Facile Syntheses of Brassinosteroids: Brassinolide, Castasterone, Teasterone and Typhasterol†

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Received January 19, 1987

(22*R*,23*R*,24*S*)-3 α ,5-Cyclo-22,23-diacetoxy-5 α -ergostan-6-one (**2b**) is a new key intermediate of some naturally occurring brassinosteroids such as brassinolide (**1a**), castasterone (**1b**), teasterone (**1c**) and typhasterol (**1d**). The cycloketone **2b** was prepared in 10 steps via (22*R*,23*R*,24*S*)-6 β -benzyloxy-3 α ,5-cyclo-22,23-dihydroxy-5 α -ergostane (**5**) from stigmaterol. **2b** was treated with a catalytic amount of *p*-toluenesulfonic acid and sodium bromide to give an enone (**7b**), which was oxidized with osmium tetroxide and derived to give a 2 α ,3 α -acetonide (**8b**). **8b** was easily separated from its isomer by the use of silica gel column chromatography. **8b** was oxidized with trifluoroacetic acid and deacetylated to give **1a**. **8b** was deacetylated and deacetonized to give **1b**. **2b** was treated with dilute sulfuric acid in acetic acid to give a 3 β -acetate (**10**). **10** was treated with sodium hydroxide to give **1c**. **2b** was treated with hydrobromic acid to give a 3 β -bromide (**12**), which was treated with silver acetate to give a 3 α -acetate (**13**). **13** was treated with sodium hydroxide to give **1d**.

Brassinolide [**1a**, (22*R*,23*R*,24*S*)-2 α ,3 α ,22,23-tetrahydroxy-*B*-homo-7-oxa-5 α -ergostan-6-one] is a steroidal compound with a plant-growth regulating activity.¹⁾ Since the discovery of brassinolide, many steroids of a brassinolide-like structure and activity have been found from a number of plants,²⁾ these being referred to as "brassinosteroids." We have already reported the synthesis of (22*R*,23*R*,24*S*)-6 β -benzyloxy-3 α ,5-cyclo-22,23-dihydroxy-5 α -ergostane (**5**), which is a new intermediate of **1a**, and the preparation of (22*R*,23*R*,24*S*)-22,23-isopropylidenedioxy-5 α -ergost-2-en-6-one (**7a**),³⁾ which is a known intermediate from the benzyl ether **5**.⁴⁾

In this paper, we report facile syntheses of four naturally occurring brassinosteroids by the use of a key intermediate, (22*R*,23*R*,24*S*)-3 α ,5-cyclo-22,23-diacetoxy-5 α -ergostan-6-one (**2b**).

The benzyl ether **5**⁴⁾ was converted into the

cycloketone **2b** by acetylation, debenzylation and oxidation in a 95% yield, the diacetyl cycloketone **2b** being more useful than our previous reported acetonide (**2a**). The enone **7a** was prepared in a 73% yield via **2a** from the benzyl ether **5**. The cycloketone **2b** was converted into an enone (**7b**) by treating with a catalytic amount of *p*-toluenesulfonic acid and sodium bromide in *N,N*-dimethylformamide. The diacetyl enone **7b** was given in a 77% yield from the benzyl ether **5**. Acetonides **8b** and **8b'**

FIG. 1

† Synthesis of Brassinolide. Part IV. For Part III, see ref. 4.

FIG. 2

FIG. 3

were prepared by osmium tetroxide oxidation and acetonidation of **7b** in 77% and 14% yields from **7b**. The mixture showed 2 spots on TLC (R_f 0.41 and 0.20, silica gel, ethyl acetate-*n*-hexane = 1:2), and the desired $2\alpha,3\alpha$ -isomer **8b** could be easily purified by column chromatog-

raphy. A bisacetone mixture of **8a** and **8a'**, which was prepared from **2a**, also showed 2 spots on TLC (R_f 0.32 and 0.27, silica gel, ethyl acetate-*n*-hexane = 1:5), but the separation of these isomers was not easy by column chromatography.

