and valine which required double couplings. The coupling progress was followed by the ninhydrin test.[10] The oxime resin was capped following each coupling cycle. Following the successful coupling of the final cysteine, it was deprotected, neutralized and acetylated.

Cyclic Disulfide AcCys-Val-Val-Gly-Tyr-Ile-Gly-Glu-Arg-Cys-NH-Et (1b). **3** (1 g) was stirred for 2.5 h with $EtNH_2$-saturated DCM. The cleaved peptide resin mixture was filtered, triturated in sequence with DCM, MeOH and TFA. The TFA-containing solution was concentrated to give 239 mg of crude protected peptide **4**. This material was subjected to HF/*p*-cresol (8:2) at 0 C for 1 h, concentrated in vacuo, taken up in 20 mL of 50% aqueous HOAc and 1% butanedithiol, and precipitated in diethyl ether (100 mL). The precipitate was washed sequentially with diethyl ether and EtOAc and taken up in 100 mL of 0.1 M sodium phosphate buffer (pH 8.0) and 20 mg of DTT to give **2b**. After 10 min ICH_2CH_2I in MeOH was added in aliquots[11a] until no free thiol remained (negative Ellman's test[13]). The reaction was quenched by the addition of glacial acetic acid. The crude peptide **1b** was purified on a RP18 column (gradient: 100/0 isocratic 30 min, 100/0 to 70/30 over 30 min, 70/30 to 60/40 over 180 min H_2O/CH_3CN + 0.1% TFA). The appropriate fractions were pooled, concentrated, redissolved in H_2O, and lyophilized. Anal. HPLC: one peak (R_f = 11.86 min, k' = 4.93). Amino Acid Anal.: Glu (1.03), Gly (2.08), Val (1.79), Ile (1.06), Tyr (1.09). FAB/MS: $(M + H)^+$ at *m/z* 1165 for mol wt 1164.

Cyclic Disulfide AcCys-Val-Val-Gly-Tyr-Ile-Gly-Glu-Arg-Cys-NH-Chx (1c). **3** (1.3 g) was treated with 1 mL of cyclohexylamine in DCM (50 mL) for 5.5 h at room temperature to give **5**. This peptide (294 mg crude), worked up as described for **4** above, was sequentially treated with HF to produce **2c**, oxidized to produce crude **1c**, and then purified on a RP18 column (gradient: 100/0 30 min, 100/0 to 60/40 over 30 min, 60/40 to 40/60 over 180 min H_2O/CH_3CN + 0.1% TFA). The peak tubes were pooled and rechromatographed in the same solvents (gradient 60/40 for 30 min, 60/40 to 40/60 over 240 min). The appropriate fractions were pooled, concentrated, redissolved in H_2O and lyophilized. Anal. HPLC: one peak (R_f = 13.45 min, k' = 7.41). Amino Acid Anal.: Glu (1.05); Gly (2.02); Cys/2 (0.88); Ile (1.03); Val (1.83); Tyr (1.2); Arg (1.11). FAB/MS: $(M + H)^+$ at *m/z* 1219 for mol wt 1218.

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An Improved Synthesis of Plant Growth Regulating Steroid Brassinolide and Its Congeners

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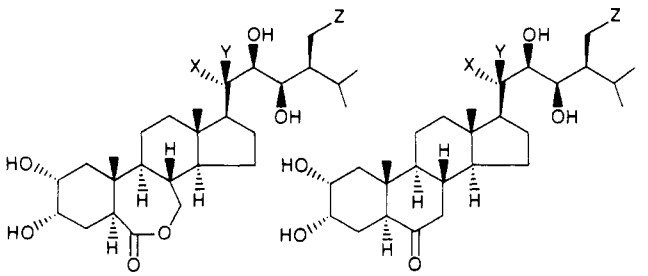
Brassinosteroids including brassinolide and castasterone have been synthesized by using pregnenolone as starting material.

Brassinolide (**1**), isolated from rape pollen (*Brassica napus* L.) is known to exhibit plant growth regulating activity. Because of its interesting physiological activity and its novel structural features, much effort has been devoted in recent years to develop the synthetic route to brassinolide and its analogues.[1]

Recently we succeeded in the stereoselective syntheses of brassinolide,[1] its enantiomer (22*S*,23*S*,24*R*)-22,23,24-epibrassinolide,[1] and 26,27-bisnorbrassinolide[2] by using a stereoselective reduction of the corresponding 5-ylidenetetronates as a key step to control the stereochemistry at the C-20, -22, -23, and -24 positions. In those syntheses, 6β-methoxy-3α,5-cyclopregnan-20-one was employed as a starting material, and the steroidal nucleus was manipulated after construction of the side chain. We here wish to report improved syntheses of brassinolide and its congeners including castasterone, where the steroidal nucleus was modified before construction of the side chain.

Chart I



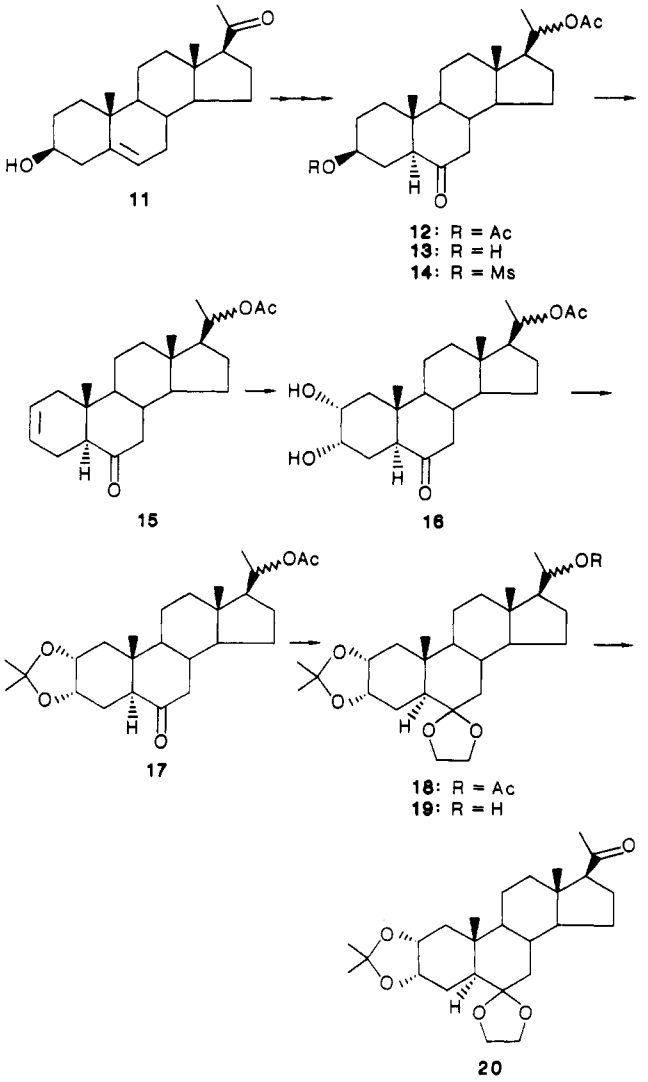
1, brassinolide (X = Me, Y = Z = H)
2, 28-hydroxybrassinolide (X = Me, Y = H, Z = OH)
3, 20,28-dihydroxybrassinolide (X = Me, Y = Z = OH)
4, 20-hydroxybrassinolide (X = Me, Y = OH, Z = H)
5, 20-epibrassinolide (X = Z = H, Y = Me)
6, castasterone (X = Me, Y = Z = H)
7, 28-hydroxycastasterone (X = Me, Y = H, Z = OH)
8, 20,28-dihydroxycastasterone (X = Me, Y = Z = OH)
9, 20-hydroxycastasterone (X = Me, Y = OH, Z = H)
10, 20-epicastasterone (X = Z = H, Y = Me)

The structure–activity relationships of brassinosteroids have been investigated by several groups,[3] whose results

(1) Kametani, T.; Katoh, T.; Tsubuki, M.; Honda, T. *J. Am. Chem. Soc.* **1986**, *108*, 7055 and references cited therein.
(2) Kametani, T.; Katoh, T.; Tsubuki, M.; Honda, T. *Chem. Pharm. Bull.* **1987**, *35*, 2334.

0022-3263/88/1953-1982$01.50/0

Scheme I

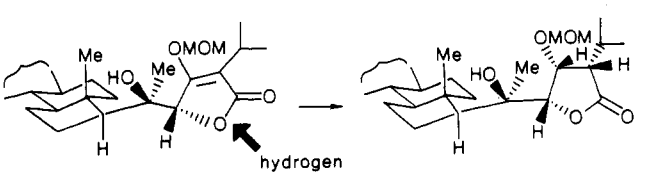


indicated that the 2α,3α-diol in the A-ring and 6-oxo or 7-oxa lactone moiety in the B-ring is required to show biological activity and suggested the possibility of finding of a new potent substance by modification of the side chain.

Given these considerations, we sought to prepare new derivatives of brassinosteroids bearing another hydroxy group(s) at the C-20 or at the C-28 position of the side chain, although such derivatives are hitherto unknown in nature (Chart I).

Thus, pregnenolone was first converted into an A,B-ring-functionalized 20-oxo steroid as follows. The diacetate **12**,[4] derived from pregnenolone **11** was selectively hydrolyzed with 5% potassium hydroxide to give alcohol **13**, whose mesylation with mesyl chloride in pyridine afforded

Chart II



mesylate **14**. Treatment of the mesylate with lithium bromide in refluxing *N,N*-dimethylformamide furnished olefin **15** in 80% yield from **12**. Osmylation of **15**, followed by acetonide formation was carried out in the usual manner to provide the acetonide **17** via the glycol **16** in 88% yield from **15**. After ketalization of **17** by transketalization with 2,2-dimethyl-1,3-dioxolane,[5] the resulting acetate **18** was hydrolyzed with 5% methanolic potassium hydroxide to yield alcohol **19**, whose oxidation with pyridinium chlorochromate afforded the desired 20-oxo steroid **20** in 84% yield from **17** (Scheme I).

With the requisite starting material available, construction of the side chain was investigated. Chelation-controlled addition reaction[1] of 3-isopropyltetronic acid to the 20-oxo steroid **20** followed by treatment of the adducts with chloromethyl methyl ether afforded the MOM ethers **21** and **23** in 79% and 9% yields, respectively. Syn dehydration of the tertiary alcohol **21** via the corresponding trifluoroacetate **22** by adopting the procedure developed by us[1] provided the olefins **24** and **25**, in 71% and 20% yields, respectively. Catalytic reduction of the major olefin **24** over 5% rhodium on alumina in ethyl acetate under 7 atm of hydrogen afforded the lactone **26** as a sole product in 92% yield. Lactone **26** was converted into 28-hydroxybrassinolide (**2**) as follows. Lithium aluminum hydride reduction of lactone **26**, followed by the acetylation of the alcohol function afforded the diacetate **28** via the diol **27**. Cleavage of the MOM and acetonide groups and deketalization of **28** was carried out in one step on treatment with aqueous acetic acid at refluxing temperature to provide the glycol, which without purification was acetylated with acetic anhydride in pyridine in the presence of (*N,N*-dimethylamino)pyridine (DMAP) to afford the pentaacetate **29** in 85% yield from **26**. Hydrolysis of the pentaacetate **29** with 5% methanolic potassium hydroxide at reflux for 1 h yielded the 28-hydroxycastasterone (**7**) in 95% yield. On the other hand, Baeyer–Villiger oxidation of **29** was achieved by adopting the well-established procedure[6] using trifluoroperacetic acid in dichloromethane at 0 °C to furnish the lactone **30**, which on hydrolysis of the acetoxy groups gave 28-hydroxybrassinolide (**2**) in 80% yield from **29**.

Thus, the new derivatives bearing a hydroxy group at the C-28 position of brassinosteroids were synthesized stereoselectively in good yield (Scheme II).

