A New Synthesis of Brassino Steroids: Plant Growth Promoting Steroids

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(22*R*,23*R*)- and (22*S*,23*S*)-2α,3α,22,23-Tetrahydroxy-B-homo-7-oxa-5α-ergostan-6-one, two physiologically active brassino steroids, have been synthesized *via* key intermediate brassicasterol obtained in a very simple way by reduction with lithium dissolved in ethylamine of the 1,4-cycloadduct of ergosterol and 4-phenyl-1,2,4-triazoline-3,5-dione.

Brassinolide (1)[1] a plant-growth stimulator is a naturally occurring steroidal lactone which promotes cell division, cell elongation, and plant growth.[2,3] Recently some brassino steroids, synthetic analogues of (1), were also found to possess strong activity in the rice-lamina inclination assay.[4a] The strongest activity among brassino steroids was shown by (22*R*,23*R*)-2α,3α,22,23-tetrahydroxy-B-homo-7-oxa-5α-ergostan-6-one (2) and (22*S*,23*S*)-2α,3α,22,23-tetrahydroxy-B-homo-7-oxa-5α-ergostan-6-one (3).[4a]

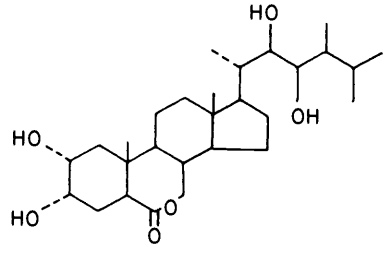
The biological activity of (2) and (3) stimulated our present work in which we report a new synthesis of brassino steroids starting from ergosterol acetate (4) (Scheme). The acetate (4) was treated with 4-phenyl-1,2,4-triazoline-3,5-dione to afford the Diels-Alder adduct (5) which by reduction with ethylamine dissolved lithium [5] afforded a 3 : 2 mixture of (22*E*)-ergosta-5,22-dien-3β-ol (6a) (brassicasterol) and of (22*E*)-5α-ergosta-7,22-dien-3β-ol (7a). Brassicasterol (6a) and its isomer (7a) are separated by chromatography on $AgNO_3$-impregnated silica. However since we wanted to elaborate the nuclear functionalities of brassino steroids *via* formation of an *i*-sterol which could be formed by solvolysis of (6b) alone, we decided to utilize in the successive reaction the mixture of (6a) and (7a).

Solvolysis of the tosylate (6b) in mixture with (7b) gave (22*E*)-3α,5-cyclo-5α-ergost-22-en-6β-ol (8a) [6] and unchanged (7b). The *i*-sterol (8a) is easily separated by chromatography on silica and oxidized by Jones reagent to the corresponding (22*E*)-3α,5-cyclo-5α-ergost-22-en-6-one (8b).

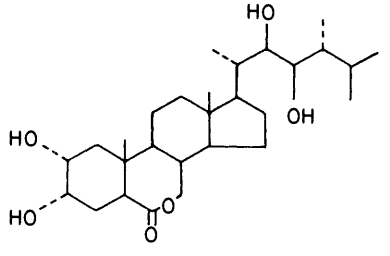
In order to introduce the 2α,3α-dihydroxy-function the cyclopropyl ketone (8b) had to be converted into the isomeric Δ^2-ene. Similar transformations have been achieved by acid catalyzed opening of the cyclopropane ring to give a 3β-acetoxy-6-ketone followed by saponification, tosylation, and detosylation to the required Δ^2-ene [4a,7] or, in one step, by refluxing the cyclopropyl ketone with a little toluene-*p*-sulphonic acid in sulpholane.[4b,8] In our hands the more successful isomerization was achieved by quantitative conversion of (8b) into (22*E*)-3β-chloro-5α-ergost-22-en-6-one (9) by treatment with hydrochloric acid in acetic acid [9] and successive dehydrohalogenation of (9) to (22*E*)-5α-ergosta-2,22-dien-6-one (10) by heating with lithium bromide in dimethylformamide. Treatment of (10) with *N*-methylmorpholine *N*-oxide in the presence of a catalytic amount of osmium tetraoxide [4b,10] afforded a mixture of known (22*R*,23*R*)-2α,3α,22,23-tetrahydroxy-5α-ergostan-6-one (11) and (22*S*,23*S*)-2α,3α,22,23-tetrahydroxy-5α-ergostan-6-one (12). Separation of the tetrahydroxy-ketones was achieved by rapid chromatography.†