Castasterone (**1b**) was given by deacetylation and deacetonidation from **8b** in a quantitative yield. The acetonide **8b** was converted into brassinolide (**1a**) in a similar manner to that described from tetraacetylcastasterone.⁵⁾

The cycloketone **2b** was converted into a 3 β -acetate (**10**) in a similar manner to that described for 3 β -acetoxy-5 α -ergost-22-en-6-one.⁶⁾ A small amount of a 3 β -alcohol compound was produced in the reaction, so the product was acetylated and then purified. The 3 β -acetate **10** was deacetylated to give **1c**. Teasterone (**1c**)⁷⁾ was given in an 82% yield from **2b**.

The cycloketone **2b** was converted into a bromide (**12**) by treating with concentrated hydrobromic acid in a quantitative yield. The previous enone **7b** could be obtained from the bromide **12** under reflux in *N,N*-dimethylformamide without catalysts in an 81% yield. The 3 β -bromide **12** was converted into a 3 α -acetate (**13**, 56%) and the enone **7b** (33%) by treating with silver acetate in acetic acid at 80°C. The 3 α -acetate **13** was converted in typhasterol (**1d**)^{8,9)} in a similar manner to that described for **1c**.

We thus prepared four brassinosteroids, brassinolide (**1a**, 15 steps, 8.5% yield), castasterone (**1b**, 14 steps, 12.3% yield), teasterone (**1c**, 12 steps, 16.3% yield) and typhasterol (**1d**, 13 steps, 9.2% yield), *via* the acetyl cycloketone **2b** from stigmasterol (**3a**) by short steps in high yields.

EXPERIMENTAL

Melting points (mp) were determined with a Yanagimoto hot-stage microscope and were uncorrected. IR spectra were obtained with a JASCO A-102 spectrometer. NMR spectra were recorded at 200 MHz with TMS as an internal standard on a Varian XL-200 spectrometer. Mass spectra were taken on a JEOL JMS-D300. Column chromatography was normally effected with silica gel 60F, and silica gel 60F₂₅₄ (0.25 mm thickness, Merck) was used for analytical thin-layer chromatography, this analysis being made with a Shimadzu CS-930 TLC scanner. Work-up refers to dilution with water, extraction with an organic solvent, washing to neutrality, drying over anhydrous magnesium sulfate, filtration and

removal of the solvent under reduced pressure. THF refers to tetrahydrofuran, AcOEt to ethyl acetate, ether to diethyl ether and DMF to *N,N*-dimethylformamide.

(22*R*,23*R*,24*S*)-3 α ,5-Cyclo-22,23-diacetoxy-5 α -ergostan-6-one (**2b**). Acetic anhydride (14.5 ml) and 4-(*N,N*-dimethylamino)pyridine (0.3 g) were added to a stirred solution of the benzyl ether **5**¹⁴⁾ (15.69 g) in pyridine (150 ml). The solution was stirred at room temperature for 5 hr. Working-up gave a crude **6b**, which was dissolved in AcOEt (600 ml). 10% Pd-C (50% wet, 9.0 g) was added to the solution, before the mixture was stirred at room temperature for 7 hr in a hydrogen atmosphere and then filtered. The filtrate was cooled at 5°C, and Jones reagent (10 ml) was added dropwise to the stirred solution, stirring being continued for 1 hr at 5°C. After adding 15% NaCl aq., working-up gave crude **2b** (15.30 g). The crude **2b** (5.10 g) was chromatographed on SiO₂ with AcOEt-benzene=1:5 as the eluant to give 4.92 g of **2b**, mp 175.5~176.5°C (from MeOH); ¹H-NMR δ (CDCl₃): 2.00 (3H, s), 2.02 (3H, s), 5.20 (1H, d), 5.37 (1H, d); EI/MS *m/z*: 514 (M⁺), 454, 394, 372, 329, 311, 299, 270, 83. *Anal.* Found: C, 74.82; H, 9.85. Calcd. for C₃₂H₅₀O₅: C, 74.67; H, 9.79.

