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7α,11β-Dimethyl-19-nortestosterone: a potent and selective androgen response modulator with prostate-sparing properties

C. Edgar Cook* and John A. Kepler*

Organic and Medicinal Chemistry, Science and Engineering Group, Research Triangle Institute, PO Box 12194, Research Triangle Park, NC 27709, USA

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Abstract—7α,11β-Dimethyl-19-nortestosterone, made by 1,6-methyl addition to 17β-acetoxy-11β-methylestra-4,6-dien-3-one, was a highly potent and selective androgen response modulator, with enhanced androgen receptor binding, androgenic activity and anabolic:androgenic ratio over its two monomethyl homologs.

Androgenic hormones (testosterone and its esters) are currently used in androgen hormone replacement therapy. Although questions must be answered about the need and effectiveness of androgen supplementation in aging men for middle-aged blues, fatigue, or declining sexual desire,[1] androgens have a definite role in treatment of hypogonadal men and beneficial effects in prevention of osteoporosis.[2] Androgen supplementation is sometimes used for women, as well.[3] Androgen use in male contraception has long been investigated and is now being studied commercially.

One significant concern about testosterone treatment in men is the potential effect on benign prostatic hyperplasia and prostate cancer. Dihydrotestosterone (DHT) is thought to be the main hormone affecting the prostate, and a recent report shows that co-administration of testosterone and finasteride (to inhibit reduction of testosterone to DHT) to older men with low serum testosterone increased bone mineral density significantly ($p < 0.001$) over placebo without increasing PSA over baseline.[4] 7α-Methyltestosterone (MENT)[5] has been reported not to undergo metabolic reduction at the 4,5-position and to maintain muscle mass with minimal effect on ventral prostate in castrated male rats[6] and monkeys.[7]

Introduction of an 11β-methyl substituent into steroid hormones has been reported to have significant effects on biological activity. In rabbits, 11β-methyl-17α-ethynyl-17β-hydroxyestr-4-en-3-one has 25-fold the progestational activity of norethindrone (its 11-nor homolog). The 11β-methyl compound also has some estrogenicity (ca. 16% of estrone in rats).[8] 11β-Methylestradiol has 124% the relative binding affinity of estradiol for the rat estrogen receptor and is equivalent to estradiol in uterotropic activity.[9] Baran et al. also report in a patent that 11β-methyl-19-nortestosterone has androgenic activity, although data are not given.[10] We have now examined the effect on androgen receptor binding and activity resulting from introduction of both 7α- and 11β-methyl groups into 19-nortestosterone, and found that the combination results in enhancement of activity together with increased separation of effects on muscle and ventral prostate compared with the two monomethyl homologs.

The synthesis of the 11β-methyl analog is outlined in Scheme 1.[18] Reaction of **1**[10] with isopropenyl acetate[11] gave 3,17β-diacetoxy-11β-methylestra-3,5-diene (**2**). Formation of the 3,5-diene rather than the 3,5(10) isomer was shown by ^{1}H NMR, in which two peaks assigned to the 4-H and 6-H vinyl protons were in the ratio of 1:1. Bromination–dehydrobromination[12] of **2** led to 4,6-dien-3-one **3**. The ^{1}H NMR spectrum of **3** had two apparent singlets at δ 5.67 and δ 6.02 with ratio 1:2, consistent with the 4-H and superimposed 6- and 7-H.

Conjugate addition of a methyl group was then carried out utilizing lithium dimethyl cuprate reagent prepared

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* Corresponding authors. Fax: +1 540 885 8920 (C.E.C.); fax: +1 919 541 8868 (J.A.K.); e-mail: cec@rti.org; jak@rti.org

