Agric. Biol. Chem., **47** (1), 97～102, 1983

Synthesis of Brassinolide Analogs with or without the Steroidal Side Chain†

Michitada KONDO and Kenji MORI*

Department of Agricultural Chemistry, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113, Japan

Received August 23, 1982

Four brassinolide analogs were synthesized starting from cholesterol, stigmasterol or pregnenolone. An analog possessing a hydroxyl group at C-17 instead of the steroidal side chain was only 0.001% as active as brassinolide upon lamina-inclination testing with rice seedlings, while other analogs were 1～2% as active as brassinolide. This indicates the indispensable role of the side chain for the plant growth-promoting activity of brassino-steroids.

The plant growth-promoting activity of brassinolide **1** as reported by Grove *et al.* aroused much interest among synthetic chemists.[1] We have already reported the synthesis of brassinolide **1**,[2,3] (22*R*,23*R*)-homobrassinolide **2**,[4] (22*S*,23*S*)-homobrassinolide **3**,[3,5] and (22*S*,23*S*)-28-norbrassinolide **4**[6] (Fig. 1). A bioassay of our materials by lamina-inclination testing with rice seedlings[7] revealed that the order of bioactivity was **1**>**2**>**3**>**4**, the demethyl analog **4** being only about 1% as active as brassinolide **1**.[2～6] This result suggested the importance of the structure of the side chain in determining the biological activity.

In order to further define the structure-activity relationship among brassino-steroids,[8～10] we synthesized four new brassinolide analogs **5**～**8**. The most obviously interesting point is to know the bioactivity of **8**

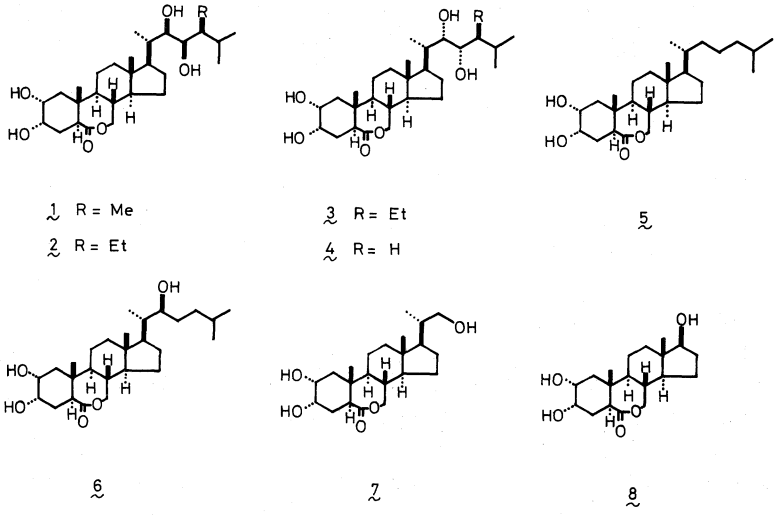

FIG. 1. Structures of Brassino-steroids.

† Brassinolide and Its Analogs. Part IV. For Part III, see ref. 6. The experimental part of this work was taken from the M. Sc. thesis of M. K. (March, 1982). This work was presented as a part of K. M.'s lecture at the 9th Conference on Isoprenoids, Prague, Czechoslovakia, on September 9, 1981.

* Address correspondence to this author.