Since castasterone (**6**),[7] one of the naturally occurring brassinosteroids, also exhibits a strong plant growth regulating activity, we were interested in the conversion of the diol **27** to **6**. Selective mesylation of the primary alcohol of **27** with mesyl chloride and triethylamine in dichloromethane at 0 °C yielded the mesylate **31**, whose reduction with lithium aluminum hydride in ether resulted in the construction of C-24 methyl function to give rise to the alcohol **32** in 82% yield from **27**. Acetylation of **32**

(3) (a) Takatsuto, S.; Ikekawa, N. *J. Chem. Soc., Perkin Trans. 1* **1984**, 439. (b) Anastasia, M.; Allevi, P.; Ciuffreda, P.; Fiecchi, A.; Scala, A. *J. Org. Chem.* **1985**, *50*, 321. (c) Kohout, L.; Strnad, M. *Collect. Czech. Chem. Commun.* **1986**, *51*, 447. (d) Černý, V.; Strnad, M.; Kaminet, M. *Ibid.* **1986**, *51*, 687. (e) Kerb, U.; Eder, U.; Krähmer, H. *Agric. Biol. Chem.* **1986**, *50*, 1359. (f) Anastasia, M.; Allevi, P.; Ciuffreda, P.; Fiecchi, A.; Scala, A. *J. Chem. Soc., Perkin Trans. 1* **1986**, 2117. (g) Kishi, T.; Wada, K.; Marumo, S.; Mori, K. *Agric. Biol. Chem.* **1986**, *50*, 1821. (h) Takatsuto, S.; Ikekawa, N.; Morishita, T.; Abe, H. *Chem. Pharm. Bull.* **1987**, *35*, 211. (i) Černý, V.; Zajíček, J.; Strnad, M. *Collect. Czech. Chem. Commun.* **1987**, *52*, 215. (j) Kohout, L.; Velgová, H.; Strnad, M. *Ibid.* **1987**, *52*, 476. (k) Kohout, L.; Černý, V.; Strnad, M. *Ibid.* **1987**, *52*, 1026.

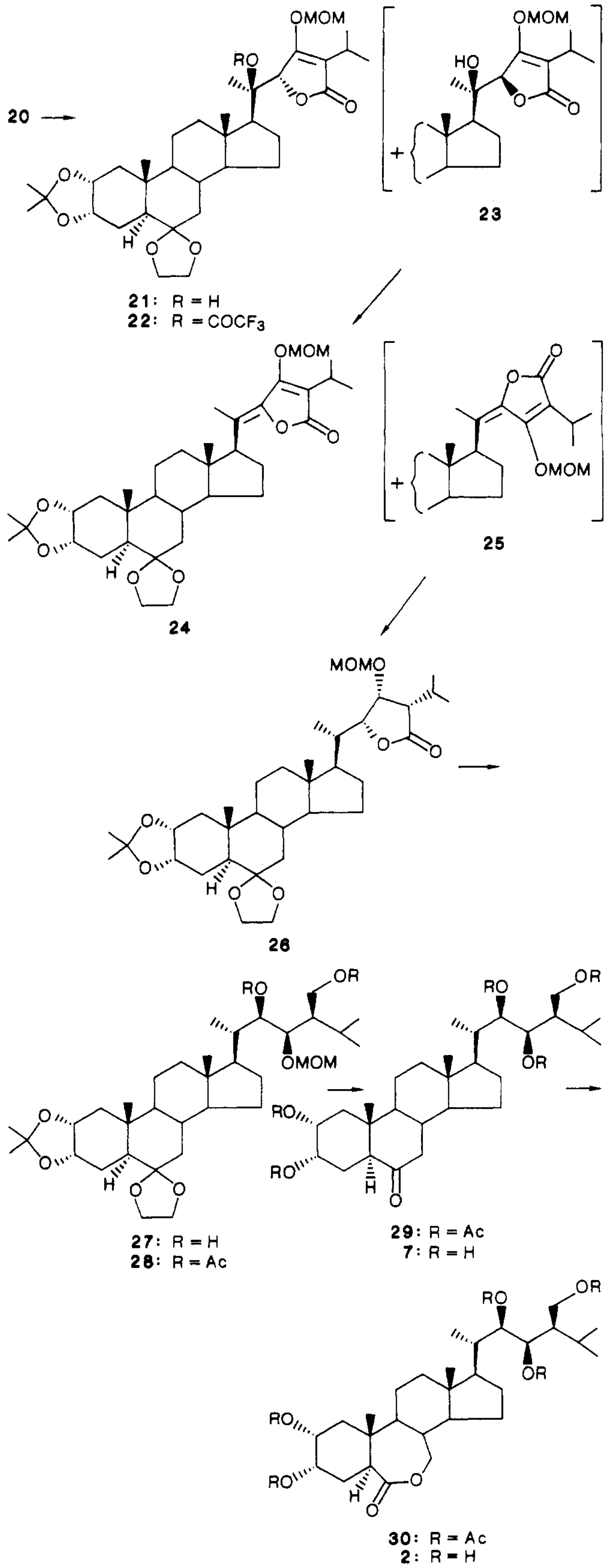
(4) Kametani, T.; Tsubuki, M.; Higurashi, K.; Honda, T. *J. Org. Chem.* **1985**, *51*, 2932.

(5) Aburatani, M.; Takeuchi, T.; Mori, K. *Agric. Biol. Chem.* **1985**, *49*, 3557.

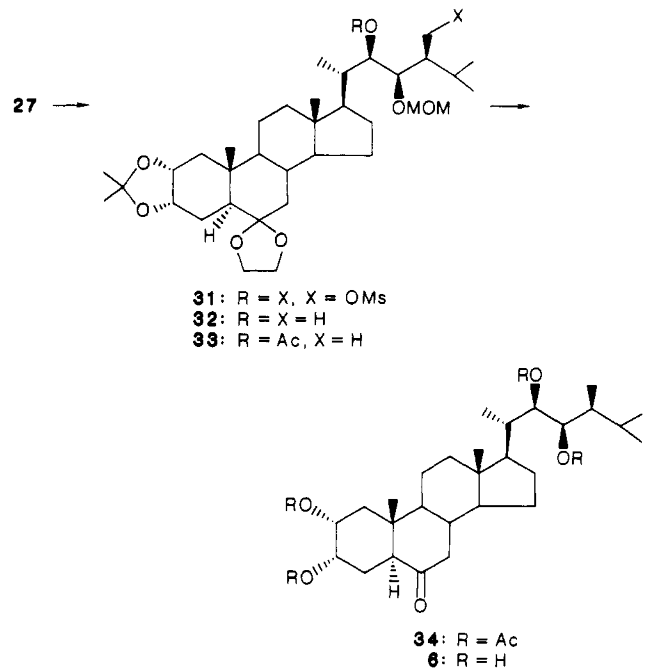
(6) Takatsuto, S.; Ikekawa, N. *Tetrahedron Lett.* **1983**, *24*, 917.

(7) Yokota, T.; Arima, M.; Takahashi, N. *Tetrahedron Lett.* **1982**, *23*, 1275.

Scheme II



Scheme III



followed by subsequent treatment with aqueous acetic acid and with 5% methanolic potassium hydroxide afforded castasterone (**6**) in 80% overall yield from **32** in three steps, via **33** and **34**. The physicochemical properties of the synthetic castasterone (**6**) were identical with those reported (Scheme III).[7,8]

Since the conversion of castasterone (**6**) into brassinolide (**1**) has already been achieved by several groups,[8b,9] this synthesis constitutes its formal synthesis.

Having accomplished an improved synthesis of brassinolide and castasterone, we next turned our attention to the preparation of the 20-hydroxybrassinosteroids, expecting to obtain an active substance as stated earlier.

The addition product **21** was reduced over 5% rhodium on alumina in ethyl acetate under 7 atm of hydrogen to furnish lactone **35**, in 93% yield, as a single product. The observed stereoselectivity indicated that the reduction occurred preferentially from the less hindered side with the stereochemistry at the C-22 position playing an important role. In other words, the reduction occurred from the side of the hydrogen at the C-22 position (Chart II). Reduction of the lactone **35** with lithium aluminum hydride in tetrahydrofuran gave the triol **36**, which was transformed into the diacetate **37** as described above in 97% yield. Deprotection of the acetonide, ketal, and MOM groups of **37** was successfully achieved by treatment with aqueous acetic acid without dehydration of the tertiary alcohol at the C-20 position, and the resulting tetrol was protected as pentaacetate **38** in 88% yield from **37**. Hydrolysis of **38** by using the reaction condition for the preparation of **6** afforded 20,28-dihydroxycastasterone (**8**) in 97% yield. Treatment of **38** with trifluoroperacetic acid brought about the desired Baeyer–Villiger oxidation to lactone **39**, which on hydrolysis of the acetoxy groups gave 20,28-dihydroxybrassinolide (**3**) in 76% yield from **38** (Scheme IV).

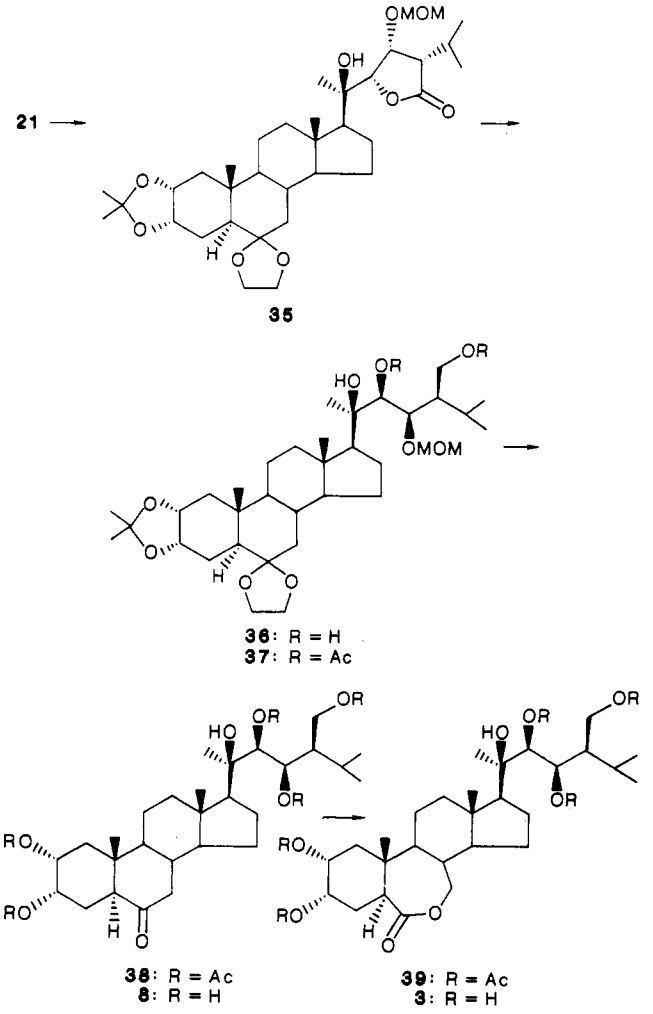
Furthermore, 20-hydroxycastasterone (**9**) and 20-hydroxybrassinolide (**4**) were prepared as follows.

Deoxygenation reaction of the primary alcohol of **36** was accomplished in two steps involving selective mesylation with mesyl chloride and triethylamine, followed by re-

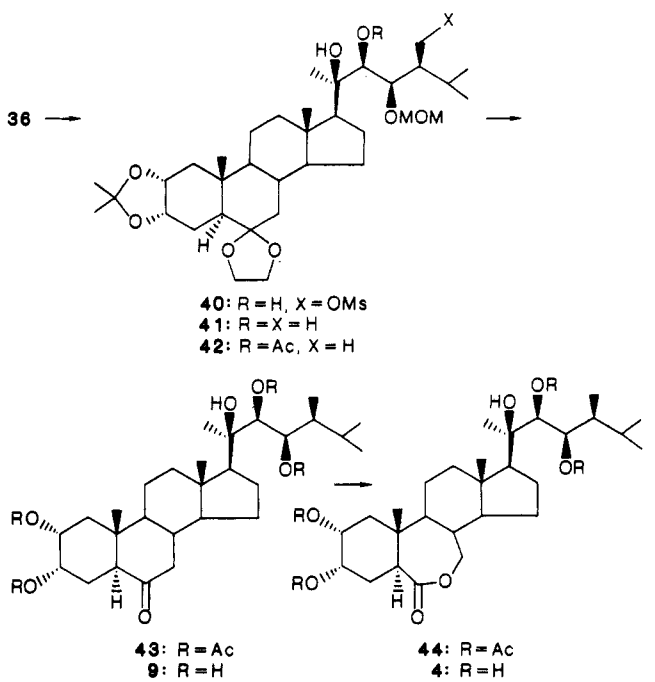
(8) (a) Mori, K.; Sakakibara, M.; Ichikawa, Y.; Ueda, H.; Okada, K.; Umemura, T.; Yabuta, G.; Kuwahara, S.; Kondo, M.; Minobe, M.; Sogabe, A. *Tetrahedron* **1982**, *38*, 2099. (b) Takatsuto, S.; Yazawa, N.; Ishiguro, M.; Morisaki, M.; Ikekawa, M. *J. Chem. Soc., Perkin Trans. 1* **1984**, 139.

