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Ergosteroids II: Biologically active metabolites and synthetic derivatives of dehydroepiandrosterone

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An improved procedure for the synthesis of 3β-hydroxyandrost-5-ene-7,17-dione, a natural metabolite of dehydroepiandrosterone (DHEA) is described. The synthesis and magnetic resonance spectra of several other related steroids are presented. Feeding dehydroepiandrosterone to rats induces enhanced formation of several liver enzymes among which are mitochondrial sn*-glycerol 3-phosphate dehydrogenase (GPDH) and cytosolic malic enzyme. The induction of these two enzymes, that complete a thermogenic system in rat liver, was used as an assay to search for derivatives of DHEA that might be more active than the parent steroid. Activity is retained in steroids that are reduced to the corresponding 17β-hydroxy derivative, or hydroxylated at 7α or 7β, and is considerably enhanced when the 17-hydroxy or 17-carbonyl steroid is converted to the 7-oxo derivative. Several derivatives of DHEA did not induce the thermogenic enzymes whereas the corresponding 7-oxo compounds did. Both short and long chain acyl esters of DHEA and of 7-oxo-DHEA are active inducers of the liver enzymes when fed to rats. 7-Oxo-DHEA-3-sulfate is as active as 7-oxo-DHEA or its 3-acetyl ester, whereas DHEA-3-sulfate is much less active than DHEA. Among many steroids tested, those possessing a carbonyl group at position 3, a methyl group at 7, a hydroxyl group at positions 1, 2, 4, 11, or 19, or a saturated B ring, with or without a 4-5 double bond, were inactive.* (*Steroids* **63**:158–165, 1998) © *1998 by Elsevier Science Inc.*

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Introduction

Dehydroepiandrosterone (3β-hydroxyandrost-5-en-17-one; hereafter DHEA), the most abundant steroid in human blood, is an intermediate in the biosynthesis of testosterone and estrogens but also exerts several physiological effects independent of the sex hormones.[1] It is an anti-obesity agent for genetically obese[2] and normal[3] animals but does not affect food intake; it is probably not effective in humans.[4] It decreases blood cholesterol concentration in several species,[5–7] lessens the severity of diabetes in genetically predisposed mice,[8] enhances the immune system,[9,10] suppresses tumor development,[11] and improves memory in aged mice.[12]

It is unlikely that these widely different metabolic responses are elicited by a single steroid molecule for which no receptor has been found in liver.[13] Another reason to doubt that DHEA, per se, is responsible is that most of these effects can be observed only when large amounts (0.4–0.6% of the diet) of this steroid are administered. The possible clinical utility of DHEA is limited by its low activity and by its conversion to sex hormones. It can be used by women in only small doses and for limited periods of time, for reasonable doses lead to greatly elevated blood testosterone and dihydrotestosterone concentrations.[14] In female rats DHEA administration leads to excess estrogen and androgen synthesis and the production of polycystic ovaries.[15,16]

To search for possible metabolites of DHEA that might have greater biological activity, greater specificity, and less propensity to form sex hormones, we initiated a program of synthesizing steroids that are derivable from DHEA. The activity of synthesized compounds was monitored by measuring the induction of thermogenic enzymes in rats.[17,18] Hepatic mitochondrial *sn*-glycerol-3-phosphate dehydrogenase and cytosolic malic enzyme function to bypass electron transport from mitochondrial NADH to ubiquinone. The result is a decreased efficiency of coupling phosphorylation to the oxidations of the tricarboxylic acid cycle,[17,19,20] greater heat production, and decreased efficiency of food utilization for growth, fat synthesis or work output.[21]

There are several cytochrome P450 enzymes that hydroxylate steroids at specific positions, and products of such reactions on DHEA have been isolated from tissues, blood and urine of normal and tumor-bearing humans and other mammals.[22–28] Hydroxylations of DHEA in tissue homog-

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enates and cell fractions have been reported from many laboratories.[29–37] 7α-Hydroxy-DHEA[23,26,27] and 7-oxo-DHEA[38–41] are known to occur naturally, mainly as esters. Both 7α- and 7β-hydroxylated DHEA are produced[23,29–33,36] and converted to 7-oxo-DHEA[42–46] by cell fractions.

After testing some commercially available steroids[18,47] we prepared several monohydroxylated derivatives of DHEA [4α, 5α, 7α, 7β, 11β, 16α, 19] and of 5-androstene-3β, 17β-diol [7α, 19]. Only the 7-hydroxy derivatives were active. Because 7-hydroxy-DHEA was fully as active as DHEA[48] and 7-oxo-DHEA was more active[49] we suggested that these compounds might be the first members of a metabolic sequence to more active hormone(s). This hypothesis is consistent with the finding that agents that induce the formation of cytochrome P_{450} 2A1, a steroid 7α-hydroxylase,[37,50] enhance the response of rats to DHEA.[18] None of these 7-substituted structures is convertible to compounds with androgenic or estrogenic activity and they are therefore potentially useful medications for women whereas DHEA is not because, in large doses, it causes masculinization. Because of the ability of these 7-oxygenated steroids to induce thermogenic enzymes that provide a pathway for heat production,[17,20] we have named them ergosteroids.[49]

Experimental

The ability of steroids to induce the formation of liver cytosolic malic enzyme and mitochondrial *sn*-glycerol-3-phosphate dehydrogenase in rats was used as a criterion of DHEA-like activity. Steroids to be assayed were finely pulverized and ground with a small amount of Purina rat chow, then mixed with the requisite amount of chow to give the desired steroid concentration. Records of daily food consumption were kept to ensure that dietary restriction was not influencing the response of the malic enzyme.[51] In some cases the steroid was dissolved in olive oil and injected intraperitoneally.