(22*R*,23*R*,24*S*)-22,23-Diacetoxy-5 α -ergost-2-en-6-one (**7b**). *p*-Toluenesulfonic acid (0.70 g), sodium bromide (0.95 g) and DMF (100 ml) were added to the crude cycloketone **2b** (10.20 g), which had been prepared from the benzyl ether **5** without purification of its intermediates. The mixture was stirred and then heated under reflux for 3 hr. Working-up (AcOEt for extraction) gave crude **7b**, and chromatography on SiO₂ with AcOEt-benzene=1:20 as the eluant provide crystalline **7b** (7.94 g), mp 218~222°C (from MeOH); ¹H-NMR δ (CDCl₃): 2.01 (3H, s), 2.03 (3H, s), 5.20 (1H, d), 5.38 (1H, d), 5.55~5.85 (2H, m); EI/MS *m/z*: 514 (M⁺), 499, 486, 394, 372, 329, 311, 83. *Anal.* Found: C, 74.90; H, 9.88. Calcd. for C₃₂H₅₀O₅: C, 74.67; H, 9.79%.

(22*R*,23*R*,24*S*)-22,23-Diacetoxy-2 α ,3 α -isopropylidenedioxy-5 α -ergostan-6-one (**8b**). *N*-Methylmorpholine *N*-oxide (6.1 g) and osmium tetroxide (0.30 g) were added to a solution of the enone **7b** (7.74 g) in *t*-butyl alcohol-THF-water (10:10:1; 120 ml). The solution was stirred for 18 hr at room temperature, before a saturated sodium bisulfite solution (60 ml) was added to the solution. The mixture was stirred for 1 hr, and working-up (AcOEt for extraction) gave 22,23-diacetoxy-2,3-dihydroxy-5 α -ergostan-6-one. The diol was dissolved with CHCl₃ (150 ml), and acetone dimethylacetal (4.8 ml) and *p*-toluenesulfonic acid (0.1 g) were then added to the solution. The mixture was stirred for 1 hr at room temperature, and then sat. sodium bicarbonate solution (50 ml) was added to it. Working-up gave crude **8b**, which was chromatographed on SiO₂ with AcOEt-*n*-hexane=1:3 as the eluant to provide crystalline **8b** (6.84 g) and the

2 β ,3 β -isomer **8b'** (1.25 g). **8b**: mp 181~181.5°C (from AcOEt-*n*-hexane); ¹H-NMR δ (CDCl₃): 2.33 (1H, dd), 2.55 (1H, dd), 4.13 (1H, m), 4.30 (1H, br. s), 5.19 (1H, d), 5.36 (1H, d); EI/MS m/z : 587 (M⁺ - 1), 573, 513, 387, 345, 327, 83. **8b'**: mp 191~193°C (from AcOEt-*n*-hexane); ¹H-NMR δ (CDCl₃): 2.25~2.85 (2H, m), 4.05 (1H, m), 4.24 (1H, m), 5.19 (1H, d), 5.36 (1H, d). EI/MS m/z : 588 (M⁺), 573, 387, 345, 327, 83.

