[Chem. Pharm. Bull. 35(2) 829—832 (1987)]

Synthesis of Homodolichosterone and Related 2-Deoxysteroids

SUGURU TAKATSUTO*,[a] and NOBUO IKEKAWA[b]

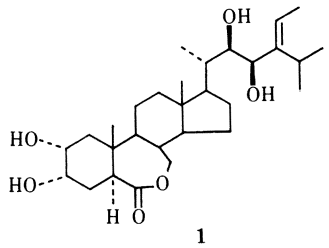
Department of Chemistry, Joetsu University of Education,[a] Joetsu, Niigata 943, Japan and Department of Chemistry, Tokyo Institute of Technology,[b] Ookayama, Meguro-ku, Tokyo 152, Japan

(Received July 14, 1986)

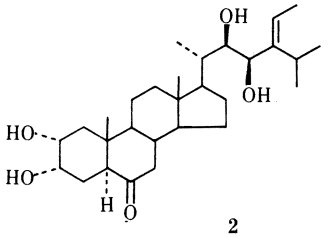
Homodolichosterone (**2**) and the related 2-deoxysteroids **9** and **13** were synthesized from (22*R*,23*R*,24*S*)-3β-acetoxy-22,23-epoxy-5α-stigmastan-6-one (**3**). Reaction of the (22*R*,23*R*)-epoxide **4** with phenylselenyl anion followed by heating with 30% H_2O_2 afforded a mixture of the allylic alcohols **5** and **6**, which were then epoxidized with *m*-chloroperbenzoic acid. The isolated hydroxyepoxide **7** was heated with aluminum isopropoxide to yield the 3β,22,23-triol **9**. Acetonide formation of **9** and mesylation gave the sulfonate **10**, which was refluxed with lithium carbonate and dimethylformamide and then saponified to give the 3α-ol **11** and the 2,24(28)-diene **12**. Acid hydrolysis of **11** provided the 3α,22,23-triol **13**. Selective α-face hydroxylation of **12** with osmium tetroxide and deprotection gave homodolichosterone (**2**).

Keywords——brassinolide; brassinosteroid; homodolicholide; homodolichosterone; plant growth promoter; 2-deoxysteroid

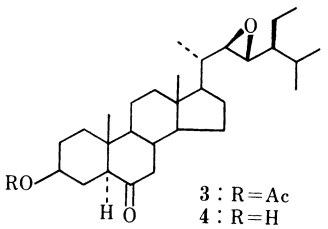
Since the discovery of brassinolide and castasterone as plant growth promoters,[1] the related 2-deoxysteroids, typhasterol (2-deoxycastasterone) and teasterone, the 3β-isomer of typhasterol, have been isolated and identified in several higher plants.[2] Similarly, in connection with homodolicholide (**1**)[3] and homodolichosterone (**2**),[4] it is possible that the related 2-deoxysteroids **9** and **13**, which correspond to teasterone and typhasterol, respectively, may exist in nature. As a part of our program for the identification and characterization of brassinosteroids from plant sources, we required standard samples of **2**, **9**, and **13**. In this paper we describe the synthesis of these heretofore unknown 2-deoxysteroids **9** and **13** and of homodolichosterone (**2**).



1



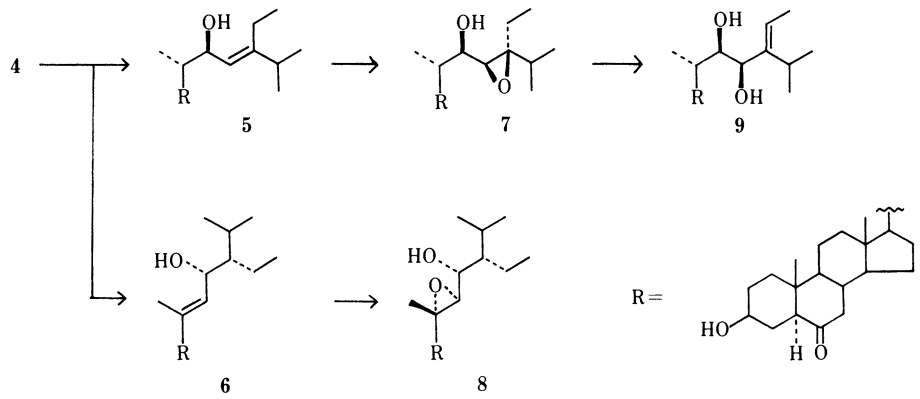
2



3 : R=Ac
4 : R=H

For the construction of the side chain part of **2**, **9**, and **13**, the method[5] developed by Sakakibara and Mori for the synthesis of homodolichosterone (**2**) seems to be convenient. Thus, we applied their method to the isomerically pure (22*R*,23*R*)-epoxide (**4**). In their synthesis of the side chain of **2**,[5] an inseparable mixture of the (22*R*,23*R*)- and (22*S*,23*S*)-epoxy compounds was used as an intermediate so that the subsequent reactions and purification were complicated. In our case, the isomerically pure compound was employed in order to avoid these problems. Reaction of **4**, derived from the known acetate **3**,[6] with an

