8, 45.0, 44.6, 43.3, 38.0, 37.5, 35.5, 28.7, 27.0, 25.3, 20.0 (CH_2(s), CH(s), quaternary-C, and OCH_3) 21.0 (acetate- CH_3), 16.79 (19-CH_3), 11.30 (18-CH_3).

*3.1.13. 3β-Methoxyandrost-5-ene-7,17-dione (**20**)*

A solution of 3β-tosyloxyandrost-5-en-17-one (**18,** 4.0 g, 9.05 mmol) in anhydrous methanol (120 ml) was refluxed for 2 h. The reaction mixture was concentrated under vacuum. On cooling, 3β-methoxyandrost-5-en-17-one (**19,** Table 1) was obtained as a white solid. Recrystallization from methanol afforded shining crystals (2.57 g, 94%), m.p. 133–5°C (lit [21] m.p. 118–120°C aq. MeOH). ^{1}H NMR ($CDCl_3$, 200 MHz): δ 5.39 (1H, d, J = 5.3 Hz, 6-H), 3.37 (3H, s, OCH_3), 3.09 (1H, m, 3α-H), 1.04 (3H, s, 19-CH_3), 0.90 (3H, s, 18-CH_3).

Air was passed through a refluxing solution of **19** (2.0 g, 6.62 mmol) and N-hydroxyphthalimide (1.5 g, 9.2 mmol) in a mixture of acetone-ethyl acetate (1:1, 50 ml). 1,1′azobis(cyclohexanecarbonitrile) (0.08 g) was added to the reaction mixture [20] and the reaction continued for 10–12 h. After the usual work up as described in the general oxidation procedure, the crude product was dissolved in pyridine (5.0 ml) and stirred with acetic anhydride (3.0 ml) at room temperature for 3 h. The reaction mixture was poured into water and stirred for 1 h. The steroid was extracted with toluene and the organic layer washed with saturated bicarbonate solution and water. Toluene was distilled off and the residue was chromatographed on silica gel (ethyl acetate-hexane, 1:3, v/v) and crystallized from methanol. 3β-Methoxyandrost-5-ene-7,17-dione (**20**, Table 1) was obtained in 67% yield (1.4 g), m.p. 227–29°C. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 5.76 (1H, d, J = 1.8 Hz, 6-H), 3.39 (3H, s, OCH_3), 3.2 (1H, m, 3α-H), 1.22 (3H, s, 19-CH_3), 0.90 (3H, s, 18-CH_3). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 220.3 (17-C), 201.0 (7-C), 166.2 (5-C), 125.9 (6-C), 78.79, 55.9, 50.1, 45.7, 44.3 (3-C, CH(s), and OCH_3), 47.8, 38.7 (quaternary-C), 38.7 (CH_2), 36.1 (CH_2), 35.6 (CH_2), 30.7 (CH_2), 27.5 (CH_2), 24.1 (CH_2), 20.5 (CH_2), 17.4 (19-CH_3), 13.7 (18-CH_3). LC-MS (API-ES, positive): m/z 339.1 ($M+N_a$, 100%), 317.3 (M+1, 10%), 301.1 (M, -CH_3, 3%), 285.1 (M+1, -CH_3OH, 3%). LC-UV (DAD) λmax: 242 nm.

*3.1.14. 3β-Methoxy-17,17-ethylenedioxyandrost-5-en-7β- and 7α-ol (**22** and **23**)*

3β-Methoxyandrost-5-en-17-one (**19,** Table 1) was ketalized using ethylene glycol and p-toluene sulfonic acid in toluene, refluxed 7 h, yield 95%, m.p. 138–40°C. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 5.35 (1H, d, J = 4.88 Hz, 6-H), 3.9 (5H, m, O-CH_2-CH_2-O and 7α-H), 3.36 (3H, s, OCH_3), 3.05 (1H, m, 3α-H), 1.0 (3H, s, 19-CH_3), 0.86 (3H, s, 18-CH_3).

The ketalized product was oxidized at the allylic 7 position, using sodium periodate and t-butylhydroperoxide [22]. A small amount of sodium bicarbonate (2.0 mol equivalent) was incorporated into the reaction mixture in order to minimize deketalization. 3β-Methoxy-17,17-ethylenedioxyandrost-5-en-7-one (**21**, Table 1) was obtained in 62% yield; m.p. 190–192°C. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 5.7 (1H, d, J = 1.96 Hz, 6-H), 3.89 (4H, m, O-CH_2-CH_2-O), 3.38 (3H, s, OCH_3), 3.2 (1H, m, 3α-H), 1.19 (3H, s, 19-CH_3), 0.87 (3H, s, 18-CH_3).

Subsequent reduction of the 7 carbonyl group of product **21** with sodium borohydride in a mixture of methanol-methylene chloride (9:1) at room temperature afforded a mixture of diastereomeric 7-hydroxy derivatives in 90% yield with 7β- and 7α- forms in 8:2 ratio (^{1}H NMR). The diastereomers were separated by column chromatography on silica gel (eluent, ethyl acetate:hexane, 3:7). 3β-Methoxy-17,17-ethylenedioxyandrost-5-en-7β-ol (**22**, Table 1), m.p. 173–75°C. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 5.29 (1H, t, J = 1.95, 6-H), 3.89 (5H, m, O-CH_2-CH_2-O and 7α-H), 3.36 (3H, s, OCH_3), 3.14 (1H, m, 3α-H), 1.05 (3H, s, 19-CH_3), 0.88 (3H, s, 18-CH_3). HRMS: m/z 362.2505 for $C_{22}H_{34}O_4$ requires 362.2457 (M^+, 8.7%), 344.2338 for $C_{22}H_{32}O_3$ requires 344.2351 (M^+, -H_2O, 2.4%), 329.2170 for $C_{21}H_{29}O_3$ requires 329.2117 (M^+, -H_2O, -CH_3, 7.2%), 261.1886 for $C_{17}H_{25}O_2$ requires 261.1854 (M^+, -101 (loss of D-ring), 100%), 229.1628 for $C_{16}H_{21}O$ requires 229.1592 (M^+, -101 (loss of D-ring), -CH_3OH 8.3%), 211 for $C_{16}H_{19}$ (M^+, -101 (D-ring), CH_3OH, -H_2O, 2%). 3β-Methoxy-17,17-ethylenedioxyandrost-5-en-7α-ol (**23**, Table 1) melted at 183–84°C. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 5.62 (1H, dd, J = 1.96, 5.62 Hz, 6-H), 3.89 (4H, m, O-CH_2-CH_2-O), 3.89 (1H, 7β-H, merged with ketal protons), 3.36 (3H, s, OCH_3), 3.14 (1H, m, 3α-H), 0.99 (3H, s, 19-CH_3), 0.87 (3H, s, 18-CH_3). HRMS m/z: 362.2447 for $C_{22}H_{34}O_4$ requires 362.2457 (M^+, 7%), 344.2347 for $C_{22}H_{32}O_3$ requires 344.2351 (M^+, -H_2O, 3.5%), 330.2228 for $C_{21}H_{30}O_3$ requires 330.2195 (M^+, -CH_3OH, 1.6%), 329.2149 for $C_{21}H_{29}O_3$ requires 329.2117 (M^+, -H_2O, -CH_3, 4.2%), 261.1838 for $C_{17}H_{25}O_2$ requires 261.1854 (M^+, -101 (loss of D-ring), 100%), 229.1594 for $C_{16}H_{21}O$ requires 229.1592 (M^+, -101, -CH_3OH, 10%), 211 for $C_{16}H_{19}$ (M^+, -101 (loss of D-ring), -CH_3OH, -H_2O, 2%).

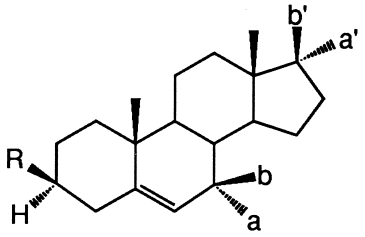
*3.1.15. 3β-Methoxyandrost-5-ene-7β,17β -diol (**24**)*

The desired product was prepared by treating a methanolic solution of 3β-methoxyandrost-5-en-7,17-dione **20** (Table 1), with sodium borohydride and cerium(III)chloride heptahydrate at 0–5°C. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 5.29 (1H, t, J = 1.95, 6-H), 3.84 (1H, dt, J = 8.3 Hz, 7α-H), 3.65 (1H, t, J = 8.3 Hz, 17α-H), 3.36 (3H, s, OCH_3), 3.09 (1H, m, 3α-H), 1.06 (3H, s, 19-CH_3), 0.78 (3H, s, 18-CH_3).