Structures (11) and (12) were in accordance with the observed physicochemical properties of the compounds. The orientation of the hydroxy-groups at C-22 and C-23 in the side chain were assigned by comparison of the optical rotations and melting points observed for the compounds with those reported by Thompson *et al.*[4a] for compounds whose structure was derived by *X*-ray analyses of the products of Baeyer-Villiger oxidation of the 6-ketone. In addition Baeyer-Villiger oxidation [4b] of (11) and (12) with trifluoroperacetic acid affords, after saponification, the brassino steroids (2) and (3). Physico-chemical properties of (2) and (3) are in complete agreement with those reported for the compounds of *X*-ray assigned structure.[4a]

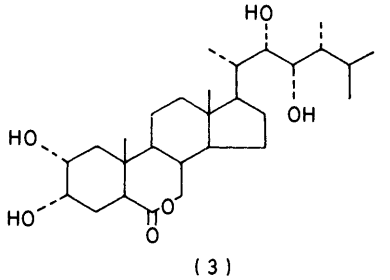
Our own synthesis of (2) and (3) has the merit of a relative brevity and simplicity of elaborating in a new way the nuclear functionalities of brassino steroids starting from ergosterol.



(1)

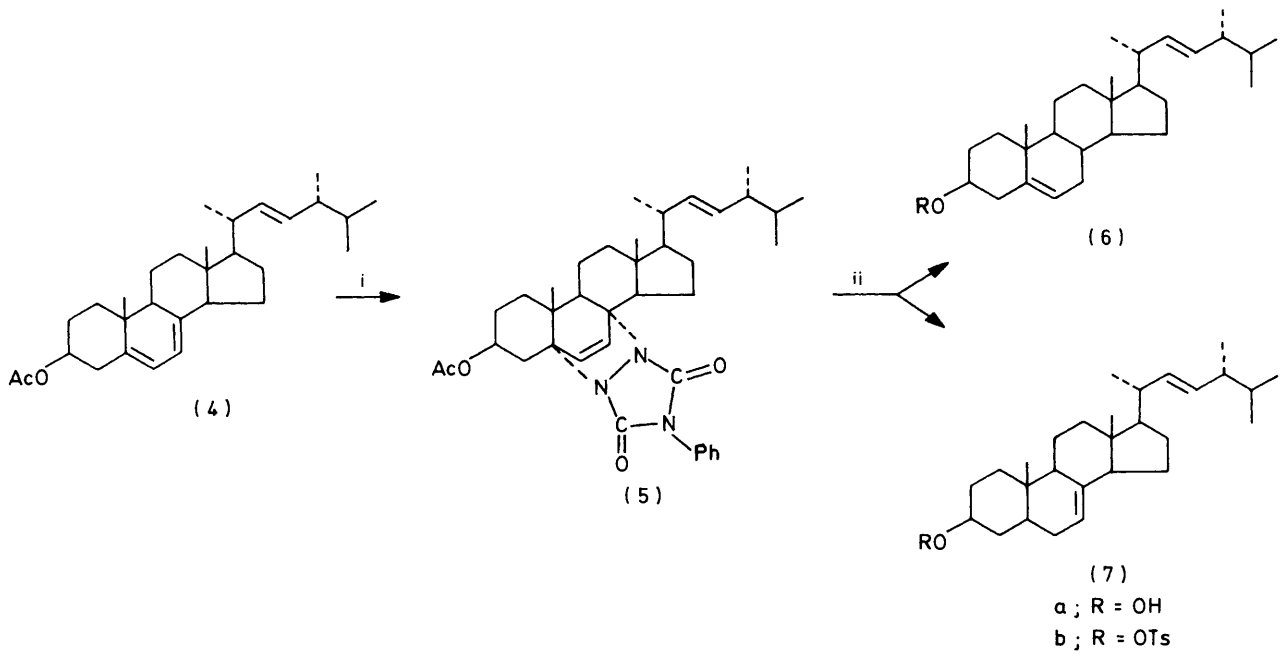


(2)

