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Short-Step Synthesis of Plant Growth-Promoting Brassinosteroids

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Brassinolide analogues, (22*R*, 23*R*, 24*R*)-2α,3α,22,23-tetrahydroxy-*B*-homo-7-oxa-5α-ergostan-6-one (24-epibrassinolide) (**10**) and (22*S*, 23*S*, 24*R*)-2α,3α,22,23-tetrahydroxy-*B*-homo-7-oxa-5α-ergostan-6-one (**9**), were synthesized from brassicasterol (**3a**) in five steps and with *ca.* 20% overall yield. The key steps are the direct formation of (22*E*, 24*R*)-3α,5-cyclo-5α-ergost-22-en-6-one (**4**) from brassicasterol mesylate (**3b**), the acid-catalyzed rearrangement of **4** to (22*E*, 24*R*)-5α-ergosta-2,22-dien-6-one (**6**), and the Baeyer–Villiger oxidation of the tetrahydroxy-5α-ergostan-6-ones **7** and **8**.

Keywords——brassinolide; brassinosteroid; plant growth hormone; brassicasterol; Baeyer–Villiger oxidation

Brassinolide (**1**),[1] (22*R*,23*R*,24*S*)-2α,3α,22,23-tetrahydroxy-*B*-homo-7-oxa-5α-ergostan-6-one, and castasterone (**2**),[2] (22*R*,23*R*,24*S*)-2α,3α,22,23-tetrahydroxy-5α-ergostan-6-one, are naturally occurring plant growth-promoting steroids. Brassinolide (**1**) showed a wide variety of biological activities in a number of bioassay systems.[3] Among the brassinolide analogues already synthesized, (22*R*,23*R*,24*R*)-2α,3α,22,23-tetrahydroxy-*B*-homo-7-oxa-5α-ergostan-6-one, 24-epibrassinolide (**10**), and (22*S*,23*S*,24*R*)-2α,3α,22,23-tetrahydroxy-*B*-homo-7-oxa-5α-ergostan-6-one (**9**) are promising candidate compounds for applications in agriculture[4] not only because these steroidal lactones were found to be highly active (comparable to brassinolide (**1**)) in a number of bioassays,[5] but also because an efficient synthesis in gram quantities from the commercially available ergosterol, (22*E*,24*R*)-ergosta-5,7,22-trien-3β-ol, has been developed by USDA scientists.[6] The high biological activity of brassinosteroids **9** and **10** prompted us to report our short-step synthesis of **9** and **10** from brassicasterol, (22*E*,24*R*)-ergosta-5,22-dien-3β-ol (**3a**).

In order to introduce 2α,3α- and 22,23-*vicinal* diol functions, (22*E*,24*R*)-5α-ergosta-2,22-dien-6-one (**6**) had to be elaborated from ergosterol or brassicasterol *via* the key intermediate, (22*E*,24*R*)-3α,5-cyclo-5α-ergost-22-en-6-one (**4**). Thompson *et al.*[6] have transformed ergosterol into the 2,22-dien-6-one **6** in eight steps. Anastasia *et al.*[7] have obtained brassicasterol (**3a**) and its Δ^7 isomer in a ratio of 3 : 2 from the 1,4-cycloadduct of ergosterol acetate and 4-phenyl-1,2,4-triazoline-3,5-dione by reduction with lithium and ethylamine. They transformed brassicasterol (**3a**) into the 2,22-dien-6-one **6** in five steps. The common transformations to obtain the 3,5-cyclo-6-one **4** are as follows; tosylation, solvolysis to the cyclopropyl-6β-ol, and oxidation (and reduction of the C-7 (8) double bond).