Male Sprague–Dawley rats (140–160 g) were fed diets containing steroids for 6 days. On Day 7 (at about 200 g), animals were sacrificed, and the large left lobe of the liver was excised and placed in an ice-cold medium containing 250 mM mannitol, 70 mM sucrose, and 3 mM Hepes at pH 7.4. The liver samples were weighed, washed, homogenized and mitochondrial and cytosolic fractions were separated.[52] Mitochondrial glycerol-3-phosphate dehydrogenase was assayed by the procedure of Wernette et al.[53] and cytosolic malic enzyme by that of Hsu and Lardy.[54] Enzyme activities in control rats are expressed as nmol/(min times mg protein). The activities in rats fed steroids are reported as a percent of the activity found in control rats treated similarly but without steroid supplementation. We wish to emphasize that because of variation in the enzyme activity of both control and treated groups from one experiment to another the reported activities are not highly precise—they serve mainly as a guide for further syntheses. Compounds are considered inactive unless they increased the enzyme activity at least 50% above that of the livers of control rats. Maximum increases obtained are about five-fold for glycerophosphate dehydrogenase and ten-fold for malic enzyme. To improve sensitivity, assays were designed with amounts of steroids that gave about half maximal responses; the variability between experiments was found to be relatively greater under these conditions than when maximally effective doses were given.

Dehydroepiandrosterone and some other steroids were purchased from Steraloids, Wilton, New Hampshire; some were from Sigma, St. Louis and Research Plus, Bayonne, New Jersey. Some steroids, other reagents and anhydrous solvents were from Aldrich, Milwaukee, Wisconsin. Many of the compounds tested were prepared by procedures in the literature. We record here the synthesis of only new compounds and improved procedures for some known structures. To save space trivial names are used for well known compounds.

The progress of chemical reactions was monitored by TLC. Melting points (°C) were determined in open capillaries in an electrically heated and stirred Thiele-type bath. They are uncorrected. Nuclear magnetic resonance spectra were acquired with Bruker 200, 300, and 500 MHz spectrometers. Tetramethyl silane (TMS) in $CDCl_3$ was used as internal standard. Chemical shifts are reported on the δ scale with peak multiplicities: d, doublet; dd, double doublet; m, multiplet; q, quartet; s, singlet; and t, triplet. Magnetic resonance data are included for some known compounds where such data were not previously reported.

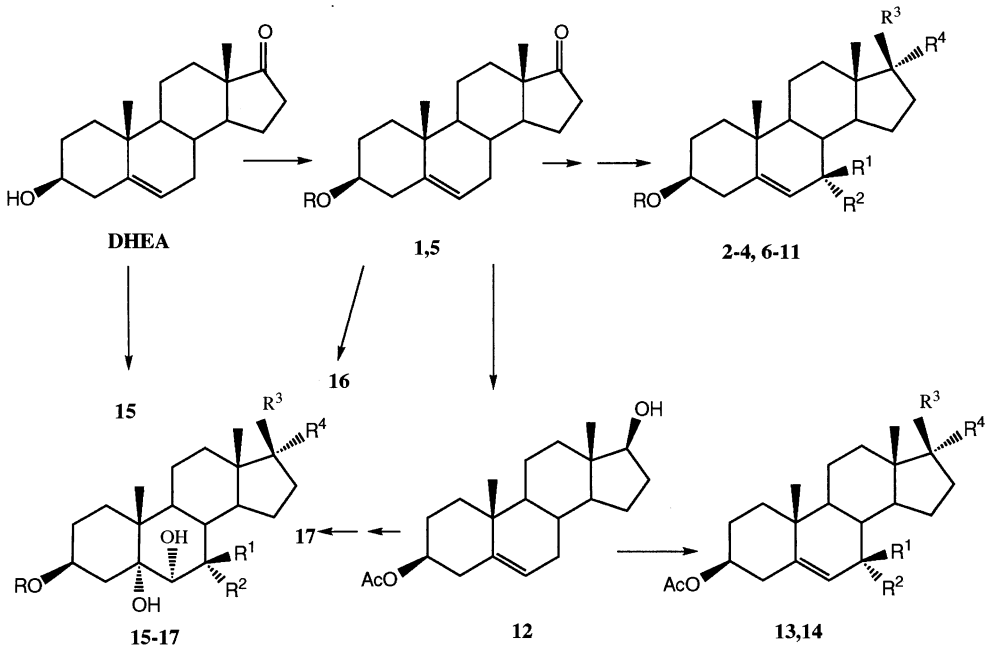
Syntheses

*3β,7α-Dihydroxyandrost-5-en-17-one (**2**)*

This compound was initially synthesized according to the method of Starka.[55] However, the low yields and lack of generality of that method prompted a search for another route to the 7-hydroxy steroids. In the method described below DHEA acetate is subjected to allylic bromination by a procedure developed by Confalone et al.[56] to prepare 7α derivatives of cholesterol. The mixture of 7α- and β-bromo DHEA acetate that is formed may be isomerized to predominantly the 7α-bromo compound and then subjected to a stereospecific oxidative debromination in the presence of acetic acid and silver acetate.

