Synthesis of fluorescent 4,6,8(14)-trien-3-one steroids via 3,5,7-trien-3-ol ethers. Important probes for steroid-protein interactions

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*A general synthesis of fluorescent 4,6,8(14)-trien-3-one steroids with and without an aliphatic side chain is described via 3-alkoxy-3,5,7-trienes as intermediates. The advantages of this method are general applicability, good yields, limited number of reaction steps (up to four), and ready availability of starting materials (at low cost). a) Starting from ergosterol **(1)** or cholesta-5,7-dien-3-ol **(2)** and oxidizing them to ergosta-4,7,22-trien-3-one **(3)** or cholesta-4,7-dien-3-one **(4)** we synthesized the enol ethers **(5)** and **(6)**. Subsequent treatment with DDQ gave the 4,6,8(14),22-tetraen-3-one **(7)** and the 4,6,8(14)-trien-3-one **(8)**. b) Similarly 17β-(1-oxopropoxy)-androsta-4,6,8(14)-trien-3-one **(13)** was obtained, but the required enol ether was synthesized via the 4,6-dien-3-one **(11)**.* (*Steroids* **59**:265–269, 1994)

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Introduction

In recent publications we demonstrated that the 4,6,8(14)-trien-3-one steroids show some very interesting fluorescence properties which were hitherto almost unknown:

a) Trienones without an aliphatic side chain in position C-17 (which are derived from the androstane and pregnane series) are highly fluorescent in aqueous solutions but not in aprotic solvents.[1] This fluorescence disappears completely upon binding to proteins, such as specific antibodies[2] or binding proteins (cortisol-binding globulin)[3] and reappears by competitive displacement with non-fluorescent steroids, making those fluorescent steroids useful probes for the investigation of steroid-protein interactions.[4]

b) Trienones with aliphatic side chain (derived from ergosterol and cholesterol) show only slight fluorescence in aqueous solutions due to micelle formation. Destroying these micelles—for example, in a 30:70 v/v-mixture of ethanol:water or by treatment with β-or γ-cyclodextrins—leads to a marked increase in fluorescence. Therefore these steroids are useful intrinsic labels in studying micellar systems and membranes[5] and in the investigation of the kinetics of micellation (Kempfle, Böhme, Winkler: to be published).

These characteristics can be used to study hydrophobic interactions of steroids as well with proteins and cyclodextrins[3,6] as with membrane systems[7] by measuring fluorescence and fluorescence quenching.

As the required steroids are not generally available, we had to synthesize them but all attempts to apply methods given in the literature did not lead to satisfactory results: either these procedures gave very small amounts only, or are not generally applicable, or are very complex (see Discussion).

Here we present a synthesis of 4,6,8(14)-trien-3-one steroids which is generally suitable for steroids with and without an aliphatic side chain. This is achieved by the synthesis of 3,5,7-trien-3-ol ethers via 4,7-dien-3-ones or 4,6-dien-3-ones.

Experimental

Melting points (mp) were determined on a Gallenkamp melting point apparatus and are uncorrected. Flash chromatography was carried out on Baker (Phillipsburg, USA) Silica Gel