(9) (a) Thompson, M. J.; Mandava, N. B.; Mendt, W. J.; Lusby, W. R.; Spaulding, D. W. *Steroids* **1981**, *38*, 567. (b) Mori, K.; Sakakibara, M.; Okada, K. *Tetrahedron* **1984**, *40*, 1767.

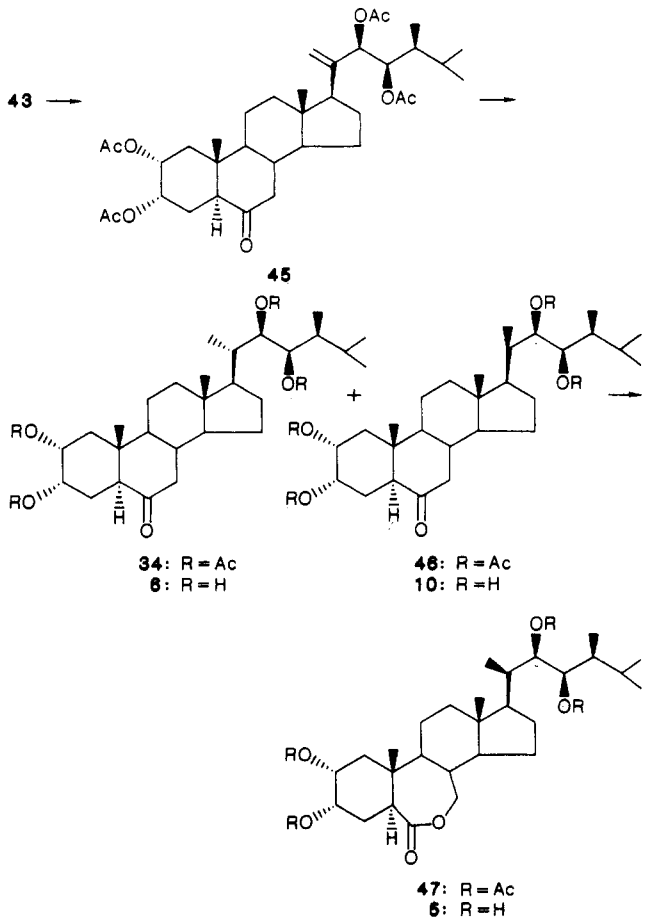
Scheme IV



Scheme V



Scheme VI



duction of the mesylate **40** with lithium aluminum hydride to give the MOM ether **41** in 80% yield. Acetylation of the secondary alcohol of **41** followed by deprotection of **42** with aqueous acetic acid and by acetylation of the resulting tetrol afforded the tetraacetate **43** in 77% yield. The tetraacetate **43** was hydrolyzed to give 20-hydroxycastasterone (**9**) in 97% yield, whereas Baeyer–Villiger oxidation and subsequent hydrolysis of the acetoxy groups for **43** provided 20-hydroxybrassinolide (**4**) in 72% yield (Scheme V).

In light of the biological activity displaced by the 20*R* derivative, the preparation of additional members of this class is of interest, although all naturally occurring brassinosteroids possess the 20*S* configuration. In order to synthesize 20*R* derivatives, the tertiary alcohol **43** was dehydrated with thionyl chloride in pyridine at 0 °C to afford the exo-olefin **45** in 90% yield. From an examination of a molecular models, catalytic reduction of the exo-olefin **45** would be expected to give an epimeric mixture at the C-20 position. Indeed, reduction of **45** over 5% rhodium on alumina in ethyl acetate under 3.2 atm of hydrogen afforded tetraacetylcastasterone **34** and its C-20 epimer in 92% yield in the ratio of 49:51. The physicochemical properties of **34**, convertible to castasterone (**6**) by hydrolysis, were identical with those reported.[8] Therefore, the stereochemistry at the C-22, C-23, and C-24 positions of the acetate **45** were unambiguously determined as 22*R*, 23*R*, and 24*S*. Hydrolysis of the C-20 epimer **46** with 5% methanolic potassium hydroxide gave rise to 20-epicastasterone (**10**) in quantitative yield. Synthesis of 20-epibrassinolide (**5**) was achieved by Baeyer–Villiger oxidation of **46** with trifluoroperacetic acid, followed by hydrolysis of the lactone **47** in 80% yield (Scheme VI).

Thus, a number of new brassinosteroids bearing hydroxy group(s) at the C-20 and C-28 positions were stereoselectively synthesized by using a stereoselective reduction of the tetronate derivatives. This synthesis indicated that the stereoselectivity of the catalytic reduction of the te-

tronate derivatives was clearly affected by the stereochemistry at the C-22 position.

The biological activity of the synthesized compounds are under investigation.

Experimental Section

Infrared (IR) spectra were taken on a Hitachi 260-10 spectrophotometer. Nuclear magnetic resonance (NMR) spectra were recorded for solutions in $CDCl_3$, unless otherwise noted, on JEOL JNM-FX-100 or JNM-GX-400 spectrometers with tetramethylsilane as an internal standard. Low- and high-resolution mass spectra (MS) were taken on a JEOL JMS-D-300 spectrometer. Melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. Optical rotations were measured with JASCO DIP-181 or DIP-360 instruments. Column chromatography was carried out on Wakogel C-200 (silica gel).

20-Acetoxy-5α-pregn-2-en-6-one (15). Potassium hydroxide–water (5%; 60 mL) was added to a stirred solution of 3β,20-diacetoxy-5α-pregnan-6-one (**12**) (20 g, 49.8 mmol) in methanol–dichloromethane (3:1 v/v) (650 mL) at room temperature. After being stirred for 1 h, the reaction mixture was treated with 6 M hydrochloric acid (60 mL) and extracted with ethyl acetate. The extract was washed with brine and dried over Na_2SO_4. Evaporation of the solvent gave 20-acetoxy-3β-hydroxy-5α-pregnan-6-one (**13**) (18.1 g), whose solution in dry pyridine (240 mL) was treated with methanesulfonyl chloride (18.6 mL, 0.24 mol) for 1 h at room temperature. The reaction mixture was diluted with water (200 mL) and extracted with ethyl acetate. The extract was washed with aqueous potassium hydrogen sulfate solution, aqueous sodium hydrogen carbonate solution, and brine and dried over Na_2SO_4. Evaporation of the solvent gave 20-acetoxy-3β-[(methylsulfonyl)oxy]-5α-pregnan-6-one (**14**) (21.7 g), whose solution in dry *N*,*N*-dimethylformamide (300 mL) was treated with lithium bromide (10 g, 95.4 mmol) for 1 h at 140 °C. The reaction mixture was extracted with ethyl acetate, and the extract was washed with aqueous potassium hydrogen sulfate solution, aqueous sodium hydrogen carbonate solution and brine, and dried over Na_2SO_4. Evaporation of the solvent gave a pale yellow solid, which was purified by chromatography on silica gel (300 g) using benzene containing 5% ethyl acetate as the eluant, to give the 2-en-6-one compound **15** (14.1 g, 80%) as colorless plates: mp 212.5–214.5 °C (acetone); IR ($CHCl_3$) 1720, 1700 cm^{-1}; ^1H NMR (400 MHz) δ 0.64 and 0.65 (3 H, each s, 18-H_3), 0.71 and 0.78 (3 H, each s, 19-H_3), 1.16 (3 H, d, J = 7 Hz, 21-H_3), 2.01 and 2.02 (3 H, each s, acetyl), 4.85 (1 H, s, 20-H), 5.56 (1 H, br d, J = 10 Hz, 2-H or 3-H), 5.69 (1 H, br d, J = 10 Hz, 2-H or 3-H); MS, m/z 358 (M^+), 343 (M^+ – Me), 298 ($M^+ - CH_3CO_2H$). Anal. Calcd for $C_{23}H_{34}O_3$: C, 77.05; H, 9.56. Found: C, 77.06; H, 9.80.

20-Acetoxy-2α,3α-(isopropylidenedioxy)-5α-pregnan-6-one (17). Osmium tetraoxide (1 g, 3.93 mmol) in tetrahydrofuran (50 mL) was added dropwise to a stirred solution of the 2-en-6-one compound **15** (14 g, 39.1 mmol) in *tert*-butyl alcohol–tetrahydrofuran–water (10:8:1 v/v) (400 mL) containing *N*-methylmorpholine *N*-oxide (16 g, 0.137 mmol) at room temperature. After the reaction mixture was stirred for 2 h at the same temperature, saturated aqueous sodium hydrogen sulfide solution (200 mL) was added, and the resulting solution was further stirred for 30 min and diluted with ethyl acetate (400 mL). The organic layer was washed with water and brine and dried over Na_2SO_4. Evaporation of the solvent gave 20-acetoxy-2α,3α-dihydroxy-5α-pregnan-6-one (**16**) (14.8 g), whose solution in acetone (300 mL) was treated with a catalytic amount of *p*-toluenesulfonic acid (500 mg) for 2 h at room temperature. The reaction mixture was diluted with ethyl acetate (300 mL), and the resulting solution was washed with aqueous sodium hydrogen carbonate solution and brine and dried over Na_2SO_4. Evaporation of the solvent gave a white solid, which was purified by chromatography on silica gel (300 g) using benzene containing 5% ethyl acetate as the eluant to give the acetonide **17** (14.9 g, 88%) as colorless needles: mp 198–200 °C (MeOH); IR ($CHCl_3$) 1720, 1710 cm^{-1}; ^1H NMR (400 MHz) δ 0.62 and 0.68 (3 H, each s, 18-H_3), 0.67 and 0.68 (3 H, each s, 19-H_3), 1.16 and 1.23 (3 H, each d, J = 7 Hz, 21-H_3), 1.34 (3 H, s, acetonide), 1.50 (3 H, s, acetonide), 2.01 and 2.02 (3 H, each s, acetyl), 4.06–4.13 (1 H, m, 2-H), 4.27 (1 H, br s, $W_{1/2}$ = 8.4 Hz, 3-H), 4.80–4.88 and 4.90–4.97 (1 H, each m, 20-H); MS, m/z 432 (M^+), 417 (M^+ – Me), 372 ($M^+ - CH_3CO_2H$). Anal. Calcd for $C_{26}H_{40}O_5$: C, 72.19; H, 9.32. Found: C, 72.17; H, 9.55.

6-(Ethylenedioxy)-2α,3α-(isopropylidenedioxy)-5α-pregnan-20-one (20). A solution of the acetonide **17** (14 g, 32.4 mmol) in 2,2-dimethyl-1,3-dioxolane[5] (80 mL) containing a catalytic amount of *p*-toluenesulfonic acid (400 mg) was refluxed for 2 h. After cooling, the reaction mixture was diluted with ethyl acetate (200 mL), and the resulting solution was washed with aqueous sodium hydrogen carbonate solution and brine and dried over Na_2SO_4. Evaporation of the solvent gave 20-acetoxy-6-(ethylenedioxy)-2α,3α-(isopropylidenedioxy)-5α-pregnane (**18**) (14.0 g), whose solution in 5% potassium hydroxide–methanol (200 mL) was refluxed for 1 h. After cooling, the reaction mixture was diluted with ethyl acetate (200 mL), and the resulting solution was washed with water and brine and dried over Na_2SO_4. Evaporation of the solvent gave 20-hydroxy-6-(ethylenedioxy)-2α,3α-(isopropylidenedioxy)-5α-pregnane (**19**) (12.7 g). A solution of **19** in dichloromethane (400 mL) was treated with pyridinium chlorochromate (12.4 g, 57.5 mmol) for 2 h at room temperature. The reaction mixture was washed with aqueous sodium hydrogen carbonate solution and brine and dried over Na_2SO_4. Evaporation of the solvent gave a yellow solid, which was purified by chromatography on silica gel (250 g) using benzene containing 10% ethyl acetate as the eluant to give the 20-oxo compound **20** (11.7 g, 84%) as colorless plates: mp 199–200 °C (MeOH); $[\alpha]^{23}_D$ +104.19° (*c* 0.43, $CHCl_3$); IR ($CHCl_3$) 1700 cm^{-1}; ^1H NMR δ (400 MHz) 0.62 (3 H, s, 18-H_3), 0.84 (3 H, s, 19-H_3), 1.32 (3 H, s, acetonide), 1.48 (3 H, s, acetonide), 2.12 (3 H, s, 21-H_3), 3.72–4.00 (4 H, m, OCH_2CH_2O), 4.04–4.14 (1 H, m, 2-H), 4.27 (1 H, br s, $W_{1/2}$ = 8.4 Hz, 3-H); MS, m/z 432 (M^+). Anal. Calcd for $C_{26}H_{40}O_5$: C, 72.19; H, 9.32. Found: C, 72.34; H, 9.53.