*3.1.16. 3β-Acetoxy-7-methoxyandrost-5-en-17-one (**26**)*

A mixture of N-bromosuccimimide (2.15 g, 0.012 mol) and 1,1′azobis(cyclohexanecarbonitrile) (50 mg), a radical initiator, was added in one lot to a refluxing solution of 3β-acetoxyandrost-5-en-17-one (3.3 g, 0.01 mol) and 1,1′azobis(cyclohexanecarbonitrile) (50 mg) in carbon tetrachloride (70 ml). The mixture was refluxed for 20 min,

Table 1
Influence of the steroids on the induction of mitochondrial glycerophosphate dehydrogenase and cytosolic malic enzyme in rat livers



#	R	b/a	b′/a′	Conc. & in diet	Enzymes	
					GPDH	Malic
					% of control	
*	AcO	O	O	0.040	336	338
9	Glu-ester	O	O	0.085	166	306
10	Glu-triAc-ester	O	O	0.107	164	234
12	AcO	H/H	Glu-ester	0.083	128	229
13	AcO	O	Glu-ester	0.093	204	363
16	AcO	Glu-ester/H	AcO	0.071	241	367
17	AcO	H/Glu-triAc-ester	O	0.046	81	93
18	OTs	H/H	O			
19	MeO	H/H	O	0.085	241	312
20	MeO	O	O	0.055	323	424
21	MeO	O	O-CH_2-CH_2-O	0.042	194	311
22	MeO	OH/H	O-CH_2-CH_2-O	0.063	395	595
23	MeO	H/OH	O-CH_2-CH_2-O	0.063	262	324
24	MeO	OH/H	OH/H	0.055	403	444
25	AcO	H/Br	O			
26	AcO	MeO,H**	O	0.064	328	459
27	MeO	H/H	OH/H			
28	MeO	O	OH/H	0.055	322	307
29	MeO	O	AcO/H	0.06	188	166
30	*tert*-BuO	H/H	O			
31	*tert*-BuO	O	O	0.04	93	116
32	TBDMSO	O	O	0.036	241	313
33	TBDMSO	O	TBDMSO/H	0.085	168	380
34	AcO	OH/H	OH/H	0.046	497	508
35	AcO	TBDMSO/H	TBDMSO/H	0.082	123	100
36	AcO	O	TBDMSO/H	0.07	375	382
37	Tetrahydropyran	O	O	0.064	280	357
38	Dodecanoxy	H/H	O			
39	Dodecanoxy	O	O	0.068	156	189
40	(Ethoxy)ethyl	H/H	O			
41	(Ethoxy)ethyl	O	O	0.05	168	275
42	Carbomethoxy	O	O	0.1	500	786
43	Carboallyloxy	O	O	0.067	440	384
44	Carboethoxy	O	O	0.045	265	373
45	Carbo,iso-butyloxy	O	O	0.048	253	515
46	Carbomethoxy	O	Carbomethoxy/H	0.051	295	292
47	Carbooctyloxy	O	O	0.055	256	312
48	Carbo(9-fluorene) methoxy	O	O	0.09	218	310
49	Carbomethoxy	OH, H**	OH/H	0.042	377	359
50	Carboethoxy	OH/H	OH/H	0.044	277	321
51	Carbooctyloxy	OH/H	OH/H	0.054	343	260

Each compound was tested in a group of two or three rats. Enzyme activity in the livers of rats fed the stock diet without supplementation is termed 100%. The abbreviation TBDMSO = tert-butyldimethylsilyl oxy.

* Mean values from ten experiments (20 rats) in which 7-oxo-DHEA was the comparative standard. ** Products tested as diestereomeric mixture (% ratio of β and α, **26**, 55:45, **49**, 80:20).

The following compounds did not induce the liver enzymes: 17-oxoA-3β-glucopyranoside Me ester (**8**), 3β-acetoxy-17-oxoA-7-glucopyranoside Me ester (**17**), 3β-*tert*-butoxyandrost-5-en-17-one (**30**), 3β-acetoxyandrost-5-ene-7β,17β-di-*tert*-butyldimethylsilyl ether (**35**); A = androst-5-enc.

cooled and solid succinimide was removed by filtration. The clear filtrate was concentrated at 20°C, triturated with petroleum ether and cooled to 0°C to obtain the off white crystalline 7α-bromo derivative **25** (Table 1); yield 2.8 g (68.6%). ^{1}H NMR ($CDCl_3$, 200 MHz): δ 5.78 (1H, d, J = 5.13 Hz, 6-H), 4.76 (1H, m, 7β-H), 4.68 (1H, m, 3α-H), 2.07 (3H, s, $OCOCH_3$), 1.07 (3H, s, 19-CH_3), 0.91 (3H, s, 18-CH_3).

Silica gel (2.0 g, 70–230 mesh) was added to a clear solution of 3β-acetoxy-7α-bromoandrost-5-en-17-one (**25**, 0.5 g) in methanol (20 ml) and the mixture was stirred for 2 h at room temperature. The methanolic solution was cooled, neutralized with saturated sodium bicarbonate solution, and filtered on a thin bed of celite. The clear filtrate was cooled to 0°C to obtain the white crystalline 7-methoxy derivative **26**. Yield 0.32 g (72.7%), m.p. 175–78°C. The ^{1}H NMR spectrum of the white solid showed 55% 7β-methoxy- and 45% 7α-methoxy-compound. Spectral data described below were deduced from the spectrum of the mixture. The biologic activity was assayed as the mixture (Table 1).

3β-Acetoxy-7α-methoxyandrost-5-en-17-one (**26**, Table 1): ^{1}H NMR ($CDCl_3$, 200 MHz): δ 5.79 (1H, d, J = 5.13 Hz, 6-H), 4.65 (1H, m, 3α-H), 3.49 (1H, m, 7β-H), 3.37 (3H, s, OCH_3), 2.05 (3H, s, $OCOCH_3$), 1.03 (3H, s, 19-CH_3), 0.87 (3H, s, 18-CH_3). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 223.5 (17-C), 171 (CO acetate), 146.5 (5-C), 121.0 (6-C), 81.3 (3-C), 72.29 (7-C), 56.5 (OCH_3), 44.4 (CH), 42.9 (CH), 37.0 (CH), 38.1 (CH_2), 36.6 (CH_2), 35.7 (CH_2), 30.9 (CH_2), 27.4 (CH_2), 21.8 (CH_2), 20.1 (CH_2), 21.3 ($OCOCH_3$), 18.2 (19-CH_3), 13.1 (18-CH_3). 3β-Acetoxy-7β-methoxyandrost-5-en-17-one: ^{1}H NMR ($CDCl_3$, 200 MHz): δ 5.53 (1H, s, 6-H), 4.65 (1H, m, 3α-H), 3.5 (1H, m, 7α-H), 3.34 (3H, s, OCH_3), 2.05 (3H, s, $OCOCH_3$), 1.08 (3H, s, 19-CH_3), 0.89 (3H, s, 18-CH_3). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 223.5 (17-C), 171 (CO acetate), 143.0 (5-C), 121.8 (6-C), 81.3 (3-C), 73.2 (7-C), 55.0 (OCH_3), 51.3 (CH), 48.2 (CH), 37.0 (CH), 37.8 (CH_2), 36.4 (CH_2), 35.9 (CH_2), 31.2 (CH_2), 27.6 (CH_2), 23.7 (CH_2), 20.4 (CH_2), 21.32 ($OCOCH_3$), 18.95 (19-CH_3), 13.55 (18-CH_3). LC-MS (API-ES, positive): m/z 383.3 (M+Na, 100%), 299.3 (M, -AcOH, 47%), 269.2 (M, -AcOH, -CH_3OH, 4%).