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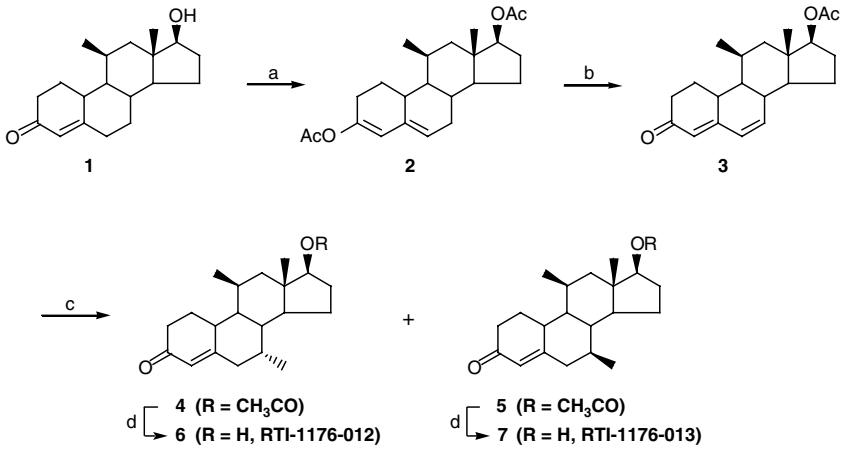

Scheme 1. Synthetic route. Conditions: (a) iso-propenyl acetate, benzene, *p*-toluenesulfonic acid; (b) (i) *N*-bromosuccinimide, (ii) Li_2CO_3, LiBr, *N*,*N*-dimethylformamide, reflux; (c) Me_2S, MeLi; (d) KOH, MeOH.

from methyl lithium and dimethyl sulfide/cuprous complex.[13] The crude product was converted into the corresponding 7-methyl-4-en-3-one **4** by treatment with acid. In contrast to the parent estrane series,[14] the 1,6-addition of the methyl group led to a mixture of stereoisomers at the 7-position (**4** and **5**). These two compounds were separated by chromatography in pure state but in low yield (12% for compound **4** and 23% for compound **5**).

The configuration of the 7-methyl was determined by comparing the ^{1}H NMR spectra of **4** and **5** to those of the known 7α- and 7β-methyltestosterone acetates. In the testosterone acetate series it was reported that the chemical shift for the 7α-methyl was 0.78 ppm (d, $J = 7$ Hz) while the chemical shift for the 7β-methyl was 1.06 ppm (d, $J = 6$ Hz).[14] The ^{1}H NMR spectrum of compound **4** had a doublet at 0.78 ppm ($J = 7$ Hz) and that of compound **5** had a doublet at 1.0 ppm ($J = 8$ Hz). Based on this comparison, the compound which eluted first (doublet at 0.78 ppm) was assigned the 7α-configuration (**4**).

The two acetates, **4** and **5**, were treated with methanol–water–potassium hydroxide to generate the corresponding 17-alcohol. The chemical shifts in the ^{1}H NMR spectra of these products, **RTI-1176-012** and **RTI-1176-013**, also correspond to those reported in the literature[14] for 7-methyltestosterones.

Receptor binding affinity was determined by use of the androgen receptor from cytosol of the ventral prostate of castrated male rats (homogenized in buffer of 0.05 M Tris–HCl, 0.05 M Tris Base, 1 mM EDTA and 0.15 mM dithiothreitol) incubated for 24 h at 0–4 °C with the test or standard compounds at 1×10^{-10} to 10^{-5} M (7 concentrations) and 25,000 cpm of dihydrotestosterone-1,2-^{3}H. Separation of free/bound radioligand was achieved with charcoal (0.5% Norit A/0.5% dextran-T-70). For estrogen binding, the estrogen receptor from the uterus of 15-day-old rats was used in an identical manner, with estradiol-2,4,6,7-^{3}H as radioligand. **RTI-1176-012** had a relative binding affinity for the androgen receptor of 194 ± 24 (SE, $n = 4$) relative to DHT = 100, compared with 162 ± 35 ($n = 3$) for MENT and only 22 for the 7β,11β-dimethyl analog **RTI-1176-013**. Estrogen receptor binding for **RTI-1176-012** was very low (0.6 ± 0.56, $n = 3$).

Compound **RTI-1176-012** (**-012**) (10, 40, and 160 μg total dose, given subcutaneously in 10% ethanol in sesame oil) was compared directly with testosterone (100, 400 and 1600 μg) and 11β-methyl-19-nortestosterone (10, 40, and 160 μg) in immature (22 days) castrated male rats injected daily for 7 days[15] for effects on the ventral prostate (VP) and seminal vesicles (SV). For VP (see Fig. 1), the dose–response regression lines (weight as a function of log dose) were parallel for compound **-012** and testosterone, with **-012** being 12.2 times as potent [95% confidence interval 8.2–18.4]. 11β-Methyl-19-nortestosterone had a shallow dose–response slope and was estimated to be only 0.1–0.2 times as potent as **-012**. Seminal vesicle weights showed similar differences in potency, but with less parallelism between **-012** and testosterone.