Brassicasterol (**3a**), present in rapeseed oil at 5—19% of the free sterol fraction[8] and now commercially available, is the most suitable starting material for the synthesis of brassinolide analogues **9** and **10**. In our case the preparation of the 3,5-cyclo-6-one **4** was achieved by treatment of the brassicasterol mesylate (**3b**) with sodium acetate[9] or potassium acetate in dimethyl sulphoxide at 90—100 °C in *ca.* 50% yield. The by-product (*ca.* 10%) was (22*E*,24*R*)-ergosta-4,22-dien-3-one (**5**). The use of triethylamine instead of sodium acetate failed to yield the cyclopropyl ketone **4**. The major product in this case was the dienone **5**. Acid-catalyzed

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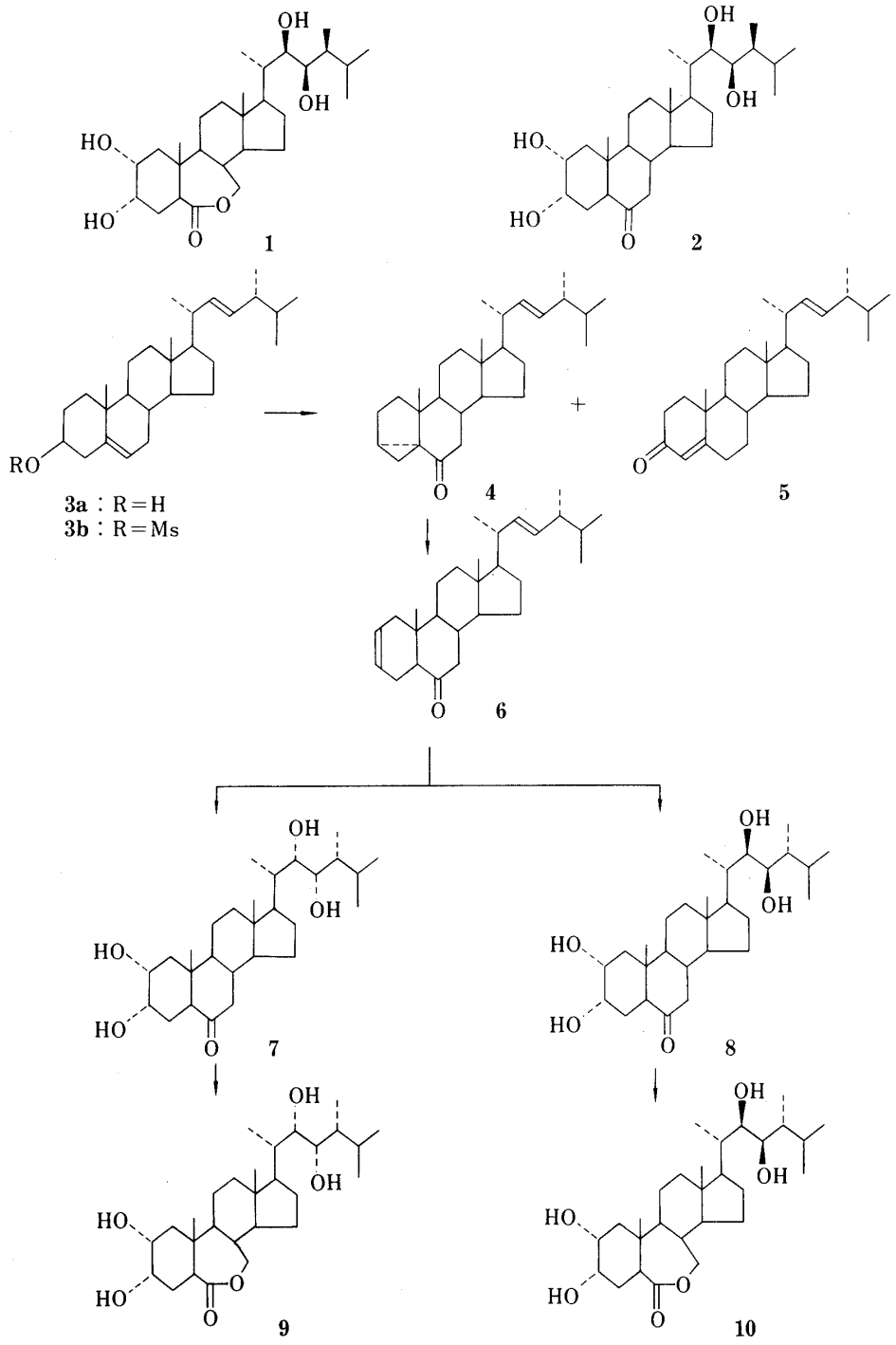