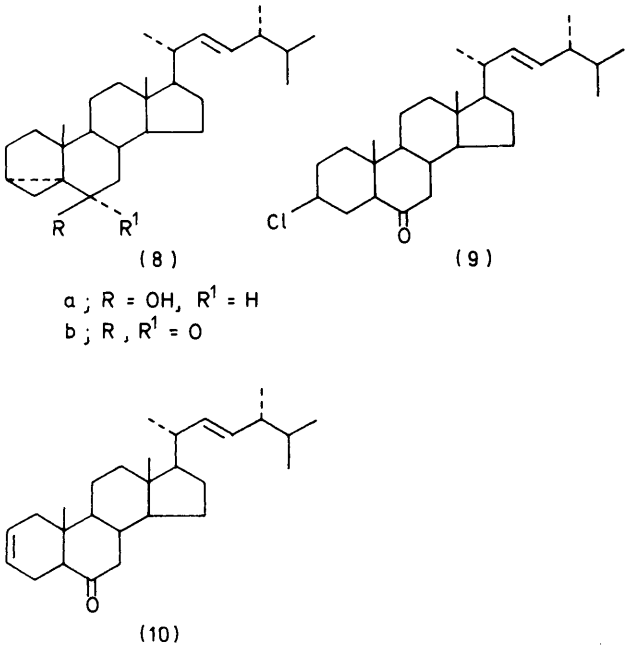


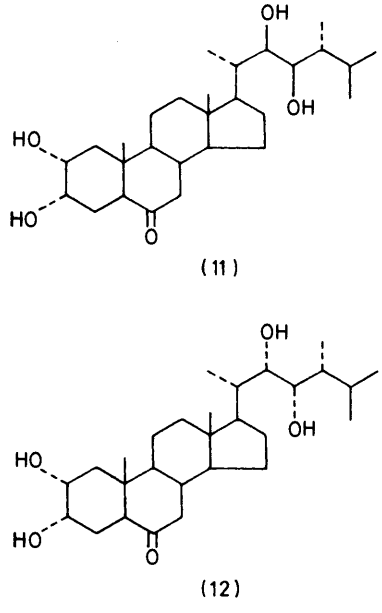
(3)

† During the preparation of this paper it was reported [11] that compounds (11) and (12) had been obtained in a mixture containing other isomers.



Scheme. *Reagents*: i, 4-phenyl-1,2,4-triazoline-3,5-dione; ii, Li, $EtNH_2$





Experimental

All m.p.s are uncorrected. I.r. spectra were recorded for solution in chloroform or for Nujol mulls.

^1H N.m.r. spectra were recorded on a Varian XL-100 spectrometer in [^2H]chloroform solutions with $SiMe_4$ as internal standard. Mass spectra were recorded on a Varian 122 S mass spectrometer (direct inlet). The progress of all reactions and column chromatographies (silica 230—400 mesh) was monitored by t.l.c. on E. Merck silica gel HF_{254} plates visualized by spraying with 70% sulphuric acid followed by heating. 1,4-Cycloaddition of 4-phenyl-1,2,4-triazoline-3,5-dione to ergosterol acetate (4) to yield the Diels-Alder adduct (5) was performed as reported by Barton *et al.*[12]

Synthesis of (22E)-*Ergosta*-5,22-*dien*-3β-*ol* (6a) *and of* (22E)-5α-*Ergost*-7,22-*dien*-3β-*ol* (7a).—The cyclic adduct (5) (8 g) dissolved in ethylamine (40 ml), was treated with lithium (1.6 g) and the mixture was stirred at reflux for 30 min longer than required for the initial appearance of a blue colour. Work-up afforded after extraction with chloroform a crude 3 : 2 mixture (5.1 g) of (22*E*)-ergosta-5,22-dien-3β-ol (6a) and (22*E*)-5α-ergosta-7,22-dien-3β-ol (7a). In one case the isomers (6a) and (7a) were separated by chromatography on silica gel G-celite (1 : 1 : 0.3) with 30% ethyl acetate–hexane as eluant to afford (i) (6a) (2.0 g), m.p. 149—150 °C (from ethanol), $[\alpha]_D^{20}$ −60° (lit.,[4a] m.p. 148°, $[\alpha]_D$ −64°); δ 0.69 (3 H, s, 18-H), 1.00 (3 H, s, 19-H), 3.6 (1 H, m, 3-H), 5.25 (m, 2 H, 22-H and 23-H); *m/z* 398 (Found: C, 84.6; H, 11.6. Calc. for $C_{28}H_{46}O$: C, 84.35; H, 11.63%); (ii) the isomer (7a) (1.4 g), m.p. 172—173 °C (from ethyl acetate), $[\alpha]_D^{20}$ −20°

(lit.,[13] m.p. 174—175 °C, $[\alpha]_D$ −21°); δ 0.55 (3 H, s, 18-H), 0.88 (3 H, s, 19-H), 3.6 (1 H, m, 3-H), and 5.25 (2 H, m, 22-H and 23-H); *m/z* 398 (Found: C, 84.5; H, 11.65. Calc. for $C_{28}H_{46}O$: C, 84.35; H, 11.63%).

It was unnecessary to separate the mixture of (6a) and (6b) which was thus used in the following reaction.

