Studies on Steroidal Plant-Growth Regulators: A New Synthesis of Brassinosteroids

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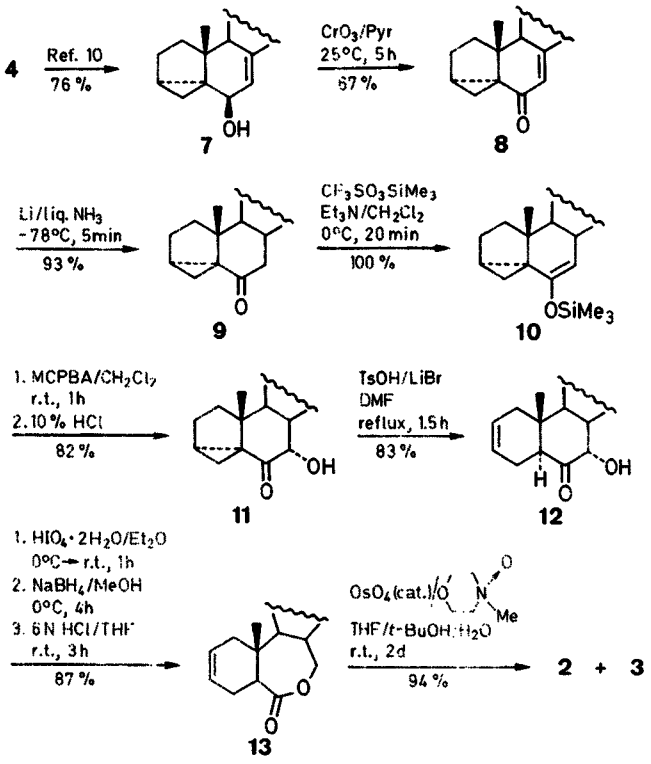
Two brassinosteroids (stereoisomers of brassinolide), (22*R*,23*R*,24*R*)-2α,3α,22,23-tetrahydroxy-B-homo-7-oxa-5α-ergostan-6-one (**2**) and (22*S*,23*S*,24*R*)-2α,3α,22,23-tetrahydroxy-B-homo-7-oxa-5α-ergostan-6-one (**3**), were synthesized from ergosterol in eight steps in ca. 30% overall yield. The key step is the highly regioselective formation of the 7-oxalactone ring by oxidation of an enol silyl ether with 3-chloroperoxybenzoic acid.

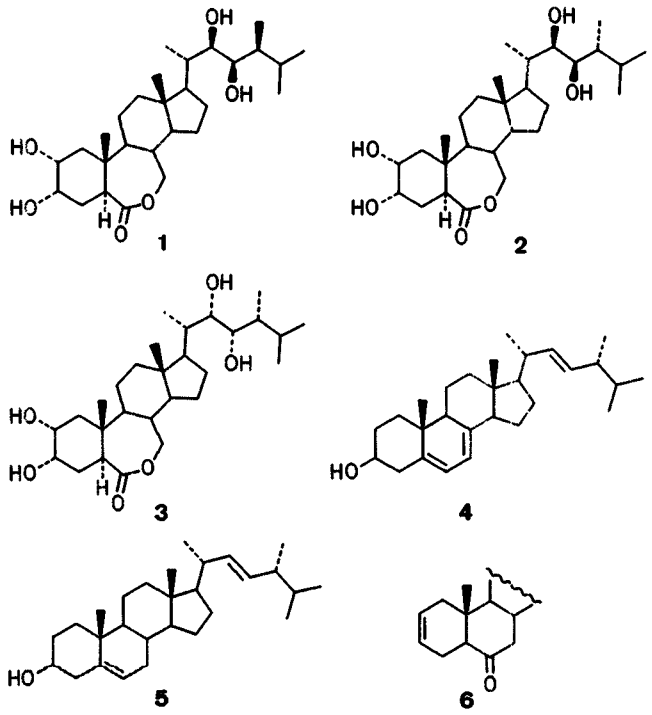
Brassinolide (**1**) is a plant promoting steroid.[1] The importance of its biological activities and novel chemical structure stimulated many laboratories to engage in the synthesis of this substance and its anologues[2] and to investigate the relationship between its structure and biological activity.[3] Recently, two stereoisomers of brassinolide, **2** and **3**, which possess strong activity in the rice-lamina inclination test, have been synthesized.[4-6] Very recently, compound **2** was also isolated from the bee pollen of the broad bean (*Vicia faba* L.) by Ikekawa and his co-workers.[7] The high biological activity of brassinosteroids **2** and **3** prompted us to report our efficient synthesis of them from ergosterol (**4**).