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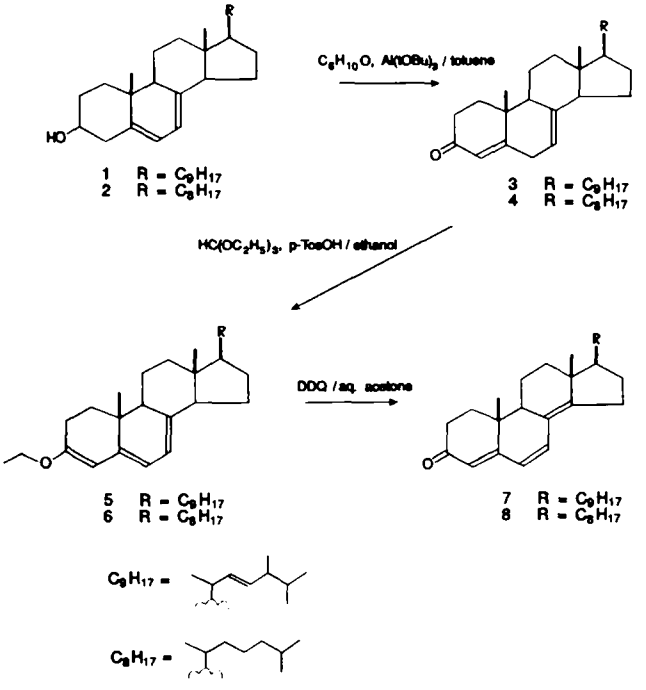
(40 μm), thin-layer chromatography (TLC) analyses on precoated silica gel F_{254} plates 0.2 mm thick (Merck, Darmstadt, Germany). Ergosterol and cholesta-5,7-dien-3β-ol were purchased from Fluka AG (Buchs, Switzerland), testosterone propionate from Serva (Heidelberg, Germany). Due to light sensitivity of the steroids with three conjugated C-C double bonds the reactions were carried out in subdued light. All (pure) trienol ethers showed blue to blue-green fluorescence at 365 nm in two spots on TLC (slight decomposition on silica gel). The conjugated trienones fluoresce strongly at 365 nm excitation on TLC (sterols: blue green, androstatrienone: blue).

Proton nuclear magnetic resonance (^{1}H NMR) spectra were measured on a Bruker AMX 500 spectrometer (Karlsruhe, Germany) in $CDCl_3$ solutions. Proton chemical shifts are referenced to the residual $CHCl_3$ signal (7.26 ppm). Coupling constants are expressed in Hertz. (Additional help for assignment of the signals was obtained by the ^{13}C NMR spectra and 2D experiments. The complete assignment of all protons and carbons for compounds **(1)–(13)** and others is in preparation and will be published later). High resolution electron impact mass spectra were recorded on a Kratos Analytical Instruments (Karlsruhe, Germany) AEI MS 50 spectrometer. Ultraviolet (UV) spectra in ethanol were recorded on a Shimadzu UV-160 UV-Vis recording spectrophotometer (Kyoto, Japan).

Steroids with aliphatic side chain (Scheme 1)

Ergosta-4,7,22-trien-3-one (Ergosterone) (3). Oppenauer oxidation of ergosterol **(1)** (50 mmol) was performed according to the literature,[8] but using aluminium *tert*-butylate and omitting the steam distillation. Yield 13.86 g, 70.3%, mp 130–131 C (acetone), reported 128–130 C (lit. 8) and 134 C (lit. 9) (petrol ether/acetone).

^{1}H NMR: δ ppm 0.60 (3H, s, 18-CH_3), 0.82 and 0.83 (3H/3H, d/d, J = 7.0/7.0 Hz, 27-CH_3/28-CH_3), 0.91 (3H, d, J = 7.0 Hz, 25-CH_3), 1.02 (3H, d, J = 6.9 Hz, 21-CH_3), 1.17 (3H, s, 19-CH_3), 1.47 (1H, octet, J = 6.5 Hz, 26-H), 2.66 (1H, dm, J = 19.3 Hz, 6-H_a), 3.14 (1H, dm, J = 19.3 Hz, 6-H_b), 5.16 (1H, dd, J = 15.0 and 8.0 Hz, 22-H), 5.22 (1H, dd, J = 15.0 and 7.2 Hz, 23-H), 5.78 (1H, d, J = 1.9 Hz, 4-H). MS m/z 394,3240 (M^+, calculated for $C_{28}H_{42}O$ 394.3236, 100%). UV λ_{max} 237.8 nm (ε 13000), reported 240 nm (ε 13300).[9]



Scheme 1 Synthetic route to 4,6,8(14)-trien-3-one with side chain R

Cholesta-4,7-dien-3-one (4). Cholesta-4,7-dien-3-one **(4)** was prepared from cholesta-5,7-dien-3-ol **(2)** (30 mmol) in the same way as ergosterone **(3)**. Yield 6.36 g, 55.5%, mp 86–88 C (acetone), reported 87–89 C (lit. 10). ^{1}H NMR data according to literature (lit. 10). MS m/z 382.3231 (M^+, calculated for $C_{27}H_{42}O$ 382.3236, 100%). UV λ_{max} 237.6 nm (ε 12700); reported 238 nm (ε 15500) (ethanol) (lit. 10).