excess of phenylselenyl anion[7] was carried out in refluxing 1-butanol for 5 d. The resulting α-hydroxyselenides were then heated with 30% H_2O_2 to effect the *syn*-elimination reaction. The regioisomeric alcohols **5** and **6** thus obtained were epoxidized with *m*-chloroperbenzoic acid and the products were easily separated by column chromatography to give the less polar hydroxyepoxide **8** [28%, δ_H 3.05 (1H, d, $J=8$ Hz, 22-H)] and the more polar isomer **7** [21%, δ_H 2.88 (1H, d, $J=7$ Hz, 23-H)], along with recovery of **4** (40%). The stereochemistry of **7** and **8** was confirmed by comparison of the proton nuclear magnetic resonance (^{1}H-NMR) data with those of reference compounds.[5] Treatment of **7** with aluminum isopropoxide in refluxing toluene[8] provided (22*R*,23*R*,24(28)*E*)-3β,22,23-trihydroxy-5α-stigmast-24(28)-en-6-one (**9**), mp 228—230 °C, in 22% yield and the starting material **7** was recovered (25%). The rearrangement of the epoxide into the allylic alcohol proceeded in low yield. The reason for this might be ascribed to the presence of the free 3β-hydroxyl group, judging from the reported results, in which the 2α,3α-diol of the substrate was protected as the acetonide.[5] Improvement of the yield was not attempted since we simply wanted to obtain standard sample of **2**, **9**, and **13**.

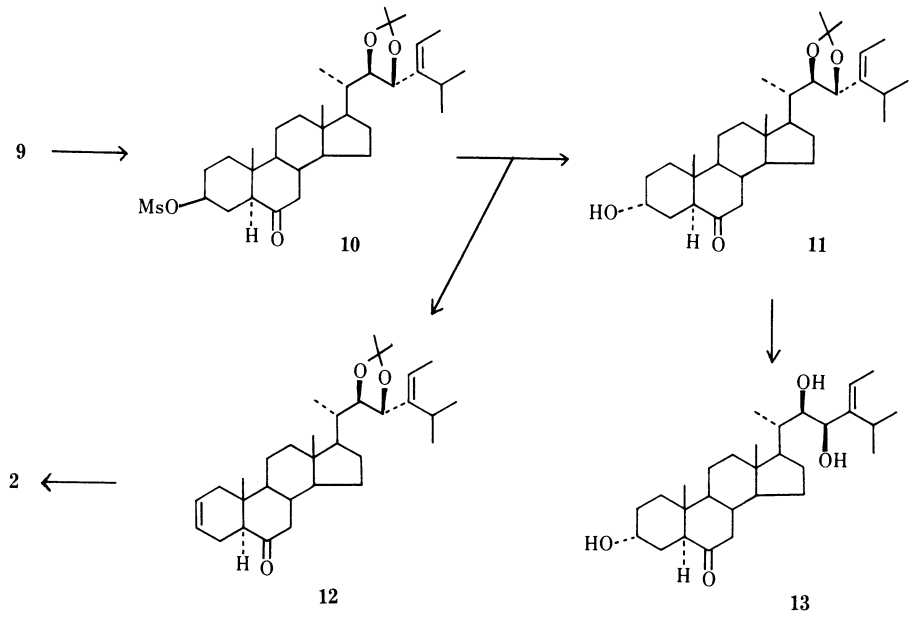


Transformation of the 3β,22,23-triol **9** into its 3α-isomer **13** and homodolichosterone (**2**) was achieved according to our procedure[6] as follows. The triol **9** was submitted to acetonide formation and then mesylation to give the mesylate **10**, which was further treated with lithium carbonate and dimethylformamide under reflux. The resulting products were saponified and then purified by chromatography on silica gel to afford the 2,24(28)-diene **12** and the 3α-ol **11** in 44 and 35% yields, respectively. Heating of **11** with 80% aqueous AcOH provided (22*R*,23*R*,24(28)*E*)-3α,22,23-trihydroxy-5α-stigmast-24(28)-en-6-one (**13**), mp 209—210 °C, in 91% yield. Regio- and stereoselective *cis*-hydroxylation of the diene **12** was carried out with a catalytic amount of OsO_4 and *N*-methylmorpholine *N*-oxide in *tert*-BuOH–tetrahydrofuran(THF)–H_2O (10:3:1). Removal of the protecting group of the resulting 2α,3α-diol gave homodolichosterone (**2**), mp 217—219 °C, sinter at 208 °C (lit.,[5] mp 218—219°C, sinter at 208 °C), in 70% yield. Its spectral data were in good agreement with the reported data.[4,5]

In conclusion, we were able to synthesize homodolichosterone (**2**) and the related 2-deoxysteroids **9** and **13**, which were required as standard samples for studies to identify them in plant sources.