Ten g of DHEA acetate (**1;** 0.03 mol) and 13.6 g of $NaHCO_3$ (0.016 mol) were stirred with 1 L hexane (b.p. 69–71°C) and heated to reflux under N_2. Dibromantin (1,3-dibromo-5,5-dimethylhydantoin (6.11 g; 0.021 mol) was added and the heating continued for 30 min. The mixture was then cooled to room temperature and filtered. The residue was extracted with 50 mL of CH_2Cl_2 and the combined organic phases were rotary evaporated to near dryness at less than 35°C. The creamy white product is unstable to storage and should be used immediately.

The 7α- and 7β-bromo derivatives were dissolved in 80 mL CH_2Cl_2, and 8 g anhydrous LiBr in 320 mL ice-cold acetone was added. The mixture was shielded from light and stirred on ice for 3 h during which time it was converted predominantly to the 7α-bromo enantiomer. Silver acetate (26 g) suspended in 320 mL CH_2Cl_2 and 80 mL of glacial acetic acid was stirred for 20 min at room temperature and then poured into the solution of 7-bromo-DHEA acetate. The mixture darkened as it was stirred for 30 min. The mixture was filtered and the solids were rinsed with CH_2Cl_2. The combined filtrate was extracted 3 times with 1 L H_2O, residual acid was removed with 5% $NaHCO_3$, extracted twice with water and rotary evaporated to dryness. The ester was dissolved in 500 mL methanol and heated to reflux under N_2 with stirring; 250 mL of 5% Na_2CO_3 were added and refluxing continued for 45 min. The methanol was evaporated and the solution carefully neutralized with acetic acid. Extraction into CH_2Cl_2 was followed by evaporation and azeotropic drying with absolute ethanol and twice with acetone. The solid 7α-hydroxy DHEA was dissolved in a minimum amount of warm acetone and hexane was added to incipient turbidity. Crystals form at room temperature (m.p. 187–189°C) and a second crop may be obtained from the filtrate by storing at lower temperature. Depending on the scale of the reaction the yield is from 50 to 80% based on DHEA acetate. The recrystallized compound melted at 192–193° (lit.[29] 182–183°C). In the event that crystallization does not occur the solution may be evaporated, dried as above and the product purified by chroma-



Scheme 1

tography on silica gel (petroleum ether:AcOEt 2.5:1, 1.5:1, then 1:1). An alternative route to either 3β-acetoxy-7α— or 3β-acetoxy-7β-hydroxyandrost-5-en-17-one has been reported recently from this laboratory.[57]

*3β-Acetoxyandrost-5-ene-7,17-dione (**3**)*

The synthesis of the 7-oxo-DHEA acetate used in the bioassays described in this paper was based on a patent by Forischer et al.[58] All starting material should be thoroughly purified and free of any acidic or basic impurities; if not, reaction time increases and the yield of product decreases.

In a two-necked flask *N*-hydroxyphthalimide (10.86 g, 0.066 mol) was taken up in a mixture of acetone and ethyl acetate (1:1, 200 mL). 3β-Acetoxyandrost-5-en-17-one (**1;** 22.0 g, 0.066 mol) and azobis (cyclohexane-carbonitrile, 1.1 g) were added in succession. Subsequently, a weak stream of compressed air was passed into the mixture which was allowed to reflux for 9 h. After completion of the reaction (as monitored by TLC: ethyl acetate: hexane 1:1.5) the mixture was evaporated to dryness and the resultant mass taken up in 150 mL of toluene. Stirring at 50°C for 30 min followed by cooling at room temperature gave a white crystalline precipitate of *N*-hydroxyphthalimide which was filtered off, washed with two 10 mL portions of toluene, and dried under suction. Thereby, 10 g (90%) of *N*-hydroxyphthalimide was recovered and may be reused.

The organic layer was washed thoroughly with saturated sodium bicarbonate solution until the aqueous layer was colorless, then with water. Toluene is distilled off under suction and the residue was heated to dissolve in 130 mL of methanol. On cooling the 7-oxo compound crystallized. It was collected on a filter, washed with cold methanol and dried under vacuum. 3β-Acetoxyandrost-5-ene-7,17-dione (**3**) was thus obtained as an off white colored compound (13.2 g, 56.7% yield). It was further purified by recrystallization from acetone-hexane, which gave 11.5 g of white crystalline product (50% yield), with a melting point of 185–187°C.

The mother liquor, when chromatographed on silica gel (eluent–ethyl acetate/hexane 1:5) yielded another 5.0 g of pure 3β-acetoxyandrost-5-ene-7,17-dione (combined yield 72%). U.V.: max 235 nM, E = 13,700 (c = 0.032 g/L, ethanol). Infra red: 1741, 1724, 1669, 1240 cm. ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.78 (1H, s, 6-H), 4.74 (1H, m, 3α-H), 2.08 (3-H, s, CH_3-acetate), 1.24 (s, 3H, 19-CH_3), 0.90 (s, 3H, 18-CH_3). ^{13}C NMR (200 MHz, $CDCl_3$): δ 221 (C-17), 200 (C-7), 170 (C═O, acetate), 164.5 (C-5), 126.3 (C-6), 71.8 (C-3), and 50, 47.7, 45.8, 44.3, 38.4, 37.8, 35.9, 35.4, 30.7, 27.2, 24.0, 21.0, 20.5, 17.3, 13.6.