isomerization of the 3,5-cyclo-ketone **4** to (22*E*,24*R*)-5α-ergosta-2,22-dien-6-one (**6**) was effected by heating with *p*-toluenesulphonic acid and sulpholane[10] in 65% yield. The 2,22-dien-6-one **6** in *tert*-BuOH–THF–H_2O (10:3:1) was treated with a catalytic amount of osmium tetroxide and 6.5 molar equivalents of *N*-methylmorpholine *N*-oxide[11] at room temperature for 3 days to give a separable mixture of the tetraols **7** and **8**. Flash chromatography on silica gel provided the less polar (22*S*,23*S*,24*R*)-2α,3α,22,23-tetrahydroxy-5α-ergostan-6-one (**7**, 50%), mp 184—185 °C, (lit.[7] mp 184—185 °C) and the more polar (22*R*,23*R*,24*R*)-2α,3α,22,23-tetrahydroxy-5α-ergostan-6-one (**8**, 30%), mp 241—242 °C (lit.[7] mp 241—242 °C). Baeyer–Villiger oxidation[12] of **7** and **8** with trifluoroperacetic acid followed by recrystallization of the product provided (22*S*,23*S*,24*R*)-2α,3α,22,23-

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tetrahydroxy-*B*-homo-7-oxa-5α-ergostan-6-one (**9**), mp 193—195 °C (lit.[7] mp 193—195 °C), and (22*R*,23*R*,24*R*)-2α,3α,22,23-tetrahydroxy-*B*-homo-7-oxa-5α-ergostan-6-one (**10**), mp 256—257 °C (lit.[7] mp 256—257 °C), respectively, in *ca.* 80% yield. The overall yield of the five-step synthesis of both **9** and **10** was *ca.* 20%.

Experimental

Melting points were determined with a hot-stage microscope and are uncorrected. Infrared (IR) spectra were recorded on a Hitachi 260-10 spectrometer. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were taken with a Hitachi R-24 (60 MHz) spectrometer with tetramethylsilane as an internal standard. Mass spectra (MS) were taken with a Shimadzu LKB-9000S or a Shimadzu GC-MS 6020 mass spectrometer. Column chromatography was done on Kieselgel 60 F_{254} (70—230 mesh, E. Merck). Analytical thin layer chromatography (TLC) was carried out on precoated Kieselgel 60 F_{254} (0.25 mm thickness, E. Merck). The usual work-up refers to dilution with water, extraction with the organic solvent indicated in parentheses, washing of the extract to neutrality, drying over $MgSO_4$, filtration, and removal of the solvent by evaporation under a vacuum. The following abbreviations are used for ^{1}H-NMR data; s, singlet; d, doublet; dd, double doublet; m, multiplet.

(22*E*,24*R*)-3α,5-Cyclo-5α-ergost-22-en-6-one (4)——Brassicasterol (**3a**) (4.1 g, 10.3 mmol) was treated with methanesulphonyl chloride (3 ml) and pyridine (20 ml) at room temperature for 3 h, then ice–water was added. The whole was extracted with ethyl acetate. The usual work-up gave the mesylate **3b** (4.9 g). This was treated with sodium acetate (5.0 g, 60.98 mmol) and dimethyl sulphoxide (160 ml) at 90—100 °C for 5 h. The usual work-up (ether) gave a crude product, which was applied to a column of silica gel (50 g). Elution with benzene provided (22*E*,24*R*)-3α,5-cyclo-5α-ergost-22-en-6-one (**4**) (2.1 g, 51%), mp 109—111 °C (lit.[7] mp 110—111 °C) (from aqueous acetone). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1710. ^{1}H-NMR ($CDCl_3$) δ: 0.72 (3H, s, 18-H_3), 0.96 (3H, s, 19-H_3), 5.20 (2H, m, 22-H and 23-H). *Anal.* Calcd for $C_{28}H_{44}O$: C, 84.78; H, 11.18. Found: C, 84.66; H, 11.30.