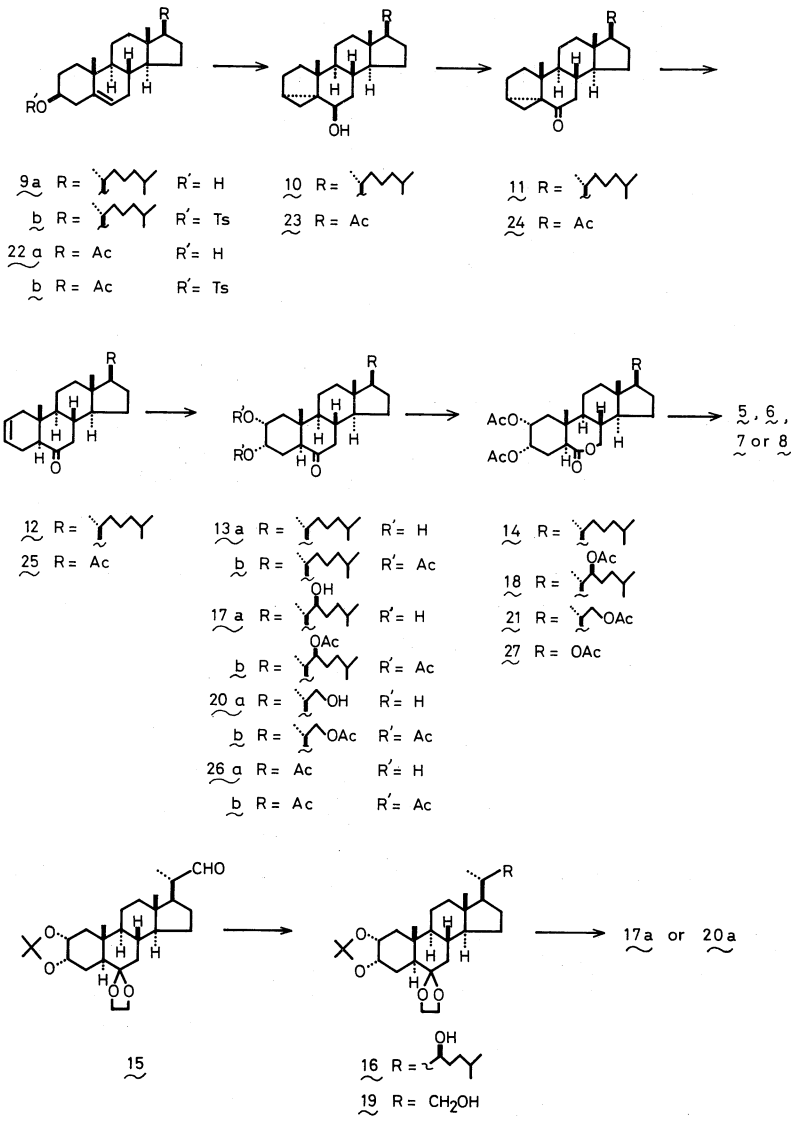

FIG. 2. Synthetic Routes to Brassinolide Analogs.

lacking the steroidal side chain.

The synthetic routes to these four analogs were similar to that employed for the synthesis of (22*S*,23*S*)-homobrassinolide **3**[3,5] as shown in Fig. 2. The starting material for the synthesis of **5** was cholesterol **9a**. This was converted by the known procedure to a ketone **11** *via* **9b** and **10**.[11~13] Treatment of **11** with *p*-toluenesulfonic acid in sulfolane gave **12**. Hydroxylation of **12** with osmium tetroxide and *N*-methylmorpholine *N*-oxide yielded a diol **13a**. The corresponding acetate **13b** was submitted to the Baeyer-Villiger oxidation to give a lactone **14**. This was converted to the desired 22,23-bisdeoxy-28-norbrassinolide **5** in the conventional manner. For the synthesis of **6** and **7**, a known aldehyde **15** was employed as the starting material, which in turn was prepared from stigmasterol.[3] Addition of isoamylmagnesium bromide to **15** afforded **16**. The (*S*)-configuration tentatively assigned to the newly generated hydroxyl group at C-22

was based on the assumption that Cram's rule was applicable in this particular case as it was in others.[14,15] After removing the acetonide protective group, a triol **17a** was obtained. The Baeyer-Villiger oxidation of the corresponding acetate **17b** yielded a lactone **18**. This gave 23-deoxy-28-norbrassinolide **6**, mp 216.5~219°C, after alkaline hydrolysis and acidification. To synthesize the third analog **7**, the aldehyde **15** was reduced with lithium aluminum hydride to **19**. Removal of the protecting group yielded a triol **20a**, whose acetate **20b** was oxidized with trifluoroperacetic acid to give a lactone **21**. This gave the brassinolide analog **7**, mp 242~247°C, with a shortened side chain.[16] The synthesis of the fourth analog **8** started from pregnenolone **22a**. Solvolysis of pregnenolone tosylate **22b** yielded **23**.[12] This was oxidized to a ketone **24**. Acid treatment of **24** gave an unsaturated ketone **25**. This yielded a glycol **26a** upon oxidation with osmium tetroxide. The corresponding diacetoxy diketone was submitted to the Baeyer–Villiger oxidation to give a triacetoxy lactone **27**. This gave the nuclear analog **8**, mp 144~146°C, of brassinolide in the usual manner.