(20*R*,22*R*)- and (20*R*,22*S*)-6-(Ethylenedioxy)-20,22-dihydroxy-2α,3α-(isopropylidenedioxy)-23-(methoxymethoxy)-5α-23-ergosten-28-oic Acid γ-Lactone (21 and 23). A solution of the 20-oxo compound **20** (2.4 g, 5.56 mmol) in anhydrous tetrahydrofuran (24 mL) was added to a stirred solution of the dianion [prepared from 3-isopropyltetronic acid[1] (3.84 g, 27 mmol) in anhydrous tetrahydrofuran (24 mL) and lithium diisopropylamide (54 mmol) in anhydrous tetrahydrofuran (36 mL)] at −78 °C under a current of nitrogen, and the reaction mixture was then stirred for 1 h at the same temperature. After being quenched with aqueous ammonium chloride solution (40 mL), the resulting solution was extracted with ethyl acetate, and the extract was washed with aqueous sodium hydrogen carbonate solution and brine and dried over Na_2SO_4. Evaporation of the solvent gave a pale yellow solid (3.18 g), whose solution in dry *N*,*N*-dimethylformamide (42 mL) was treated with potassium carbonate (1.54 g, 11.1 mmol) for 2 h at 100 °C, and then chloromethyl methyl ether (0.464 mL, 6.12 mmol) was added at 50 °C. The reaction mixture was stirred for 10 min at the same temperature and diluted with ethyl acetate (100 mL). The organic layer was washed with water and brine and dried over Na_2SO_4. Evaporation of the solvent gave two products, which were separated by chromatography on silica gel (150 g) using dichloromethane containing 15% chloroform as the eluant to give the 20*R*,22*R* compound **21** (less polar; 2.71 g, 79%) as a colorless amorphous powder [$[\alpha]^{22}_D$ +33.07° (*c* 1.01, $CHCl_3$); IR ($CHCl_3$) 1750, 1660 cm^{-1}; ^1H NMR (400 MHz) δ 0.83 (3 H, s, 18-H_3), 0.87 (3 H, s, 19-H_3), 1.19 (3 H, s, 21-H_3), 1.23 (6 H, d, J = 7 Hz, 26-H_3 and 27-H_3), 1.33 (3 H, s, acetonide), 1.49 (3 H, s, acetonide), 2.85–2.95 (1 H, m, 25-H), 3.53 (3 H, s, OCH_3), 3.70–4.00 (4 H, m, OCH_2CH_2O), 4.05–4.12 (1 H, m, 2-H), 4.27 (1 H, br s, $W_{1/2}$ = 8.4 Hz, 3-H), 4.65 (1 H, s, 22-H), 5.06 and 5.44 (each 1 H, each d, J = 6 Hz, OCH_2OCH_3); MS, m/z 618 (M^+), 603 (M^+ – Me), 600 ($M^+ - H_2O$), 585 (M^+ – Me – H_2O), 433 (M^+ – 185, C_{22}–C_{23} cleavage); exact mass calcd for $C_{35}H_{54}O_9$ 618.3765, found 618.3759] and the 20*R*,22*S* compound **23** (more polar; 0.309 g, 9%) as a colorless amorphous powder: $[\alpha]^{22}_D$ +30.18° (*c* 1.13, $CHCl_3$); IR ($CHCl_3$) 1750, 1640 cm^{-1}; ^1H NMR (400 MHz) δ 0.84 (3 H, s, 18-H_3), 0.87 (3 H, s, 19-H_3), 1.18 (3 H, s, 21-H_3), 1.23 (3 H, d, J = 7 Hz, Me), 1.24 (3 H, d, J = 7 Hz, Me), 1.33 (3 H, s, acetonide), 1.47 (3 H, s, acetonide), 2.88–2.98 (1 H, m, 25-H), 3.55 (3 H, s, OCH_3), 3.70–3.97 (4 H, m, OCH_2CH_2O), 4.06–4.13 (1 H, m, 2-H), 4.27 (1 H, br s, $W_{1/2}$ = 8.4 Hz, 3-H), 4.54 (1 H, s, 22-H), 5.14 and 5.30 (each 1 H, each d, J = 6 Hz, OCH_2OCH_3); MS, m/z 618 (M^+),

603 (M^+ – Me), 600 (M^+ – H_2O), 585 (M^+ – Me – H_2O), 433 (M^+ – 185, C_{22}–C_{23} cleavage); exact mass calcd for $C_{35}H_{54}O_9$ 618.3768, found 618.3784.

(20*Z*)-6-(Ethylenedioxy)-2α,3α-(isopropylidenedioxy)-23-(methoxymethoxy)-5α-ergosta-20(22),23-dieno-28,22-lactone (24). Trifluoroacetic anhydride (1.35 mL, 9.56 mmol) was added dropwise to a stirred solution of the alcohol **21** (1 g, 1.62 mmol), triethylamine (0.933 mL, 6.69 mmol), and 4-pyrrolidinopyridine (141 mg, 0.951 mmol) in anhydrous dichloromethane (20 mL) at room temperature under a current of nitrogen. After being stirred for 1 h, the reaction mixture was diluted with chloroform (40 mL), and the resulting solution was washed with aqueous sodium carbonate solution and brine and dried over Na_2SO_4. Evaporation of the solvent gave the trifluoroacetate **22** (1.07 g, 93%): IR ($CHCl_3$) 1780, 1750, 1650 cm^{-1}; ^1H NMR (400 MHz) δ 0.82 (6 H, s, 18-H_3 and 19-H_3), 1.24 (6 H, d, J = 7 Hz, 26-H_3 and 27-H_3), 1.32 (3 H, s, acetonide), 1.47 (3 H, s, acetonide), 1.73 (3 H, s, 21-H_3), 2.98–3.08 (1 H, m, 25-H), 3.53 (3 H, s, OCH_3), 3.70–3.97 (4 H, m, OCH_2CH_2O), 4.05–4.12 (1 H, m, 2-H), 4.27 (1 H, br s, $W_{1/2}$ = 8.4 Hz, 3-H), 5.14 and 5.18 (each 1 H, each d, J = 6 Hz, OCH_2OCH_3), 5.30 (1 H, s, 22-H), MS, m/z 714 (M^+), 699 (M^+ – Me), 600 (M^+ – CF_3CO_2H), 585 (M^+ – Me – CF_3CO_2H); exact mass calcd for $C_{37}H_{53}O_{10}F_3$ 714.3589, found 714.3579. A solution of the trifluoroacetate **22** (1.07 g, 1.5 mmol) in anhydrous benzene (64 mL) containing DBU (0.456 mL, 3 mmol) was refluxed for 30 min. After cooling, the reaction mixture was washed with aqueous sodium carbonate solution and brine and dried over Na_2SO_4. Evaporation of the solvent gave a pale yellow solid, which was purified by chromatography on silica gel (30 g) using dichloromethane containing 5% chloroform as the eluant to give the 20*Z* compound **24** (638 mg, 71%) as a colorless amorphous powder: $[\alpha]^{23}_D$ –27.80° (*c* 1.09, $CHCl_3$); IR ($CHCl_3$) 1730, 1600 cm^{-1}; ^1H NMR (400 MHz) δ 0.67 (3 H, s, 18-H_3), 0.83 (3 H, s, 19-H_3), 1.25 (3 H, d, J = 7 Hz, Me), 1.28 (3 H, d, J = 7 Hz, Me), 1.32 (3 H, s, acetonide), 1.48 (3 H, s, acetonide), 2.04 (3 H, s, 21-H_3), 2.90–3.05 (1 H, m, 25-H), 3.56 (3 H, s, OMe), 3.70–4.00 (4 H, m, OCH_2CH_2O), 4.04–4.15 (1 H, m, 2-H), 4.27 (1 H, br s, $W_{1/2}$ = 8.4 Hz, 3-H), 5.17 and 5.20 (each 1 H, each d, J = 6 Hz, OCH_2OCH_3); MS, m/z 600 (M^+) 585 (M^+ – Me), 557 (M^+ – C_3H_7), 542 (M^+ – Me – C_3H_7); exact mass calcd for $C_{35}H_{52}O_8$ 600.3660, found 600.3645. Further elution gave the 20*E* compound **25** (180 mg, 20%) as a colorless amorphous powder: IR ($CHCl_3$) 1730, 1600 cm^{-1}; ^1H NMR (400 MHz) δ 0.70 (3 H, s, 18-H_3), 0.85 (3 H, s, 19-H_3), 1.28 (6 H, d, J = 7 Hz, 26-H_3 and 27-H_3), 1.32 (3 H, s, acetonide), 1.47 (3 H, s, acetonide), 1.94 (3 H, s, 21-H_3), 2.90–3.05 (1 H, m, 25-H), 3.55 (3 H, s, OMe), 3.70–4.00 (4 H, m, OCH_2CH_2O), 4.04–4.15 (1 H, m, 2-H), 4.27 (1 H, br s, $W_{1/2}$ = 8.4 Hz, 3-H), 5.14 and 5.21 (each 1 H, each d, J = 6.4 Hz, OCH_2OCH_3); MS, m/z 600 (M^+).

(22*R*,23*R*,24*S*)-6-(Ethylenedioxy)-2α,3α-(isopropylidenedioxy)-23-(methoxymethoxy)-5α-ergostano-28,22-lactone (26). A solution of the 20*Z* compound **24** (500 mg, 0.833 mmol) in ethyl acetate (20 mL) was hydrogenated over 5% rhodium on alumina (500 mg) for 13 h under medium pressure (7.0 atm) of hydrogen. The catalyst was filtered off and the filtrate was evaporated to afford the lactone **26** (460 mg, 92%) as a colorless amorphous powder: $[\alpha]^{23}_D$ +43.33° (*c* 0.57, $CHCl_3$); IR ($CHCl_3$) 1770 cm^{-1}; ^1H NMR (400 MHz) δ 0.71 (3 H, s, 18-H_3), 0.83 (3 H, s, 19-H_3), 1.07 (3 H, d, J = 7 Hz, Me), 1.14 (3 H, d, J = 7 Hz, Me), 1.24 (3 H, d, J = 7 Hz, Me), 1.33 (3 H, s, acetonide), 1.48 (3 H, s, acetonide), 2.10–2.20 (1 H, m, 25-H), 2.28 (1 H, dd, J = 8, 3.5 Hz, 24-H), 3.41 (3 H, s, OMe), 3.70–4.00 (4 H, m, OCH_2CH_2O), 4.05–4.13 (1 H, m, 2-H), 4.22 (1 H, dd, J = 3.5, 1.5 Hz, 22-H), 4.27 (1 H, br s, $W_{1/2}$ = 8.4 Hz, 3-H), 4.32 (1 H, dd, J = 5, 3.5 Hz, 23-H), 4.67 and 4.73 (each 1 H, each d, J = 6 Hz, OCH_2OCH_3); MS, m/z 604 (M^+), 589 (M^+ – Me); exact mass calcd for $C_{35}H_{60}O_8$ 604.3973, found 604.3972.