3-Ethoxy-ergosta-3,5,7,22-tetraene (5). Ergosta-4,7,22-trien-3-one **(3)** (3.97 g, 10 mmol) was suspended in dry ethanol (130 mL) at 40 C and cooled to room temperature. The fine suspension was vigorously stirred under argon (mechnical stirrer) and triethyl orthoformate (3.35 mL, 20 mmol) and *p*-toluenesulfonic acid (50 mg) were added. Two to five minutes after the addition of the acid the enol ether began to precipitate and the suspension became a thick pulp. Stirring was continued for 45 min, then pyridine (30 drops) in methanol (10 mL) was added. After stirring in an ice-bath for additional 2 h the solid was filtered by suction and crystallized from *n*-hexane. The yield was 2.64 g, 62.5%; mp 121–122 C (*n*-hexane).

^{1}H NMR: δ ppm 0.65 (3H, s, 18-CH_3), 0.83/0.85 (3H/3H, d/d, J = 7.0/6.9 Hz, 27-CH_3/28-CH_3), 0.93 (3H, d, J = 6.9 Hz, 25-CH_3), 0.95 (3H, s, 19-CH_3), 1.05 (3H, d, J = 6.8 Hz, 21-CH_3), 1.32 (3H, t, J = 7.0 Hz, ether CH_3), 1.48 (1H, octet, J = 6.7 Hz, 26-H), 1.86 (1H, sextet, J = 6.8 Hz, 24-H), 2.14 (1H, ddd, J = 17.4 and 5.6 and 1.8 Hz, 2-H_a), 2.43 (1H, ddd, J = 17.4 and 12.8 and 5.0 Hz, 2-H_b), 3.81 (1H, dq, J = 9.4 and 7.0 Hz, ether CH_aO), 3.84 (1H, dq, J = 9.4 and 7.0 Hz, ether CH_bO), 5.19 (1H, dd, J = 15.0 and 7.5 Hz, 22-H), 5.21 (1H, d, J = 1.8 Hz, 4-H), 5.24 (1H, dd, J = 15.0 and 6.9 Hz, 23-H), 5.46 (1H, d, J = 5.9 Hz, 6-H), 5.50 (1H, dt, J = 5.9 and 2.7 Hz, 7-H). MS m/z 423.3593 ($C_{30}H_{47}O$, M^++H, 26%), 422.3555 (M^+, calculated for $C_{30}H_{46}O$ 422.3547, 100%), 407 (M^+-CH_3, 4%), 297 (M^+-C_9H_{17} side chain, 7%), 251 (M^+-C_9H_{17}-C_2H_5OH, 3%), 69 ($C_4H_5O^+$, 82%). UV λ_{max} 214 nm (ε 7200), 321.5 nm (ε 14500), 336-337 nm sh (ε 10500).

3-Ethoxy-cholesta-3,5,7-triene (6). A solution of cholesta-4,7-dien-3-one **(4)** (6 mmol) in dry ethanol (55 mL) was treated as described for 3-ethoxy-ergosta-3,5,7,22-tetraene **(5)**. Yield: 1.61 g, 65.4%, mp 126–127 C (*n*-hexane), reported 120-130 C (lit. 11). ^{1}H NMR data were according to literature (lit 11). MS m/z 411.3581 ($C_{29}H_{47}O$, M^+ + H, 26%), 410.3550 (M^+, calculated for $C_{29}H_{46}O$ 410.3549, 100%). UV λ_{max}: 321.3 nm (ε 19500), 337 nm sh (ε 14100); reported 322 nm (ε 17500), 337 nm (ε 11600) (lit. 11).