Further elution with benzene–ethyl acetate (50 : 1) gave (22*E*,24*R*)-ergosta-4,22-dien-3-one (**5**) (0.49 g, 12%), mp 150—151 °C (from methanol). ^{1}H-NMR ($CDCl_3$) δ: 0.74 (3H, s, 18-H_3), 1.15 (3H, s, 19-H_3), 5.20 (2H, m, 22-H and 23-H), 6.14 (1H, s, 4-H). *Anal.* Calcd for $C_{28}H_{44}O$: C, 84.78; H, 11.18. Found: C, 84.70; H, 11.21.

(22*E*,24*R*)-5α-Ergosta-2,22-dien-6-one (6)——The cyclopropyl ketone **4** (2.1 g, 5.28 mmol) was treated with *p*-toluenesulphonic acid (100 mg) and sulpholane (16 ml) at 160 °C for 1.5 h. The usual work-up (ether) gave a crude product, which was applied to a column of silica gel (50 g). Elution with hexane–benzene (1 : 5) provided (22*E*,24*R*)-5α-ergosta-2,22-dien-6-one (**6**) (1.3 g, 65%), mp 123—124 °C (lit.[7] mp 123—124 °C) (from methanol). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1705. ^{1}H-NMR ($CDCl_3$) δ: 0.68 (3H, s, 18-H_3), 0.70 (3H, s, 19-H_3), 5.20 (2H, m, 22-H and 23-H), 5.60 (2H, m, 2-H and 3-H). *Anal.* Calcd for $C_{28}H_{44}O$: C, 84.78; H, 11.18. Found: C, 84.72; H, 11.27.

(22*S*,23*S*,24*R*)-2α,3α,22,23-Tetrahydroxy-5α-ergostan-6-one (7) and (22*R*,23*R*,24*R*)-2α,3α,22,23-Tetrahydroxy-5α-ergostan-6-one (8)——The diene **6** (1.3 g, 3.43 mmol) in *tert*-BuOH–THF–H_2O (10 : 3 : 1, 20 ml) was treated with osmium tetroxide (20 mg) and *N*-methylmorpholine *N*-oxide (3.0 g) at room temperature for 3 d, then sat. $NaHSO_3$ solution (20 ml) was added. The mixture was stirred at room temperature for 1 h. The usual work-up (dichloromethane) gave two separable products (1.5 g), which were purified by flash chromatography. Then 500 mg of the products was applied to a column of silica gel (3.5 cm i.d. × 15 cm, Kieselgel 60, 230—400 mesh, E. Merck). Elution with chloroform–methanol (15 : 1) provided the less polar (22*S*,23*S*,24*R*)-2α,3α,22,23-tetrahydroxy-5α-ergostan-6-one (**7**) (253 mg), mp 184—185 °C (lit.[7] mp 184—185 °C) (from ethyl acetate). EI-MS *m/z*: 446 ($M^+ - 18$), 394, 393 ($M^+ - 71$, C_{23}–C_{24} fission), 364 ($M^+ - 101$, C_{22}–C_{23} fission + H, base peak), 363, 345, 327, 287, 263, 245, 175, 173, 155, 147, 107, 101, 95, 43. Emitter CI-MS (isobutane) *m/z*: 465 ($M^+ + 1$, base peak), 447, 429. *Anal.* Calcd for $C_{28}H_{48}O_5$: C, 72.41; H, 10.43. Found: C, 72.28; H, 10.50. Tetraacetate of **7**; ^{1}H-NMR ($CDCl_3$) δ: 0.67 (3H, s, 18-H_3), 1.93 (3H, s, acetyl), 2.03 (9H, s, three acetyls), 4.50—5.50 (4H, m, 2-H, 3-H, 22-H, and 23-H).

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 (**8**) (152 mg), mp 241—242 °C (lit.[7] mp 241—242 °C) (from ethyl acetate). EI-MS *m/z*: 446 ($M^+ - 18$), 394, 393, 364, 363, 345, 327, 287, 263, 245, 175, 173, 155, 147, 107, 101, 95, 43. Emitter CI-MS (isobutane) *m/z*: 465 ($M^+ + 1$), 447, 429. *Anal.* Calcd for $C_{28}H_{48}O_5$: C, 72.41; H, 10.43. Found: C, 72.40; H, 10.47. Tetraacetate of **8**; ^{1}H-NMR ($CDCl_3$) δ: 0.67 (3H, s, 18-H_3), 1.95 (3H, s, acetyl), 2.00 (6H, s, two acetyls), 2.05 (3H, s, acetyl), 4.65—5.45 (4H, m, 2-H, 3-H, 22-H, and 23-H). The purification procedure was repeated three times. The total amounts of **7** and **8** were 760 and 457 mg (total yield, 80%), respectively.