3.1.17. 3β-Methoxy-17β-hydroxyandrost-5-en-7-one (28)

3β-Methoxyandrost-5-en-17-one (**19**, 0.7 g, 2.3 mmol) was dissolved in dry tetrahydrofuran (20 ml) and the solution was cooled to 0°C. Lithium tri-t-butoxyaluminum hydride (1.2 g) was added to the cooled solution and the mixture was stirred at the same temperature for 2.5 h. Excess base was neutralized with dilute acetic acid and the product was extracted with methylene chloride. 3β-Methoxyandrost-5-en-17β-ol (**27**) was crystallized from methanol, yield 0.6 g (86%), m.p. 145–47°C. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 5.35 (1H, d, J = 5.3 Hz, 6-H), 3.65 (1H, t, J = 7.5 Hz, 17α-H), 3.36 (3H, s, OCH_3), 3.07 (1H, m, 3α-H), 1.02 (3H, s, 19-CH_3), 0.76 (3H, s, 18-CH_3).

3β-Methoxyandrost-5-en-17β-ol (**27**, Table 1) was subjected to air oxidation in the presence of N-hydroxyphthalimide and a radical initiator, and the product 3β-methoxy-17β-hydroxyandrost-5-en-7-one (**28**, Table 1) was obtained in 53% yield. An analytical sample was obtain by recrystallization from acetone-hexane, m.p. 202–4°C. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 5.7 (1H, d, J = 1.47, 6-H), 3.36 (1H, t, J = 7.8, 17α-H), 3.38 (3H, s, OCH_3), 3.2 (1H, m, 3α-H), 1.21 (3H, s, 19-CH_3), 0.77 (3H, s, 18-CH_3). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 202 (7-C), 165.9 (5-C), 125.8 (6-C), 80.8, 78.9 (3, 17-C), 55.9, 50.03, 45.22, 44.9, 43.32, 38.72, 38.58, 36.22, 35.63, 30.46, 27.56, 25.78, 20.85 (CH(s), CH_2(s), quaternary-C and OCH_3), 17.3 (19-CH_3), 11.1 (18-CH_3). LC-MS (API-ES, positive): m/z: 659.5 (2M+Na, 18%), 341.2 (M+Na, 100%), 319.2 (M+1, 22%). LC-UV (DAD): λmax 241.5.

3.1.18. 3β-Methoxy-17β-acetoxyandrost-5-en-7-one (29)

3β-Methoxy-17β-hydroxyandrost-5-en-7-one (**28**, Table 1) was acetylated in pyridine-acetic anhydride mixture at room temperature to obtain product **29** (Table 1), m.p. 168–70°C. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 5.71 (1H, d, J = 1.73 Hz, 6-H), 4.63 (1H, t, J = 7.1 Hz, 17α-H), 3.41 (3H, s, OCH_3), 3.21 (1H, m, 3α-H), 2.08 (3H, s, $OCOCH_3$), 1.2 (3H, s, 19-CH_3), 0.81 (3H, s, 18-CH_3). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 201.3 (7-C), 171 (CO, acetate), 165.7 (5-C), 125.8 (6-C), 81.9, 78.9 (3, 17-C), 55.9, 49.9, 44.9, 44.6 (CH(s) and OCH_3), 38.6 (CH_2), 36.2 (CH_2), 35.8 (CH_2), 30.46 (CH_2), 27.5 (CH_2), 25.8 (CH_2), 20.7 (CH_2), 21.1 (CH_3, acetate), 17.3 (19-CH_3), 12.0 (18-CH_3). HRMS m/z: 360.2311 for $C_{22}H_{32}O_4$ requires 360.23 (M^+, 100%), 329.2152 for $C_{21}H_{29}O_3$ requires 329.2117 (M^+, -OCH_3, 42%), 328.2101 for $C_{21}H_{28}O_3$ requires 328.2038 (M^+, -CH_3OH, 9.5%), 300.2061 for $C_{20}H_{28}O_2$ requires 300.2089 (M^+, -CH_3COOH, 12%), 268.1840 for $C_{19}H_{24}O$ requires 268.1827 (M^+, -CH_3OH, -CH_3COOH, 6%), 253.1640 for $C_{18}H_{21}O$ requires 253.1592 (M^+, -CH_3OH, -CH_3COOH, -CH_3, 20%).

3.1.19. 3β-tert-Butoxyandrost-5-en-17-one (30)

The product was prepared by the procedure of Armstrong et al. [23] utilizing *tert*-butyl trichloroacetimidate and borontrifluoroetherate in a solution of dichloromethane and cyclohexane, in 67.2% yield; m.p. 185–87°C. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 5.34 (1H, d, J = 4.89 Hz, 6-H), 3.32 (1H, m, 3α-H), 1.2 (9H, s, $(CH_3)_3$), 1.02 (3H, s, 19-CH_3), 0.89 (3H, s, 18-CH_3).

3.1.20. 3β-tert-Butoxyandrost-5-ene-7,17-dione (31)

3β-*tert*-Butyloxyandrost-5-en-17-one (**30**, Table 1) was oxidized, utilizing a new procedure [24], at the allylic 7 position to obtain 3β-tert-Butyloxyandrost-5-ene-7,17-dione (**31**, Table 1) in 87% yield, m.p. 189–91°C. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 5.71 (1H, s, 6-H), 3.48 (1H, m, 3α-H), 1.2 (9H, s, $(CH_3)_3$), 1.2 (3H, s, 19-CH_3), 0.89 (3H, s, 18-CH_3). HRMS: m/z 358.2502 for $C_{23}H_{34}O_3$ requires

358.2508 (M^+, 2.4%), 302.1866 for $C_{19}H_{26}O_3$ requires 302.1882 (M^+, -$(CH_3)_2C = CH_2$, 52%), 284.1798 for $C_{19}H_{24}O_2$ requires 284.1776 (M^+, -$(CH_3)_2C = CH_2$, -H_2O, 17%), 274.1965 for $C_{18}H_{26}O_2$ requires 274.1933 (M^+, -CO, -$(CH_3)_2C = CH_2$, 21%), 256.1843 for $C_{18}H_{24}O$ requires 256.1827 (M^+, -H_2O, -CO, -$(CH_3)_2C = CH_2$, 25%), 246.1563 for $C_{16}H_{22}O_2$ requires 246. M^+, -CO, -$CH_2 = CH_2$, -$(CH_3)_2C = CH_2$, 100%), 231.1379 for $C_{15}H_{19}O_2$ requires 231.1385 (M^+, -CO, -CH_3, -$CH_2 = CH_2$, -$(CH_3)_2C = CH_2$, 9%), 228.1551 for $C_{16}H_{20}O$ requires 228.1514 (M^+, -CO, -$CH_2 = CH_2$, -H_2O, -$(CH_3)_2C = CH_2$, 43%), 213.1383 for $C_{15}H_{17}O$ requires 231.1446 (M^+, -CO, -H_2O, -$CH_2 = CH_2$, -CH_3, -$(CH_3)_2C = CH_2$, 8%).

*3.1.21. 3β-tert-Butyldimethylsilyloxyandrost-5-ene-7,17-dione (**32**)*

3β-Hydroxyandrost-5-ene-7,17-dione (**2**, Scheme I) (0.3 g, 0.99 mmol) was dissolved in anhydrous dimethyl formamide (10.0 ml), imidazol (0.6 g, 8.82 mmol) and *tert*-butyldimethylsilyl (TBDMS) chloride (0.3 g, 1.99 mmol) were added in sequence, and the mixture was stirred at room temperature under argon for 3 h. The mixture was poured into cold water and the product was extracted into ether and washed with dilute acetic acid, saturated bicarbonate solution, then by water and brine. The solution was dried over $MgSO_4$ and the solvent was removed. The crude compound was crystallized from aqueous methanol to afford white crystals of 3β-*tert*-butyldimethylsilyloxyandrost-5-ene-7,17-dione [24] (**32**, Table 1) in 92.6% yield (0.37 g), m.p. 135–6°C. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 5.73 (1H, s, 6-H), 3.61 (1H, m, 3α-H), 1.22 (3H, s, 19-CH_3), 0.89 (12H, s, 18-CH_3 and $(CH_3)_3$), 0.07 (6H, s, Si$(CH_3)_2$). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 220.5 (17-C), 201 (7-C), 166.8 (5-C), 125.6 (6-C), 71.1 (3-C), 50.2 (CH), 45.7 (CH), 44.3 (CH), 42.6 (CH_2), 36.4 (CH_2), 35.6 (CH_2), 31.7, 30.7 (CH_2), 24.2 (CH_2), 20.6 (CH_2), 25.8 ($(CH_3)_3$), 17.4 (19-CH_3), 13.7 (18-CH_3), -4.0 (Si $(CH_3)_2$). LC-MS (APCI, positive) m/z: 417.3 (M+1, 71%), 285.2 (M+1, -TBDMSiOH, 100%), 267.2 (M+1, -TBDMSiOH, -H_2O, 14%), 249.3 (M+1, -TBDMSiOH, -2× H_2O, 1%), 243.2 (M+1, -CH_2 = C = O, 6%). LC-UV (DAD) λmax: 240 nm.