Introduction of the 2α,3α-dihydroxy-B-homo-7-oxa-5α-6-oxo-moiety of **2** and **3** had to be achieved from the key intermediate **6** obtained from ergosterol (**4**) or brassicasterol (**5**) via osmylation and Baeyer–Villiger oxidation. However, the Baeyer–Villiger oxidation gave also a structural isomer, a 2α, 3α-dihydroxy-B-homo-6-oxa-5α-7-oxo-compound, as by-product.[8] In the light of our report[9] on the highly regioselective formation of a 7-oxalactone ring (B-homo-7-oxa-6-oxo-moiety) by oxidation of an enol silyl ether (cf. compound **10**) with ozone, we now wish to report the use of this highly regioselective method to prepare the 7-oxalactone ring of brassinosteroids.

i-Ergosterol (**7**) was obtained from ergosterol (**4**) in 76% yield by a known method.[10] Oxidation of **7** to **8** with chromic acid in pyridine in 67% yield, followed by reduction of **8** with lithium in liquid ammonia at −78°C for 5 min, yielded compound **9** in 93% yield. Deprotonation of **9** with triethylamine in the presence of trimethylsilyl triflate provided enol silyl ether **10** quantitatively. Oxidation of **10** with 1.1 equivalent of 4-chloroperoxybenzoic acid (MCPBA) gave α-ketol **11** in 82% yield. Opening of the cyclopropane ring of **11** with lithium bromide/*p*-toluenesulfonic acid gave the Δ^2-α-ketol **12** in 83% yield. Oxidation of **12** with periodic acid followed by reduction with sodium borohydride and acidification gave the $\Delta^{2,23}$-7-oxo-compound **13** in 87% yield. Compound **13** was treated with a catalytic amount of osmium tetroxide in the presence of *N*-methylmorpholine *N*-oxide to give a separable mixture of the tetraols **2** and **3**, respectively, in 94% yield (ratio of 3:4). The overall yield of the eight-step synthesis of both **2** and **3** based on ergosterol as starting material was ca. 30%. This is the highest overall yield reported until now. Furthermore, the hydroxylated products **2** and **3** were easily obtained with high purity, since no structurally isomeric B-homo-6-oxa-7-oxo compound was formed using our highly regioselective reaction for the construction of the 7-oxalactone.

Using the commercially available brassicasterol (**5**) as starting material, the number of steps could be reduced. In addition, a reduction step, **8** → **9**, could thereby be avoided. The synthesis of **2** and **3** from brassicasterol (**5**) is in progress.





All m'ps are uncorrected. Optical rotations were measured on an Autopol III polarimeter; IR spectra were recorded as Nujol mull on a IR-440 spectrophotometer; ^{1}H-NMR spectra were recorded on Varian-360L (60 MHz) or on Varian XL-200 (200 MHz) spectrometers, using TMS as internal standard. Only relevant signals are given. Mass spectra were obtained by the Analytical Department of this Institute. Silica gel H (10–40 μ) was used for the flash chromatography. PE refers to petroleum ether (bp 60–90°C).

(22*E*,24*R*)-3α,5-Cyclo-5α-ergost-7,22-dien-6-ol (8):
To a solution of **7** (8.3 g, 21 mmol) obtained from ergosterol (**4**)[10] in dry pyridine (65 mL), chromic acid (6.2 g, 62 mmol) is added with vigorous stirring at room temperature for 5 h. The mixture is diluted with ether (200 mL) and filtered. The ethereal solution is washed with water, 10% HCl, brine, dried (Na_2SO_4), and concentrated to dryness under reduced pressure. The residue is recrystallized from acetone to afford **8**; yield: 5.6 g (67%); mp 168–169°C (Lit.[10] mp 168–169°C).

(22*E*,24*R*)-3α,5-Cyclo-5α-ergost-7,22-dien-6-one (9):
A solution of **8** (3.44 g, 8.73 mmol) in EtOAc (120 mL) is added dropwise to a solution of liquid ammonia (70 mL) containing lithium (200 mg, 28.6 mmol) at −78°C with vigorous stirring under N_2. After the addition is completed, the dark blue color of the solution persisted for ca. 5 min. Then the excess Li is destroyed with solid NH_4Cl. The mixture is allowed to warm to room temperature (evaporation of ammonia); the residue is diluted with water (20 mL) and extracted with ether (3 × 15 mL). The organic phase is washed with 10% HCl, brine, and dried (Na_2SO_4). After removal of the solvent, the crude product is purified by flash chromatography (EtOAc/PE, 1:100) to afford **9**; yield: 3.2 g (93%); mp 108–110°C (Lit.[10] mp 108–110°C).