Experimental

Melting points were determined on a Yazawa hot stage microscope and are uncorrected. ^{1}H-NMR spectra were



run on a Hitachi R-24B (60 MHz) spectrometer unless otherwise noted. All NMR spectra were taken in $CDCl_3$ solution with tetramethylsilane as an internal standard. Mass spectra (MS) were obtained with a Shimadzu LKB-9000S mass spectrometer. Thin-layer chromatography (TLC) was carried out on precoated plates of silica gel (Merck, Kieselgel $60F_{254}$, 0.25 mm thickness) and column chromatography on Kieselgel $60F_{254}$ (70—230 mesh, Merck). Work-up refers to dilution with water, extraction with an organic solvent, washing of the extract to neutrality, drying over $MgSO_4$, filtration, and removal of the solvent under reduced pressure.

(20*R*,22*S*,23*R*,24*S*)-20,22-Epoxy-3β,23-dihydroxy-5α-stigmastan-6-one (7) and (22*R*,23*S*,24*R*)-23,24-Epoxy-3β,22-dihydroxy-5α-stigmastan-6-one (8)——Sodium borohydride (2.4 g, 63.2 mmol) was added to a suspension of diphenyl diselenide (9.9 g, 31.7 mmol) in 1-butanol (150 ml) and the mixture was stirred at room temperature for 20 min. A solution of **4** (2.3 g, 5.14 mmol), derived from the known acetate **3**,[6] in THF (10 ml) was added to the reagent solution. The mixture was refluxed for 5 d. THF (100 ml) and 30% H_2O_2 (30 ml) were added to the cooled reaction mixture and it was further stirred at 80 °C for 2 h. Work-up (ether) gave crude products, which showed four spots of *Rf* 0.42, 0.33, 0.27, and 0.19 on TLC (benzene–EtOAc, 1 : 1, developed once). The spot of *Rf* 0.42 was identical with **4**. The mixture in CH_2Cl_2 (100 ml) was treated with *m*-chloroperbenzoic acid (300 mg) at room temperature for 1 h. TLC analysis of the reaction mixture showed no spot of *Rf* 0.33. $Ca(OH)_2$ (1.0 g, powder) was added to the mixture and it was stirred for 1 h. Filtration and evaporation of the solvent gave crude products (2.1 g), which were applied to a column of silica gel (2.5 cm i,d, × 28 cm). Elution with benzene–EtOAc (3 : 1) gave **4** (917 mg, 40% recovery), which was identified by ^{1}H-NMR. Further elution with benzene–EtOAc (2.5 : 1) gave **8** (671 mg, 28%), mp 185—187 °C (EtOAc), *Rf* 0.27. ^{1}H-NMR δ: 0.68 (3H, s, 18-H_3), 0.72 (3H, s, 19-H_3), 1.32 (3H, s, 21-H_3), 3.05 (1H, d, J = 8 Hz, 22-H), 3.20—3.80 (2H, m, 3-H and 23-H). *Anal.* Calcd for $C_{29}H_{48}O_4$: C, 75.60; H, 10.50. Found: C, 75.35; H, 10.34. Further elution with benzene–EtOAc (2 : 1) provided **7** (498 mg, 21%), mp 209—211 °C (EtOAc), *Rf* 0.19. ^{1}H-NMR δ: 0.68 (3H, s, 18-H_3), 0.73 (3H, s, 19-H_3), 2.90 (1H, d, J = 7 Hz, 23-H), 3.20—3.80 (2H, m, 3-H and 22-H). *Anal.* Calcd for $C_{29}H_{48}O_4$: C, 75.60; H, 10.50. Found: C, 75.42; H, 10.34.

