Improved Synthesis of 24-Epibrassinolide from Ergosterol

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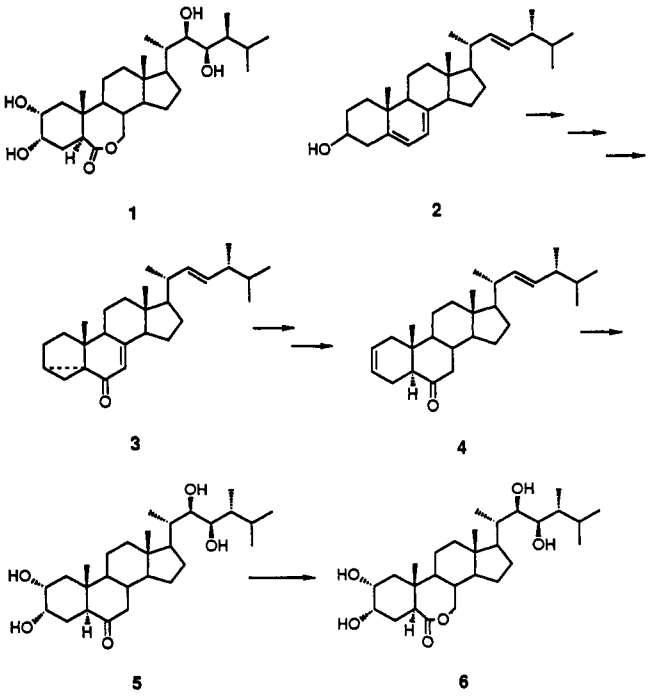
The discovery of brassinolide (1), the steroidal plant growth hormone, by scientists at the USDA laboratories has led to a resurgence of interest in the natural products chemistry of steroids.[1] Many analogs of brassinolide have been isolated from a wide variety of plants, and there has been intense activity in synthesis of these brassinosteroids, particularly because they hold promise for applications in agriculture.

We report here an improved method for converting inexpensive ergosterol to 24-epibrassinolide (6). The method is essentially the same as that reported by Thompson and co-workers,[2] but it has been shortened and two steps in particular can now be carried out reproducibly and in high yield.

Ergosterol (2) was converted to the mesylate at 10 °C and the product transformed to the *i*-sterol. The latter, which is sensitive to traces of acid, was oxidized to the corresponding ketone 3 with chromium trioxide–pyridine. Reduction with lithium in liquid ammonia afforded a cyclopropyl ketone which was isomerized to (22*E*,24*R*)-5α-ergosta-2,22-dien-6-one (4) in 80% yield by heating with pyridinium hydrochloride and lithium bromide in dimethylacetamide at 160 °C.[3] Several ways of effecting this isomerization have been published,[4] but we find the aforementioned method to be the most convenient.



Hydroxylation of 4 to give the tetrahydroxy ketone 5 can be carried out with osmium tetraoxide and *N*-methylmorpholine *N*-oxide. The 22*R*,23*R* diol was obtained in 30% yield and the isomeric 22*S*,23*S* product in 50% yield. By employing the method of asymmetric dihydroxylation reported recently[5] (OsO_4, $K_3Fe(CN)_6$ with the chiral ligand dihydroquinidine (DHQD) 4-chlorobenzoate in 2-methyl-2-propanol–H_2O (1:1)), the yield of the desired 22*R*,23*R* isomer (5) increased to 80%.

Use of the newly reported phthalazine ligand $(DHQD)_2$-PHAL[6] afforded further slight improvement in the ratio of the yield of 22*R*,23*R* diol to 22*S*,23*S* diol (from 9:1 to 10:1). The reaction rate was increased substantially in the presence of $CH_3SO_2NH_2$.[6]

Direct conversion of the tetrahydroxy ketone to 24-epibrassinolide (6) was accomplished in 80% yield with trifluoroperoxyacetic acid. The overall yield of 24-epibrassinolide from ergosterol was 26% in seven steps which is a considerable improvement over published methods.