*3β-Hydroxyandrost-5-ene-7,17-dione (**4**)*

3β-Hydroxyandrost-5-ene-7,17-dione (**4**) was prepared from the acetate (**3**) by saponification with Na_2CO_3 in methanol M.p. 236–239°C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.74 (d, *J* = 1.47 Hz, 6-H), 3.69 (m, 3α-H), 1.23 (s, 19-CH_3), 0.90 (s, 18-CH_3). ^{13}C NMR (200 MHz, $CDCl_3$): δ 219.8 (C-17), 200.7 (C-7), 166.2 (C-5), 125.7 (C-6), 70.2 (C-3), 50.3, 47.8, 45.9, 44.4, 41.9, 38.4, 36.4, 35.5, 31.14, 30.8, 24.1, 20.6, 17.4, 13.71.

*Acyl esters of 3β-hydroxyandrost-5-ene-7,17-dione and related steroids (**5–13**)*

Propionyl, butyryl, and long-chain fatty acyl esters of DHEA have been described in the literature and some are items of commerce. Isobutyryl DHEA and propionyl, butyryl, and isobutyryl esters of 7-oxo-DHEA were prepared by reacting the parent steroid with a 10% molar excess of the corresponding acid anhydride in anhydrous pyridine; acid chlorides were used for preparing the long chain esters. When acylation was completed the mixture was poured into ice-water, carefully neutralized with HCl, collected by filtration, washed with water, dried and crystallized from ethanol or acetone-hexane. A second crop was usually obtained by storing the filtrate at 4°C. Some esters of 7-oxo-DHEA were also prepared by oxidizing the corresponding ester of DHEA.

3β-isoButyroxyandrost-5-en-17-one (5). M.p. 187–188°C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.4 (1H, d, J = 5.0 Hz, 6-H), 4.59 (1H, m, 3α-H), 1.18 (3H, s, CH_3), 1.14 (3H, s, CH_3), 1.05 (3H, s, 19-CH_3), 0.89 (3H, s, 18-CH_3).

3β-Butyroxyandrost-5-ene-7,17-dione (6). M.p. 219–220°C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.76 (1H, d, J = 1.4 Hz, 6-H), 4.75 (1H, m, 3α-H), 1.24 (3H, s, 19-CH_3), 0.96 (3H, t, J = 7.4 Hz, CH_3), 0.90 (3H, s, 18-CH_3).

3β-isoButyroxyandrost-5-ene-7,17-dione (7). M.p. 185–186°C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.78 (1H, s, 6-H), 4.74 (1H, m, 3α-H), 1.22 (3H, s, 19-CH_3), 1.18 (6H, s, $(CH_3)_2$), 0.9 (3H, s, 18-CH_3).

3β-Dodecanoyloxyandrost-5-ene-7,17-dione (8). M.p. 90–91°C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.77 (1H, s, 6H), 4.73 (1H, m, 3α-H), 1.24 (s, 19-CH_3), 0.9 (s, 18-CH_3).

3β-Hexadecanoyloxyandrost-5-ene-7,17-dione (9). M.p. 87–88°C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.76 (1H, s, 6H), 4.73 (1H, m, 3α-H), 1.2 (s, 19-CH_3), 0.9 (s, 18-CH_3).

3β-Octadecanoyloxyandrost-5-ene-7,17-dione (10). M.p. 87–88°C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.76 (1H, s, J = 1.4 Hz, 6H), 4.74 (1H, m, 3α-H), 0.90 (3H, s, 18-CH_3).

3β-Hemisuccinoyloxyandrost-5-ene-7,17-dione (11). M.p. 265–265.5°C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.76 (1H, s, J = 1.6 Hz, 6-H), 4.76 (1H, m, 3α-H), 1.24 (3H, s, 19-CH_3), 0.9 (3H, s, 18-CH_3).

3β-Acetoxyandrost-5-en-17β-octadecanoate (13). To 0.85 g of 3β-acetoxyandrost-5-en-17β-ol (**12**) in 10 mL dry pyridine, 1 g of stearoyl chloride was added at room temperature. Ninety percent of the starting steroid had reacted within 30 min and none could be detected the following day when the reaction mixture was worked up as described above. The crystalline product, 1.394 g (91%) melted at 82–83°C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.37 (1H, s, J = 5.4 Hz, 6H), 4.6 (2H, m, 3α-H + 17α-H), 2.04 (3H, s, $COCH_3$), 1.03 (3H, s, 19-CH_3), 0.80 (3H, s, 18-CH_3).