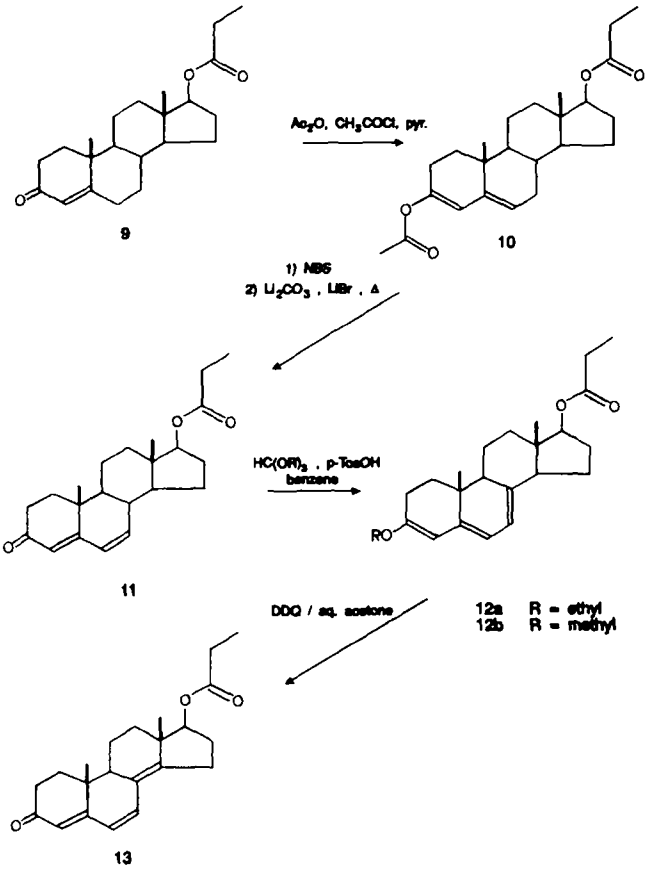
Ergosta-4,6,8(14),22-tetraen-3-one (7). 3-Ethoxyergosta-3,5,7,22-tetraene **(5)** (2.11 g, 5 mmol) in aqueous acetone (95%) (150 mL) was dissolved by stirring at 40–45 C. After cooling to room temperature and flushing the reaction flask with argon a freshly prepared solution of 2,3-dichloro-5,6-dicyano-p-benzoquinone (DDQ, 1.19 g, 5.24 mmol) in aqueous acetone (95%; 35 mL) was added through a dropping funnel within 4 min. After stirring for another 4–5 min the red solution (if necessary after filtration) was poured into diethyl ether (300 mL) and washed with brine. The aqueous layer was extracted once with ether. The combined organic layers were washed with the following solutions to remove excess quinone and hydroquinone: sodium disulfite 3% (2 × 300 mL), sodium

hydrogencarbonate 2% (3 × 300 mL), brine (2 × 300 mL), and water (2 × 300 mL). After drying over Na_2SO_4 and evaporating the solvents under reduced pressure, the remaining yellow solid was crystallized from methanol resulting in pale yellow plates, yield 1.55 g, 79.0%, mp 115 C (114–115 C; lit. 12).

^{1}H NMR data were according to literature[13] and additional (higher resolution): δ ppm 1.86 (1H, sextet, J = 6.7 Hz, 24-H), 2.02 (1H, ddd, J = 12.6 and 5.0 and 2.2 Hz, 1-H_b), 2.53 (1H, ddd, J = 17.7 and 14.1 and 5.0 Hz, 2-H_b), 5.19 (1H, dd, J = 15.4 and 8.0 Hz, 22-H), 5.25 (1H, dd, J = 15.4 and 7.4 Hz, 23-H). MS m/z 392.3077 (M^+, calculated for $C_{28}H_{40}O$ 392.3079, 24%), 268.1807 ($C_{19}H_{24}O$, M^+-C_9H_{17} side chain + H, 100%). UV λ_{max} 239 nm (ε 5800), 282 nm sh (ε 8500), 384.0 nm (ε 33500); reported 237 nm (ε 4700), 282 nm (ε 7100), 350 nm (ε 27100) (ethanol).[12]