*3.1.22. 3β,17β-di-tert-Butyldimethylsilyloxy-androst-5-en-7-one (**33**)*

The product **33** (Table 1) was prepared in the same fashion as described for product **32**, utilizing 3β,17β-dihydroxyandrost-5-en-7-one as the starting steroid. M.p. 99–101°C. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 5.65 (1H, s, 6-H), 3.60 (2H, m, 3α-H and 17α-H), 1.18 (3H, s, 19-CH_3), 0.74 (3H, s, 18-CH_3), 0.87 (18H, s, 2X $(CH_3)_3$), 0.04 (12H, s, 2X $(CH_3)_2$).

*3.1.23. 3β-Acetoxyandrost-5-ene-7β,17β-di-tert-butyldimethylsilyl ether (**35**)*

The product **35** (Table 1) was prepared from 3β-acetoxyandrost-5-ene-7β,17β-diol (**34**, Table 1), tert-butyldimethylsilyl chloride and imidazole in dichloromethane in 80% yield, m.p. 85–87°C. LC-MS (APCI, positive) m/z: 577.3 (M+1, 0.6%), 576.3 (M^+, 1.6%), 575.25 (M^+, -H, 4.4%), 515.35 (M^+, -H, -CH_3COOH, 40%), 385.3 (M+1, -AcOH, -TBDMSiOH, 11%), 253.15 (M+1, -AcOH, -2× TBDMSiOH, 29%), 238.15 (M+1, -AcOH, -2× TBDMSiOH, -CH_3, 7%).

*3.1.24. 3β-Acetoxy-17β-tert-butyldimethylsilyloxyandrost-5-en-7-one (**36**)*

Prepared from 3β-acetoxy-17β-hydroxyandrost-5-en-7-one (**4**, Scheme I) in 76% yield, m.p. 202–4°C. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 5.7 (1H, d, J = 1.3 Hz, 6-H), 4.72 (1H, m, 3α-H), 3.56 (1H, dd, J = 1.7 Hz, 17α-H), 2.07 (3H, s, $OCOCH_3$), 1.22 (3H, s, 19-CH_3), 0.88 (9H, s, $(CH_3)_3$), 0.72 (3H, s, 18-CH_3), 0.009 (6H, s, $((CH_3)_2)$. ^{13}C NMR ($CDCl_3$, 300 MHz): δ 201.5 (7-C), 170.0 (CO, acetate), 164.1 (5-C), 126.5 (6-C), 80.9 (17-C), 72.1 (3-C), 50.1 (CH), 45.3 (CH), 44.5 (CH), 37.8 (CH_2), 36.0 (CH_2), 35.9 (CH_2), 30.9 (CH_2), 27.3 (CH_2), 26.2 (CH_2), 20.87 (CH_2), 25.93 (2× CH_3), 25.82 (CH_3), 21.2 (acetate CH_3), 17.3 (19-CH_3), 11.3 (18-CH_3), -4.5 (CH_3), -4.9 (CH_3).

*3.1.25. 3β-(2-Tetrahydropyranyloxy)-androst-5-ene-7,17-dione (**37**)*

3β-Hydroxyandrost-5-ene-7,17-dione (**2**, Scheme I) (0.5 g, 1.65 mmol) was placed in a dried, argon-flushed flask containing dry dichloromethane (20.0 ml). Pyridinium toluene-*p*-sulfonate (0.043 g, 0.17 mmol) was added followed by 3,4-dihydro-2H-pyran (0.23 ml, 2.5 mmol) and the solution was stirred at room temperature under an atmosphere of argon for 3 h. The reaction was quenched by adding water and the product was extracted thrice with ether (3 × 20 ml). The combined ether extracts were washed with dilute acetic acid, water and bicarbonate solution and dried ($MgSO_4$). Solvent was removed and the crude product was chromatographed on silica gel (ethyl acetate-hexane, 1.5: 8.5, v/v). The compound first eluted was identified as androsta-3,5-diene-7,17-dione (0.150 g), m.p. 167–8°C.

Further elution with the same solvent gave a mixture of diastereomeric 3β-(2-tetrahydropyranyloxy)androst-5-ene-7,17-dione (**37**, Table 1), as a white solid which was crystallized from methanol (0.43 g, 68%), m.p. 137–39°C. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 5.76, 5.73* (1H, dd, J = 2 Hz, 6-H), 4.7 (1H, m, 2-H pyran), 3.91 (1H, m, 6-H pyran), 3.67 (1H, m, 3α-H), 3.51 (1H, m, 6-H pyran), 1.23 (3H, s, 19-CH_3), 0.90 (3H, s, 18-CH_3). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 221 (17-C), 201 (7-C), 167, 166.4* (5-C), 125.8 (6-C), 97.4, 97.1*, 74.7, 74.53* (3 and 2-C (Pyranyl)), 62.86 (6-C (Pyranyl)), 50.05 (CH), 45.69 (CH), 44.26 (CH), 47.82 (quaternary-C), 38.62 (quaternary-C), 40.10 (CH_2), 38.80 (CH_2), 36.41 (CH_2), 36.15 (CH_2), 35.59 (CH_2), 31.07 (CH_2), 30.68 (CH_2), 29.31 (CH_2), 27.64 (CH_2), 25.35 (CH_2), 24.14, 20.52 (CH_2), 19.83 (CH_2)*, 17.35 (19-CH_3), 13.71 (18-CH_3). LC-MS (APES, positive): m/z 795.5

(2M+Na, 100%), 409.1 (M+Na, 85%), 285.1 (M, -101 (pyran-2-O), 14%). LC-UV (DAD): λmax 238 nm.

* Extra peaks are due to the diastereomers which are formed at 2-C of the pyranyl moiety as a result of the formation of the ether linkage.

*3.1.26. 3β-Dodecanoxyandrost-5-ene-7,17-dione (**39**)*

A solution of DHEA (**1**) (2.88 g, 0.01 mol) in anhydrous tetrahydrofuran (20 ml) was added to a mixture of sodium hydride (0.5 g, 60% in oil, washed twice with pentane) in anhydrous tetrahydrofuran (10 ml). The mixture was refluxed for 2 h, cooled to room temperature, and a solution of 1-bromododecane (2.74 g, 0.11 mol) was added slowly (10 min). The solution was refluxed for 4 h, cooled and poured into ice water and the product was extracted with ether-ethyl acetate mixture (1:1). 3β-Dodecanoxyandrost-5-en-17-one (**38**, Table 1) was purified by column chromatography on silica gel (eluent, ethyl acetate-petroleum ether, 1:9), yield 0.6 g (43%, based on 30.5% conversion). ^{1}H NMR ($CDCl_3$, 200 MHz): δ 5.38 (1H, d, J = 5.12 Hz, 6-H), 3.45 (2H, t, J = 6.6, 6.83 Hz, -CH_2-O), 3.14 (1H, m, 3α-H), 1.03 (3H, s, 19-CH_3), 0.89 (3H, s, 18-CH_3). Further elution with the same solvents (4:6) afforded unreacted DHEA (2.0 g, 69.5%).