(22*R*,23*R*,24*R*)-22,28-Dihydroxy-6-(ethylenedioxy)-2α,3α-(isopropylidenedioxy)-23-(methoxymethoxy)-5α-ergostane (27). Lithium aluminum hydride (86 mg, 2.27 mmol) was added in small portions to a stirred solution of the lactone **26** (460 mg, 0.762 mmol) in anhydrous tetrahydrofuran (30 mL) under nitrogen at room temperature. After the reaction mixture was stirred for 30 min, 25% aqueous sodium hydroxide solution (4 mL) was added, and then the resulting solution was stirred for 10 min and extracted with ethyl acetate. The extract was washed with brine and dried over Na_2SO_4. Evaporation of the solvent gave the diol **27** (463 mg, 100%) as a colorless amorphous powder $[\alpha]^{22}_D$ +20.35° (*c* 1.07, $CHCl_3$); IR ($CHCl_3$) 3400 cm^{-1}; ^1H NMR (400 MHz) δ 0.66 (3 H, s, 18-H_3), 0.83 (3 H, s, 19-H_3), 0.87 (3 H, d, J = 7 Hz, Me), 1.00 (3 H, d, J = 7 Hz, Me), 1.06 (3 H, d, J = 7 Hz, Me), 1.32 (3 H, s, acetonide), 1.48 (3 H, s, acetonide), 3.45 (3 H, s, OMe), 3.75–4.00 (8 H, m, 22-H, 23-H, 28-H_2 and OCH_2CH_2O), 4.05–4.13 (1 H, m, 2-H), 4.27 (1 H, br s, $W_{1/2}$ = 8.4 Hz, 3-H), 4.67 and 4.81 (each 1 H, each d, J = 6 Hz, OCH_2OCH_3); MS, m/z 605 (M^+ – 3), 562 (M^+ – 3 – C_3H_7), 447 (M^+ – 161, C_{22}–C_{23} cleavage); exact mass calcd for $C_{35}H_{57}O_8$ 605.4052, found 605.4082.

(22*R*,23*R*,24*R*)-22,28-Diacetoxy-6-(ethylenedioxy)-2α,3α-(isopropylidenedioxy)-23-(methoxymethoxy)-5α-ergostane (28). The diol **27** (350 mg, 0.576 mmol) was acetylated with acetic anhydride (1.34 mL, 13.1 mmol) and pyridine (7 mL) containing a catalytic amount of 4-(*N*,*N*-dimethylamino)pyridine (50 mg) for 10 h at room temperature. The reaction mixture was poured into water (20 mL), the resulting solution was extracted with ethyl acetate, and the extract was washed with aqueous sodium hydrogen carbonate solution and brine and dried over Na_2SO_4. Evaporation of the solvent gave a pale yellow solid, which was purified by chromatography on silica gel (10 g) using dichloromethane containing 40% chloroform as the eluant to give the acetate **28** (390 mg, 97%) as a colorless amorphous powder: $[\alpha]^{23}_D$ +39.60° (*c* 0.79, $CHCl_3$); IR ($CHCl_3$) 1730 cm^{-1}; ^1H NMR (400 MHz) δ 0.69 (3 H, s, 18-H_3), 0.83 (3 H, s, 19-H_3), 0.96 (3 H, d, J = 7 Hz, Me), 0.99 (3 H, d, J = 7 Hz, Me), 1.01 (3 H, J = 7 Hz, Me), 1.32 (3 H, s, acetonide), 1.47 (3 H, s, acetonide), 2.06 (3 H, s, acetyl), 2.07 (3 H, s, acetyl), 3.33 (3 H, s, OMe), 3.70–3.97 (4 H, m, OCH_2CH_2O), 3.75 (1 H, d, J = 9 Hz, 23-H), 4.05–4.13 (1 H, m, 2-H), 4.16 and 4.32 (each 1 H, each dd, J = 11, 6 Hz, 28-H_2), 4.27 (1 H, br s, $W_{1/2}$ = 8.4 Hz, 3-H), 4.54 and 4.67 (each 1 H, each d, J = 6 Hz, OCH_2OCH_3), 5.32 (1 H, d, J = 9 Hz, 22-H); MS, m/z 692 (M^+), 677 (M^+ – Me); exact mass calcd for $C_{39}H_{64}O_{10}$ 692.4497, found 692.4491.

(22*R*,23*R*,24*R*)-2α,3α,22,23,28-Pentaacetoxy-5α-ergostan-6-one (29). A solution of the diacetate **28** (390 mg, 0.564 mmol) in acetic acid–water (3:1 v/v) (8 mL) was refluxed for 4 h. After cooling, the reaction mixture was diluted with ethyl acetate (60 mL), and the resulting solution was washed with aqueous sodium hydrogen carbonate solution and brine and dried over Na_2SO_4. Evaporation of the solvent gave the white solid (330 mg), which was acetylated with acetic anhydride (1.6 mL, 15.7 mmol) and pyridine (8 mL) containing a catalytic amount of 4-(*N*,*N*-dimethylamino)pyridine (50 mg) for 10 h at room temperature. The resulting mixture was poured into water (20 mL), the resulting solution was extracted with ethyl acetate, and the extract was washed with aqueous sodium carbonate solution, aqueous potassium hydrogen sulfate solution, and brine and dried over Na_2SO_4. Evaporation of the solvent gave a yellow solid, which was purified by chromatography on silica gel (15 g) using dichloromethane containing 45% chloroform as the eluent to give the pentaacetate **29** (338 mg, 88%) as a colorless amorphous powder: $[\alpha]^{21}_D$ –3.28° (*c* 0.64, $CHCl_3$); IR ($CHCl_3$) 1730 cm^{-1}; ^1H NMR (400 MHz) δ 0.71 (3 H, s, 18-H_3), 0.83 (3 H, s, 19-H_3), 0.92 (3 H, d, J = 7 Hz, Me), 0.98 (3 H, d, J = 7 Hz, Me), 1.04 (3 H, d, J = 7 Hz, Me), 1.98 (3 H, s, acetyl), 1.99 (3 H, s, acetyl), 2.02 (3 H, s, acetyl), 2.08 (3 H, s, acetyl), 2.09 (3 H, s, acetyl), 4.10 and 4.35 (each 1 H, each dd, J = 11, 6 Hz, 28-H_2), 4.92–4.98 (1 H, m, 2-H), 5.30 (1 H, d, J = 9 Hz, 22-H), 5.35 (1 H, d, J = 9 Hz, 23-H), 5.38 (1 H, br s, $W_{1/2}$ = 8 Hz, 3-H); MS, m/z 690 (M^+), 630 (M^+ – CH_3CO_2H), 570 (M^+ – $2CH_3CO_2H$), 510 (M^+ – $3CH_3CO_2H$); exact mass calcd for $C_{38}H_{58}O_{11}$ 690.3997, found 690.3979.

(22*R*,23*R*,24*R*)-2α,3α,22,23-Pentaacetoxy-*B*-homo-7-oxa-5α-ergostan-6-one (30). A solution of the pentaacetate **29** (300 mg, 0.435 mmol) in dichloromethane (7 mL) was added dropwise to a stirred solution of trifluoroperacetic acid (17.6 mmol) [prepared from 30% H_2O_2 (2 mL) and trifluoroacetic anhydride (11 mL) in dichloromethane (12 mL)] at 0 °C. The reaction mixture was then stirred for 1 h at room temperature and diluted with dichlorometane (50 mL), and the resulting solution was washed with aqueous sodium carbonate solution, aqueous sodium hydrogen sulfite solution, and brine and dried over Na_2SO_4. Evaporation of the solvent gave a white solid, which was purified by chromatography on silica gel (10 g) using dichloromethane containing 20% chloroform as the eluant to give the lactone **30**

(264 mg, 86%) as a colorless amorphous powder: $[\alpha]^{22}_D$ +25.35° (*c* 0.68, $CHCl_3$); IR ($CHCl_3$) 1730 cm^{-1}; ^1H NMR (400 MHz) δ 0.75 (3 H, s, 18-H_3), 0.92 (3 H, d, *J* = 7 Hz, Me), 0.98 (3 H, d, *J* = 7 Hz, Me), 0.99 (3 H, s, 19-H_3), 1.02 (3 H, d, *J* = 7 Hz, Me), 1.99 (3 H, s, acetyl), 2.00 (3 H, s, acetyl), 2.01 (3 H, s, acetyl), 2.09 (3 H, s, acetyl), 2.11 (3 H, s, acetyl), 4.00–4.15 (3 H, m, 7-H_2 and 28-H), 4.35 (1 H, dd, *J* = 11, 6 Hz, 28-H), 4.84–4.92 (1 H, m, 2-H), 5.28 (1 H, d, *J* = 9 Hz, 22-H), 5.34 (1 H, d, *J* = 9 Hz, 23-H), 5.37 (1 H, br s, $W_{1/2}$ = 8.4 Hz, 3-H); MS, *m/z* 706 (M^+), 646 (M^+ – CH_3CO_2H), 586 (M^+ – $2CH_3CO_2H$), 526 (M^+ – $3CH_3CO_2H$), 505 (M^+ – 201, C_{22}–C_{23} cleavage); exact mass calcd for $C_{38}H_{58}O_{12}$ 706.3927, found 706.3947.

(22*R*,23*R*,24*R*)-2α,3α,22,23,28-Pentahydroxy-*B*-homo-7-oxa-5α-ergostan-6-one (28-Hydroxybrassinolide) (2). A solution of the lactone **30** (264 mg, 0.374 mmol) in 5% potassium hydroxide–methanol (13 mL) was refluxed for 1 h. The reaction mixture was cooled to room temperature, and 6 M hydrochloric acid (13 mL) was added. The resulting solution was stirred for 10 min and extracted with ethyl acetate. The extract was washed with brine and dried over Na_2SO_4. Evaporation of the solvent gave the white solid, which was purified by chromatography on silica gel (10 g) using chloroform containing 5% methanol as the eluant to give 28-hydroxybrassinolide (**2**) (172 mg, 93%) as colorless leaflets: mp 298–300 °C (MeOH–EtOH); $[\alpha]^{22}_D$ –4.50° (*c* 0.60, EtOH); IR (KBr) 3400, 1725, 1700 cm^{-1}, ^1H NMR (400 MHz, [$^2H_5$]pyridine) δ 0.68 (3 H, s, 18-H_3), 1.02 (3 H, s, 19-H_3), 1.16 (3 H, d, *J* = 7 Hz, Me), 1.19 (3 H, d, *J* = 7 Hz, Me), 1.20 (3 H, d, *J* = 7 Hz, Me), 3.57 (1 H, dd, *J* = 13, 4.5 Hz, 5-H), 3.98–4.11 (3 H, m, 2-H and 7-H_2), 4.17–4.28 (4 H, m, 22-H, 23-H and 28-H_2), 4.39 (1 H, br s, $W_{1/2}$ = 8.4 Hz, 3-H); MS, *m/z* 493 (M^+ – 3), 460 (M^+ – $2H_2O$), 453 (M^+ – C_3H_7), 379 (M^+ – 117, C_{22}–C_{23} cleavage). Anal. Calcd for $C_{28}H_{48}O_7$: C, 67.71; H, 9.74. Found: C, 67.37; H, 9.96.

(22*R*,23*R*,24*R*)-2α,3α,22,23,28-Pentahydroxy-5α-ergostan-6-one (28-Hydroxycastasterone) (7). The same procedure as for 28-hydroxybrassinolide (**2**) was applied to the pentaacetate **29** (50 mg, 0.0724 mmol) to afford 28-hydroxycastasterone (**7**) (33 mg, 95%) as colorless plates: mp 273–274.5 °C (EtOH–H_2O); $[\alpha]^{22}_D$ –4.54° [*c* 0.44, EtOH–H_2O (7:1)]; IR (KBr) 3400, 1700 cm^{-1}; ^1H NMR (400 MHz, [$^2H_5$]pyridine) δ 0.70 (3 H, s, 18-H_3), 0.83 (3 H, s, 19-H_3), 1.17 (3 H, d, *J* = 7 Hz, Me), 1.20 (3 H, d, *J* = 7 Hz, Me), 1.25 (3 H, d, *J* = 7 Hz, Me), 3.12 (1 H, dd, *J* = 13, 3.5 Hz, 5-H), 4.01–4.09 (1 H, m, 2-H), 4.25 (4 H, br s, $W_{1/2}$ = 18 Hz, 22-H, 23-H, and 28-H_2), 4.42 (1 H, br s, $W_{1/2}$ = 8.4 Hz, 3-H); MS, *m/z* 363 (M^+ – 117, C_{22}–C_{23} cleavage), 345 (M^+ – 117 – H_2O). Anal. Calcd for $C_{28}H_{48}O_6$: C, 69.96; H, 10.07. Found: C, 69.97; H, 10.25.