Table 1 Enzyme Induction by Esters of DHEA and 7-Oxo-DHEA

Compound	Concentration in diet	No. of rats	GPDH % of control	ME % of control
DHEA	0.05	5	232	295
DHEA-acetate	0.05	13	210	276
7-Oxo-DHEA-acetate	0.052	13	238	378
DHEA-propionate	0.052	6	240	292
7-Oxo-DHEA-propionate	0.054	6	250	379
DHEA	0.05	3	202	311
DHEA-acetate	0.057	5	263	351
DHEA-butyrate	0.062	2	175	196
7-Oxo-DHEA-butyrate	0.013	3	119	196
7-Oxo-DHEA-butyrate	0.065	4	291	459
DHEA-isobutyrate	0.062	6	97	125
7-Oxo-DHEA-isobutyrate	0.064	6	208	285
DHEA-acetate	0.05	6	152	261
DHEA-laurate	0.08	6	159	228
7-Oxo-DHEA-laurate	0.08	3	158	221
DHEA-palmitate	0.08	5	194	235
7-Oxo-DHEA-palmitate	0.05	2	146	154
7-Oxo-DHEA-palmitate	0.09	5	238	292
7-Oxo-DHEA-acetate	0.02	2	209	396
DHEA-stearate	0.096	2	202	205
7-Oxo-DHEA-stearate	0.036	2	151	228
7-Oxo-DHEA-sulfate	0.067	3	305	356
3β-Acetoxy-A-17β-stearate	0.035	2	120	143
3β-Acetoxy-17β-stearoyl-A-7-one	0.036	2	134	165
DHEA-acetate	0.05	8	220	346
DHEA-hemisuccinate	0.06	4	205	353
7-Oxo-DHEA-acetate	0.05	2	268	429
7-Oxo-DHEA-hemisuccinate	0.012	2	172	177
7-Oxo-DHEA-hemisuccinate	0.03	2	206	213
7-Oxo-DHEA-hemisuccinate	0.06	6	333	488

In each assay, enzyme activities of rats fed the new esters for six days were compared with rats fed DHEA or DHEA-acetate and with non-supplemented controls. All acyl groups are at the 3β position unless designated at 17β. GPDH, sn glycerol 3-phosphate dehydrogenase; ME, malic enzyme. In this table -A- is an abbreviation for androst-5-ene.

*3β-Acetoxy-17β-octadecanoyloxyandrost-5-en-7-one (**14**)*

M.p. 87–90°C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.71 (1H, s, J = 1.0 Hz, 6-H), 4.71 (1H, m, 3α-H), 4.64 (1H, m, 17α-H), 2.05 (3H, s, $COCH_3$), 1.22 (3H, s, 19-CH_3), 0.81 (3H, s, 18-CH_3).

*3β,5α,6α-Trihydroxyandrostan-17-one (**15**)[59]*

M.p. 239–242°C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 4.05 (m, 3α-H), 3.73, 3.67 (dd, J = 5.4, 11.2 Hz, 6β-H), 0.96 (s, 19-CH_3), 0.84 (18-CH_3). ^{13}C NMR (300 MHz, $CDCl_3$): δ 210 (C-17), 70.2, 67.1 (C-3,6), 50.9, 44.4, 33.3 (C-methines), 38.4, 35.8, 33.8, 31.5, 31.1, 30.6, 21.7, 20.4 (C-methylenes), 15.5, 13.8 (C-methyls).

*3β-Acetoxy-5α,6α-dihydroxyandrostane-7,17-dione (**16**)*

A solution of 1.15 g of 3β-acetoxyandrost-5-ene-7,17-dione (**3**) in 10 mL of pyridine was treated with 0.98 g of OsO_4 in 15 mL of pyridine and stirred for 2.5 h at room temperature. A solution of 1.8 g of sodium bisulfite in 30 mL of water and 20 mL of pyridine was added to cleave the osmate ester. After stirring for 20 min the mixture was extracted repeatedly with dichloromethane (100, 50, 50, 25 mL) which was washed with saline and water, and dried over $MgSO_4$. When concentrated under reduced pressure the product crystallized, was collected by filtration and dried in vacuo. Yield 930 mg. M.p. 238–240°C. The mother liquor yielded 191 mg (m.p. 237–238°C) for an overall yield of 88%. ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.05 (m, 3α-H), 4.12 (d, J = 1.00 Hz, 6β-H), 2.03 (s, $OCOCH_3$), 1.32 (s, 19-CH_3), 0.88 (s, 18-CH_3). ^{13}C NMR (300 MHz, $CDCl_3$): δ 219.6 (C-17), 209.3 (C-7), 170.5 (C═O acetate), 81.3 (C-5), 77.1, 70.0 (C-3,6), 47.1, 46.7, 44.3 (C-methines), 35.5, 34.9, 30.7, 30.6, 26.5, 22.6, 21.0 (C-methylenes), 21.3, 16.0, 13.8 (methyls).

*3β-17β-Diacetoxy-5α,6α-dihydroxyandrostan-7-one (**17**)*

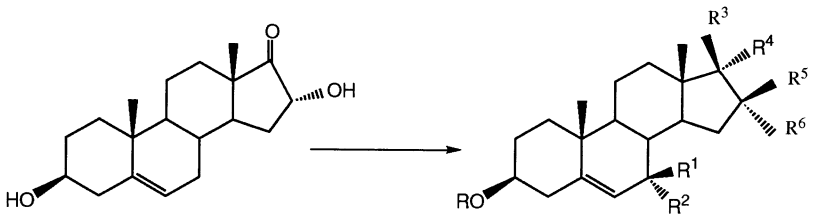
This compound, prepared as for the diketone above, melted at 159–160°C when recrystallized. ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.06 (m, 3α-H), 4.63 (t, J = 8.3 Hz, 17α-H), 4.08 (d, J = 2.9 Hz,

Table 2 Steroids that Induce the Synthesis of Mitochondrial Glycerophosphate Dehydrogenase and Cytosolic Malic Enzyme in Rats' Livers