3β-Dodecanoxyandrost-5-en-17-one (**38**) was subjected to oxidation by our new procedure [24] and the product, 3β-dodecanoxyandrost-5-ene-7,17-dione (**39**, Table 1) was obtained in 82.8% yield, m.p. 70–72°C. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 5.74 (1H, d, J = 1.71 Hz, 6-H), 3.47 (2H, t, J = 6.35, 6.83 Hz, -CH_2-O), 3.27 (1H, m, 3α-H), 1.22 (3H, s, 19-CH_3), 0.9 (3H, s, 18-CH_3). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 220 (17-C), 200 (7-C), 166.0 (5-C), 125.8 (6-C), 77.45 (3-C), 68.58 (-CH_2-O), 50.14 (CH), 45.75 (CH), 44.3 (CH), 39.19 (CH_2), 36.31 (CH_2), 35.58 (CH_2), 31.9 (CH_2), 30.7 (CH_2), 30.1 (CH_2), 29.59 (CH_2), 29.46 (CH_2), 29.34 (CH_2), 28.07 (CH_2), 26.16 (CH_2), 24.15 (CH_2), 22.66 (CH_2), 20.55 (CH_2), 17.35 (CH_3), 14.1 (19-CH_3), 13.7 (18-CH_3). HR-MS: m/z 470.3758 for $C_{31}H_{50}O_3$ requires 470.3760 (M^+, 23%), 301.1783 for $C_{19}H_{25}O_3$ requires 301.1804 (M^+, -$C_{12}H_{25}$, 10%), 285.1870 for $C_{19}H_{25}O_2$ requires 285.1854 (M^+, -$C_{12}H_{15}O$, 12%), 284.1610 for $C_{19}H_{24}O_2$ requires 284.1776 (M^+, -$C_{12}H_{15}OH$, 5.6%).

*3.1.27. 3β-(1′-ethoxy)ethoxyandrost-5-ene-7,17-dione (**41**)*

To a solution of DHEA (**1**, 1.5 g, 0.005 mol) and *p*-toluene sulfonic acid (35 mg) in methylene dichloride (20 ml), a solution of ethyl vinyl ether (1.17 g, 0.016 mol) in methylene dichloride was added slowly during 2 h at room temperature. The solution was stirred for an additional 6 h and poured into cold water. The organic layer was separated and the product was subjected to column chromatography on silica gel (eluent, ethyl acetate-petroleum ether, 1:9) to afford 3β-(1′-ethoxy)ethoxyandrost-5-en-17-one (**40**, Table 1) as a viscous mass (0.92 g, 84% based on 55% conversion). ^{1}H NMR ($CDCl_3$, 200 MHz): δ 5.37 (1H, s, 6-H), 4.78 (1H, q, J = 5.37 Hz, O-CH-O), 3.68 (2H, m, -CH_2-O), 3.5 (1H, m, 3α-H), 1.31 (3H, d, J = 5.13, CH_3), 1.2 (3H, m, CH_3), 1.03 (3H, s, 19-CH_3), 0.89 (3H, s, 18-CH_3). 3β-(1′-Ethoxy)ethoxyandrost-5-en-17-one was oxidized at the allylic 7 position of the steroid using air and N-hydroxyphthalimide [20] as described before to obtain 3β-(1′-ethoxy)ethoxyandrost-5-ene-7,17-dione (**41**, Table 1) in 50% yield. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 5.74 (1H, s, 6-H), 4.79 (1H, m, O-CH-O), 3.58 (3H, m, -CH_2-O and 3α-H), 1.34 (3H, d, J = 5.13 Hz, CH_3), 1.22 (3H, m, CH_3), 1.22 (3H, s, 19-CH_3), 0.9 (3H, s, 18-CH_3). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 220 (17-C), 200 (7-C), 166.0 (5-C), 125.77 (6-C), 98.42 (O-CH-O), 73.89 (3-C), 60.19 (O-CH_2), 50.06 (CH), 45.69 (CH), 44.27 (CH), 41.88 (CH_2), 40.03 (CH_2), 35.58 (CH_2), 30.67 (CH_2), 29.1 (CH_2), 24.1 (CH_2), 20.5 (CH_2), 20.7 (CH_3), 17.34 (CH_3), 15.28 (19-CH_3), 13.71 (18-CH_3).

*3.1.28. 3β-Carbomethoxyandrost-5-ene-7,17-dione (**42**)*

3β-Hydroxyandrost-5-ene-7,17-dione (2, 0.5 g, 0.0016 mol) was dissolved in dry pyridine (6 ml) and the solution was cooled to 0–5°C. Methyl chloroformate (0.19 g, 0.002 mol) was added dropwise during 15 min and the mixture was stirred at the same temperature for 3 h. The reaction mixture was quenched with water and the product was extracted with methylene dichloride. The organic layer was washed, dried and solvent was removed. The crude product was chromatographed on silica gel (eluent: acetone-hexane, 2:8) to afford 0.42 g (89%, based on 80% conversion) of white solid which was crystallized from methanol, m.p. 168–70°C. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 5.77 (1H, d, J = 1.7 Hz, 6-H), 3.78 (3H, s, OCH_3), 4.62 (1H, m, 3α-H) 1.24 (3H, s, 19-CH_3), 0.90 (3H, s, 18-CH_3). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 220.1 (17-C), 200.2 (7-C), 164 (5-C), 155 (O-C-O), 126.7 (6-C), 75.7 (3-C), 54.8 (OCH_3), 49.9 (CH), 45.7 (CH), 44.3 (CH), 37.7 (CH_2), 35.8 (CH_2), 35.6 (CH_2), 30.7 (CH_2), 27.2 (CH_2), 24.1 (CH_2), 20.6 (CH_2), 17.4 (19-CH_3), 13.7 (18-CH_3). HRMS: m/z CH_3 360.1929 for $C_{21}H_{28}O_5$ requires 360.1937 (M^+, 1.7%), 284.1789 for $C_{19}H_{24}O_2$ requires 284.1776 (M^+, -CH_3OH, -CO_2, 100%), 269.1521 for $C_{18}H_{21}O_2$ requires 269.1541 (M^+,-CH_3OH, -CO_2, -CH_3, 5.4%), 256.1856 for $C_{18}H_{24}O$ requires 256.1827 (M^+, -CH_3OH, -CO_2, -CO, 25.6%), 241.1623 for $C_{17}H_{21}O$ requires 241.1592 (M^+, -CH_3OH, -CO_2, -CH_3, -CO, 11%), 227.1461 for $C_{16}H_{19}O$ requires 227.1463 (M^+, -CH_3OH, -CO_2, -CH_3, -CO, -CH_2, 5.6%).

*3.1.29. 3β-Carboallyloxyandrost-5-ene-7,17-dione (**43**)*

Compound **43** (Table 1) was prepared from 7-oxo-DHEA (**2**, 1.0 mmol) and allylchloroformate (4.0 mmol) in tetrahydrofuran-pyridine mixture at 0–5°C. After 6 h of stirring only 30% conversion had occurred. The product was isolated in 78% yield (based on 30% conversion) by column chromatography of the resultant mixture, m.p. 159–61°C. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 5.92 (1H, m, -CH =), 5.74 (1H, d, J = 1.7 Hz, 6-H), 5.38 (q), 5.29 (m), 5.23 (m) (2H, J = 18.5, 11.6, 1.47 Hz, CH_2 =), 4.62 (2H, dt, J = 5.86,

1.22, 1.47 Hz, -CH_2-O), 4.62 (1H, m, 3α-H), 1.21 (3H, s, 19-CH_3), 0.87 (3H, s, 18-CH_3). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 219.2 (17-C), 200 (7-C), 164 (5-C), 154 (O-C-O), 131.5 (= C-), 126.5 (6-C), 118.7 (-CH_2 =), 75.7 (3-C), 68.24 (O-CH_2), 50.0 (CH), 45.8 (CH), 44.3 (CH), 47.64 (quaternary-C), 38.33 (quaternary-C), 37.7 (CH_2), 35.8 (CH_2), 35.4, 30.7 (CH_2), 27.2 (CH_2), 24.02 (CH_2), 20.53 (CH_2), 17.3 (19-CH_3), 13.7 (18-CH_3). LC-MS (API-ES, positive): m/z 409.1 (M+Na, 100%), 307.1 (M+Na, -102 (CH_2 = CH-CH_2OH, CO_2), 80%), 285 (M, -101, 52%), 256 (M, -102, -28 (CO), 15%). LC-UV (DAD): λmax 234 nm.