(22*E*,22*R*)-6-Trimethylsiloxy-3α,5-cyclo-5α-ergost-6,22-diene (10):
To a solution of **9** (3.22 g, 8.13 mmol) in CH_2Cl_2 (50 mL) and Et_3N (4 mL), is added dropwise trimethylsilyl triflate (3 mL, 15.5 mmol) at 0°C in 20 min. Then the organic phase is washed with brine to neutrality, dried (Na_2SO_4), and concentrated to dryness *in vacuo* to give **10** in quantitative yield. $[\alpha]_D^{20}$ −48° (*c* = 0.531, $CHCl_3$).

$C_{31}H_{52}OSi$	calc.	C 79.42	H 11.17
(468.8)	found	79.51	11.01

MS (EI, 70 eV): *m/z* (%) = 469 (M^+ + 1, 1); 396 (M^+ + 1 − $SiMe_3$, 18).

IR: *ν* = 1650 cm^{-1} (C=C).

^{1}H-NMR ($CDCl_3$): *δ* = 0.70 (s, 3H, H-18); 0.99 (s, 3H, H-19), 4.37 (m, 1H, H-7); 5.20 (m, 2H, H-22, H-23).

(22*E*,24*R*)-7α-Hydroxy-3α,5-cyclo-5α-ergost-22-en-6-one (11):
Compound **10** (2 g, 4.27 mmol) in CH_2Cl_2 (94 mL) is treated with MCPBA (1.1 equiv) at room temperature for 1 h. Then sat. $NaHSO_3$ (10 mL) is added to destroy excess MCPBA. The organic phase is washed with 10% HCl, sat. $NaHCO_3$, brine, dried (Na_2SO_4), and concentrated under reduced pressure. The crude product is chromatographed (EtOAc/PE, 1:30) to afford **12**; yield: 1.47 g (82%); mp 145–147°C; $[\alpha]_D^{20}$ −3.8° (*c* = 0.524, $CHCl_3$).

$C_{28}H_{44}O_2$	calc.	C 81.49	H 10.74
(412.7)	found	81.21	10.95

MS (EI, 70 eV): *m/z* (%) = 413 (M^+ + 1, 67), 395 (M^+ + 1 − H_2O, 19).

IR: *ν* = 3360 (OH), 1700 cm^{-1} (CO).

^{1}H-NMR ($CDCl_3$): *δ* = 0.70 (s, 3H, H-18); 1.10 (s, 3H, H-19); 3.80 (m, 1H, H-7); 5.23 (m, 2H, H-22, H-23)

(22*E*,24*R*)-7α-Hydroxy-5α-ergost-2,22-dien-6-one (12):
To a mixture of TsOH (80 mg) and LiBr (50 mg) in DMF (32 mL) is added **11** (1.1 g, 2.66 mmol). The mixture is refluxed for 1.5 h. The solvent is removed *in vacuo*. The residue is diluted with water (10 mL) and extracted with ether (3 × 15 mL). The ethereal extracts are washed with water, sat. $NaHCO_3$, brine, dried (Na_2SO_4), and concentrated at reduced pressure to give a residue, which is chromatographed (PE/EtOAc, 80:1) to afford **13**; yield: 940 mg (83%); mp 128–130°C; $[\alpha]_D^{20}$ +10.8° (*c* = 0.70, $CHCl_3$).

$C_{28}H_{44}O_2$	calc.	C 81.49	H 10.74
(412.7)	found	81.50	10.92

MS (EI, 70 eV): *m/z* (%) = 413 (M^+ + 1, 5), 395 (M^+ + 1 − H_2O, 49), 379 (M^+ − H_2O − CH_3, 100).

IR: *ν* = 3400 (OH), 1700 cm^{-1} (CO).

^{1}H-NMR ($CDCl_3$): *δ* = 0.68 (s, 3H, H-18); 0.71 (s, 3H, H-19); 3.50 (m, 1H, H-7); 5.20 (m, 2H, H-22, H-23); 5.60 (m, 2H, H-2, H-3).