Steroid	Concentration in diet	No. of rats	GDPH	ME
			% of control	
3β-ol-A-17-one (DHEA)	0.01	8	124	107
	0.025	3	157	178
	0.05	135	253 ± 56	327 ± 76
	0.1	68	295 ± 68	428 ± 86
	0.2	4	285	645
3β-acetoxy-A-17-one	0.023	3	148	285
	0.057	42	241 ± 55	329 ± 75
	0.1	9	285 ± 64	455 ± 105
3β-ol-A-7,17-dione (7-oxo-DHEA)	0.0105	6	168 ± 17	244 ± 61
	0.026	5	290 ± 32	499 ± 47
	0.052	11	341 ± 88	507 ± 71
	0.105	6	366	704
3β-acetoxy-A-7,17-dione	0.02–0.03	9	215 ± 66	341 ± 85
	0.052–0.06	19	304 ± 107	392 ± 40
	0.1–0.12	15	270 ± 59	463 ± 113
3β,7α-diol-A-17-one	0.033	2	308	374
	0.05	2	292	423
3β-acetoxy-7β-ol-A-17-one	0.05	3	219	339
A-3β,17β-diol	0.05	4	234	261
	0.2	3	275	494[a]
3β,17β-diol-A-7-one	0.01	8	216	219
	0.05	5	378	416
	0.1	6	231	652
3β-acetoxy-A-17β-ol	0.058	2	222	338
3β-acetoxy-17β-ol-A-7-one	0.13	3	232	452
3β,17β-diacetoxy-A	0.065	8	114	131
3β,17β-diacetoxy-A-7-one	0.068	9	290	274
3β,16α-diol-A-17-one	0.05	5	99	135
A-3β,7α,17β-triol	0.1	2	227	611
3β,16α,17α-triacetoxy-A	0.075	3	145	107
3β,16α,17α-triacetoxy-A-7-one	0.078	3	198	164
3β,16α,17α-triol-A-7-one	0.056	3	102	227
3β,16α-diacetoxy-7α-ol-A-17-one	0.05	2	246	270
3β,16β-diacetoxy-7β-ol-A-17-one	0.05	2	228	507
3β,16β-diacetoxy-7α-ol-A-17-one	0.05	5	242	340
3β-propionoxy-7α-F-A-17-one	30 mg/day times 3	3	213	164
3β-acetoxy-7α-F-A-17-one	30 mg/day times 3	3	157	290
3β-acetoxy-7β-F-A-17-one	30 mg/day times 3	3	167	258
3β-acetoxy-7α-F-A-17-one	20 mg/day times 3	3	185	270
3β-acetoxy-7β-F-A-17-one	20 mg/day times 3	3	224	231

In this table, -A- is an abbreviation for androst-5-en. Enzyme activity in the livers of rats fed the stock diet without supplementation is termed 100%.
[a]Data from Su and Lardy (18).

6β-H), 3.83 (d, OH), 2.04, 2.02 (2s, 3,17-acetate), 1.31 (s, 19-CH_3), 0.80 (s, 18-CH_3). ^{13}C NMR (300 MHz, $CDCl_3$): δ 209.5 (C-7), 171.05, 170.48 (C═O, acetates), 81.27 (C-5), 81.78, 77.02, 70.16, (C-3,6,17), 47.27, 46.86, 43.48 (C-8,9,14), 35.74, 34.81, 30.7, 27.48, 26.46, 24.3, 21.22 (C-1,2,4,11,12,15,16), 21.31, 21.1 (acetate-CH_3), 15.96, 12.08 (C-18,19).



18-21

Scheme 2

Table 3 Compounds that Do Not Induce Mitochondrial Glycerophosphate Dehydrogenase or Cytosolic Malic Enzyme in Rats' Livers

Androst-4-ene-3β,17β-diol; androst-4-ene-3,17-dione; androst-4-ene-3,11,17-trione (0.052); 4-hydroxyandrost-4-ene-3,17-dione (0.015); 11β-hydroxyandrost-4-ene-3,17-dione (0.05); 19-hydroxyandrost-4-ene-3,17-dione (0.024); 7α-hydroxytestosterone; 17α-ethynyltestosterone; 17-hydroxyandrosta-4,6-dien-3-one; 3β-acetoxy-5α-ol-androst-6-en-17-one (0.05).

Androst-5-en-17-one; androst-5-ene-7,17-dione; 3β-hydroxy-7-methylandrost-5-en-17-one; 4β-hydroxy-DHEA; 16α-hydroxy-DHEA; 16α-bromo-DHEA; 19-ol-DHEA; androst-5-ene-3β,7α,19-triol; 1α,3β-diacetoxyandrost-5-ene-7,17-dione; 2α,3β-diacetoxyandrost-6-en-17-one; androst-5-ene-3β,16α-diol; 3β,17β-dihydroxyandrost-5-en-11-one (0.053); 3β-acetoxy-5α-hydroxyandrost-6-en-17-one; androst-5-ene-3β,19,17β-triol; 2β,3β,17β-triacetoxyandrost-5-en-7-one; 3β,7α-dihydroxy-16β-acetoxyandrost-5-en-17-one (0.05); 3β-hydroxyandrost-5-ene-17-carboxylic acid; 3β-acetoxyandrosta-1,5-diene-7,17-dione; androsta-3,5-diene-7,17-dione (0.05); 17β-hydroxyandrosta-3,5-dien-7-one; 3β-hydroxyandrosta-5,7-dien-17-one; androsta-5,16-dien-3β-ol, and its 3-acetate.