(22*R*,23*R*,24(28)*E*)-3β,22,23-Trihydroxy-5α-stigmast-24(28)-en-6-one (9)——A mixture of **8** (410 mg, 0.891 mmol) and aluminum isopropoxide (410 mg, 2.01 mmol) in toluene (30 ml) was refluxed for 1 h. Work-up (CH_2Cl_2) gave crude products, which were applied to a column of silica gel (1.5 cm i.d. × 20 cm). Elution with $CHCl_3$–MeOH (15 : 1) recovered **8** (103 mg, 25%), which was identified by ^{1}H-NMR and TLC. Further elution with the same solvent gave **9** (92 mg, 22%), mp 228—230 °C (EtOAc). ^{1}H-NMR δ: 0.62 (3H, s, 18-H_3), 0.74 (3H, s, 19-H_3), 1.69 (3H, d, J = 7 Hz, 29-H_3), 2.74 (1H, m, 25-H), 3.55 (1H, m, 3-H), 3.63 (1H, d, J = 8 Hz, 22-H), 3.92 (1H, d, J = 8 Hz, 23-H), 5.46 (1H, q, J = 7Hz, 28-H). EI-MS (as the methaneboronate–TMS derivative)[9] *m/z* (20 eV): 556 (M^+, 14%), 541 (18), 527 (8), 513 (80), 466 (6), 167 (100). *Anal.* Calcd for $C_{29}H_{48}O_4$: C, 75.60; H, 10.50. Found: C, 75.32; H, 10.44.

(22*R*,23*R*,24(28)*E*)-22,23-Isopropylidenedioxy-5α-stigmasta-2,24(28)-dien-6-one (11) and (22*R*,23*R*,24(28*E*)-3α-Hydroxy-22,23-isopropylidenedioxy-5α-stigmast-24(28)-en-6-one (12)——A solution of **9** (117 mg, 0.255 mmol) in acetone (10 ml) was treated with *p*-TsOH (5 mg) at room temperature for 1 h. Work-up (ether) gave a crude product, which was taken up in pyridine (2 ml) and reacted with MsCl (0.1 ml) at room temperature for 0.5 h.

Work-up (EtOAc) gave the mesylate **10** (147 mg). ^{1}H-NMR δ: 0.63 (3H, s, 18-H_3), 0.76 (3H, s, 19-H_3), 1.37 and 1.40 (6H, s×2, acetonide), 1.72 (3H, d, J=7 Hz, 29-H_3), 2.98 (3H, s, mesyl), 3.72 (1H, d, J=9 Hz, 22-H), 4.08 (1H, d, J=9 Hz, 23-H), 4.60 (1H, m, 3-H), 5.50 (1H, q, J=7 Hz, 28-H). A mixture of **10** (147 mg), dimethylformamide (3 ml), and lithium carbonate (100 mg) was refluxed for 1 h. Work-up (EtOAc) gave crude products, which were treated with 5% KOH/MeOH (5 ml) at room temperature for 0.5 h. Work-up (ether) and chromatography on silica gel (1.5 cm i.d. × 14 cm) with benzene–EtOAc (40 : 1) gave **12** (55 mg, 44%), mp 205—206 °C (MeOH). ^{1}H-NMR δ: 0.66 (3H, s, 18-H_3), 0.73 (3H, s, 19-H_3), 1.41 and 1.43 (6H, s×2, acetonide), 1.75 (3H, d, J=7 Hz, 29-H_3), 2.70 (1H, m, 25-H), 3.80 (1H, d, J=9 Hz, 22-H), 4.35 (1H, d, J=9 Hz, 23-H), 5.30—5.93 (3H, 2-H, 3-H, and 28-H). *Anal.* Calcd for $C_{32}H_{50}O_3$: C, 79.62; H, 10.44. Found: C, 79.55; H, 10.39. Further elution with benzene–EtOAc (20 : 1) gave **11** (46 mg, 35%), mp 189—190 °C (MeOH). ^{1}H-NMR δ: 0.65 (3H, s, 18-H_3), 0.73 (3H, s, 19-H_3), 1.41 and 1.43 (6H, s×2, acetonide), 1.75 (3H, d, J=7 Hz, 29-H_3), 2.50—2.95 (2H, m, 5α-H and 25-H), 3.80 (1H, d, J=9 Hz, 22-H), 4.35 (1H, d, J=9 Hz, 23-H), 4.37 (1H, m, $W_{1/2}$=8 Hz, 3β-H), 5.57 (1H, q, J=7 Hz, 28-H). *Anal.* Calcd for $C_{32}H_{52}O_4$: C, 76.75; H, 10.47. Found: C, 76.68; H, 10.53.