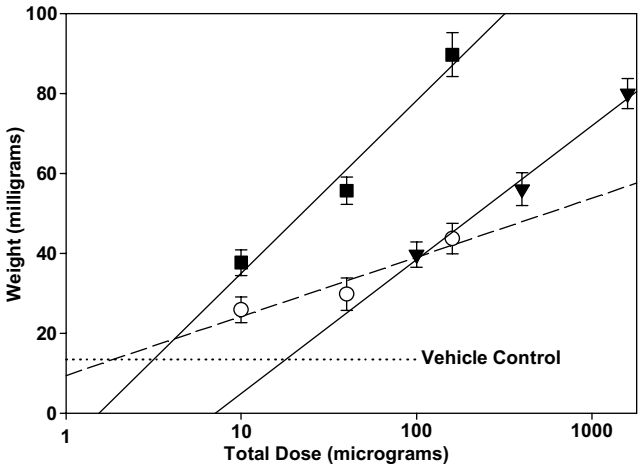

Figure 1. Ventral prostate weight as a function of dose, with linear regression. Squares—compound **6**; circles—compound **1**; triangles—testosterone. Error bars are SE ($n = 10$).

Anabolic activity of **-012** was also assessed. Over the dose range of 0.25–1 mg total dose in a 10 day Hershberger assay **-012** showed 136-fold the activity of testosterone on levator ani (LA, muscle) weight, 37-fold activity for SV and 14-fold activity for VP. Over a similar dose range, MENT had values of 25-fold, 8-fold and 6-fold for the three tissues. Literature values for MENT in immature male rats were 15-fold the activity of testosterone for LA and 4-fold for VP.[16] For four assays over a total dose range of 0.008–1 mg, **-012** averaged 64-fold testosterone for LA, 21-fold for SV and 14-fold for VP. In the assay versus 11β-methyl-19-nortestosterone, anabolic response was not measured directly, but it was found that the final weights of the rats were higher than control weights ($p < 0.05$) for the 40 and 160 μg doses of **-012** and the 1600 μg dose of testosterone (one-way ANOVA followed by Bonferroni's modified *t*-test; initial body weights were equalized among treatment groups). This result is indicative of anabolic activity,[17] but not observed for any doses of 11β-methyl-19-nortestosterone.

Finally, **-012** had estrogenic (uterotropic) activity only 0.8% that of estradiol in 21-day-old female rats injected subcutaneously over 3 days with 6.4, 12.8 or 25.6 μg **-012** or 0.08, 0.16, or 0.32 μg total dose estradiol.

Because of the ability of receptors to modify their conformation in response to ligands, combining two substituents that reportedly contribute to a specific biological activity may or may not enhance the response. Thus it was gratifying that our expectation of enhanced activity upon combining 7α-methyl and 11β-methyl substituents in 19-nortestosterone led to a potent and selective compound. In our tests, this modification resulted in at least a 5-fold increase in androgenic potency over the compound with only the 11α-methyl group when measured by the effect on VP. The 2-fold difference observed over the 7α-methyl compound for this same endpoint was less striking, but still indicated at least equal if not increased potency. More importantly, the increase in anabolic potency for the 7α,11β-dimethyl modification appears to be markedly greater, and the LA:VP ratio is 5-fold more than that of the selective 7α-methyl analog at similar doses, indicating a more selective anabolic:androgenic ratio for **-012**.

In conclusion, **-012** appears an attractive candidate for a potent and selective androgen response modulator in hormonal contraception and replacement therapy. Development will require not only more biological study, but also a more efficient route of synthesis, which will be addressed in subsequent publications.