(22*R*,23*R*,24*S*)-22-Acetoxy-6-(ethylenedioxy)-2α,3α-(isopropylidenedioxy)-23-(methoxymethoxy)-5α-ergostane (33). Methanesulfonyl chloride (0.01 mL, 0.13 mmol) was added slowly to a stirred solution of the diol **27** (75 mg, 0.121 mmol) in anhydrous dichloromethane (2 mL) containing triethylamine (0.02 mL, 0.14 mmol) under nitrogen at 0 °C. After stirring for 10 min at the same temperature, the reaction mixture was diluted with dichloromethane (20 mL), and the resulting solution was washed with aqueous sodium hydrogen carbonate solution and brine and dried over Na_2SO_4. Evaporation of the solvent gave (22*R*,23*R*,24*R*)-6-(ethylenedioxy)-22-hydroxy-2α,3α-(isopropylidenedioxy)-23-(methoxymethoxy)-28-[(methylsulfonyl)oxy]-5α-ergostane (**31**) (83 mg), whose solution in anhydrous ether (3 mL) was treated with lithium aluminum hydride (23 mg, 0.606 mmol) under nitrogen for 30 min at room temperature. To the reaction mixture was added 25% aqueous sodium hydroxide solution (0.5 mL), and then resulting solution was stirred for 10 min and extracted with ethyl acetate. The extract was washed with brine and dried over Na_2SO_4. Evaporation of the solvent gave (22*R*,23*R*,24*S*)-6-(ethylenedioxy)-22-hydroxy-2α,3α-(isopropylidenedioxy)-23-(methoxymethoxy)-5α-ergostane (**32**) (65 mg), which was acetylated with acetic anhydride (0.4 mL) and pyridine (2 mL) containing a catalytic amount of 4-(*N*,*N*-dimethylamino)pyridine (8 mg) for 10 h at room temperature. The reaction mixture was poured into water (5 mL), and isolation of the product by ethyl acetate extraction gave a pale yellow solid, which was purified by chromatography on silica gel (5 g) using dichloromethane containing 20% chloroform as the eluant to give the acetate **33** (64.9 mg, 82%) as a colorless amorphous powder: $[\alpha]^{23}_D$ +47.89 (*c* 0.57, $CHCl_3$); IR ($CHCl_3$) 1725 cm^{-1}; ^1H NMR (400 MHz) δ 0.68 (3 H, s, 18-H_3), 0.83 (3 H, s, 19-H_3), 0.93 (3 H, d, *J* = 7 Hz, Me) 0.94 (3 H, d, *J* = 7 Hz, Me), 0.97 (3 H, d, *J* = 7 Hz, Me), 1.32 (3 H, s, acetonide), 1.47 (3 H, s, acetonide), 2.07 (3 H, s, acetyl), 3.33 (3 H, s, OMe), 3.70–4.00 (4 H, m, OCH_2CH_2O), 3.73 (1 H, d, *J* = 9 Hz, 23-H), 4.05–4.12 (1 H, m, 2-H), 4.27 (1 H, br s, $W_{1/2}$ = 8.4 Hz, 3-H), 4.54 and 5.14 (each 1 H, each d, *J* = 6 Hz, OCH_2OCH_3), 5.15 (1 H, d, *J* = 9 Hz, 22-H); MS, *m/z* 634 (M^+), 619 (M^+ – Me), 591 (M^+ – C_3H_7), 574 (M^+ – CH_3CO_2H); exact mass calcd for $C_{37}H_{62}O_8$ 634.4466, found 634.4444.

(22*R*,23*R*,24*S*)-2α,3α,22,23-Tetraacetoxy-5α-ergostan-6-one (34). A solution of the acetate **33** (48 mg, 0.0736 mmol) in acetic acid–water (3:1 v/v) (1.35 mL) was refluxed for 4 h. After cooling, the reaction mixture was diluted with ethyl acetate (30 mL), and the resulting solution was washed with aqueous sodium hydrogen carbonate solution and brine and dried over Na_2SO_4. Evaporation of the solvent gave a white solid (34 mg), which was acetylated with acetic anhydride (0.4 mL) and pyridine (2 mL) containing a catalytic amount of 4-(*N*,*N*-dimethylamino)pyridine (6 mg) for 10 h at room temperature. The reaction mixture was poured into water (6 mL), and isolation of the product by ethyl acetate extraction gave a pale yellow solid, which was purified by chromatography on silica gel (4 g) using dichloromethane containing 20% chloroform as the eluant to give the tetraacetate **34** (40.9 mg, 88%) as colorless needles: mp 216–218 °c (MeOH) (lit.[8b] mp 215–217 °C; lit.[9b] mp 215–218 °C; lit.[8a] mp 221–224 °C); $[\alpha]^{22}_D$ +1.62° (*c* 0.76, $CHCl_3$), [lit.[8a] $[\alpha]^{22}_D$ +6.81° ($CHCl_3$); lit.[8b] $[\alpha]^{22}_D$ –6.82° ($CHCl_3$); IR ($CHCl_3$) 1730 cm^{-1}; ^1H NMR (100 MHz) δ 0.70 (3 H, s, 18-H_3), 0.83 (3 H, s, 19-H_3), 0.95 (6 H, d, *J* = 7 Hz, 2 × Me), 1.99 (6 H, s, two acetyls), 2.01 (3 H, s, acetyl), 2.08 (3 H, s, acetyl), 4.91–5.41 (4 H, m, 2-H, 3-H, 22-H and 23-H); MS, *m/z* 632 (M^+). The spectral data were identical with those reported.[8]

(22*R*,23*R*,24*S*)-2α,3α,22,23-Tetrahydroxy-5α-ergostan-6-one (Castasterone) (6). The same procedure as for 28-hydroxybrassinolide (**2**) was applied to the tetraacetate **34** (40.9 mg, 0.0647 mmol) to afford castasterone (**6**) (28.2 mg, 94%) as colorless needles: mp 257–258 °C ($CHCl_3$–MeOH), [lit.[8b] mp 250–252 °C; lit.[8a] mp 258–260 °C; lit.[7] mp 259–261 °C]; $[\alpha]^{23}_D$ +0.08° (*c* 0.85, $CHCl_3$–MeOH (10:1)], [lit.[8a] $[\alpha]_D$ +0.03° ($CHCl_3$–MeOH)]; IR ($CHCl_3$) 3400, 1705 cm^{-1}; ^1H NMR (400 MHz) δ 0.69 (3 H, s, 18-H_3), 0.76 (3 H, s, 19-H_3), 0.85 (3 H, d, *J* = 7 Hz, Me), 0.91 (3 H, d, *J* = 7 Hz, Me), 0.95 (3 H, d, *J* = 7 Hz, Me), 0.97 (3 H, d, *J* = 7 Hz, Me), 2.69 (1 H, dd, *J* = 13, 4 Hz, 5-H), 3.56 (1 H, d, *J* = 9 Hz, 22-H), 3.68–3.79 (1 H, m, 2-H), 3.72 (1 H, d, *J* = 9 Hz, 23-H), 4.05 (1 H, br s, $W_{1/2}$ = 8.4 Hz, 3-H); MS, *m/z* 464 (M^+). The spectral data were identical with those reported.[7,8]

(20*R*,22*S*,23*R*,24*S*)-6-(Ethylenedioxy)-20-hydroxy-2α,3α-(isopropylidenedioxy)-23-(methoxymethoxy)-5α-ergostano-28,22-lactone (35). The same procedure as for the lactone **26** was applied to the 20*R*,22*R* compound **21** (1 g, 1.62 mmol) to afford the lactone **35** (933 mg, 93%) as a colorless amorphous powder: $[\alpha]^{22}_D$ +34.70° (*c* 0.66, $CHCl_3$); IR ($CHCl_3$) 3450, 1770 cm^{-1}, ^1H NMR (400 MHz) δ 0.83 (3 H, s, 18-H_3), 0.88 (3 H, s, 19-H_3), 1.03 (3 H, d, *J* = 7 Hz, Me), 1.25 (3 H, d, *J* = 7 Hz, Me), 1.33 (3 H, s, acetonide), 1.48 (3 H, s, acetonide), 1.53 (3 H, s, 21-H_3), 3.44 (3 H, s, OMe), 3.70–4.00 (4 H, m, OCH_2CH_2O), 4.08 (1 H, d, *J* = 3.5 Hz, 22-H), 4.08–4.15 (1 H, m, 2-H), 4.27 (1 H, br s, $W_{1/2}$ = 8.4 Hz, 3-H), 4.48 (1 H, dd, *J* = 5, 3.5 Hz, 23-H), 4.70 and 4.88 (each 1 H, each d, *J* = 6 Hz, OCH_2OCH_3); MS, *m/z* 605 (M^+ – Me), 602 (M^+ – H_2O), 587 (M^+ – Me – H_2O), 433 (M^+ – 187, C_{20}–C_{22} cleavage); exact mass calcd for $C_{26}H_{41}O_5$ 433.2952, found 433.2910.

(20*R*,22*S*,23*R*,24*R*)-6-(Ethylenedioxy)-2α,3α-(isopropylidenedioxy)-23-(methoxymethoxy)-20,22,28-trihydroxy-5α-ergostane (36). The same procedure as for the diol **27** was applied to the lactone **35** (900 mg, 1.45 mmol) to afford the triol **36** (906 mg, 100%) as a colorless amorphous powder: $[\alpha]^{23}_D$ +31.50° (*c* 1.00, $CHCl_3$); IR ($CHCl_3$) 3400 cm^{-1}; ^1H NMR (400 MHz) δ 0.83 (3 H, s, 18-H_3), 0.86 (3 H, s, 19-H_3), 0.97 (3 H, d, *J* = 7 Hz, Me), 1.02 (3 H, d, *J* = 7 Hz, Me), 1.28 (3 H, s, 21-H_3), 1.32 (3 H, s, acetonide), 1.48 (3 H, s, acetonide), 3.44 (3 H, s, OMe), 3.56–4.08 (8 H, m, 22-H, 23-H, 28-H_2 and OCH_2CH_2O), 4.06–4.13 (1 H, m, 2-H), 4.27 (1 H, br s, $W_{1/2}$ = 8.4 Hz, 3-H), 4.75 and 4.78 (each 1 H, each d, *J* = 6 Hz, OCH_2OCH_3); MS, *m/z* 609 (M^+ – Me), 591 (M^+ – Me – H_2O), 571 (M^+ – Me – $2H_2O$), 433 (M^+ – 191, C_{20}–C_{22} cleavage); exact mass calcd for $C_{26}H_{41}O_5$ 433.2952, found 433.2940.

(20*R*,22*S*,23*R*,24*R*)-22,28-Diacetoxy-6-(ethylenedioxy)-20-hydroxy-2α,3α-(isopropylidenedioxy)-23-(methoxymethoxy)-5α-ergostane (37). The same procedure as for the diacetate **28** was applied to the triol **36** (400 mg, 0.641 mmol) to afford the diacetate **37** (440 mg, 97%) as a colorless amorphous powder: $[\alpha]^{23}_D$ +50.96° (*c* 0.71, $CHCl_3$); IR ($CHCl_3$) 3400, 1720 cm^{-1}; ^1H NMR (400 MHz) δ 0.83 (3 H, s, 18-H_3), 0.86 (3 H, s, 19-H_3), 0.95 (3 H, d, *J* = 7 Hz, Me), 1.00 (3 H, d, *J* = 7 Hz, Me), 1.32 (3 H, s, acetonide), 1.41 (3 H, s, 21-H_3), 1.46 (3 H, s, acetonide), 2.05 (3 H, s, acetyl), 2.08 (3 H, s, acetyl), 3.37 (3 H, s, OMe), 3.72–3.96 (4 H, m, OCH_2CH_2O), 3.99 (1 H, dd, *J* = 12, 6 Hz, 28-H), 4.05–4.13 (2 H, m, 2-H and 23-H), 4.27 (1 H, br s, $W_{1/2}$ = 8.4 Hz, 3-H), 4.47 (1 H, dd, *J* = 12, 3.3 Hz, 28-H), 4.50 and 4.67 (each 1 H, each d, *J* = 6 Hz, OCH_2OCH_3), 5.13 (1 H, d, *J* = 6 Hz, 22-H); MS, *m/z* 693 (M^+ – Me), 648 (M^+ – CH_3CO_2H), 633 (M^+ – Me – CH_3CO_2H), 433 (M^+ – 275, C_{20}–C_{22} cleavage); exact mass calcd for $C_{38}H_{61}O_{11}$ 693.4214, found 693.4214.