(22*R*,23*R*,24*S*)-2 α ,3 α ,22,23-Tetrahydroxy-7-oxa-*B*-homo-5 α -ergosteran-6-one (brassinolide, **1a**). A solution of trifluoroacetic anhydride (18.5 ml) to an ice-cooled 60% aq. H₂O₂ solution (5.1 ml) in methylene chloride (56 ml). The peracid solution was added to a solution of **8b** (3.28 g) in methylene chloride (280 ml) containing powdered Na₂HPO₄ (21.6 g) with stirring. The mixture was stirred at 5°C for 1 hr and then at 30°C for 3 hr. Water was added to the mixture and the organic layer was separated. The organic layer was washed with water and aq. NaHCO₃, dried over MgSO₄ and concentrated *in vacuo* to give a mixture of **9b** and **9b'** (3.26 g). A 10*N*-NaOH aqueous solution (5.6 ml) was added to a solution of product (3.26 g) in methanol (224 ml). The mixture was stirred and heated under reflux for 3 hr, and after cooling, 6*N*-HCl (14 ml) was added. The mixture was then stirred at 30°C for 2 hr, before the solvent was removed *in vacuo*. To the residue were added chloroform (300 ml) and water (150 ml). The organic layer was washed with aq. NaHCO₃, dried over MgSO₄ and concentrated *in vacuo* to give crude **1a**, which was recrystallized from methanol to give 1.79 g of **1a**, mp 274~278°C. The ¹H-NMR, IR and EI/MS spectra were identical with our previous synthesized sample.¹⁰⁾

(22*R*,23*R*,24*S*)-2 α ,3 α ,22,23-Tetrahydroxy-5 α -ergosteran-6-one (castasterone, **1b**). A 10*N*-NaOH aqueous solution (1.0 ml) was added to a solution of **8b** (589 mg) in methanol (50 ml). The mixture was stirred and was heated under reflux for 3 hr, and after cooling, 6*N*-HCl (2.5 ml) was added. The mixture was then stirred at 40°C for 2 hr, before the solvent was removed *in vacuo*. To the residue were added chloroform (50 ml) and water (25 ml). The organic layer was washed with aq. NaHCO₃, dried over MgSO₄ and concentrated *in vacuo* to give **1b** (458 mg), mp 255~257°C (from AcOEt-MeOH). The ¹H-NMR, IR and EI/MS spectra were identical with our previous synthesized castasterone (**1b**).¹⁰⁾

(22*R*,23*R*,24*S*)-3 β ,22,23-Triacetoxo-5 α -ergosteran-6-one (**10**). 3*N*-H₂SO₄ (1.34 ml) was added to a solution of **2b** (1.03 g) in AcOH (20 ml). The mixture was stirred and heated under reflux for 1 hr, and the solution was then concentrated *in vacuo*. To the residue were added AcOEt (50 ml) and water (25 ml). The organic layer was washed with water (25 ml $\times$ 3 times), aq. NaHCO₃ and aq. NaCl, dried over MgSO₄ and concentrated *in vacuo*. The residue

was dissolved in pyridine (10 ml), before acetic anhydride (1.0 ml) and 4-(*N,N*-dimethylamino)pyridine (10 mg) were added to the solution. The mixture was stirred at room temperature for 2 hr, and working-up gave crude **10**, which was chromatographed on SiO₂ with AcOEt-benzene = 1:8 as the eluant to give crystals of **10** (1.02 g), mp 222.5~223.5°C (from AcOEt); ¹H-NMR δ (CDCl₃): 2.00 (3H, s), 2.03 (6H, s), 2.22~2.4 (2H, m), 4.6~4.8 (1H, m), 5.20 (1H, d), 5.37 (1H, d); EI/MS m/z : 574 (M⁺), 514, 454, 389, 383, 371, 329, 311, 83.

(22*R*,23*R*,24*S*)-3 β ,22,23-Trihydroxy-5 α -ergosteran-6-one (teasterone, **1c**). A 10*N*-NaOH aqueous solution (3.0 ml) was added to a solution of **10** (1.00 g) in methanol (10 ml) and tetrahydrofuran (10 ml). The mixture was stirred at 30°C for 3 hr, before chloroform (100 ml) was added to the solution. The mixture was washed with water, dried over MgSO₄ and concentrated *in vacuo*. The residue was chromatographed on SiO₂ with MeOH-CHCl₃ = 1:30 as the eluant to give crystals of **1c** (0.72 g), mp 200~201°C (from AcOEt); TLC *R*_f 0.22 (MeOH-CHCl₃ = 1:20); ¹H-NMR δ (CDCl₃): 2.18~2.42 (2H, m), 3.5~3.7 (2H, m), 3.75 (1H, d); EI/MS m/z : 449, 448 (M⁺), 430, 415, 377, 348, 346, 329, 318, 311. IR ν (KBr): 1702 cm⁻¹. Acetonide **11**; TLC *R*_f 0.23 (AcOEt-*n*-hexane = 1:1).