Experimental Section

^1H NMR spectra were obtained at 300 MHz. Spectra were taken as solutions in $CDCl_3$ with Me_4Si as internal standard. Melting points were determined with a Kofler hot stage apparatus. Column chromatography was carried out with silica gel (Davisil 100–200 mesh and 230–425 mesh, Fisher Scientific). Analytical TLC was carried out on Whatman 4410 222 silica gel plates. Reactions were routinely monitored by TLC.

(22*E*,24*R*)-3α,5-Cyclo-5α-ergosta-7,22-dien-6-one (3). To a stirred solution of recrystallized erosterol (2, 50 g, 0.126 mol) in anhyd pyridine at 10 °C was added methanesulfonyl chloride (48.9 mL, 0.631 mol, 5 equiv) dropwise. After being stirred at 10 °C for 1 h the reaction mixture was poured onto crushed ice–water with vigorous stirring. The resulting precipitate was collected by filtration, washed well with water, and dried under suction overnight to afford 60 g of ergosterol mesylate (>90% pure, by ^1H NMR) which was used without further purification: ^1H NMR ($CDCl_3$) δ 0.63 (3 H, s, 18-H), 0.82 (3 H, d, J = 6.9 Hz, 26-H), 0.84 (3 H, d, J = 6.9 Hz, 27-H), 0.92 (3 H, d, J = 6.9 Hz, 28-H), 0.96 (3 H, s, 19-H), 1.04 (3 H, d, J = 6.9 Hz, 21-H), 1.22–2.15 (m, not assigned), 3.02 (3 H, s, SO_3CH_3), 4.63 (1 H, m, 3-H), 5.2 (2 H, m, 22-H and 23-H), 5.39 (1 H, m, 7-H), 5.6 (1 H, m, 6-H); MS m/z 474 (M^+), 378, 363, 253, 199, 157, 105, 91, 69, 43.

The finely powdered mesylate (50 g, 0.106 mol) was added in portions to a refluxing solution of $KHCO_3$ (10.56 g, 0.106 mol) in water (300 mL) and acetone (1.2 L). After the addition of mesylate was complete, acetone was distilled off until the mixture became cloudy, whereupon it was cooled in an ice bath and ice–water (500 mL) was added. Residual acetone was removed under reduced pressure at rt to precipitate out the *i*-sterol which was collected by filtration, washed with water several times, and dried overnight under suction to afford 38 g of *i*-sterol (>90% pure, by ^1H NMR) which was used without further purification. A small portion of *i*-sterol was recrystallized from MeOH: mp 131–132 °C [lit.[7] mp 131–133 °C (acetone)]; ^1H NMR ($CDCl_3$) 0.48 (2 H, m, 4-H), 0.64 (3 H, s, 18-H), 0.83 (3 H, d, J = 6.9 Hz, 26-H), 0.84 (3 H, d, J = 6.9 Hz, 27-H), 0.92 (3 H, d, J = 6.9 Hz, 28-H),

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1.03 (3 H, d, J = 6.9 Hz, 21-H), 1.08 (3 H, s, 19-H), 1.2–2.1 (m, not assigned), 3.4 (1 H, brs, OH), 5.2 (2 H, m, 22-H and 23-H), 5.47 (1 H, m, 7-H).

To a solution of *i*-sterol (30 g, 0.076 mol) in anhyd pyridine (300 mL) at rt was added CrO_3 (30.4 g, 0.304 mol, 4 equiv) in anhyd pyridine (300 mL) [prepared at rt by adding CrO_3 in portions to pyridine with good stirring. Caution: Do not add pyridine to CrO_3 as the mixture usually inflames.] The mixture was stirred at rt for 24 h. After dilution with Et_2O (1 L), the mixture was filtered and the filtrate washed several times with water, dried, and concentrated. The crude product was purified by flash chromatography on silica gel (hexane–EtOAc) to give enone **3** (24 g, 80%): mp 168–169 °C (ether) [lit.[7] mp 168–169 °C (ether)]; ^{1}H NMR ($CDCl_3$) δ 0.68 (3 H, s, 18-H), 0.76 (1 H, t, J = 4.5 Hz, 3-H), 0.83 (3 H, d, J = 6.9 Hz, 26-H), 0.84 (3 H, d, J = 6.9 Hz, 27-H), 0.92 (3 H, d, J = 6.9 Hz, 28-H), 1.05 (3 H, d, J = 6.9 Hz, 21-H), 1.25–2.3 (m, not assigned), 5.22 (2 H, m, 22-H and 23-H), 5.8 (1 H, brs, 7-H).