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18. *3,17*β*-Diacetoxy-11*β*-methylestra-3,5-diene* (**2**). The procedure of Halkes et al.[11] was followed with some modification. 11β-methyl-19-nortestosterone (**1**, 600 mg, 2.09 mmol) was dissolved in benzene (200 mL) with tosyl acid (325 mg). Traces of water were removed by distillation with a Dean–Stark trap. Isopropenyl acetate (15 mL) was added and benzene (30 mL) was distilled off over a period of 4 h. The cooled reaction mixture was then poured into ice-cold saturated sodium bicarbonate solution (75 mL) and extracted with ethyl acetate (3×50 mL), dried (Na_2SO_4) and concentrated to give a yellow solid which was chromatographed (silica gel, 60 g, benzene–ethyl acetate 97.5:2.5) to give 652.5 mg (84% yield) of pure product. A small amount of the compound was recrystallized from acetone–hexane (some decomposition): mp 143–147 °C; IR (CH_2Cl_2) 1730 cm^{-1} (broad); ^1H NMR ($CDCl_3$) δ 0.9 (s, 3, 18-CH_3), 2.0 (s, 3, 17-OCO–CH_3), 2.1 (s, 3, 3-OCO–CH_3), 4.53 (t, 1, $J = 8$ Hz, 17α-H), 5.37 (s, 1, 5-H), 5.7 ppm (s, 1, 4-H); mass spectrum *m/e* 372 (M^+, 8), 330 ($M^+ - CH_2CO$, 100), 315 ($M^+ - CH_2CO–CH_3$, 2), 270 ($M^+ - CH_3COOH–CH_2CO$, 5).

17β-Acetoxy-11β-methylestra-4,6-dien-3-one (**3**). This compound was prepared by treating enol acetate **2** with *N*-bromosuccinimide (NBS), followed by refluxing with a mixture of lithium carbonate, lithium bromide and DMF according to the method of Grunwell et al.[12] Yield: 67%: UV_{max} (CH_3OH) 283 nm; IR (CH_2Cl_2), 1740 (ester), 1660 cm^{-1} (3-ketone); ^1H NMR ($CDCl_3$) δ 0.93 (s, 3, 18-CH_3), 1.04 (d, 3, J = 7 Hz, 11-CH_3), 2.0 (s, 3, 17-CCH_3), 4.27 (t, 1, 17-H), 5.67 (s, 1, 4-H), 6.03 ppm (s, 3, 2-, 6- and 7-H); mass spectrum *m/e* 328 (M^+, 44), 286 (M^+ –CH_2CO, 58), 268 ($M^+ - CH_3COOH$, 100); TLC R_f 0.56 (20% ethyl acetate in chloroform).

17β-Acetoxy-7,11β-dimethylestr-4-en-3-one (**4** and **5**). Under nitrogen, dimethyl sulfide/cuprous bromide complex (136.3 mg, 0.66 mmol) was stirred with dry ether (5 mL) and 1 drop of dimethyl sulfide at room temperature. Methyl lithium in ether (0.34 mL of 1.88 M solution, 0.64 mmol) was added dropwise to the mixture (with rapid stirring) until the last trace of yellow precipitate dissolved.[13] A solution of **3** (102.8 mg, 0.31 mmol) in dry tetrahydrofuran (3 mL) was added to the vigorously stirred yellow solution. The solution was stirred for an additional 45 min at room temperature and poured into an ice-cold saturated ammonium chloride solution (75 mL) with vigorous stirring. A deep blue solution resulted. The mixture was extracted with ethyl acetate (4 × 30 mL). The organic layers were combined and washed twice with ammonium chloride solution, dried (Na_2SO_4) and concentrated to give a yellow gum. TLC showed a trace of starting material with the remainder of the material being a non-UV-quenching, faster moving spot (silica gel, 20% ethyl acetate in chloroform). Treatment of the crude 7-methyl-5-en-3-one with methanol/HCl gave 110 mg of 7-methyl-4-en-3-one. GC analysis indicated a mixture of 7α and 7β isomers. Careful TLC showed two spots with R_f values of 0.58 and 0.53 after three developments (silica gel, 2.5% ethyl acetate in chloroform). The crude product was chromatographed (silica gel, EM pre-packed size A column). A gradient of benzene (200 mL) to 15% ethyl acetate–benzene (200 mL) eluted **4** (12 mg, 12%) in the 345–375 mL fraction and **5** (23 mg, 23%) in the 395–500 mL fraction.