(22*E*,22*R*)-B-Homo-7-oxa-5α-ergost-2,22-dien-6-one (13):
A solution of **12** (350 mg, 0.85 mmol) in dry ether (50 mL) is treated with $HIO_4 \cdot 2H_2O$ (210 mg, 0.92 mmol) at 0°C for 1 h under vigorous stirring. The mixture is then allowed to warm to room temperature for 2 h. The solid is filtered off, and the filtrate is concentrated *in vacuo*. The residue is dissolved in MeOH (20 mL) and treated with $NaBH_4$ (100 mg, 2.65 mmol) at 0°C for 4 h. The solvent is removed, and the residue is acidified with 6N HCl (10 mL) in THF (10 mL) at room temperature for 3 h. After concentration, the residue is extracted with ether (3 × 5 mL). The organic phase is washed with sat. $NaHCO_3$, brine, dried (Na_2SO_4), and concentrated *in vacuo*. The crude product is purified by chromatography (PE/EtOAc, 100:1) to afford **13**; yield: 300 mg (87%); mp 149–150°C; $[\alpha]_D^{20}$ +6° (*c* = 0.33, $CHCl_3$).

$C_{28}H_{44}O_2$	calc.	C 81.49	H 10.74
(412.7)	found	81.30	10.75

MS (EI, 70 eV): *m/z* (%) = 413 (M^+ + 1, 30).

IR: *ν* = 1720 (lactone), 1660 cm^{-1} (CH=CH).

^{1}H-NMR ($CDCl_3$): *δ* = 0.69 (s, 3H, H-18); 0.71 (s, 3H, H-19); 4.04 (m, 2H, H-7); 5.20 (m, 2H, H-22, H-23); 5.61 (m, 2H, H-2, H-3).

(22*R*,23*R*,24*R*)-2α,3α,22,23-Tetrahydroxy-B-homo-7-oxa-5α-ergostan-6-one (2) and (22*S*,23*S*,24*R*)-2α,3α,22,23-Tetrahydroxy-B-homo-7-oxa-5α-ergostan-6-one (3):
Compound **13** (200 mg, 0.49 mmol) is dissolved in a solvent mixture of THF/H_2O/*t*-BuOH (10:3:3.5 mL). To the solution is added OsO_4 (15 mg) and *N*-methylmorpholine *N*-oxide (500 mg) at room temperature, and the mixture is stirred for 2d. Then sat. $NaHSO_3$ (2 mL) is added within 30 min. The mixture is extracted with $CHCl_3$ (3 × 5 mL). The extracts are washed with brine, dried over (Na_2SO_4), and concentrated under reduced pressure. The residue is purified by chromatography ($CHCl_3$/MeOH, 9:1) to give **3** (130 mg) and **2** (90 mg) in 94% overall yield **2**; mp 255–256°C; $[\alpha]_D^{27}$ +31° (*c* = 0.90, MeOH) (Lit.[5] mp 256–258°C; $[\alpha]_D^{21}$ +32°).

$C_{28}H_{48}O_6$	calc.	C 69.93	H 10.25
(480.7)	found	69.96	10.06

MS (EI, 70 eV): *m/z* (%) = 481 (M^+ + 1, 4); 445 (M^+ + 1 − $2H_2O$, 2); 409 (M^+ + 1-$4H_2O$, 2); 379 (M^+ − $Me_2CHCHMeCHOH$, 15).

IR: *ν* = 3450 (OH), 1720 cm^{-1} (lactone).

^{1}H-NMR (pyridine-d_5), 200 MHz): *δ* = 0.72 (s, 3H, H-18); 0.96 (s, 3H, H-19); 1.02 (d, 3H, *J* = 7 Hz, H-25); 1.03, 1.05 (2d, 6H, *J* = 6.7 Hz, H-26 + H-27); 1.24 (d, 3H, *J* = 6, 8 Hz, H-21); 3.94–4.10 (m, 4H, H-2, 3, 22 + H-23); 4.13, 4.42 (2m, 2H, H-7).

3; mp 194–195°C; $[\alpha]_D^{27}$ +30 (*c* = 0.11, MeOH) (Lit.[5] mp 194–196°C; $[\alpha]_D^{21}$ +30°).

$C_{28}H_{48}O_6$	calc.	C 69.93	H 10.25
(480.7)	found	69.89	10.18

MS (EI, 70 eV): *m/z* (%) = 481 (M^+ + 1, 2); 409 (M^+ + 1 − $4H_2O$, 4); 379 (M^+ − $Me_2CHCHMeCHOH$, 20).

IR: *ν* = 3400 (OH), 1720 cm^{-1} (lactone).