(22*E*,24*R*)-5α-Ergosta-2,22-dien-6-one (4). Li (2.49 g, 0.356 mol, 7 equiv) was added in small pieces to anhyd NH_3 (500 mL). After the dark blue solution was stirred for 15 min, a solution of enone **3** (20 g, 0.0508 mol) in anhyd THF (20 mL) was added dropwise. The reaction mixture which was still dark blue was stirred for 10 min. Anhydrous NH_4Cl was cautiously added in small portions until the blue color disappeared. NH_3 was allowed to evaporate overnight, and THF was removed in vacuo. To the residue was added water, and the mixture was extracted with CH_2Cl_2 (4 × 75 mL). The combined organic extracts were washed with water, dried, concentrated, and recrystallized from aqueous acetone to give *i*-sterone (17.1 g, 80%): mp 110–111 °C. [Lit.[4] mp 110–111 °C (acetone)]; ^{1}H NMR ($CDCl_3$) δ 0.72 (3 H, s, 18-H), 0.81 (3 H, d, J = 6.9 Hz, 26-H), 0.83 (3 H, d, J = 6.9 Hz, 27-H), 0.91 (3 H, d, J = 6.9 Hz, 28-H), 1.0 (3 H, s, 19-H), 1.01 (3 H, d, J = 6.9 Hz, 21-H), 1.09–2.07 (m, not assigned), 5.18 (2 H, m, 22-H and 23-H).

A mixture of *i*-sterone (15 g, 0.038 mol), pyridinium hydrochloride (0.88 g, 7.6 mmol, 0.2 equiv), anhyd LiBr (1.65 g, 0.019 mol, 0.5 equiv), and N,N-dimethylacetamide (150 mL) was heated at 160 °C in an argon atmosphere for 3 h. The reaction mixture was allowed to come to rt and then poured onto crushed ice–water. The precipitate was collected by filtration, washed well with water, and dried under suction. Recrystallization from MeOH afforded olefin **4** (12 g, 80%): mp 123–124 °C (MeOH) [lit.[4] mp 122–124 °C (MeOH)]; ^{1}H NMR ($CDCl_3$) δ 0.68 (3 H, s, 18-H), 0.71 (3 H, s, 19-H), 0.82 (3 H, d, J = 6.9 Hz, 26-H), 0.83 (3 H, d, J = 6.9 Hz, 27-H), 0.91 (3 H, d, J = 6.9 Hz, 28-H), 1.01 (3 H, d, J = 6.9 Hz, 21-H), 1.1–2.4 (m, not assigned), 5.18 (2 H, m, 22-H and 23-H), 5.57 (1 H, m, 2-H), 5.68 (1 H, m, 3-H).

24-Epicastasterone (5). A mixture of olefin (3 g, 7.58 mmol), $K_3Fe(CN)_6$ (14.97 g, 45.5 mmol, 6 equiv), anhyd K_2CO_3 (6.29 g, 45.5 mmol, 6 equiv), methanesulfonamide (1.44 g, 15.2 mmol, 2 equiv), dihydroquinidine 4-chlorobenzoate (0.71 g, 1.52 mmol, 0.2 equiv), and OsO_4 (0.075 g, 0.3 mmol, 0.04 equiv) in *t*-BuOH–water (1:1, 300 mL) was stirred at rt for 6 days (Without methane sulfonamide, reaction was only 50% complete after stirring for 8 days). Solid sodium sulfite (6 g) was added, and the mixture was stirred at rt for 18 h. *t*-BuOH was removed under reduced pressure, and the residue was extracted with EtOAc (6 × 50 mL). Combined organic extracts were washed with water (1 × 50 mL), 0.25 M H_2SO_4 (3 × 50 mL) to recover the ligand, and brine (1 × 50 mL), dried, and concentrated. The crude product was purified by flash chromatography on silica gel. Elution with $CHCl_3$–EtOH (9:1) provided the less polar (22*S*,23*S*,24*R*)-2α,3α,-22,23-tetrahydroxy-5α-ergostan-6-one (0.3 g, 8.5%): mp 184–185 °C (EtOAc) [lit.[4] mp 184–185 °C (EtOAc)]; ^{1}H NMR ($CDCl_3$) δ 0.67 (3 H, s, 18-H), 0.72 (3 H, s, 19-H), 0.85 (3 H, d, J = 6.9 Hz, 26-H), 0.87 (3 H, d, J = 6.9 Hz, 27-H), 0.94 (3 H, d, J = 6.9 Hz, 28-H), 1.0 (3 H, d, J = 6.9 Hz, 21-H), 1.03–2.71 (m, not assigned), 3.56 (1 H, brs), 3.69 (2 H, m), 3.99 (1 H, brs).