For compound **4**: IR (CH_2Cl_2), 1730 (ester), 1665 cm^{-1} (3-ketone); ^1H NMR ($CDCl_3$) δ 0.78 (d, 3, J = 7 Hz, 7α-CH_3), 0.92 (s, 3, 18-CH_3), 1.05 (d, 3, J = 7 Hz, 11β-CH_3), 2.01 (s, 3, 17-O(CO)CH_3), 4.56 (s, 1, 17α-H), 5.84 ppm (s, 1, 4-H); mass spectrum *m/z* 344 (M^+, 100), 329 (M^+ $-CH_3$, 5), 302 ($M^+ - CH_2CO$, 20), 284 ($M^+ - CH_3COOH$, 52); TLC R_f 0.58 (2.5% ethyl acetate in chloroform, developed three times), 0.48 (10% ethyl acetate in benzene, developed four times).

Anal. Calcd for $C_{22}H_{32}O_3$, *m/z* 344.2351. Found: *m/z* 344.2354.

For compound **5**: IR (CH_2Cl_2), 1730 (ester), 1665 cm^{-1} (3-ketone); ^1H NMR ($CDCl_3$) δ 0.92 (s, 3, 18-CH_3), 1.00 (d, 3, J = 8 Hz, 7β-CH_3), 1.05 (d, 3, J = 7 Hz, 11β-CH_3), 2.01 (s, 3, 17-O(CO)CH_3), 4.52 (t, 1, 17α-H), 5.80 ppm (s, 1, 4-H); mass spectrum 344 (M^+, 100), 329 ($M^+ - CH_3$, 6), 302 ($M^+ - CH_2CO$, 23), 284 ($M^+ - CH_2COOH$, 76); TLC R_f 0.53 (2.5% ethyl acetate in chloroform, developed three times), 0.44 (10% ethyl acetate in benzene, developed four times).

Anal. Calcd for $C_{22}H_{32}O_3$, *m/z* 344.2351. Found: *m/z* 344.2354.

7α,11β-Dimethyl-17β-hydroxyestr-4-en-3-one (**RTI-1176-012**). Acetate **4** (12 mg) was dissolved in methanol (2 mL) with water (0.2 mL) and potassium hydroxide (50 mg) at room temperature for 2 h. The reaction mixture was then diluted with water (10 mL), acidified with 3 N hydrochloric acid and extracted with ethyl acetate (3 × 10 mL). The organic layers were combined, washed with water and saturated sodium chloride solution. Removal of the solvents gave a gum which was purified by thick layer chromatography (chloroform–ethyl acetate 1:1) to give 7.9 mg (75% yield) of -**012**; IR (CH_2Cl_2), 3500 (OH), 1660 cm^{-1} (3-ketone); ^1H NMR ($CDCl_3$) δ 0.78 (d, 3, J = 7 Hz, 7α-CH_3), 0.88 (s, 3, 18-CH_3), 1.07 (d, 3, J = 8 Hz, 11β-CH_3), 3.64 (t, 1, 17α-H), 5.85 ppm (s, 1, 4-H); mass spectrum 302 (M^+, 100), 287 ($M^+ - CH_3$, 5), 284 ($M^+ - H_2O$, 13), 260 ($M^+ - CH_2CO$, 20).

Anal. Calcd for $C_{20}H_{30}O_2$, *m/z* 302.2247. Found: *m/z* 302.2243.

7β,11β-Dimethyl-17β-hydroxyestr-4-en-3-one (**RTI-1176-013**). Treatment of **5** (15 mg) with methanol–water–potassium carbonate afforded 7.2 mg (53% yield) of the corresponding 17β-alcohol (**7**) after purification by thick layer chromatography (chloroform–ethyl acetate 1:1); IR (CH_2Cl_2) 3600 (OH), 1660 cm^{-1} (3-ketone); ^1H NMR ($CDCl_3$) δ 0.88 (s, 3, 18-CH_3), 0.98 (d, 3, J = 7 Hz, 7β-CH_3), 1.07 (d, 3, J = 7 Hz, 11β-CH_3), 3.60 (m, 1, 17α-H), 5.82 ppm (s, 1, 4-H); mass spectrum 302 (M^+, 100), 287 ($M^+ - CH_3$, 11), 284 ($M^+ - H_2O$, 21), 260 (M^+ $-CH_2CO$, 20).

Anal. Calcd for $C_{20}H_{30}O_2$, *m/z* 302.2247. Found: *m/z* 302.2243.