*3.1.30. 3β-Carboethoxyandrost-5-ene-7,17-dione (**44**)*

Prepared from 7-oxo-DHEA (**2**) and ethylchloroformate in pyridine at 0–5°C. Yield 90%, m.p. 187–8°C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.77 (1H, d, J = 1.65 Hz, 6-H), 4.6 (1H, m, 3α-H), 4.2 (2H, q, J = 7.2 Hz, -CH_2-O), 1.32 (3H, t, J = 7.2 Hz, CH_3), 1.23 (3H, s, 19-CH_3), 0.9 (3H, s, 18-CH_3). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 220.2 (17-C), 200.7 (7-C), 164.4 (5-C), 154.3 (O-C-O), 126.7 (6-C), 75.4 (3-C), 64.1 (O-CH_2), 49.9 (CH), 45.7 (CH), 44.3 (CH), 47.8 (quaternary-C), 38.4 (quaternary-C), 37.7 (CH_2), 35.8 (CH_2), 35.6 (CH_2), 30.7 (CH_2), 27.2 (CH_2), 24.1 (CH_2), 20.5 (CH_2), 14.4 (Ac-CH_3), 14.2 (19-CH_3), 13.8 (18-CH_3). LC-MS (API-ES, positive): m/z 397.2 (M+Na, 100%), 285.2 (M+1, -90 (C_2H_5OH, CO_2), 10%). LC-UV (DAD): λmax 238 nm.

*3.1.31. 3β-Carboiso-butoxyandrost-5-ene-7,17-dione (**45**)*

Prepared from 3β-hydroxyandrost-5-en-7,17-dione (2) and *iso*-butylchloroformate in pyridine at 0–5°C. Yield 78%, m.p. 132–3°C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.77 (1H, d, J = 1.66 Hz, 6-H), 4.6 (1H, m, 3α-H), 3.92 (2H, d, J = 6.8 Hz, -CH_2-O), 1.24 (3H, s, 19-CH_3), 0.96 (6H, d, J = 6.6 Hz, $(CH_3)_2$), 0.9 (3H, s, 18-CH_3). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 220.2 (17-C), 200.7 (7-C), 164.4 (5-C), 154.5 (O-C-O), 126.6 (6-C), 75.5 (3-C), 74.1 (O-CH_2), 49.9 (CH), 45.7 (CH), 44.3 (CH), 27.7 (CH), 37.8 (CH_2), 35.8 (CH_2), 35.6 (CH_2), 30.7 (CH_2), 27.2 (CH_2), 24.1 (CH_2), 20.6 (CH_2), 18.9 (CH_3), 18.9 (CH_3), 17.4 (19-CH_3), 13.7 (18-CH_3). LC-MS (API-ES, positive): m/z 425.2 (M+Na, 100%), 301.1 (M+1, -102 (HCOO-*iso*-butyl), 90%), 285.2 (M+1, -118 (*iso*-butyl alcohol, CO_2), 10%), 269.0 (M+1, -118, -CH_3, 3%). LC-UV (DAD): λmax 236 nm.

*3.1.32. 3β,17β-Dicarbomethoxyandrost-5-en-7-one (**46**)*

Prepared from 3β,17β-dihydroxyandrost-5-en-7-one and methyl chloroformate in pyridine at 0–5°C. Yield 81%, m.p. 167–9°C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.73 (1H, d, J = 1.66 Hz, 6-H), 4.57 (2H, m, 3α-H, 17α-H), 3.79 (3H, s, CH_3O), 3.77 (3H, s, CH_3O), 1.2 (3H, s, 19-CH_3), 0.84 (3H, s, 18-CH_3). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 201.0 (7-C), 163. 9 (5-C), 155.7 (O-C-O), 154.9 (O-C-O), 126.6 (6-C), 85.7 (17-C), 75.8 (3-C), 54.7 (OCH_3), 54.6 (OCH_3), 49.7 (CH), 44.9 (CH), 44.6 (CH), 43.0 (quaternary-C), 38.3 (quaternary-C), 37.7 (CH_2), 35.8 (CH_2), 35.7 (CH_2), 27.4 (CH_2), 27.2 (CH_2), 25.7 (CH_2), 20.7 (CH_2), 17.3 (19-CH_3), 11.9 (18-CH_3). LC-MS (API-ES, positive): m/z 443.2 (M+Na, 50%), 345.1 (M+1, -MeOH, -CO_2, 10%), 301.1 (M+1, -120 (2× $HCOOCH_3$, 100%)) 269.1 (M+1, -152 (2× CH_3OH, 2× CO_2), 5%). LC-UV (DAD): λmax 236 nm.

*3.1.33. 3β-Carbooctyloxyandrost-5-ene-7,17-dione (**47**)*

Prepared from 3β-hydroxyandrost-5-ene-7,17-dione (**2**, Scheme I) and octylchloroformate in pyridine at 0–5°C. The product (**47**, Table 1) was purified by column chromatography on silica gel. Yield 65%, m.p. 72–3°C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.77 (1H, d, J = 1.66 Hz, 6-H), 4.6 (1H, m, 3α-H), 4.13 (2H, t, J = 6.6 Hz, -CH_2-O), 1.23 (3H, s, 19-CH_3), 0.88 (3H, s, 18-CH_3). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 219.8 (17-C), 200.4 (7-C), 164.3 (5-C), 154.3 (O-C-O), 126.5 (6-C), 75.3 (3-C), 68.1 (OCH_2), 49.8 (CH), 45.6 (CH), 44.2 (CH), 47.7 (quaternary-C), 38.3 (quaternary-C), 37.7 (CH_2), 35.7 (CH_2), 35.5 (CH_2), 31.7 (CH_2), 30.6 (CH_2), 29.1 (CH_2), 29.0 (CH_2), 28.5 (CH_2), 27.2 (CH_2), 25.6 (CH_2), 24.1 (CH_2), 22.5 (CH_2), 20.5 (CH_2), 14.0 (CH_3), 17.3 (19-CH_3), 13.7 (18-CH_3). LC-MS (APCI, positive): m/z 459.2 (M+1, 8%), 285.1 (M+1, -174 ($CH_3(CH_2)_6CH_2OH$, CO_2, 100%)).

*3.1.34. 3β-Carbo(9-fluorenyl)methoxyandrost-5-ene-7,17-dione (**48**)*

A mixture of 7-oxo-DHEA (**2**) (0.3 g, 1 mmol) and 9-fluorenylmethylchloroformate (0.285 g, 1.1 mmol) in pyridine (4.0 ml) was stirred at room temperature for 1 h. The product (**48**, Table 1) was purified by column chromatography on silica gel. Yield 57% (0.3 g), m.p. 98–103°C. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 7.77 (2H, d, J = 7.11 Hz, Ar-H), 7.62 (2H, d, J = 6.83 Hz, Ar-H), 7.37 (4H, m, Ar-H), 5.77 (1H, d, J = 1.46 Hz, 6-H), 4.61 (1H, m, 3-H), 4.43 (2H, d, J = 6.96 Hz, AB system, OCH_2), 4.26 (1H, t, J = 7.08 Hz, AB system, Ar-H), 1.25 (3H, s, 19-CH_3), 0.90 (3H, s, 18-CH_3); LC-MS (API-ES) m/z: 547 (M+Na, 100%), 285 (M+1, -240 ((9-fluorenyl)methanol, CO_2), 3%). LC-UV (DAD): λmax 254, 264, 290 and 300 nm.

*3.1.35. 3β-Carbomethoxyandrost-5-ene-7,17β-diol (**49**)*

3β-Carbomethoxyandrost-5-ene-7,17-dione **(42)** (0.5 g, 0.0014 mol) was dissolved in a mixture of dichloromethane (5 ml) and methanol (10 ml). The mixture was stirred at room temperature and finely powdered sodium borohydride (0.16 g, 0.004 mol) was added slowly. After 15 min the reaction mixture was quenched with water and the product was extracted with dichloromethane. The organic layer was washed, dried and solvent was removed. The product was crystallized from acetone-petroleum ether to afford 0.35 g (70%) of 3β-carbomethoxyandrost-5-ene-7,17β-diol (**49**, Table 1**)** as a white crystalline solid, m.p. 150–52°C. LC-MS and ^{1}H NMR analysis of the product showed a 8:2 ratio of diastereomeric 7β-hydroxy and 7α-hydroxy compounds. ^{1}H NMR for 7β-hydroxy isomer ($CDCl_3$, 200 MHz): δ 5.33 (1H, s, 6-H), 4.49 (1H, m, 3α-H), 3.77 (3H,

s, OCH_3), 3.84 (1H, d, J = 7.08 Hz, 7α-H), 3.64 (1H, t, J = 8.05 Hz, 17α-H), 1.07 (3H, s, 19-CH_3), 0.77 (3H, s, 18-CH_3); the 7α-hydroxy isomer showed different chemical shifts for 6 and 19 protons, deduced from the spectrum of the mixture; δ 5.65 (1H, d, J = 4.89 Hz, 6-H), 1.02 (3H, s, 19-CH_3). LC-MS (API-ES) m/z: 387.2 (M+Na, 100%), 311.2 (M+Na, -76 (MeOH, CO_2), 25%), 271.3 (M+1, -76, -18 (H_2O), 5%), 253 (M+1, -76, -18, -18, 3%).