The biological activity of these analogs was kindly estimated by the lamina-inclination test[7,17] with rice seedlings by Professor S. Marumo and Dr. K. Wada, Nagoya University. The relative activity of our new analogs as compared with brassinolide **1** and (22*S*,23*S*)-homobrassinolide **3** was **1** : **3** : **5** : **6** : **7** : **8** = 100 : 10 : 1~2 : 2 : 2 : 0.001. Details of the bioassay will be published elsewhere.

In conclusion, the existence of the steroidal side chain with a proper array of the substituents is indispensable for the high plant growth-promoting activity of brassino-steroids.

EXPERIMENTAL

All melting points were uncorrected. IR spectra were determined as films for oils or as nujol mulls for solids on a Jasco A-102 spectrometer. NMR spectra were recorded at 60 MHz with TMS as an internal standard on a Hitachi R-24A spectrometer unless otherwise stated. Optical rotations were measured on a Jasco DIP-140 polarimeter.

3α,5-Cyclo-5α-cholestan-6-one **11**. This was prepared in an 87.4% yield from **9a** by the Jones oxidation of **10** as prisms from 99% ethanol, mp 101~102°C, $[\alpha]_D^{21}$ +43.1° (c=1.61, $CHCl_3$) [lit.[13] mp 96~97°C, $[\alpha]_D$ +42° ($CHCl_3$)]; IR ν_{max} cm^{-1}: 1680 (s), 1410 (m), 1300 (s), 840 (w); *Anal.* Found: C, 84.57; H, 11.43. Calcd. for $C_{27}H_{44}O$: C, 84.31; H, 11.53%.

5α-Cholest-2-en-6-one **12**. *p*-Toluenesulfonic acid (1.2 g) was added to a mixture of **11** (39.5 g) in sulfolane (220 ml). The mixture was stirred and heated under argon for 130 min at 160°C. The cooled mixture was then poured into iced-water and extracted with benzene–ether (1 : 1). The extract was washed with water, saturated sodium bicarbonate solution and brine, dried over magnesium sulfate and concentrated *in vacuo* to give an oil. This was chromatographed over silica gel (Merck Kiesekgel 60) to give 19.7 g (50%) of **12**, mp 105.5~106.5°C, $[\alpha]_D^{21}$ +34.04° (c=2.045, $CHCl_3$); IR ν_{max} cm^{-1} 1695 (s), 1230 (m), 990 (m); NMR δ (CCl_4) 0.45~1.0 (15H), 1.0~2.4 (27H), 5.47 (2H); *Anal.* Found: C, 84.56; H, 11.57. Calcd. for $C_{27}H_{44}O$: C, 84.31; H, 11.53%.

(2R,3S)-2,3-Diacetoxy-5α-cholestan-6-one **13b**. A solution of osmium tetroxide (1 g) in *t*-butyl alcohol (40 ml), *N*-methylmorpholine *N*-oxide (20 g) and water (30 ml) was added to a solution of **12** (12.3 g) in acetone (550 ml). The mixture was stirred overnight under argon at room temperature. The excess oxidant was then reduced by the addition of 30% sodium bisulfite solution (40 ml). The mixture was filtered through Celite and the filtrate was concentrated *in vacuo*. The residue was extracted with chloroform. The extract was washed with dilute hydrochloric acid, dried over potassium carbonate and concentrated *in vacuo* to give crude **13a** (24.6 g), IR ν_{max} cm^{-1}: 3350 (s), 1710 (s). Acetic anhydride (100 ml) was added to a solution of **13a** (24.6 g) in pyridine (300 ml) and the mixture was stirred overnight at room temperature. This was poured into iced-hydrochloric acid and extracted with ether. The extract was washed with dilute hydrochloric acid, water, saturated sodium bicarbonate solution and brine, dried over magnesium sulfate and concentrated *in vacuo*. The residue was chromatographed over silica gel (Merck Kieselgel 60) to give 12.4 g (77% from **12**) of **13b**. This was recrystallized from ethyl acetate–petroleum ether to give needles, mp 150.5~152.5°C, $[\alpha]_D^{21}$ +0.69° (c=1.31, $CHCl_3$); IR ν_{max} cm^{-1}: 1745 (s), 1720 (s); NMR δ ($CDCl_3$) 0.5~1.05 (15H), 2.0 (3H, s), 2.1 (3H, s), 4.95 (1H), 5.37 (1H); *Anal.* Found: C, 74.01; H, 10.17, Calcd. for $C_{31}H_{50}O_5$: C, 74.06; H, 10.02%.