11β-hydroxyandrosterone; 11-oxo-androsterone (0.04); 3β-acetoxy-5α-androstane-7α,17β-diol (0.01); 3β-acetoxy-5α,6α-dihydroxyandrostane-7,17-dione; 3β,5α,6α-trihydroxyandrostan-17-one (0.056); 3β,17β-diacetoxy-5α,6α-dihydroxyandrostan-7-one; 3β,5α,6α,16α,17α-pentacetoxyandrostane; 2α,3α-dihydroxy-5α-androstane-7,17-dione; 3β,5α,6β-trihydroxyandrostan-17-one; 17β-hydroxy-5α-androstane-3,7-dione (0.027).

5β-androstane-3α,17β-diol (0.067); 5β-androstane-3β,17β-diol (0.077), and its 3-acetate (0.05); 3β-hydroxy-5β-androstan-17-one (0.077) and its 3-acetate (0.077); 5β-androstane-3,17-dione (0.077); 5β-androstane-3α,7α,17β-triol (0.043); 7α,17β-dihydroxy-5β-androstan-3-one-(0.05); 3α,7α,16α-trihydroxy-5β-androstan-17-one (0.08), and its 16-acetate (0.05); 7α-hydroxy-16α-acetoxy-5β-androstane-3,17-dione (0.05).

7-hydroxyestrone (0.01); androsta-1,4,6-triene-3,7-dione; 17α-ethyl-17β-ol-19-nor androst-4-en-3-one; 3α,6α-dihydroxy-5β-pregnan-20-one (0.05); 3α-hydroxy-5β-pregnan-20-one (0.05); 16α-hydroxyprogesterone (0.06); pregnenolone; 7α-hydroxypregnenolone; 3β-acetoxypregnene-7,20-dione (0.06); 3β,17α-diacetoxypregnen-7,20-dione.

These steroids were fed at 0.1 or 0.2% of the diet unless a lower concentration is indicated. For comparative purposes, some additional inactive compounds are reported in Tables 1 and 2.

*3β,16α-diacetoxyandrost-5-en-17-one (**18**)*

Four g of 3β,16α-dihydroxyandrost-5-en-17-one[60] in 24 mL Ac_2O and 16 mL pyridine was stirred for 12 h at room temperature. After removal of solvents in vacuo, the residue was purified over silica gel ($CHCl_3$: Me_2CO = 60:1) to yield compound **18** (3 g, 67%) as a white solid which was crystallized from AcOEt/pet ether. M.p. 165–167°C. ^{1}HMR (300 MHz, $CDCl_3$) δ 5.20 (m, 2 H, 6-H and 16-H), 4.60 (m, 1 H, 3-H), 2.09 (s, $OCOCH_3$), 2.02 (s, $OCOCH_3$), 1.06 (s, CH_3), 1.01 (s, CH_3).

*3β,16α-diacetoxy-7-α-hydroxyandrost-5-en-17-one (**19**) and 3β,7α,16α-triacetoxyandrost-5-en-17-one (**20**)*

Compound **18** (3 g) was brominated, treated with LiBr and subsequently with AgOAc as in the procedure for compound **2** except that toluene was the solvent to which the LiBr solution was added. Thirty min after the addition of AgOAc, solids were removed by filtration and washed; the filtrate was concentrated and 300 mL H_2O was added to the residue. The pH was brought to neutral with $NaHCO_3$ and the solution was extracted with AcOEt (150 mL times 5). The combined organic solution was washed with brine, dried and concentrated to dryness. The crude product was purified over silica gel (AcOEt:pet ether = 1:3, 1:2, and then 1:1) to yield 1.5 g of diacetate **19** (48%) and 700 mg of triacetate **20** (20%). Both compounds were crystallized from Et_2O.

Compound **19.** M.p. 155–158°C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.64 (d, 1H, J = 4 Hz, 6-H), 5.44 (d, 1H, J = 5 Hz, 16-H), 4.63 (m, 1H, 3-H), 3.92 (m, 1H, 7-H), 2.15 (s, $OCOCH_3$), 2.04 (s, $OCOCH_3$), 1.02 (s, CH_3), 0.97 (s, CH_3).

Compound **20.** M.p. 170–172°C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.61 (d, 1H, J = 2 Hz, 6-H), 5.40 (d, 1H, J = 4 Hz, 16-H), 5.02 (dd, 1H, J = 2 Hz, 7-H), 4.70 (m, 1H, 3-H), 2.10 (s, $OCOCH_3$), 2.02 (s, $OCOCH_3$), 1.02 (s, CH_3), 0.98 (s, CH_3).

*3β,7α,17β-Trihydroxyandrost-5-en-16-one (**21**)*

Diacetate **19** (400 mg) was stirred with 342 mg K_2CO_3 in 25 mL MeOH at room temperature for 2 h. After removal of insoluble salt the filtrate was concentrated in vacuo and the residue was purified over silica gel. The white solid was crystallized from MeOH/Et_2O to yield triol **20** (180 mg, 56%. M.p. > 230°C. ^{1}H NMR (300 MHz, DMSO) δ 5.42 (d, 1H, J = 2 Hz, 6-H), 5.30 (br s, 1H, OH, D_2O exch.), 4.68 (br s, 1H, OH, D_2O exch.), 4.26 (d, 1H, J = 2 Hz, OH, D_2O exch.), 3.64 (s, 1 H, 17-H), 3.60 (br s, 1H, 7-H), 3.32 (m, 3-H), 0.92 (s, CH_3), 0.61 (s, CH_3).