*3.1.36. 3β-Carboethoxyandrost-5-ene-7β,17β-diol (**50**)*

3β-Carboethoxyandrost-5-ene-7,17-dione (**44**) (0.3 g, 0.8 mmol) in 5.0 ml dichloromethane and 10.0 ml methanol was treated with cerium(III) chloride heptahydrate (0.3 g, 0.8 mmol). To this cold solution, finely powdered sodium borohydride (0.09 gm, 2.4 mmol) was added slowly. After 15 min the solvent was evaporated to complete dryness, the solid was taken up in dichloromethane, stirred for 30 min and filtered on a small bed of celite. The organic layer was washed once with water, dried and solvent was removed. The product was crystallized from acetone-petroleum ether to afford 0.22 g (73%) of 3β-carboethoxyandrost-5-ene-7β,17β-diol (50, Table 1) as a white solid, m.p. 170–71°C. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 5.33 (1H, s, 6-H), 4.48 (1H, m, 3α-H), 4.23 (2H, q, J = 7.08, OCH_2), 3.84 (1H, d, J = 7.57 Hz, 7α-H), 3.64 (1H, t, J = 8.3 Hz, 17α-H), 1.31 (3H, t, J = 7.08, CH_3), 1.07 (3H, s, 19-CH_3), 0.77 (3H, s, 18-CH_3). LC-MS (API-ES): m/z 401.2 (M+Na, 100%), 311.1 (M+Na, -90 (EtOH, CO_2), 15%), 271.0 (M+1, -90, -18 (H_2O), 3%), 253.1 (M+1, -90, -18, -18, 5%).

*3.1.37. 3β-Carbooctyloxyandrost-5-ene-7β,17β-diol (**51**)*

3β-Carbooctyloxyandrost-5-ene-7,17-dione **(47)** (0.3 g, 0.65 mmol) was converted to product 51 as described for compound 49. The product was crystallized from petroleum ether to afford 0.23 g (77%) of 3β-carbooctyloxy-7β,17β-dihydroxyandrost-5-ene **(51**, Table 1**)** as a white solid, m.p. 86–87°C. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 5.33 (1H, s, 6-H), 4.48 (1H, m, 3α-H), 4.12 (2H, t, J = 6.6, OCH_2), 3.89 (1H, d, J = 7.54 Hz, 7α-H), 3.65 (1H, t, J = 8.3 Hz, 17α-H), 0.88 (3H, t, CH_3), 1.07 (3H, s, 19-CH_3), 0.77 (3H, s, 18-CH_3). LC-MS (API-ES): m/z 485 (M+Na, 100%), 311 (M+Na, -174 ($C_8H_{17}OH$, CO_2), 3%), 293 (M+23, -174, -18 (H_2O), 2%).

4. Results and discussion

The 7-oxygenated steroids that are more active than DHEA have been converted to glucuronides, ethers and alkylcarbonate esters in an attempt to produce more active, longer lasting derivatives for possible therapeutic use.

Glucuronides are generally considered as the end products of metabolism that facilitate excretion of steroid hormones, bile acids and xenobiotics. DHEA glucuronide, however, is not exclusively an end product; it is converted mostly to DHEA sulfate and only a small fraction (~15%) is excreted in the urine [26]. The D-glucuronic moeity of UDP-glucuronic acid is transferred to hydroxyl, carboxyl, amino, imino and sulfhydryl groups of various compounds by hepatic glucuronyl transferases [27]. Our attention was directed toward the synthesis of glucuronides of 7-oxygenated steroids and to assess their induction of thermogenic enzymes in rat liver. Monoglucuronides of steroids at positions 3, 7, or 17 (Scheme I) were synthesized as their methyl ester in an attempt to increase the bioavailability of the parent compounds and to slow the metabolism and elimination process. Glucuronides of steroids substituted at the 3 and 17 positions have been prepared [28] and studied in the past but neither their 7-oxygenated derivatives (**9**, **10**, **13**, **14**, Scheme I) nor 7-O-glucuronides (**15–17**, Scheme I) have been reported previously.

Attempts to synthesize glucuronides of 7-oxygenated androgens by reacting the appropriately protected 7-oxygenated derivatives with bromo glucuronate followed by selective hydrolysis to remove acetate protection on the glucose hydroxyls gave poor yields of the targeted compounds because of the strong sensitivity of 7-oxygenated steroids under acidic or basic conditions to undergo elimination of 3-hydroxyl or 3-glucuronide to give androst-3,5-diene-7,17-dione. Better yields were obtained when acetylated glucuronides of steroids were prepared first, acetates were hydrolyzed selectively and the resulting Δ-5 steroid glucuronates were oxygenated at the allylic 7-position.

Synthesis of β-pyranoside forms of steroid glucuronides (as their tri-O-acetyl methyl esters) was carried out by condensation of a specific, open hydroxyl group of the steroid with methyl 2,3,4-tri-O-acetyl-1-bromo-1-deoxy-α−D-glucopyranuronate utilizing the well known Koenigs-Knorr [29] reaction. Acid acceptors such as silver carbonate or cadmium carbonate in anhydrous benzene were used to promote the reaction and freshly pulverized dry calcium sulfate was included as internal desiccant. Reactions were run in the dark (when silver carbonate was used), at room temperature for 24–48 h, during which time ~40–60% conversion took place (identified by the ^{1}H-NMR spectrum of the reaction mixture). The acetylated glucuronide methyl esters were isolated by column chromatography on silica gel and/or by crystallization. Fractional crystallization was the major technique employed for isolating the Koenigs-Knorr product from the reaction mixture and it was found to be quite satisfactory in providing high purity glucuronides. Only in cases where isomeric ortho esters or other side products were formed in larger quantities, were the desired products purified by column chromatography on silica gel.

The structures of acetylated glucuronide methyl esters were confirmed by ^{1}H-NMR, ^{13}C-NMR and mass spectrometry. The anomeric proton signal (1H) of the glucuronic moiety appeared as a doublet at 4.58–4.69 ppm (J = 8.0–8.4 Hz) indicating β-glucuronoside linkage. The position of the olefinic 6-H of the 7-keto steroids, usually occurred at 5.65–5.70 ppm and showed a down field shift of 0.35–0.4 ppm from 6-H of non-7-keto steroids; this was another

significant point in the characterization of 7-keto glucuronides. β-Anomers of these steroid glucuronopyranosides were obtained in good to excellent yield when corrected for recovered unreacted steroids. Glucuronidation of 7-oxo-DHEA was an exception where formation of a dehydration product androst-3,5-diene-7,17-dione, as well as formation of an ortho ester (~25%), reduced the yield of the desired glucuronide to 25%.

Deacetylation of acetylated glucuronide methyl esters, with or without a 7-keto group was performed with freshly prepared sodium methoxide in anhydrous methanol at ambient temperature yielding methyl esters of steroid glucuronides as the major product in good to excellent yield except again in case of the 3-glucuronide of 7-keto compounds. ^{1}H-NMR spectra of deacetylated steroid glucuronides exibited the anomeric proton as a doublet at 4.4–4.48 ppm (J = 7.4–8.3 Hz) thereby confirming the retention of the β-linkage. High resolution mass spectra (HRMS-EI) of steroid glucuronides helped confirm the structure of the products but the expected molecular ion was absent. The molecular ion was obtained as a sodium adduct base peak in the FAB-MS spectra of the steroid glucuronides. Finally allylic oxidation at the 7 position of Δ^5-steroid glucuronides **8** and **12** was performed utilizing the aerobic oxidation procedure of Foricher et al. [20] in the presence of N-hydroxy phthalimide and a radical initiator to afford the corresponding 7-oxo derivatives of steroid glucuronides **9** and **13**.