(2R,3S)-2,3-Diacetoxy-B-homo-7-oxa-5α-cholestan-6-one **14**. A solution of trifluoroperacetic acid in methylene chloride (10 ml) was prepared from trifluoroacetic anhydride (7.1 ml) and 90% hydrogen peroxide (1.1 ml). This was added to a stirred and ice-cooled mixture of **13b** (1 g) and disodium hydrogen phosphate (6 g) in methylene

chloride (60 ml). After the addition, the mixture was stirred and heated under reflux for 2 hr. It was then poured into iced-water and extracted with methylene chloride. The extract was washed with saturated sodium bicarbonate, dried over magnesium sulfate and concentrated *in vacuo*. The residue was chromatographed over silica gel (Mallinckrodt SILICAR CC-7). Elution with *n*-hexane–ethyl acetate (6:1) gave 464 mg (45%) of **14**. This was recrystallized from ethyl acetate–petroleum ether to give prisms, mp 185.5~187.5°C, $[\alpha]_D^{21}$ +49.4° (c=1.107, $CHCl_3$); IR ν_{max} cm^{-1}: 1780 (m), 1740 (s), 1720 (s), 1240 (s), 1180 (s), 1135 (s), 1050 (s), 1025 (s), 740 (s); NMR δ ($CDCl_3$) 0.45~1.05 (15H), 1.98 (3H, s), 2.09 (3H, s), 4.05 (2H, d, J=6 Hz), ~4.75–~5.30 (2H); *Anal.* Found: C, 72.06; H, 9.75. Calcd. for $C_{31}H_{50}O_6$: C, 71.78; H, 9.72%.

(2R,3S)-2,3-Dihydroxy-B-homo-7-oxa-5α-cholestan-6-one **5**. 50% Sodium hydroxide solution (2 ml) was added to a solution of **14** (410 mg) in methanol (20 ml). The solution was stirred for 1 hr under reflux and another 1 hr at room temperature. This was diluted with THF (15 ml) and acidified with 6 N-hydrochloric acid (6 ml). The solution was heated under reflux for 30 min and concentrated *in vacuo*. The residue was extracted with chloroform. The extract was washed with water and brine, dried over magnesium sulfate and concentrated *in vacuo*. The residue was chromatographed over silica gel (Kieselgel 60). Elution with chloroform–methanol (40:1) gave 150 mg (44%) of **5** as a gum, $[\alpha]_D^{22}$ +29.7° (c=1.08, $CHCl_3$); IR ν_{max} cm^{-1}: 3400 (br. s), 1740 (sh), 1720 (s), 1710 (s), 1215 (s), 1180 (s), 1140 (m), 1065 (s), 1025 (s), 980 (w), 910 (w), 755 (s). NMR δ ($CDCl_3$) 0.45~1.0 (15H), 1.0~3.0 (27H), 3.4~4.25 (4H); MS: m/z 434 (M^+, $C_{27}H_{46}O_4$).

(2R,3S,22S)-2,3,22-Triacetoxy-5α-cholestan-6-one **17b**. A solution of **15** (1.36 g) in dry ether (20 ml) was added dropwise to a solution of isoamylmagnesium bromide in ether (20 ml) prepared from isoamyl bromide (4.6 g) and magnesium (740 mg). The mixture was stirred overnight at room temperature. The reaction was then quenched by the addition of ammonium chloride solution under ice-cooling and the mixture was extracted with ether. The extract was washed with water, saturated sodium bicarbonate solution and brine, dried over potassium carbonate and concentrated *in vacuo* to give 1.58 g of crude **16**, IR ν_{max} cm^{-1}: 3500 (m), 1285 (s), 1220 (s), 1150 (s), 1085 (s). This was dissolved in THF (80 ml) and mixed with 35% perchloric acid (80 ml). The mixture was stirred and heated at 55°C for 1 hr. It was then diluted with water and extracted with ethyl acetate. The extract was washed with water, saturated sodium bicarbonate and brine, dried over magnesium sulfate and concentrated *in vacuo* to give 770 mg of crude **17a**, IR ν_{max} cm^{-1}: 3350 (br. s), 1710 (s), 1320 (s), 1270 (s), 1040 (s). This was dissolved in pyridine (35 ml) and mixed with acetic anhydride (15 ml). The mixture was stirred overnight at room temperature. The subsequent conventional work-up gave crude **17b** as an oil. This was chromatographed over silica gel to give 448 mg (26% from **15**) of **17b** as needles, mp 160~165°C, $[\alpha]_D^{26}$ −16.7° (c=0.94, $CHCl_3$); IR ν_{max} cm^{-1}: 1740 (s), 1710 (s), 1240 (s), 1040 (s); NMR δ ($CDCl_3$) 0.4~1.05 (15H), 1.93 (3H, s), 2.00 (3H, s), 2.04 (3H, s), 4.6~5.1 (2H, m), 5.15~5.4 (1H, m). *Anal.* Found: C, 70.71; H, 9.39. Calcd. for $C_{33}H_{52}O_7$: C, 70.68; H, 9.35%.