Results and discussion

Many therapeutically useful steroids are administered as esters and the widespread distribution of esterase activity[61] in mammalian tissues ensures the cellular availability of the free steroid. Several aliphatic esters of DHEA and of its 7-oxo analog were synthesized to ascertain their possible utility in therapy and to provide standards for metabolic studies; their activity as inducers of rat liver glycerol-3-phosphate dehydrogenase and malic enzyme is shown in Table 1. Unexpected selective hydrolyses appear to be involved in the availability of some of these steroids. Among the monoesters prepared and tested all were active except the isobutyryl derivative of DHEA; however, isobutyryl-7-oxo-DHEA was fully active. Other examples of inactive esters matched by active 7-oxo derivatives are presented in Table 2 and are discussed below. The sulfate ester of 7-oxo-DHEA, which is found in humans,[40] was approximately as active as equimolar amounts of DHEA-acetate whereas DHEA sulfate was poorly active orally and especially when injected intraperitoneally.[18]

Long chain fatty acyl derivatives of steroids occur naturally[62–66] and some may have special functions.[64] The weak enzyme induction by 17β-stearoyl derivatives of 3β-acetoxy-androstenediol and the corresponding 7-oxo compound indicates that these esters are not readily hydrolyzed by tissue esterases. Hochberg et al. have observed that fatty acyl esters of testosterone[64] and estrogens[65] also have prolonged half lives in rats.

The finding that oxygenated derivatives of dehydroepiandrosterone occur in the urine of normal subjects and of patients with adrenal tumors[22,23] led Okada et al.[23] to pos-

tulate that the parent C_{19} ketosteroid might be converted to other active hormones as well as to testosterone and estrogens. Mammalian tissue preparations hydroxylate DHEA to produce 7α, 7β, and 16α-hydroxy derivatives and 7-oxo-DHEA.[29–37] Such oxygenated analogs of DHEA had apparently not been tested for biological activity in animals until recently.[18,47–49] The fact that there is no known receptor for DHEA and that relatively massive amounts (ca. 0.5% of the diet; or ca. 500 mg/kg body wt. per day) are required to elicit its antiobesity effect in mice and rats indicates that DHEA is not an active hormone but only a precursor of an active anti-obesity factor. We felt that some of the known metabolites of DHEA might be intermediates in the biological conversion of DHEA to more active hormones that have yet to be discovered. We therefore tested these known metabolites and synthesized additional compounds that might be produced from DHEA in mammalian tissues or that might shed light on structure/activity relations.

A comparison of the structures of compounds that induce the formation of liver mitochondrial *sn*-glycerol-3-phosphate dehydrogenase and cytosolic malic enzyme in rats (Table 2) with those that do not (Table 3) is informative. The conversion of precursors of DHEA to active steroids in the brain and testes of rats apparently does not occur rapidly enough to provide DHEA to the liver for feeding pregnenolone, 7α-hydroxypregnenolone, or 7-oxo-17α-hydroxypregnenolone had no influence on the activity of these enzymes in liver. Introduction of hydroxyl groups into DHEA or 7-oxo-DHEA at position 1, 2, 4, 11, or 19 abolished the activity as did saturating the B ring or moving the double bond from 5-6 to 4-5. Introducing a second double bond at 1-2 or 7-8 abolished activity as did oxidizing the 3β-hydroxyl group to form a ketone. The 16α-hydroxy derivative of DHEA is produced in normal and tumor bearing humans. It did not induce the formation of glycerol-3-phosphate dehydrogenase and had only a minor influence on malic enzyme (Table 2); likewise the 16α-hydroxy derivative of androstenediol assayed as the triacetate was inactive but the corresponding 7-oxo derivatives of both were active. The diacetyl ester of androstenediol was inactive but 7-oxo-androstenediol diacetate was as effective as free androstenediol (Table 2).

Both 7α- and 7β-hydroxy DHEA induce the formation of the thermogenic enzymes and both are known to be oxidized to 7-oxo DHEA in tissue preparations.[29,32,44] The 7-oxo derivatives of both DHEA and androstenediol are the most active compounds tested. They effect good responses when fed at only 0.01% of the diet whereas DHEA at that concentration fails to raise the enzyme activity above our arbitrary score of 150%. To eliminate the inter-experiment variation in response to the steroids a dose-comparison assay was conducted in a single 27-rat experiment (the assay results are included in Table 2). Within that single experiment, over the range 0.01 to 0.1% of the diet, 7-oxo-DHEA was 2.5 times as active as DHEA. Some 60 steroids that do not induce the thermogenic enzymes are recorded in Table 3. Selected members of this group are being tested for other activities.

In view of the fact that rat liver does not contain the 17α-hydroxylase that participates in the conversion of pregnenolone to DHEA, and that there is not a significant amount of the latter in rat blood, it is astonishing that the rat responds so readily to administered DHEA, androstene diol and their 7-oxygenated derivatives. Because pregnenolone and 17α-hydroxy pregnenolone did not enhance the liver enzymes, it does not seem likely that small amounts of DHEA produced in brain and testes or ovaries are rapidly converted to an active steroid to which liver is responsive.

It seems likely that the 7-hydroxy derivatives of DHEA, and 7-oxo-DHEA are on an enzyme-catalyzed pathway to one or more active hormones that are the ultimate effectors of the several metabolic responses to DHEA.

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