Cholesta-4,6,8(14)-trien-3-one (8). 3-Ethoxycholesta-3,5,7-triene **(6)** (0.822 g, 2 mmol) was suspended in 80 mL acetone and stirred in a water bath at 40–50 C under argon until the solid dissolved. Water (4.25 mL, to achieve a 95% acetone solution) was added and the stirred mixture slowly cooled down to 30 C, resulting in a finely dispersed suspension (if the suspension is not fine enough the steroid will not dissolve during the reaction). DDQ (0.48 g, 2,11 mmol) in aqueous acetone (95%, 20 mL) was added within 4 min. Stirring was continued for another 5 min, giving a dark red but clear solution, which was poured into diethyl ether (200 mL) and the work-up was continued as described in the ergostatetraenone section. Crystallization from acetone at −18 C (crystal seeds) resulted in a yellow solid (0.548 g, 72.0%), mp 60–61 C (acetone); reported 62 C (lit. 10), 58–62 C (lit. 11).

^{1}H NMR data were in accordance with literature.[11] MS m/z 380.3074 (M^+, calculated for $C_{27}H_{40}O$ 380.3079, 43%). UV λ_{max} 348.2 nm (ε 26900) (ethanol); reported 348 nm (ε 25500) (acetonitrile; lit. 10).



Scheme 2 Synthetic route to 4,6,8(14)-trien-3-ones without side chain

Steroids without aliphatic side chain (Scheme 2)

3-Acetoxy-17β-(1-oxopropoxy)-androsta-3,5-diene (10). 17β-(1-Oxopropoxy)-androst-4-en-3-one (testosterone propionate) **(9)** (10.34 g, 30 mmol, dissolved in acetic anhydride 132 mL), pyridine (5.34 ml, 0.066 mol), and acetyl chloride (26.67 ml, 0.375 mol) were refluxed under argon for 3 h.[14] After cooling the precipitated solid was filtered. To remove excess acetic anhydride the filtrate was evaporated repeatedly with ethanol at reduced pressure and concentrated to give more product. The solids were recrystallized once or twice from ethanol. Yield of white needles 10.26 g, 88.5%, mp 146–147 C (ethanol).

^{1}H NMR: δ ppm 0.82 (3H, s, 18-CH_3), 1.00 (3H, s, 19-CH_3), 1.13 (3H, t, J = 7.6 Hz, propionyl CH_3), 1.85 (1H, ddd, J = 12.9 and 5.7 and 1.1 Hz, 1-H_b), 2.13 (1H, s, acetyl CH_3), 2.32 (1H, q, J = 7.6 Hz, propionyl CH_2), 2.44 (1H, dddd, J = 18.2 and 12.1 and 5.7 and 2.2 Hz, 2-H_b), 4.62 (1H, dd, J = 9.0 and 8.0 Hz, 17-H), 5.39 (1H, s, 6-H), 5.68 (1H, d, J = 2.2 Hz, 4-H). MS m/z 386.2474 (M^+ calculated for $C_{24}H_{34}O_4$ 386.2457, 2.4%), 345.2396 ($C_{22}H_{33}O_3$, M^+-C_2H_2O acetyl + H, 18%), 344.2351 ($C_{22}H_{32}O_3$ M^+-C_2H_2O, 100%). UV λ_{max} 234.4 nm (ε 19200).

17β-(1-Oxopropoxy)-androsta-4,6-dien-3-one (6-dehydrotestosterone propionate) (11). To a cooled suspension of 3-acetoxy-17β-(1-oxopropoxy)-androsta-3,5-diene **(10)** (7.73 g, 20 mmol) in dimethylformamide (30 mL) and water (0.44 mL) *N*-bromosuccinimide (3.70 g, 20.8 mmol) was added under argon in 6 portions during a period of 1 h,[15] the temperature not rising above 2 C. After stirring for another 0.5 h at 0 C lithium carbonate (3.45 g, 48 mmol) and lithium bromide (1.86 g, 21 mmol) were added and the suspension heated under argon for 3 h at 95 C.[16] The cooled suspension was poured into ice water with acetic acid (150 mL + 10 mL) and diethyl ether (200 mL). The aqueous layer was extracted twice with ether; the ether layers were washed with H_2O, 1% aqueous solution of $NaHCO_3$, and H_2O again. After drying over Na_2SO_4 the solvent was evaporated. Crystallization from ether afforded yellow prisms or powder. Yield 6.22 g, 90.8%, mp 132–133 C (ether), reported 134 C (hexane).[17]