(2R,3S,22S)-2,3,22-Triacetoxy-B-homo-7-oxa-5α-cholestan-6-one **18**. A solution of trifluoroperacetic acid in methylene chloride (5 ml) was prepared from trifluoroacetic anhydride (3.5 ml) and 90% hydrogen peroxide (0.5 ml). This was added dropwise to a stirred and ice-cooled mixture of **17b** (445 mg) and disodium hydrogen phosphate (3 g) in methylene chloride (30 ml). After the addition, the mixture was stirred and heated under reflux for 1.8 hr. The subsequent work-up was followed by chromatographic purification of the product to give 241 mg (53%) of **18** as a gum, IR ν_{max} cm^{-1}: 1725 (s), 1240 (s); NMR δ($CDCl_3$) 0.5~1.1 (15H), 1.98 (3H, s), 2.02 (3H, s), 2.09 (3H, s), 2.75~3.20 (1H, m), 3.9~4.2 (2H), 4.6~5.1 (2H), 5.2~5.5 (1H). This was directly employed for the next step.

(2R,3S,22S)-2,3,22-Trihydroxy-B-homo-7-oxa-5α-cholestan-6-one **6**. 50% Sodium hydroxide solution (1 ml) was added to a solution of **18** (241 mg) in methanol (6 ml) and the mixture was stirred for 1 hr under reflux and for another 1 hr at room temperature. Subsequent acidification and work-up gave 200 mg of crude **6**. This was chromatographed over silica gel (Mallinckrodt SILICAR CC-7). Elution with 1.5% methanol in chloroform gave 82 mg (44%) of **6** as prisms from methanol, mp 216.5~219°C, $[\alpha]_D^{22}$ +29.6° (c=0.88, $CHCl_3$); IR ν_{max} cm^{-1}: 3400 (s), 1720 (sh), 1710 (s), 1060 (s), 1020 (s); NMR δ 0.5~1.0 (15H, 0.68, 0.81, 0.90), 1.0~2.2 (22H), 2.75~3.2 (1H), 3.3~3.6 (2H), 3.7~4.2 (3H); *Anal.* Found: C, 71.47; H, 10.01. Calcd. for $C_{27}H_{46}O_5$: C, 71.96; H, 10.29%.

(2R,3S,20S)-2,3-Diacetoxy-20-acetoxymethyl-5α-pregnan-6-one **20b**. A solution of **15** (1.26 g) in dry ether (55 ml) was added dropwise to a stirred and ice-coold suspension of lithium aluminum hydride (282 mg) in dry ether (55 ml). The mixture was stirred overnight at room temperature. The excess lithium aluminum hydride was destroyed by carefully adding water with ice-cooling. The subsequent work-up gave crude **19** (1.12 g), IR ν_{max} cm^{-1}: 3450 (m), 1080 (s), 1060 (s), 1040 (s). This was dissolved in THF (80 ml) and mixed with 35% perchloric acid (80 ml). The mixture was stirred for 1 hr at 55°C. It was then diluted with water and extracted with ethyl acetate. The work-up gave 740 mg of crude **20a**, IR ν_{max} cm^{-1}: 3300 (s), 1715 (s), 1040 (s). This was dissolved in pyridine (35 ml) and mixed with acetic anhydride (15 ml). The mixture was stirred overnight at room temperature. The subsequent