(22E)-3α,5-*Cyclo*-5α-*ergost*-22-*en*-6β-*ol* (8a).—A mixture of dried (6a) and (7a) (10 g, 3 : 2) in pyridine (80 ml) was treated with toluene-*p*-sulphonyl chloride (10 g) dissolved in pyridine (25 ml) at room temperature for 24 h. After work-up the wet tosylates (3 g portions) were dissolved in acetone (1.2 l) at 20 °C and were added to a boiling solution of potassium hydrogencarbonate (7 g) in water (360 ml). The mixture was refluxed for 6 h after which the acetone was evaporated under reduced pressure and the residue diluted with water and extracted with diethyl ether. The ethereal solution was evaporated and the residue was chromatographed on silica to afford (22-*E*)-3α,5-cyclo-5α-ergost-22-en-3β-ol (8a) (2.8 g), m.p. 116—117 °C (from acetone); $[\alpha]_D^{21}$ 16° (lit.,[6] 113—115 °C, $[\alpha]_D$ 15°); $\nu_{max.}$ 3 015 and 3 060 cm^{-1}; δ 0.30—0.60 (3 H, m), 0.72 (3 H, s), 1.02 (3 H, s), 3.24 (1 H, m, 6-H), 5.10—5.30 (2 H, m, 22- and 23-H); *m/z* 398 (Found: C, 84.4; H, 11.6. Calc. for $C_{28}H_{46}O$: C, 84.35; H, 11.63%). The tosylate (7b) was recovered unchanged after chromatography.

(22E)-3α,5-*Cyclo*-5α-*ergost*-22-*en*-6-*one* (8b).—Jones reagent [14] was added at −15 °C to a solution of (8a) (500 mg) in acetone (25 ml) until present in an excess; the excess was then discharged with methanol. After work-up the crude product was crystallized from methanol to yield (22*E*)-3α,5-cyclo-5α-ergost-22-en-6-one (8b) (460 mg), m.p. 110—111 °C (from moist acetone); $[\alpha]_D^{21}$ 6° (lit.,[6] m.p. 108—110 °C, $[\alpha]_D$ 5°); $\nu_{max.}$ 1 695 cm^{-1}; δ 0.30—0.60 (3 H, m), 0.72 (3 H, s), 1.00 (3 H, s), 5.10—5.30 (2 H, m, 22- and 23-H); *m/z* 396 (Found: C, 84.5; H, 11.3. Calc. for $C_{28}H_{44}O$: C, 84.78; H, 11.18%).

(22E)-3β-*Chloro*-5α-*ergosta*-22-*en*-6-*one* (9).—The ketone (8b) (600 mg) in acetic acid (12 ml) and hydrochloric acid (0.8 ml of a 37% solution) was kept for 10 min at room temperature. At this time a crystalline compound formed which was filtered off and washed with water to yield (22E)-3β-*chloro*-5α-*ergost*-22-*en*-6-*one* (9) (500 mg), m.p. 150—151 °C (from methanol); δ 0.70 (3 H, s), 0.98 (3 H, s), 5.15—5.30 (2 H, m, 22- and 23-H); *m/z* 396 (M^+ − HCl) (Found: C, 77.2; H, 10.5; Cl, 8.2. $C_{28}H_{45}ClO$ requires C, 77.17; H, 10.44; Cl, 8.16%).

(22E)-5α-*Ergosta*-2,22-*dien*-6-*one* (10).—The chloro-ketone (9) (350 mg) was dissolved in dimethylformamide (15 ml) and lithium bromide (450 mg) was added. The mixture was refluxed for 1 h and then poured into water and extracted with diethyl ether. Work-up followed by chromatography afforded (22*E*)-5α-ergosta-2,22-dien-6-one (10) (240 mg), m.p. 123—124 °C (from methanol); $[\alpha]_D^{21}$ 2° (lit.,[4a] m.p. 123—124 °C, $[\alpha]_D^{25}$ 3°); δ (200 MHz) 0.677 (3 H, s), 0.702 (3 H, s), 1.006 (3 H, d, *J* 6.7, C-21); *m/z* 396 (Found: C, 84.7; H, 11.3. Calc. for $C_{28}H_{44}O$: C, 84.78; H, 11.18%).