^{1}H NMR: δ ppm 0.77 (3H, s, 18-CH_3), 1.11 (3H, s, 19-CH_3), 1.13 (3H, t, J = 7.7 Hz, propionyl CH_3), 1.71 (1H, td, J = 13.9 and 4.6 Hz, 1-H_a), 1.99 (1H, ddd, J = 13.2 and 5.4 and 2.0 Hz, 1-H_b), 2.32 (1H, q, J = 7.7 Hz, propionyl CH_2), 2.43 (1H, ddd, J = 18.2 and 4.6 and 2.0 Hz, 2-H_a), 2.56 (1H, ddd, J = 18.2 and 14.6 and 5.4 Hz, 2-H_b), 4.64 (1H, dd, J = 9.0 and 8.0 Hz, 17-H), 5.67 (1H, s, 4-H), 6.08 (1H, d, J = 10.0 Hz, 7-H), 6.11 (1H, dd, J = 10.0 and 2.1 Hz, 6-H). MS m/z 342.2192 (M^+ calculated for $C_{22}H_{30}O_3$ 342.2195, 100%). UV λ_{max} 282.5 nm (ε 27900).

3-Ethoxy-17β-(1-oxopropoxy)-androsta-3,5,7-triene (12a).[18] To a solution of 17β-(1-oxopropoxy)-androsta-4,6-dien-3-one **(11)** (2.91 g, 8.5 mmol) in dry benzene (50 mL) *p*-toluenesulfonic acid (18 mg) in dry ethanol (2 mL) and triethyl orthoformate (2 mL, 12 mmol, excess) were added under argon and the mixture refluxed under argon for 2.5 h (75–80 C). After cooling to room temperature pyridine (2 mL) was added to the solution, stirring for another 15 min. The benzenic solution was washed with water (4 ×), dried over sodium sulfate, and concentrated to dryness under reduced pressure (not over 40 C). The remaining orange oil (containing traces of pyridine) was dissolved in ethanol and stored at −18 C, to give a yellow solid, yield:

1.11 g, 35.2%. An analytical sample was obtained by Flash chromatography on silica gel with cyclohexane/ethyl acetate (12:1), mp 151–153 C; reported 156 to 161 (lit. 18).

^{1}H NMR similar to **(12b)** and additional signals δ ppm 1.34 (3H, t, J = 7.0 Hz, ether CH_3), 3.82 (1H, dq, J = 9.3 and 7.0 Hz, OCH_a ether), 3.86 (1H, dq, J = 9.3 and 7.0 Hz, OCH_b ether). MS m/z 371 (M^+ + H, 22%), 370.2516 (M^+ calculated for $C_{24}H_{34}O_3$ 370.2508, 100%), 355 (M^+-CH_3, 9%), 197 (M^+-173, 30%), UV λ_{max} 212 nm (ε 7600), 321 nm (ε 15300), 335 nm sh (ε 11300); reported 320.5 nm (ε 19600) (lit. 18).

Due to the small yields of the ethyl enol ether, the methoxy compound **(12b)** was synthesized, having the advantage of being less soluble and excess trimethyl orthoformate being removed easier.

3-Methoxy-17β-(1-oxopropoxy)-androsta-3,5,7-triene (12b). The methyl enol ether **(12b)** was prepared from 17β-(1-oxopropoxy)-androsta-4,6-dien-3-one **(11)** (12 mmol) in dry benzene (100 ml) similarly as described above with a slight modification: The steroid was stirred with the acid for 5 min and the trimethyl orthoformate (27 mmol) was added in a larger excess. Work-up gave an orange oil that was treated with methanol resulting in a yellow solid precipitate. More product (yellow powder) precipitated after storing at −18 C. Yield 2.69 g, 62.8%. An analytical sample was obtained by flash chromatography on silica gel with toluene/ethyl acetate (20:1), mp 145–147 C.