work-up was followed by chromatographic purification (Merck Kieselgel 60). Elution with *n*-hexane–ethyl acetate (6 : 1 ~ 4 : 1) gave 528 mg (38% from **15**) of **20b**. This was recrystallized from ethyl acetate–petroleum ether to give prisms, mp 182 ~ 185°C, $[\alpha]_D^{22}$ −7.48° (c=0.53, $CHCl_3$); IR ν_{max} cm^{-1}: 1750 (s), 1740 (s), 1710 (s), 1250 (s); NMR δ ($CDCl_3$) 0.70 (3H, s), 0.85 (3H, s), 1.04 (3H, d, J=6 Hz), 1.99 (3H, s), 2.06 (3H, s), 2.10 (3H, s), 3.6 ~ 4.3 (2H, m), 4.7 ~ 5.2 (1H, m), 5.25 ~ 5.5 (1H, m); *Anal.* Found: C, 68.77; H, 8.61. Calcd. for $C_{28}H_{42}O_7$: C, 68.54; H, 8.63%.

(2R,3S,20S)-2,3-Diacetoxy-20-acetoxymethyl-B-homo-7-oxa-5α-pregnan-6-one **21**. A solution of trifluoroperacetic acid in methylene chloride (5 ml) was prepared from trifluoroacetic anhydride (4.5 ml) and 90% hydrogen peroxide (0.7 ml). This was added dropwise to a solution of **20b** (520 mg) in methylene chloride (35 ml) suspending disodium hydrogen phosphate (3 g) with stirring and ice-cooling. After the addition, the mixture was stirred and heated under reflux for 1.8 hr. The subsequent work-up was followed by chromatographic purification over silica gel (Mallinckrodt SILICAR CC-7). Elution with *n*-hexane–ethyl acetate (6 : 1 ~ 2 : 1) gave 305 mg (57%) of **21**. This was recrystallized from ethyl acetate–petroleum ether to give needles, mp 218 ~ 220°C, $[\alpha]_D^{22}$ +34.2° (c=0.51, $CHCl_3$); IR ν_{max} cm^{-1}: 1740 (sh), 1730 (s), 1720 (sh), 1240 (s), 1050 (s), 1025 (s); NMR ($CDCl_3$) 0.72 (3H, s), 0.97 (3H, s), 0.99 (3H, d, J=6 Hz), 1.96 (3H, s), 2.00 (3H, s), 2.08 (3H, s), 2.8 ~ 3.2 (1H, m), 3.5 ~ 4.2 (4H, m), 4.6 ~ 5.0 (1H, m), 5.15 ~ 5.4 (1H, m); *Anal.* Found: C, 66.44; H, 8.27. Calcd. for $C_{28}H_{42}O_3$: C, 66.38; H, 8.36%.

(2R,3S,20S)-2,3-Dihydroxy-20-hydroxymethyl-B-homo-7-oxa-5α-pregnan-6-one **7**. 50% Sodium hydroxide solution (1.5 ml) was added to a solution of **21** (305 mg) in methanol (8 ml). The mixture was stirred for 1 hr under reflux and for 1.5 hr at room temperature. Subsequent acidification and work-up gave 84 mg (37%) of **7** as crystals. This was recrystallized from methanol to give prisms, mp 242 ~ 247°C, $[\alpha]_D^{26}$ +52.8° (c=0.716, CH_3OH); IR ν_{max} cm^{-1}: 3500 (s), 3400 (s), 3220 (s), 1720 (s), 1180 (m), 1060 (s), 1030 (m), 980 (m); NMR δ (C_5D_5N) 0.60 (3H, s), 1.00 (3H, s), 1.16 (3H, d, J=6 Hz), 3.1 ~ 4.5 (7H, m); *Anal.* Found: C, 68.78; H, 9.28. Calcd. for $C_{22}H_{36}O_5$; C, 69.44; H, 9.54%.