We next attempted to synthesize small and long chain alkyl ethers to see whether etherification of hydroxyl groups might increase absorption and/or slow the metabolism and elimination process. The methyl ether (**20**, Table 1) of 7-oxoDHEA (**2**, Scheme I) was prepared in 67% yield by allylic oxidation of 3β-methoxy-DHEA [21] (**19**) which was obtained by refluxing 3β-tosyl-DHEA (**18**) in anhydrous methanol in 94% yield. Further reduction of the product **20** with sodium borohydride in methanol-dichloromethane (usually 9:1) at 0–5°C in presence of cerium(III) chloride heptahydrate afforded 3β-methoxyandrost-5-en-7β,17β-diol (**24**). The 17 keto group of 3β-methoxy DHEA (**19**) was protected as ethylene ketal, and subsequent oxidation of the ketal derivative afforded 7-oxo compound **21** (Table 1). The 7-oxo group of product **21** was further subjected to reduction with sodium borohydride in methanol at room temperature and a mixture of isomeric 7β- and 7α-hydroxy derivatives (**22**, **23**, Table 1) was formed, and separated easily by column chromatography. Reduction of 3β-methoxy DHEA (**19**) at position 17, afforded 3β-methoxy-17β-hydroxyandrost-5-ene (**27**, Table 1) in 86% yield, which was oxidized at the 7 position using N-hydroxyphthalimide, oxygen and a radical initiator in refluxing acetone to afford product **28** (53% yield, Table 1).

3β-*tert*-Butyl ether of 7-oxo-DHEA (**31**, Table 1) was prepared in 87% yield by oxidizing 3β-*tert*-butyl-DHEA (**30**) using pyridinium dichromate and N-hydroxyphthalimide [24] a new, simple and high yielding procedure. 3β-*tert*-Butyl-DHEA was synthesized in 67% yield by the procedure of Armstrong et al. [23] using DHEA and *tert*-butyl trichloroacetimidate in a mixture of dichloromethane and cyclohexane.

Synthesis of 7α- and 7β-methoxy derivatives of DHEA was reported in 1971 by Adams and Wynne [30]. After a lengthy and tedious procedure starting from DHEA acetate, they were able to obtain 7α- and 7β-methoxy-DHEA in a very poor yield of 0.7% and 7.7% respectively. In our hands, a mixture of diastereomeric 7-methoxy ethers (**26**) was prepared in a simple, short procedure and in very good yields (73%) by stirring 7α-bromoDHEA (**25**) in methanol and silica gel at room temperature for 2 h. No attempt was made to synthesize these derivatives in pure α and β forms.

3β-Dodecanoyl ether of 7-oxo-DHEA (**39**) was prepared in 83% yield by allylic oxidation of 3β-dodecanyloxy-DHEA (**38**) using pyridinium dichromate and N-hydroxyphthalimide [24]. 3β-Dodecanyloxy-DHEA (**38**) was synthesized using a standard procedure of refluxing 1-bromododecane and DHEA solution in tetrahydrofuran in presence of sodium hydride.

Etherification of DHEA utilizing ethyl vinyl ether in dichloromethane and in presence of *p*-toluene sulfonic acid, at room temperature for 6 h afforded 3β-ethoxy ethyl ether of DHEA (**40**) in 84% yield based on 55% conversion. The product **40** was subjected to aerobic oxidation in presence of N-hydroxyphthalimide and a radical initiator in refluxing acetone to obtain 3β-(1′-ethoxy)ethoxyandrost-5-en-7,17-dione (**41**, Table 1).

3β-Tetrahydropyranyl ether of 7-oxo-DHEA (**37**, Table 1) was made to test the effect of a saturated pyran ring having two stereogenic centers thus generated, on the induction of thermogenic enzymes. This compound was easily formed utilizing dihydropyran and pyridinium *p*-toluene sulfonate in anhydrous dichloromethane and was stable to most non-acidic reagents.

Silyl protected ethers, especially *tert*-butyldimethylsilyl ether (**32**, **33**, **35** and **36**) substituted at hydroxyls 3[25], 3 and 17, 7, and 7 and 17 positions of the steroid molecule were also synthesized. TBDMS ethers are quite stable to a variety of organic reactions and have good stability toward base. Their synthesis involved use of *tert*-butyldimethylsilyl chloride and imidazole in dimethyl formamide. Dichloromethane was not appropriate for the synthesis of these specific 7-substituted derivatives since its acidic character caused the formation of dienes.

Our next interest was to prepare and investigate the effect of carbonate substitution on hydroxyls at 3 and 17 position of the corresponding sterols, with 7-position oxygenated (keto or hydroxyl) in the steroid molecule. Carbonate substitution of the hydroxyls is much more desirable than acetate protection. Acetates and carbonates both are prone to basic hydrolysis, but carbonates are less susceptible to cleavage because of the resonance effect of the additional oxygen. Various alkyl carbonates (Table 1) such as methyl (**42**), ethyl (**44**), allyl (**43**), isobutyl (**45**), and octyl (**47**) as

well as tricyclic fluorenyl carbonates (**48**) of 7-oxo-DHEA (**2**) were made by somewhat similar procedure which involved a slow addition of respective chloroformates to the ice cold solution of steroid substrates in pyridine. Conversion was total in most of the cases and product yields were generally high except in the case of the methyl carbonate (**42**), an 80% conversion gave 89% product yield and in the case of the allyl derivative (**43**) where poor conversion (30%) afforded product in 78% yield. Carbonates **42** and **43** were isolated by column chromatography on silica gel. Some of the carbonate derivatives of the steroids (**42**, **44**, **47**) were subjected to borohydride reduction in methanol-dichloromethane with cerium(III) chloride heptahydrate at 0–5°C and 7β- and 17β-hydroxy carbonate derivatives **(49-51**, Table 1) were isolated in good yields.

All these 7-oxygenated alkyl ethers as well as carbonates are new entities unless mentioned otherwise. Their structure and stereochemical assignment were made on the basis of their ^{1}H-NMR and ^{13}C-NMR spectroscopic data, high resolution mass spectroscopy, and LC-MS studies. Reaction products, as well as some precursors, when known, were also characterized by comparison of spectroscopic data with those available from the literature. Proton NMR of the 6-olefinic hydrogen of all 7-oxo derivatives showed a characteristic absorption at δ 5.7–5.75 with a corresponding ^{13}C absorption at about 200–202 ppm. On the other hand, 7-hydroxy steroids showed different ppm values for 6-olefinic protons. In case of 7β-hydroxy compounds the olefinic 6-H appeared high field and around 5.3 ppm whereas 7α-hydroxy compounds showed a doublet for olefinic 6-H down field and around 5.6 ppm.

The described new compounds were assayed for liver thermogenic enzymes induction in rats (Table 1); Some of the synthesized derivatives particularly 3-substituted methyl ethers and carbonates were found to be somewhat more effective than the equimolar amounts of 7-oxo-DHEA, with which they were compared in each experiment, in either or both enzyme induction assays. The 3-methoxy derivative (**22**) was the most active among the methyl ethers. However still better activity was achieved by derivatizing the 3β-hydroxy group of 7-oxo-DHEA (compound **42**, Table 1) with methyl carbonate. The assayable activities of some of the steroid esters are anomalous [11]. The diacetyl ester of androstenediol does not induce the liver thermogenic enzymes but the 7-oxo analog of this ester is fully active [11], the isobutyryl ester of DHEA is inactive but the introduction of a 7-oxo group or substituting a β-hydroxyl at 17 renders the steroid active [11]. Derivatization at the 17-position of the steroids either as a ketal or an acetyl group resulted in decreased enzyme induction as seen in case of 3β-methoxy-17β-acetoxyandrost-5-en-7-one (**29**, Table 1). These responses probably reflect different susceptibilities to esterase activity. Some of the glucuronides (**9**, **10**, **12**), on the other hand, displayed negligible effect on the induction of mitochondrial glycerophosphate dehydrogenase despite reasonable induction of malic enzyme. Such separation of the activities has been seen in other steroid derivatives [31].

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