Further elution with the same solvent gave the more polar (22*R*,23*R*,24*R*)-2α,3α,22,23-tetrahydroxy-5α-ergostan-6-one (**5**, 2.8 g, 80%): mp 241–242 °C (EtOAc) [lit.[4] mp 241–242 °C (EtOAc)]; ^{1}H NMR ($CDCl_3$) δ 0.68 (3 H, s, 18-H), 0.76 (3 H, s, 19-H), 0.85 (3 H, d, J = 6.9 Hz, 26-H), 0.87 (3 H, d, J = 6.9 Hz, 27-H), 0.92 (3 H, d, J = 6.9 Hz, 28-H), 0.98 (3 H, d, J = 6.9 Hz, 21-H), 1.03–2.15 (m, not assigned), 2.3 (2 H, dd, J = 12.6 and 4.8 Hz), 2.69 (1 H, dd, J = 12.6 and 3.3 Hz), 3.41 (1 H, t, J = 5.3 Hz), 3.7 (1 H, brd, J = 4.2 Hz), 3.77 (1 H, m), 4.05 (1 H, brs).

24-Epibrassinolide (6). A solution of epicastasterone (**5**, 2.5 g, 5.39 mmol) in $CHCl_3$ (150 mL) was added dropwise to a stirred solution of trifluoroperoxyacetic acid (53.9 mmol, 10 equiv) [prepared from 30% aqueous H_2O_2 (6.1 mL, 53.9 mmol) and TFAA (38.1 mL, 0.27 mol, 5 equiv) in $CHCl_3$ (50 mL)] at 0 °C. The reaction mixture was stirred for 2 h at rt and diluted with $CHCl_3$ (100 mL), and the resulting solution was washed with water (1 × 25 mL), aqueous Na_2CO_3 (2 × 25 mL), aqueous $NaHSO_3$ (2 × 25 mL), and brine and dried. Evaporation of the solvent gave a colorless solid which was recrystallized from EtOAc to give (22*R*,23*R*,24*R*)-2α,3α,22,23-tetrahydroxy-β-homo-7-oxa-5α-ergostan-6-one (**6**, 2.07 g, 80%): mp 256–257 °C (EtOAc) [lit.[4] mp 256–258 °C (EtOAc)]; ^{1}H NMR ($CDCl_3$) δ 0.71 (3 H, s, 18-H), 0.85 (3 H, d, J = 6.9 Hz, 26-H), 0.87 (3 H, d, J = 6.9 Hz, 27-H), 0.92 (3 H, d, J = 6.9 Hz, 28-H), 0.92 (3 H, s, 19-H), 0.97 (3 H, d, J = 6.9 Hz, 21 H), 1.17–2.3 (m, not assigned), 3.12 (1 H, dd, J = 12.3 and 4.8 Hz), 3.41 (1 H, t, J = 5.4 Hz), 3.69 (2 H, m), 4.03 (1 H, brs), 4.1 (1 H, m); CIMS (isobutane) m/z 481 ($M^+ + 1$, base peak), 463, 445.

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Supplementary Material Available: NMR spectral data for compounds **2–6** (7 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.