^{1}H NMR: δ ppm 0.74 (3H, s, 18-CH_3), 0.94 (3H, s, 19-CH_3), 1.14 (3H, t, J = 7.4 Hz, propionyl CH_3), 1.51 (1H, td, J = 12.8 and 5.7 Hz, 1-H_a), 1.89 (1H, ddd, J = 12.7 and 5.5 and 1.4 Hz, 1-H_b), 2.13 (1H, ddd, J = 17.1 and 5.7 and 1.4 Hz, 2-H_a), 2.33 (1H, q, J = 7.4 Hz, propionyl CH_2), 2.41 (1H, ddd, J = 17.1 and 12.9 and 5.5 Hz, 2-H_b), 3.61 (1H, s, OCH_3), 4.72 (1H, dd, J = 9.0 and 7.2 Hz, 17-H), 5.23 (1H, d, J = 1.9 Hz, 4-H), 5.49 (1H, d, J = 6.0 Hz, 6-H), 5.51 (1H, dt, J = 6.0 and 2.7 Hz, 7-H). MS m/z 357 (M^- + H, 22%), 356.2351 (M^+ calculated for $C_{23}H_{32}O_3$ 356.2351, 100%), 341 (356-CH_3, 13%). UV λ_{max} 212 nm (ε 8400), 321 nm (ε 18900), 335 nm sh (ε 13800).

Both enol ethers **(12a** and **12b)** could be used to synthesize the trienone **(13)**.

17β-(1-Oxopropoxy)-androsta-4,6,8(14)-trien-3-one (13). To 3-methoxy-17β-(1-oxopropoxy)-androsta-3,5,7-triene **(12b)** (1.87 g, 5.25 mmol) completely dissolved in 95% aqueous acetone (160 mL), DDQ (1.28 g, 5.6 mmol in acetone 95%, 40 mL) was added as described for compound **(7)**. Work-up of the greenish solution was similar and rendered a yellow oil which was dissolved in methanol. At −18 C yellow prisms crystallized. Yield 1.21 g, 67.8%, mp 89–91 C.

^{1}H NMR: δ ppm 0.96 (3H, s, 18-CH_3), 1.02 (3H, s, 19-CH_3), 1.11 (3H, t, J = 7.7 Hz, propionyl-CH_3), 1.98 (1H, ddd, J = 13.0 and 5.1 and 2.3 Hz, 1-H_b), 2.32 (2H, q, J = 7.7 Hz, propionyl-CH_2), 2.41 (1H, ddd, J = 18.0 and 5.2 and 2.3 Hz, 2-H_a), 2.49 (1H, ddd, J = 18.0 and 14.7 and 5.1 Hz, 2-H_b), 4.65 (1H, dd, J = 10.0 and 8.0 Hz, 17-H), 5.70 (1H, s, 4-H), 6.03 (1H, d, J = 9.8 Hz, 6-H), 6.54 (1H, d, J = 9.8 Hz, 7-H). MS m/z 340.2039 (M^- calculated for $C_{22}H_{28}O_3$ 340.2038, 26%), 285.1828 ($C_{19}H_{25}O_2$, M^+-C_3H_3O, 13%), 284.1779 ($C_{19}H_{24}O_2$, M^+-C_3H_4O, 100%), 267.1711 ($C_{19}H_{23}O$, M^+-$C_3H_5O_2$ side chain, 11%), 266.1674 ($C_{19}H_{22}O$, M^+-$C_3H_4O_2$ side chain-H, 72%). UV λ_{max} 239 nm (ε 5100), 280 nm sh (ε 7600), 340.4 nm (ε 23500). The propionate group can be removed easily by stirring with potassium hydroxide in methanol at room temperature to the desired androsta-4,6,8(14)-trien-3-one.

Results and discussion

Searching for a generally applicable chemical synthesis of trienone steroids starting from easily available precursors we attempted several processes described in the literature.