(22*R*,23*R*,24*S*)-3 β -Bromo-22,23-diacetoxy-5 α -ergosteran-6-one (**12**). A 47% hydrobromic acid solution (0.35 ml) was added to a solution of **2b** (1.29 g) in AcOEt (12.5 ml). The mixture was stirred at 55°C for 1 hr and then AcOEt (50 ml) was added to the mixture. The solution was washed with water, aq. NaHCO₃ and aq. NaCl, dried over MgSO₄ and concentrated *in vacuo* to give crystalline **12** (1.49 g), mp 220~222°C (from AcOEt); ¹H-NMR δ (CDCl₃): 2.00 (3H, s), 2.03 (3H, s), 3.89~4.08 (1H, m), 5.19 (2H, d), 5.36 (1H, d); EI/MS m/z : 536, 534, 514, 476, 474, 411, 409, 393, 391, 352, 351, 350, 349, 329, 83. Anal. Found: C, 64.56; H, 8.60; Br, 13.47. Calcd. for C₃₂H₅₁O₅Br: C, 64.53; H, 8.63; Br, 13.41%.

(22*R*,23*R*,24*S*)-3 α ,22,23-Triacetoxo-5 α -ergosteran-6-one (**13**). Silver acetate (0.79 g) was added to a solution of **12** (1.42 g) in acetic acid (24 ml). The mixture was stirred and heated at 80°C for 26 hr, before ethyl acetate (50 ml) was added. The mixture was filtered and concentrated *in vacuo*, and the residue was then dissolved in AcOEt (100 ml). The AcOEt solution was washed with water (50 $\times$ 3 times), aq. NaHCO₃ and aq. NaCl, before the organic layer was dried and concentrated *in vacuo*. The residue was chromatographed on SiO₂ with AcOEt-benzene = 1:10 as the eluant to provide the less-polar enone **7b** (0.41 g) as crystals and the more polar triacetate **13** (0.76 g) as an amorphous solid. **13**; ¹H-NMR δ (CDCl₃): 2.00 (3H, s), 2.03 (3H, s), 2.04 (3H, s), 2.33 (1H, dd), 2.58 (1H, dd), 5.12~5.27 (2H, m), 5.37 (1H, d). EI/MS m/z : 574 (M⁺), 514, 499, 454, 413, 371, 329, 311, 83.

(22*R*,23*R*,24*S*)-3 α -22,23-Trihydroxy-5 α -ergostan-6-one (typhasterol, **1d**). The triacetate **13** (0.64 g) was converted, in a manner similar to that described for **1c**, into crystalline **1d** (0.41 g), mp 233~236°C (from AcOEt); TLC *R_f* 0.22 (MeOH-CHCl₃ = 1:20); ¹H-NMR δ (CDCl₃): 2.33 (1H, dd), 2.75 (1H, t), 3.57 (1H, d), 3.74 (1H, d), 4.18 (1H, br. s); EI/MS *m/z*: 449, 448 (M⁺), 431, 430, 377, 348, 346, 329, 318, 311. IR ν (KBr): 1704 cm⁻¹. Acetonide **14**; TLC *R_f* 0.33 (AcOEt-*n*-hexane = 1:1).

Acknowledgments. We thank Mr. M. Hirota, Mr. M. Hanada and Ms. C. Yamaguchi (Research Institute, Daiichi Seiyaku Co., Ltd.) for their measurement of ¹H-NMR (200 MHz), EI/MS and elemental analysis.

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