5α-Pregn-2-ene-6,20-dione **25**. Pregnenolone tosylate **22b** was prepared from pregnenolone **22a** in the usual manner. A mixture of **22b** (25 g), potassium hydrogen carbonate (12 g), acetone (1.8 liters) and water (150 ml) was stirred and heated under reflux overnight. It was then concentrated *in vacuo*. The residue was extracted with ether–ethyl acetate. The subsequent work-up gave 16.4 g of crude **23**,[12] IR ν_{max} cm^{-1} 3530 (s), 1690 (s). Jones' chromic acid (8N, 30 ml) was gradually added to a stirred solution of **23** (37.4 g) in acetone (600 ml). The mixture was stirred for 10 min at room temperature. The subsequent work-up gave 25.9 g of crude **24**, IR ν_{max} cm^{-1}: 1700 (s), 1680 (sh), 1270 (s). *p*-Toluenesulfonic acid (700 mg) was added to a suspension of **24** (19.42 g) in sulfolane (150 ml) and the mixture was stirred and heated at 160°C for 1.5 hr. After cooling, it was poured into water and extracted with benzene–ether (1 : 1). The subsequent work-up gave 20 g of crude **25**. This was purified by chromatography over silica gel (Merck Kieselgel 60). Elution with *n*-hexane–ethyl acetate (10 : 1 ~ 9 : 1) yielded 7.31 g (28% from **22a**) of almost pure **25**. A portion of it was rechromatographed and recrystallized from benzene–petroleum ether to give prisms, mp 144 ~ 147°C. $[\alpha]_D^{23}$ +76.9° (c=1.026, $CHCl_3$); IR ν_{max} cm^{-1}: 1700 (s), 1660 (m), 1620 (w), 1355 (s), 1230 (s), NMR δ ($CDCl_3$) 0.62 (3H, s), 0.70 (3H, s), 2.10 (3H, s), 5.60 (2H, br. s); *Anal.* Found: C, 80.11; H, 9.71. Calcd. for $C_{21}H_{30}O_2$: C, 80.21; H, 9.62%.

(2R,3S)-2,3-Dihydroxy-5α-pregnane-6,20-dione **26a**. Osmium tetroxide (0.1 g) in *t*-butyl alcohol (3 ml), *N*-methylmorpholine *N*-oxide (1.2 g) and water (2 ml) were added to a solution of **25** (1.1 g) in acetone (60 ml). The mixture was stirred overnight under argon at room temperature. It was then concentrated *in vacuo*. The residue was extracted with chloroform. The extract was washed with dilute hydrochloric acid, dried over potassium carbonate and concentrated *in vacuo*. The residue was triturated with ether to give 550 mg (46%) of **26a** as crystals. This was recrystallized from ethanol to give prisms, mp 176 ~ 177°C, $[\alpha]_D^{23}$ +41.6° (c=0.267, $CHCl_3$); IR ν_{max} cm^{-1}: 3360 (m), 1710 (s), 1055 (m), 1040 (m); NMR δ ($CDCl_3$) 0.60 (3H, s), 0.72 (3H, s), 2.10 (3H, s), 3.4 ~ 4.1 (2H, m); *Anal.* Found: C, 71.52; H, 9.14. Calcd. for $C_{21}H_{32}O_4$: C, 72.38; H, 9.26%.

(2R,3S)-2,3-Diacetoxy-5α-pregnane-6,20-dione **26b**. Acetic anhydride (3 ml) was added to a solution of **26a** (500 mg) in pyridine (6 ml) containing *N,N*-dimethylaminopyridine (0.1 g). The mixture was left to stand overnight at room temperature. Subsequent working-up gave 500 mg (81%) of **26b**. This was chromatographed over silica gel. IR ν_{max} cm^{-1}: 1740 (s), 1705 (s), 1240 (s), 1040 (s); NMR ($CDCl_3$) 0.62 (3H, s), 0.82 (3H, s), 0.96 (3H, s), 1.06 (3H, s), 1.08 (3H, s), 4.7 ~ 5.1 (1H, m), 5.2 ~ 5.5 (1H, m). This was used directly for the next step.

(2R,3S,17S)-2,3,17-Triacetoxy-B-homo-7-oxa-5α-androstan-6-one **27**. A solution of trifluoroperacetic acid in methylene chloride was prepared from trifluoroacetic anhydride (5.2 ml) and 90% hydrogen peroxide (0.8 ml) in methylene dichloride (8 ml). This was added dropwise to a stirred and ice-cooled mixture of **26b** (500 mg) and disodium hydrogen phosphate (6 g) in methylene chloride (30 ml). The mixture was stirred and heated under reflux for 1.5 hr. Subsequent working-up was followed by chromatographic purification over silica gel (Mallinckrodt SILICAR CC-7). Elution with *n*-hexane–

ethyl acetate (9 : 1 ~ 4 : 1) gave 219 mg (41%) of **27**, IR ν_{max} cm^{-1}: 1745 (s), 1730 (s), 1240 (s), 1040 (s); NMR δ 0.80 (3H, s), 0.98 (3H, s), 1.96 (3H, s), 2.02 (3H, s), 2.08 (3H, s), 1.8 ~ 2.2 (1H, m), 3.9 ~ 4.2 (2H, m), 4.3 ~ 5.2 (2H, m), 5.35 (1H, broad s). This was employed for the next step without further purification.