^{1}H-NMR (pyridine-d_5, 200 MHz): *δ* = 0.71 (s, 3H, H-18); 0.94 (s, 3H, H-19); 1.00 (d, 3H, *J* = 6.9 Hz, H-25); 1.03, 1.14 (2d, 6H, *J* = 6.6 Hz, H-26, H-27); 1.23 (d, 3H, *J* = 6.8 Hz, 21-H); 3.92–4.10 (m, 4H, H-2, H-3, H-22, H-23); 4.14, 4.44 (2m, 2H, H-7).

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(1) Grove, M.D., Spencer, G.F., Rohwedder, W.K., Mandava, N.B., Warley, J.F., Warthen, J.D., Jr., Steffens, G.L., Flipen-Anderson, J.L., Cook, J.C., Jr. *Nature* **1979**, *281*, 216.
(2) Takatsuto, S., Ikekawa, N. *J. Chem. Soc., Perkin Trans. 1* **1980**, 2133, and references cited therein.
(3) Takatsuto, S., Yazawa, N., Ikekawa, N., Morishita, T., Abe, H. *Phytochemistry* **1983**, *22*, 1393.
Takatsuto, S., Yazawa, N., Takematsu, T., Taenchi, Y., Koguchi, M. *Phytochemistry* **1983**, *22*, 2437.
Zhou, W.S., Jiang, L.Z., Tian, W.S., Zhau, X.Y., Zheng, H. *Acta Chim. Sinica* **1988**, *46*, 1212.
(4) Thompson, M.J., Mandava, N.B., Flipen-Anderson, J.L., Worley, J.F., Dutky, S.R., Robbins, W.E., Lusby, W. *J. Org. Chem.* **1979**, *44*, 5002.
(5) Anastasia, M.C., Fiechi, A. *J. Chem. Soc. Perkin Trans. 1* **1983**, 379.
(6) Takatsuto, S., Ikekawa, N. *Chem. Pharm. Bull.* **1984**, *32*, 2001.
(7) Ikekawa, N., Nishiyama, F., Fujimoto, Y. *Chem. Pharm. Bull.* **1988**, *36*, 405.
(8) Thompson, M.J., Maudt, W.J., Mandava, N.B., Dutky, S.R., Lushy, W.R., Saulding, S.W. *Steroids* **1982**, *39*, 89.
(9) Zhou, W.S., Jiang, B., Pan, X.F. *J. Chem. Soc. Chem. Commun.* **1988**, 791.
(10) Thompson, M.J., Cohn, F., Lansater, S.N. *Steroids* **1965**, *5*, 745.

1988

- Chen, Q.-Y., He, Y.-B. *Synthesis* **1988**, 896. On page 897 the amount of $Pd(PPh_3)_2Cl_2$ in the general procedure for Arenes **3** should be 0.075 mmol.

1989

- Bhaha, S.K., Hajdu, J. *Synthesis* **1989**, 16. Throughout the paper thioacetyl should be replaced by acetylthio. Hence **7** is named 2-*S*-acetyl-1-*O*-hexadecyl-L-2-thioglycerol.

- Burger, A., Hetru, C., Luu, B. *Synthesis* **1989**, 93. On page 94 the formulae of the Horner–Emmons reagent used is:

$$(EtO)_2\overset{\overset{O}{\|}}{P}CCl_2Li$$

and the correct name in the experimental section p. 96 is: diethyl dichloromethylphosphonate.

- Schinzer, D. *Synthesis* **1989**, 179. On page 180 compound **14b** is (2*R*,3*RS*,4*SR*)-3-hydroxy-2,4,6-trimethyl-5-heptenoyltriethylsilane.

- Cristau, H.J., Fonte, M., Torreilles, E. *Synthesis* **1989**, 301. On page 301 compound **7** is 2-(2-benzylaminoethoxy)-1-[(2-methyl-1,3-dioxolan-2-yl)methyl]ethyltriphenylphosphonium iodide.

- Zhou, W.-S., Zhou, Y.-P., Jiang, B. *Synthesis* **1989**, 426. On page 427 compound **8** is (22*E*,24*R*)-3α,5-cyclo-5α-ergosta-7,22-dien-6-one and **9** is (22*E*,24*R*)-3α5-cyclo-5α-ergost-22-en-6-one.

- Stuart, J.G., Nicholas, K.M. *Synthesis* **1989**, 454. In the title abstract and text propargyl nitriles should read propargyl cyanides.

- Schick, H., Eichhorn, I., *Synthesis* **1989**, 477. On page 481 the final entry to Table 4 should read:
$CH_2CH{=}CH{-}(CH_2)_3CO_2Me$