(20*R*,22*S*,23*R*,24*R*)-20-Hydroxy-2α,3α,22,23,28-pentaacetoxy-5α-ergostan-6-one (38). The same procedure as for the pentaacetate **29** was applied to the diacetate **37** (400 mg, 0.565 mmol) to afford the pentaacetate **38** (340 mg, 88%) as a colorless amorphous powder: $[\alpha]^{22}_D$ +5.22° (*c* 0.79, $CHCl_3$); IR ($CHCl_3$) 1720 cm^{-1}; ^1H NMR (400 MHz) δ 0.83 (3 H, s, 18-H_3), 0.86 (3 H, s, 19-H_3), 0.93 (3 H, d, *J* = 7 Hz, Me), 0.95 (3 H, d, *J* = 7 Hz, Me), 1.40 (3 H, s, 21-H_3), 1.99 (3 H, s, acetyl), 2.02 (3 H, s, acetyl), 2.06 (3 H, s, acetyl), 2.07 (3 H, s, acetyl), 2.08 (3 H, s, acetyl), 4.03 and 4.42 (each 1 H, each dd, *J* = 11, 6 Hz, 28-H_2), 4.90–4.97 (1 H, m, 2-H), 5.15 (1 H, d, *J* = 7 Hz, 22-H), 5.38 (1 H, br s, $W_{1/2}$ = 8.4 Hz, 3-H), 5.47 (1 H, dd, *J* = 7, 3.5 Hz, 23-H); MS, *m/z* 688 (M^+ – H_2O), 628 (M^+ – H_2O – CH_3CO_2H), 586 (M^+ – $2CH_3CO_2H$), 568 (M^+ – H_2O – $2CH_3CO_2H$); exact mass calcd for $C_{34}H_{50}O_8$ 586.3503, found 586.3491.

(20*R*,22*S*,23*R*,24*R*)-20-Hydroxy-2α,3α,22,23,28-pentaacetoxy-*B*-homo-7-oxa-5α-ergostan-6-one (39). Trifluoroacetic anhydride (2.2 mL, 15.6 mmol) was added to a stirred solution of 90% H_2O_2 in dichloromethane (2.4 mL) at 0 °C. After the reaction mixture was stirred for 10 min, powdered disodium hydrogen phosphate (2.4 g, 16.9 mmol) and a solution of the pentaacetate **38** (120 mg, 0.17 mmol) in dichloromethane (2 mL) were added, and then the resulting mixture was stirred for 3 h at the same temperature. To the reaction mixture was added aqueous sodium hydrogen sulfite solution (4 mL) and extracted with ethyl acetate. The extract was washed with aqueous sodium hydrogen carbonate solution and brine and dried over Na_2SO_4. Evaporation of the solvent gave a white solid, which was purified by chromatography on silica gel (10 g) using dichloromethane containing 20% chloroform as the eluant to give the lactone **39** (97 mg, 79%) as a colorless amorphous powder: $[\alpha]^{22}_D$ +30.44° (*c* 0.91, $CHCl_3$); IR ($CHCl_3$) 3400, 1720 cm^{-1}; ^1H NMR (400 MHz) δ 0.89 (3 H, s, 18-H_3), 0.92 (3 H, d, *J* = 7 Hz, Me), 0.95 (3 H, d, *J* = 7 Hz, Me), 0.98 (3 H, s, 19-H_3), 1.38 (3 H, s, 21-H_3), 2.00 (3 H, s, acetyl), 2.02 (3 H, s, acetyl), 2.06 (3 H, s, acetyl), 2.10 (3 H, s, acetyl), 2.17 (3 H, s, acetyl), 2.99 (1 H, dd, *J* = 13, 5 Hz, 5-H), 4.02 and 4.42 (each 1 H, each dd, *J* = 11, 6 Hz, 28-H_2), 4.03–4.16 (2 H, m, 7-H_2), 4.83–4.90 (1 H, m, 2-H), 5.14 (1 H, d, *J* = 7 Hz, 22-H), 5.37 (1 H, br s, $W_{1/2}$ = 8.4 Hz, 3-H), 5.47 (1 H, dd, *J* = 7, 3.5 Hz, 23-H); MS, *m/z* 602 (M^+ – $2CH_3CO_2H$), 461 (M^+ – 261, C_{20}–C_{22} cleavage); exact mass calcd for $C_{26}H_{37}O_7$, 461.2537, found 461.2527.

(20*R*,22*S*,23*R*,24*R*)-2α,3α,20,22,28-Hexahydroxy-*B*-homo-7-oxa-5α-ergostan-6-one (20,28-Dihydroxybrassinolide) (3). The same procedure as for 28-hydroxybrassinolide (**2**) was applied to the lactone **39** (80 mg, 0.111 mmol) to afford the hexol **3** (54.5 mg, 96%) as colorless needles: mp 263–264.5 °C (EtOAc–MeOH); $[\alpha]^{21}_D$ +31.17° (*c* 0.62, EtOH); IR (KBr) 3400, 1710 cm^{-1}; ^1H NMR (400 MHz, [$^2H_5$]pyridine) δ 1.05 (3 H, s, 18-H_3), 1.06 (3 H, d, *J* = 7 Hz, Me), 1.08 (3 H, s, 19-H_3), 1.12 (3 H, d, *J* = 7 Hz, Me), 1.74 (3 H, s, 21-H_3), 3.62 (1 H, dd, *J* = 13, 4.5 Hz, 5-H), 3.89 (1 H, br s, $W_{1/2}$ = 5 Hz, 22-H), 4.00–4.13 (3 H, m, 2-H and 7-H_2), 4.22 (1 H, dd, *J* = 10, 3 Hz, 28-H), 4.27 (1 H, dd, *J* = 10, 7 Hz, 28-H), 4.43 (1 H, br s, $W_{1/2}$ = 8.4 Hz, 3-H), 4.78 (1 H, d, *J* = 3 Hz, 23-H). MS, *m/z* 457 (M^+ – 1 – $3H_2O$); exact mass calcd for $C_{28}H_{41}O_5$ 457.6110, found 457.6115. Anal. Calcd for $C_{28}H_{48}O_8 \cdot 0.2H_2O$: C, 65.14; H, 9.45. Found: C, 64.76; H, 9.67.

(20*R*,22*S*,23*R*,24*R*)-2α,3α,20,22,23,28-Hexahydroxy-5α-ergostan-6-one (20,28-Dihydroxycastasterone) (8). The same procedure as for 28-hydroxybrassinolide (**2**) was applied to the pentaacetate **38** (50 mg, 0.071 mmol) to afford the hexol **8** (34 mg, 97%) as colorless leaflets: mp 258–259 °C (AcOEt–MeOH); $[\alpha]^{22}_D$ –11.70° (*c* 0.47, EtOH); IR (KBr) 3400, 1700 cm^{-1}; ^1H NMR (400 MHz, [$^2H_5$]pyridine) δ 0.85 (3 H, s, 18-H_3), 1.07 (3 H, d, *J* = 7 Hz, Me), 1.10 (3 H, s, 19-H_3), 1.11 (3 H, d, *J* = 7 Hz, Me), 1.77 (3 H, s, 21-H_3), 3.14 (1 H, dd, *J* = 13, 3.5 Hz, 5-H), 3.92 (1 H, br s, $W_{1/2}$ = 6 Hz, 22-H), 4.02–4.08 (1 H, m, 2-H), 4.22 (1 H, dd, *J* = 10, 3 Hz, 28-H), 4.27 (1 H, dd, *J* = 10, 7 Hz, 28-H), 4.42 (1 H, br s, $W_{1/2}$ = 8.4 Hz, 3-H), 4.80 (1 H, d, *J* = 3 Hz, 23-H); MS, *m/z* 363 (M^+ – 133; C_{20}–C_{22} cleavage). Anal. Calcd for $C_{28}H_{48}O_7$: C, 67.71; H, 9.74. Found: C, 67.57; H, 10.04.

(20*R*,22*S*,23*R*,24*S*)-6-(Ethylenedioxy)-20,22-dihydroxy-2α,3α-(isopropylidenedioxy)-23-(methoxymethoxy)-5α-ergostane (41). Methanesulfonyl chloride (0.178 mL, 2.31 mmol) was added slowly to a stirred solution of the triol **36** (1.2 g, 1.92 mmol) in anhydrous dichloromethane (40 mL) containing triethylamine (0.322 mL, 2.31 mmol) under nitrogen at 0 °C. After being stirred for 5 min at the same temperature, the reaction mixture was diluted with dichloromethane (300 mL), and the resulting solution was washed with aqueous sodium hydrogen carbonate solution and brine and dried over Na_2SO_4. Evaporation of the solvent gave (20*R*,22*S*,23*R*,24*R*)-6-(ethylenedioxy)-20,22-dihydroxy-2α,3α-(isopropylidenedioxy)-23-(methoxymethoxy)-28-[(methylsulfonyl)oxy]-5α-ergostane (**40**) (1.36 g), whose solution in anhydrous ether (50 mL) was treated with lithium aluminum hydride (365 mg, 9.62 mmol) under nitrogen for 30 min at room temperature. To the reaction mixture was added 25% aqueous sodium hydroxide solution (5 mL), and then resulting solution was stirred for 10 min and extracted with ethyl acetate. The extract was washed with brine and dried over Na_2SO_4. Evaporation of the solvent gave a white solid, which was purified by chromatography on silica gel (30 g) using chloroform containing 40% dichloromethane as the eluant to give the diol **41** (935 mg, 80%) as a colorless amorphous powder: $[\alpha]^{22}_D$ +28.98° (*c* 0.71, $CHCl_3$); IR ($CHCl_3$) 3400 cm^{-1}; ^1H NMR (400 MHz) δ 0.83 (3 H, s, 18-H_3), 0.86 (3 H, s, 19-H_3), 0.86 (3 H, d, *J* = 7 Hz, Me), 0.87 (3 H, d, *J* = 7 Hz, Me), 0.97 (3 H, d, *J* = 7 Hz, Me), 1.28 (3 H, s, 21-H_3), 1.32 (3 H, s, acetonide), 1.48 (3 H, s, acetonide), 3.06 (1 H, d, *J* = 7 Hz, 22-H), 3.42 (3 H, s, OCH_3), 4.72–3.96 (4 H, m, OCH_2CH_2O), 3.83 (1 H, t, *J* = 3.5 Hz, 23-H), 4.05–4.13 (1 H, m, 2-H), 4.27 (1 H, br s, $W_{1/2}$ = 8.4 Hz, 3-H), 3.72 and 4.74 (each 1 H, each d, *J* = 6 Hz, OCH_2OCH_3); MS, *m/z* 606 (M^+ – 2), 593 (M^+ – Me), 433 (M^+ – 175, C_{20}–C_{22} cleavage); exact mass calcd for $C_{35}H_{58}O_8$ 606.4132, found 606.4135.

(20*R*,22*S*,23*R*,24*S*)-22-Acetoxy-6-(ethylenedioxy)-20-hydroxy-2α,3α-(isopropylidenedioxy)-23-(methoxymethoxy)-5α-ergostane (42). The same procedure as for the diacetate **28** was applied to the diol **41** (900 mg, 1.48 mmol) to afford the acetate **42** (924 mg, 96%) as a colorless amorphous powder: $[\alpha]^{22}_D$ +45.01° (*c* 0.86, $CHCl_3$); IR ($CHCl_3$) 3400, 1720 cm^{-1}; ^1H NMR (400 MHz) δ 0.83 (3 H, s, 18-H_3), 0.83 (3 H, d, *J* = 7 Hz, Me), 0.87 (3 H, s, 19-H_3), 0.88 (3 H, d, *J* = 7 Hz, Me), 0.93 (3 H, d, *J* = 7 Hz, Me), 1.32 (3 H, s, acetonide), 1.45 (3 H, s, 21-H_3), 1.46 (3 H, s, acetonide), 2.09 (3 H, s, acetyl), 3.40 (3 H, s, OMe), 3.72–3.96 (4 H, m, OCH_2CH_2O), 3.98 (1 H, t, *J* = 5 Hz, 23-H), 4.05–4.13 (1 H, m, 2-H), 4.27 (1 H, br s, $W_{1/2}$ = 8.4 Hz, 3-H), 4.60 and 4.73 (each 1 H, each d, *J* = 6 Hz, OCH_2OCH_3), 5.01 (1 H, d, *J* = 5 Hz, 22-H); MS, *m/z* 648 (M^+ – 2), 635 (M^+ – Me), 575 (M^+ – Me – CH_3CO_2H), 433 (M^+ – 217, C_{20}–C_{22} cleavage); exact mass calcd for $C_{37}H_{60}O_9$ 648.4238, found 648.4261.