(22*R*,23*R*,24(28)*E*)-3α,22,23-Trihydroxy-5α-stigmast-24(28)-en-6-one (13)——A mixture of **12** (38 mg, 0.075 mmol) and 80% aqueous AcOH (4 ml) was heated at 60 °C for 1.5 h. Removal of the solvent under reduced pressure and chromatography on silica gel (1.5 cm i.d. × 15 cm) with EtOAc gave **13** (32 mg, 91%), mp 209—210 °C (EtOAc). ^{1}H-NMR δ: 0.63 (3H, s, 18-H_3), 0.73 (3H, s, 19-H_3), 1.72 (3H, d, J=7 Hz, 29-H_3), 2.40—3.00 (2H, m, 5α-H and 25-H), 3.67 (1H, d, J=8 Hz, 22-H), 3.96 (1H, d, J=8 Hz, 23-H), 4.15 (1H, m, $W_{1/2}$=8 Hz, 3β-H), 5.52 (1H, q, J=7 Hz, 28-H). EI-MS (as the methaneboronate–TMS derivative)[9] m/z(20 eV): 556 (M^+, 23%), 541 (19), 527 (2), 513 (100), 466 (4), 167 (96).

(22*R*,23*R*,24(28*E*)-2α,3α,22,23-Tetrahydroxy-5α-stigmast-24(28)-en-6-one (2)——A solution of **11** (47 mg, 0.097 mmol) in *tert*-BuOH–THF–H_2O (10 : 3 : 1, 10 ml) was treated with a catalytic amount of OsO_4 and *N*-methylmorpholine *N*-oxide (33 mg, 0.24 mmol) at room temperature for 1 h, then sat. $NaHSO_3$ solution (5 ml) was added and the mixture was kept at room temperature for 1 h. Work-up (CH_2Cl_2) gave a crude product, which was further treated with 80% aqueous AcOH (10 ml) at 60 °C for 2 h. Removal of the solvent and chromatography on silica gel (1 cm i.d. × 24 cm) with EtOAc–MeOH (20 : 1) provided homodolichosterone (**2**) (32 mg, 70%), mp 217—219 °C, sinter at 208 °C (MeOH) (lit.,[5] mp 218—219 °C, sinter at 208 °C). ^{1}H-NMR (200 MHz) and EI-MS (as the bismethaneboronate)[9] of the synthetic **2** were in good agreement with the reported data.[4,5]

Acknowledgement The authors thank Mr. Y. Kawahata and Mr. M. Ohtsuka of Tokyo Institute of Technology for measurement of the ^{1}H-NMR (200 MHz) and MS.

References

1) *a*) M. D. Grove, G. F. Spencer, W. K. Rohwedder, N. B. Mandava, J. F. Worley, J. D. Warthen Jr., G. L. Steffens, J. L. Flippen-Anderson, and J. C. Cook Jr., *Nature* (London), **281**, 216 (1979); *b*) T. Yokota, M. Arima, and N. Takahashi, *Tetrahedron Lett.*, **23**, 1275 (1982).
2) *a*) J. A. Schneider, K. Yoshihara, K. Nakanishi, and N. Kato, *Tetrahedron Lett.*, **24**, 3859 (1983); *b*) T. Yokota, M. Arima, N. Takahashi, S. Takatsuto, N. Ikekawa, and T. Takematsu, *Agric. Biol. Chem.*, **47**, 2419 (1983); *c*) H. Abe, T. Morishita, M. Uchiyama, S. Takatsuto, and N. Ikekawa, *ibid.*, **48**, 2171 (1984); *d*) Y. Suzuki, I. Yamaguchi, and N. Takahashi, Abstracts of Symposium on Chemical Regulation of Plants, Tokyo, October 18—19, 1984, p. 28.
3) T. Yokota, J. Baba, and N. Takahashi, *Agric. Biol. Chem.*, **47**, 1409 (1983).
4) J. Baba, T. Yokota, and N. Takahashi, *Agric. Biol. Chem.*, **47**, 659 (1983).
5) M. Sakakibara and K. Mori, *Agric. Biol. Chem.*, **48**, 745 (1984).
6) S. Takatsuto and N. Ikekawa, *J. Chem. Soc., Perkin Trans. 1*, **1984**, 439.
7) K. B. Sharpless and R. F. Lauer, *J. Am. Chem. Soc.*, **95**, 2697 (1973).
8) S. Terao, M. Shiraishi, and K. Kato, *Synthesis*, **1979**, 467.
9) *a*) S. Takatsuto, B. Ying, M. Morisaki, and N. Ikekawa, *J. Chromatogr.*, **239**, 233 (1982); *b*) N. Ikekawa, S. Takatsuto, T. Kitsuwa, H. Saito, T. Morishita, and H. Abe, *ibid.*, **290**, 289 (1984); *c*) S. Takatsuto and N. Ikekawa, *Chem. Pharm. Bull.*, **34**, 3435 (1986).