(2R,3R,17S)-2,3,17-Trihydroxy-B-homo-7-oxa-5α-androstan-6-one **8**. 50% Sodium hydroxide solution (1 ml) was added to a solution of **27** (210 mg) in methanol (6 ml). The mixture was stirred and heated under reflux for 1 hr. Subsequent acidification and work-up gave crude **8**. This was chromatographed over silica gel (Mallinckrodt SILICAR CC-7) to give 100 mg (65%) of **8**. This was recrystallized from 95% ethanol to give prisms, mp 144 ~ 146°C, $[\alpha]_D^{21}$ +43.3° (c = 0.205, $CHCl_3$); IR ν_{max} cm^{-1}: 3640 (w), 3530 (s), 3500 (s), 3430 (s), 3320 (s), 1680 (s), 1062 (s), 1030 (m); NMR δ (400 MHz, C_5D_5N) 0.90 (3H, s), 1.07 (3H, s), ~2.6 (1H, m), 3.60 (1H, dd, J_1 = 4, J_2 = 12 Hz), 3.83 (1H, t, J = 8 Hz), 3.5 ~ 3.7 (3H, m), 4.45 (1H, br. s), ~6.1 (~3H, br); MS: m/z 338 (M^+, $C_{19}H_{30}O_5$); *Anal.* Found: C, 64.69; H, 8.55. Calcd. for $C_{19}H_{30}O_5 \cdot H_2O$: C, 64.02; H, 9.05%.

Acknowledgments. We are indebted to Professor S. Marumo and Dr. K. Wada, Nagoya University, for their bioassay. Our thanks are due to Dr. K. Okada and Mr. S. Kuwahara of this Laboratory for their help in purifying several analytical samples. We thank Mr. T. Uematsu for drawing the formulas.

REFERENCES

1) M. D. Grove, J. F. Spencer, W. K. Rohwedder, N. Mandava, J. F. Worley, J. D. Warthen, Jr., G. L. Steffens, J. L. Flippen-Anderson and J. C. Cook, Jr., *Nature*, **281**, 216 (1979).
2) M. Sakakibara, K. Okada, Y. Ichikawa and K. Mori, *Heterocycles*, **17**, 301 (1982).
3) K. Mori, M. Sakakibara, Y. Ichikawa, H. Ueda, K. Okada, T. Umemura, G. Yabuta, S. Kuwahara, M. Kondo, M. Minobe and T. Sogabe, *Tetrahedron*, **38**, 2099 (1982).
4) M. Sakakibara and K. Mori, *Agric. Biol. Chem.*, **46**, 2769 (1982).
5) K. Mori, *Agric. Biol. Chem.*, **44**, 1211 (1980).
6) K. Okada and K. Mori, *Agric. Biol. Chem.*, **47**, 89 (1983).
7) E. Maeda, *Physiol. Planta.*, **18**, 813 (1965).
8) M. J. Thompson, N. Mandava, J. L. Flippen-Anderson, J. F. Worley, S. R. Dutky, W. E. Robbins and W. Lusby, *J. Org. Chem.*, **44**, 5002 (1979).
9) K. Wada and S. Marumo, *Agric. Biol. Chem.*, **45**, 2579 (1981).
10) S. Takatsuto, B. Ying, M. Morisaki and N. Ikekawa, *Chem. Pharm. Bull.*, **29**, 903 (1981).
11) E. M. Kosower and S. Winstein, *J. Am. Chem. Soc.*, **78**, 4347 (1956).
12) M. Patel and W. J. Peal, *J. Chem. Soc.*, 1544 (1963).
13) M. P. Hartshorn, *J. Chem. Soc.*, 3168 (1962).
14) D. H. R. Barton, P. G. Feakins, J. P. Poyser and P. G. Sammes, *J. Chem. Soc. (C)*, 1584 (1970).
15) J. R. Wiersig, N. Waespe-Sarcevic and C. Djerassi, *J. Org. Chem.*, **44**, 3374 (1979).
16) S. Fung and J. B. Siddall, Abstracts of Papers, PEST 69, Second Chemical Congress of the North American Continent, San Francisco, August, 1980.
17) K. Wada, S. Marumo, N. Ikekawa, M. Morisaki and K. Mori, *Plant & Cell Physiol.*, **22**, 323 (1981).