(22*R*,23*R*,24*R*)-2α,3α,22,23-Tetrahydroxy-*B*-homo-7-oxa-5α-ergostan-6-one (10)——Trifluoroperacetic acid was prepared by adding trifluoroacetic anhydride (6.74 ml) to 30% aqueous H_2O_2 (1.0 g) in dichloromethane (7.4 ml) at 0 °C. Three molar equivalents of the prepared trifluoroperacetic acid solution was added to a solution of the ketone **8** (400 mg, 0.86 mmol) in dichloromethane (10 ml) at 0 °C. The mixture was stirred at 0 °C for 3 h, then sat. aqueous $NaHSO_3$ solution (10 ml) was added. The whole was extracted with dichloromethane. The usual work-up and recrystallization from ethyl acetate provided (22*R*,23*R*,24*R*)-2α,3α,22,23-tetrahydroxy-*B*-homo-7-oxa-5α-ergostan-6-

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one (**10**) (343 mg, 83%), mp 256—258 °C (lit.[7] mp 256—258 °C). EI-MS *m/z*: 465 ($M^+ - 15$), 462 ($M^+ - 18$), 447, 409, 380 ($M^+ - 101$, C_{22}–C_{23} fission + H, base peak), 379, 361, 350, 343, 331, 325, 322, 313, 307, 303, 285, 177, 173, 155, 131, 101, 71, 43. Emitter CI-MS (isobutane) *m/z*: 481 ($M^+ + 1$, base peak), 463, 445. *Anal.* Calcd for $C_{28}H_{46}O_6$: C, 70.03; H, 9.85. Found: C, 70.01; H, 9.92. Tetraacetate of **10**; ^{1}H-NMR ($CDCl_3$) δ: 0.69 (3H, s, 18-H_3), 1.98 (3H, s, acetyl), 2.02 (6H, s, two acetyls), 2.09 (3H, s, acetyl), 3.00 (1H, dd, $J = 13$ and 6 Hz, 5α-H), 4.07 (2H, m, 7-H_2), 4.70—5.50 (4H, m, 2-H, 3-H, 22-H, and 23-H). The mother liquor contained some trifluoroacetate of **10** which was recovered by saponification with 5% KOH–MeOH and relactonization with conc. HCl.

(22*S*,23*S*,24*R*)-2α,3α,22,23-Tetrahydroxy-*B*-homo-7-oxa-5α-ergostan-6-one (9)——The 6-ketone **7** (500 mg, 1.08 mmol) was oxidized, as described for **10**, to give, after recrystallization from ethyl acetate, (22*S*,23*S*,24*R*)-2α,3α,22,23-tetrahydroxy-*B*-homo-7-oxa-5α-ergostan-6-one (**9**) (415 mg, 80%), mp 193—195 °C (lit.[7] mp 193—195 °C). EI-MS *m/z*: 465 ($M^+ - 15$), 462 ($M^+ - 18$), 447, 409, 380, 379, 361, 350, 343, 331, 325, 322, 313, 307, 303, 285, 177, 173, 155, 131, 101, 71, 43. Emitter CI-MS (isobutane) *m/z*: 481 ($M^+ + 1$, base peak), 463, 445. *Anal.* Calcd for $C_{28}H_{48}O_6$: C, 70.03; H, 9.85. Found: C, 69.88; H, 9.87. Tetraacetate of **9**; ^{1}H-NMR ($CDCl_3$) δ: 0.67 (3H, s, 18-H_3), 1.93 (3H, s, acetyl), 2.03 (9H, s, three acetyls), 3.00 (1H, dd, $J = 13$ and 6 Hz, 5α-H), 4.07 (2H, m, 7-H_2), 4.50—5.50 (4H, m, 2-H, 3-H, 22-H, and 23-H). Some trifluoroacetate of **9** contained in the mother liquor was recovered as **9** by saponification and acidification as described above.

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