(22R,23R)-2α,3α,22,23-*Tetrahydroxy*-5α-*ergostan*-6-*one* (11) and (22S,23S)-2α,3α,22,23-*Tetrahydroxy*-5α-*ergostan*-6-*one* (12).—The ketone (10) (198 mg) dissolved in tetrahydrofuran (3 ml) was added to a mixture of *N*-methylmorpholine *N*-oxide hydrate (1.350 g) dissolved in water (2 ml) and tetrahydrofuran (3 ml) and osmium tetraoxide (10 mg in 5 ml of t-butyl alcohol). The mixture was then stirred at room temperature for 76 h. Methylene chloride was added and the base was eliminated by washing with dilute hydrochloric acid. The organic solution was then shaken with potassium hydroxide and mannitol (0.5 g in 5 ml of water). The crude product, isolated with the usual washing and drying procedures, was chromatographed [CH_2Cl_2–acetone, 1 : 1, (v/v)] to afford: (i) (22*S*,23*S*)-2α,3α,22,23)-tetrahydroxy-5α-ergostan-6-one (12) (75 mg), m.p. 184—185 °C (from ethyl acetate); $[\alpha]_D^{23}$ −3° lit.,[4a] 182—183°, $[\alpha]_D^{25}$ −2°); $\nu_{max.}$ 1 695 cm^{-1}; δ 0.69 (3 H, s), 0.75 (3 H, s), 2.70 (1 H, m), 3.56 (1 H, m), 3.72 (1 H, m), 3.79 (1 H, m), and 4.06 (1 H, m); *m/z* 364 (M^+ − 100) (Found: C, 72.5; H, 10.4. Calc. for $C_{28}H_{48}O_5$: C, 72.41; H, 10.43%); (ii) (22*R*,23*R*)-2α,3α,22,23-tetrahydroxy-5α-ergostan-6-one (11) (78 mg), m.p. 241—242 °C (from ethyl acetate); $[\alpha]_D^{23}$ 1° (lit.,[4a] m.p. 241—242 °C, $[\alpha]_D^{23}$ 0°); δ 0.69 (3 H, s), 0.75 (3 H, s), 2.70 (1 H, m), 3.56 (1 H, m), 3.72 (1 H, m), 3.79 (1 H, m), and 4.06 (1 H, m); *m/z* 364 (M^+ − 100) (Found: C, 72.4; H, 10.5. Calc. for $C_{28}H_{48}O_5$: C, 72.41; H, 10.43%).

(22R,23R)- and (22S,23S)-2,3,22,23-*Tetrahydroxy*-B-*homo*-7-*oxa*-5-*ergostan*-6-*one* (2) and (3).—To the tetrahydroxyketone (100 mg) dissolved in moist methylene chloride (5 ml) was added at 0 °C trifluoroperacetic acid (1.1 ml of a 0.6M solution in moist methylene chloride, trifluoroacetic acid) in the presence of Na_2HPO_4 (350 mg); the mixture was then shaken for 4 h. The crude product of the reaction was treated with 0.2 M-methanolic sodium hydroxide at reflux for 30 min. The hot solution was then acidified with 6M-hydrochloric acid and heated at reflux for 10 min. Work-up afforded: (i) (22*R*,23*R*)-2α,3α,22,23-tetrahydroxy-B-homo-7-oxa-5α-ergostan-6-one (2) (65 mg) [from (11)], m.p. 256—258 °C (from ethyl acetate); $[\alpha]_D^{21}$ 32° (lit.,[4a] m.p. 256—258 °C; $[\alpha]_D^{25}$ 30°); $\nu_{max.}$ (KBr) 3 490, 3 425, 1 705, and 1 670 cm^{-1}; δ (C_5D_5N) 0.70 (3 H, s), 1.02 (3 H, s), 3.50—3.73 (3 H, overlapping), and 4.10 (3 H, overlapping); *m/z* 462 (M^+ − H_2O) (Found: C, 70.1; H, 10.0. Calc. for $C_{28}H_{48}O_6$: C, 70.03; H, 9.85%); (ii) (22*S*,23*S*)-2α,3α,22,23-tetrahydroxy-B-homo-7-oxa-5α-ergostan-6-one (3) (68 mg) [from (12)], m.p. 193—195 °C (from ethyl acetate); $[\alpha]_D^{21}$ 30° (lit.,[4a] m.p. 194—195°C; $[\alpha]_D^{25}$ 31°); $\nu_{max.}$ (KBr) 3 490, 3 425, 1 705, and 1 670 cm^{-1}; δ (C_5D_5N) 0.68 (3 H, s), 1.03 (3 H, s), 3.50—3.73 (3 H, overlapping), and 4.10 (3 H, overlapping); *m/z* 462 (M^+ − H_2O) (Found: C, 70.15; H, 9.7. Calc. for $C_{28}H_{48}O_6$: C, 70.03; H, 9.85%).

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