Many syntheses start with 5,7-dien-3-ols, which are commercially available for the steroids with side chain at C-17, such as ergosterol or 7-dehydrocholesterol. For the steroids without side chain, the 5,7-dien-3-ols have to be synthesized (in 3 steps) from 5-en-3-ol steroids. In the most cited method of Elks[12] ergosterol is oxidized in a modified Oppenauer reaction. However, the advantages of this "one pot" reaction are diminished by difficulties in the purification and thus resulting in low yields. The Johns' reaction[19] can be used with 5,7-dien-3-ols with or without a side chain and needs a photooxygenation step to produce endoperoxides, which then can be dehydrated to the trienones. This last step requires a long reaction time and a large excess of expensive palladium catalyst. Whalley and colleagues[20] oxidized the 3-hydroxy function in 5,7-dien-3-ols, while the diene is protected by addition of 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD), which can be removed by boron trifluoride etherate. The last step gives good yields of trienone, but the introduction of the protective group requires toxic phenylseleninic anhydride[21] or expensive PTAD.

Other useful intermediates are 4,7-dien-3-ones as described by Kruger.[22] Ergosterone (ergosta-4,7,22-trien-3-one) **(3)** is readily available through Oppenauer oxidation[8,9] of ergosterol. Pelc and Kodicek[23] performed the oxidation of ergosterone with DDQ in toluene, but the yields of tetraenone were poor. Kruger used DDQ in DMSO to obtain the tetraenone from ergosterol.[24] Our attempts to oxidize ergosterone directly with either DDQ or chloranil in toluene, dioxane, or DMSO resulted in a mixture of three substances (TLC): excess of ergosterone, a small degree of product simulating a high yield by its strong fluorescence, and a yellow decomposition product, all having similar R_f-values. In a recent publication Luu and colleagues[10] use DDQ in dioxane to oxidize cholesta-4,7-dien-3-one directly to the trienone.

We followed another pathway via the enol ether. This is a very mild method to synthesize the trienol ether of $\Delta^{4,7}$-dien-3-ones of sterols in ethanol as solvent. The product precipitates from the solvent and can be isolated easily (the yields are better than with dioxane as solvent).

The resulting 3-alkoxy-3,5,7-trien-3-ols are oxidized by DDQ to render the 4,6,8(14)-trien-3-ones, similar to the easy oxidation of the enol ethers of Δ^4-3-ketosteroids to the 4,6-dien-3-ones described by Pradhan and Ringold.[25] The reaction time is short, the yields are good. An advantage of the intermediate enol ether step is the difference of the R_f-values between starting material and product. This allows an easier separation and is a good control whether the enol ether is totally converted into the trienone.

Steroid hormones with a Δ^4-3-keto structure are easily converted into 4,6-dien-3-ones. Again the two-step

process involving an enol acetate leads to a better separation between starting material and product and purification of the latter. Testosterone propionate [17β-(1-oxopropoxy)-androsta-4-en-3-one] is commercially widely available and is easily converted into its enol acetate,[14] which is brominated and dehydrobrominated[15,16] into the 6-dehydro derivative **(11)**; both steps result in high yields and well-crystallized products.

To obtain the required 4,7-dien-3-ones from the 4,6-dien-3-ones Kruger[12] deconjugated them in DMSO via the enolates. Kruger used mainly 19-hydroxy substituted steroids as this group stabilizes the enolate. But our attempts to deconjugate the 6-dehydrotestosterone propionate (C-19 = CH_3) directly led to a mixture of 4–5 products, so this pathway was abandoned. 4,6-Dien-3-one steroids can be converted directly into the enol ethers, the 3-alkoxy-3,5,7-trienes.[18] The ethoxy and the methoxy enol ethers of 6-dehydrotestosterone propionate were synthesized by us. Both enol ethers can be used in the DDQ oxidation to produce the desired trienone.

To summarize our results the desired 4,6,8(14)-trien-3-one steroids with or without side chain are prepared by oxidation of 3,5,7-trienol ethers with DDQ in aqueous acetone, where the enol ethers are obtained from readily available intermediates (4,7-dien-3-ones in case of the sterols with side chain, and 4,6-dien-3-ones in case of the androstane derivatives). The described procedure may be suitable for other 4,6,8(14)-trien-3-one steroids, e.g., 4,6,8(14)-pregnatrien-3-one from progesterone (in preparation).

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