(20*R*,22*S*,23*R*,24*S*)-20-Hydroxy-2α,3α,22,23-tetraacetoxy-5α-ergostan-6-one (43). The same procedure as for the pentaacetate **29** was applied to the acetate **42** (900 mg, 1.38 mmol) to afford the tetraacetate **43** (716 mg, 80%) as a colorless amorphous powder: $[\alpha]^{23}_D$ +9.31° (*c* 1.04, $CHCl_3$); IR ($CHCl_3$) 3400, 1730, 1710 cm^{-1}; ^1H NMR (400 MHz) δ 0.82 (3 H, s, 18-H_3), 0.83 (3 H, d, *J* = 7 Hz, Me), 0.84 (3 H, d, *J* = 7 Hz, Me), 0.85 (3 H, s, 19-H_3), 0.91 (3 H, d, *J* = 7 Hz, Me), 1.36 (3 H, s, 21-H_3), 1.99 (3 H, s, acetyl), 2.07 (3 H, s, acetyl), 2.08 (3 H, s, acetyl), 2.10 (3 H, s, acetyl), 2.56 (1 H, dd, *J* = 11.5, 4.6 Hz, 5-H), 4.91–4.98 (1 H, m, 2-H), 5.02 (1 H, d, *J* = 4.5 Hz, 22-H), 5.32 (1 H, dd, *J* = 4.5, 3.4 Hz, 23-H), 5.38 (1 H, br d, *J* = 2.3 Hz, 3-H); MS, *m/z* 588 (M^+ – CH_3CO_2H), 528 (M^+ – $2CH_3CO_2H$), 433 (M^+ – 215, C_{20}–C_{22} cleavage); exact mass calcd for $C_{25}H_{37}O_6$ 433.2585, found 433.2583.

(20*R*,22*S*,23*R*,24*S*)-2α,3α,20,22,23-Pentahydroxy-5α-ergostan-6-one (20-Hydroxycastasterone) (9). The same procedure as for 28-hydroxybrassinolide (**2**) was applied to the tetraacetate **43** (50 mg, 0.0771 mmol) to afford 20-hydroxycastasterone (**9**) (36 mg, 97%) as colorless leaflets: mp 242.5–244 °C [EtOAc–MeOH (20:1)]; $[\alpha]^{22}_D$ −3.64° (*c* 0.72, EtOH); IR (KBr) 3400, 1700 cm^{-1}; ^{1}H NMR (400 MHz, [$^2H_5$]pyridine) δ 0.85 (3 H, s, 18-H$_3$), 0.99 (3 H, d, *J* = 7 Hz, Me), 1.04 (3 H, d, *J* = 7 Hz, Me), 1.09 (3 H, s, 19-H$_3$), 1.20 (3 H, d, *J* = 7 Hz, Me), 1.72 (3 H, s, 21-H$_3$), 3.12 (1 H, dd, *J* = 11.5, 3.4 Hz, 5-H), 3.79 (1 H, br s, $W_{1/2}$ = 7 Hz, 22-H), 4.01–4.08 (1 H, m, 3-H), 4.42 (1 H, br d, *J* = 2.3 Hz, 2-H), 4.49 (1 H, d, *J* = 5.3 Hz, 23-H); MS, *m/z* 462 (M$^+$ − H$_2$O), 419 (M$^+$ − H$_2$O − C$_3$H$_7$), 349 (M$^+$ − 131, C$_{20}$–C$_{22}$ cleavage). Anal. Calcd for $C_{28}H_{48}O_6$: C, 69.96; H, 10.07. Found: C, 69.92; H, 10.32.

(20*R*,22*S*,23*R*,24*S*)-20-Hydroxy-2α,3α,22,23-tetraacetoxy-*B*-homo-7-oxa-5α-ergostan-6-one (44). The same procedure as for the lactone **39** was applied to the tetraacetate **43** (80 mg, 0.123 mmol) to afford the lactone **44** (63 mg, 77%) as a colorless amorphous powder: $[\alpha]^{23}_D$ +39.40° (*c* 0.54, CHCl$_3$); IR (CHCl$_3$) 1720 cm^{-1}; ^{1}H NMR (400 MHz) δ 0.82 (3 H, d, *J* = 7 Hz, Me), 0.83 (3 H, d, *J* = 7 Hz, Me), 0.88 (3 H, s, 18-H$_3$), 0.90 (3 H, d, *J* = 7 Hz, Me), 0.98 (3 H, s, 19-H$_3$), 1.34 (3 H, s, 21-H$_3$), 2.00 (3 H, s, acetyl), 2.07 (3 H, s, acetyl), 2.09 (3 H, s, acetyl), 2.11 (3 H, s, acetyl), 2.98 (1 H, dd, *J* = 11.5, 4.6 Hz, 5-H), 3.99–4.16 (2 H, m, 7-H$_2$), 4.82–4.89 (1 H, m, 2-H), 5.01 (1 H, d, *J* = 4.5 Hz, 22-H), 5.31 (1 H, dd, *J* = 4.5, 3.4 Hz, 23-H), 5.36 (1 H, br s, $W_{1/2}$ = 9.6 Hz, 3-H); MS, *m/z* 664 (M$^+$), 604 (M$^+$ − CH$_3$CO$_2$H), 544 (M$^+$ − 2CH$_3$CO$_2$H), 449 (M$^+$ − 215, C$_{20}$–C$_{22}$ cleavage); exact mass calcd for $C_{25}H_{37}O_7$ 449.2536, found 449.2537.

(20*R*,22*S*,23*R*,24*S*)-2α,3α,20,22,23-Pentahydroxy-*B*-homo-7-oxa-5α-ergostan-6-one (20-Hydroxybrassinolide) (4). The same procedure as for 28-hydroxybrassinolide (**2**) was applied to the tetraacetate **44** (40 mg, 0.0602 mmol) to afford 20-hydroxybrassinolide (**4**) (28 mg, 94%) as a colorless amorphous powder: $[\alpha]^{22}_D$ +27.26° (*c* 0.85, EtOH); IR (KBr) 3400, 1725, 1700 cm^{-1}; ^{1}H NMR (400 MHz, [$^2H_5$]pyridine) δ 0.98 (3 H, d, *J* = 7 Hz, Me), 1.03 (3 H, d, *J* = 7 Hz, Me), 1.04 (3 H, s, 18-H$_3$), 1.07 (3 H, s, 19-H$_3$), 1.19 (3 H, d, *J* = 7 Hz, Me), 1.68 (3 H, s, 21-H$_3$), 3.59 (1 H, dd, *J* = 13, 4.5 Hz, 5-H), 3.76 (1 H, br s, $W_{1/2}$ = 8 Hz, 22-H), 3.97–4.06 (1 H, m, 2-H), 4.06–4.12 (2 H, m, 7-H$_2$), 4.41 (1 H, br d, *J* = 2.3 Hz, 3-H), 4.47 (1 H, d, *J* = 5.3 Hz, 23-H); MS, *m/z* 496 (M$^+$), 453 (M$^+$ − C$_3$H$_7$), 365 (M$^+$ − 131, C$_{20}$–C$_{22}$ cleavage). Anal. Calcd for $C_{28}H_{48}O_7$: C, 67.71; H, 9.74. Found: C, 67.36; H, 9.96.

(22*R*,23*R*,24*S*)-2α,3α,22,23-Tetraacetoxy-5α-ergost-20(21)-en-6-one (45). Thionyl chloride (0.225 mL, 3.09 mmol) was added slowly to a stirred solution of the tetraacetate **43** (200 mg, 0.309 mmol) in anhydrous pyridine (6 mL) at 0 °C under a current of nitrogen. After being stirred for 30 min at the room temperature, the reaction mixture was diluted with ethyl acetate (60 mL), and the resulting solution was washed with aqueous sodium hydrogen carbonate solution, aqueous potassium hydrogen sulfate solution and brine and dried over Na_2SO_4. Evaporation of the solvent gave a pale yellow solid, which was purified by chromatography on silica gel (10 g) using dichloromethane containing 35% chloroform as the eluant to give the tetraacetate **45** (175 mg, 90%) as a colorless amorphous powder: $[\alpha]^{23}_D$ +37.13° (*c* 1.09, CHCl$_3$); IR (CHCl$_3$) 1720 cm^{-1}; ^{1}H NMR (400 MHz) δ 0.57 (3 H, s, 18-H$_3$), 0.81 (3 H, d, *J* = 7 Hz, Me), 0.83 (3 H, s, 19-H$_3$), 0.86 (3 H, d, *J* = 7 Hz, Me), 0.96 (3 H, d, *J* = 7 Hz, Me), 1.99 (3 H, s, acetyl), 2.01 (3 H, s, acetyl), 2.09 (3 H, s, acetyl), 2.14 (3 H, s, acetyl), 2.59 (1 H, dd, *J* = 11.5, 4.6 Hz, 5-H), 4.91–4.98 (1 H, m, 2-H), 4.96 (1 H, br s, $W_{1/2}$ = 4.6 Hz, 21-H), 5.09 (1 H, dd, *J* = 9, 1.6 Hz, 23-H), 5.13 (1 H, br s, $W_{1/2}$ = 4.6 Hz, 21-H), 5.26 (1 H, br s, $W_{1/2}$ = 8 Hz, 22-H), 5.39 (1 H, br d, *J* = 2.8 Hz, 3-H); MS, *m/z* 630 (M$^+$), 570 (M$^+$ − CH$_3$CO$_2$H), 510 (M$^+$ − 2CH$_3$CO$_2$H); exact mass calcd for $C_{36}H_{54}O_9$ 630.3765, found 630.3765.

Hydrogenation of 45. A solution of the tetraacetate **45** (140 mg, 0.222 mmol) in ethyl acetate (20 mL) was hydrogenated over 5% rhodium on alumina (40 mg) for 2 h under medium pressure (3.2 atm) of hydrogen. The catalyst was filtered off and the filtrate was evaporated to afford two products, which were separated by chromatography on silica gel (14 g) using benzene containing 5% ethyl acetate as the eluant to give (20*R*,22*R*,23*R*,24*S*)-2α,3α,22,23-tetraacetoxy-5α-ergostan-6-one (**46**) (less polar; 66 mg, 47%) as a colorless amorphous powder [$[\alpha]^{22}_D$ −8.48° (*c* 1.02, CHCl$_3$); IR (CHCl$_3$) 1720 cm^{-1}; ^{1}H NMR (400 MHz) δ 0.65 (3 H, s, 18-H$_3$), 0.84 (3 H, s, 19-H$_3$), 0.86 (3 H, d, *J* = 7 Hz, Me), 0.90 (3 H, d, *J* = 7 Hz, Me), 0.91 (3 H, d, *J* = 7 Hz, Me), 0.93 (3 H, d, *J* = 7 Hz, Me), 1.99 (3 H, s, acetyl), 2.04 (3 H, s, acetyl), 2.06 (3 H, s, acetyl), 2.09 (3 H, s, acetyl), 2.59 (1 H, dd, *J* = 11.5, 4.6 Hz, 5-H), 4.91–4.98 (1 H, m, 2-H), 5.06 (1 H, dd, *J* = 6.2, 3 Hz, 22-H), 5.28 (1 H, dd, *J* = 6.2, 4.4 Hz, 23-H), 5.39 (1 H, br d, *J* = 2.4, 3-H); MS, *m/z* 632 (M$^+$), 572 (M$^+$ − CH$_3$CO$_2$H); exact mass calcd for $C_{36}H_{56}O_9$ 632.3927, found 632.3926] and the 20*S* compound **34** (more polar; 63 mg, 45%) as colorless needles: mp 215–218 °C (MeOH) [lit.[8b] mp 215–217 °C; lit.[9b] mp 215–218 °C; lit.[8a] mp 221–224 °C]; $[\alpha]^{21}_D$ +1.51° (*c* 1.01, CHCl$_3$) [lit.[8a] $[\alpha]_D$ +6.81° (CHCl$_3$); lit.[8b] $[\alpha]_D$ −6.82° (CHCl$_3$)]; IR (CHCl$_3$) 1720 cm^{-1}; ^{1}H NMR (400 MHz) δ 0.70 (3 H, s, 18-H$_3$), 0.83 (3 H, s, 19-H$_3$), 0.91 (3 H, d, *J* = 7 Hz, Me), 0.94 (3 H, d, *J* = 7 Hz, Me), 0.96 (3 H, d, *J* = 7 Hz, Me), 1.02 (3 H, d, *J* = 7 Hz, Me), 1.99 (6 H, s, two acetyls), 2.01 (3 H, s, acetyl), 2.08 (3 H, s, acetyl), 2.56 (1 H, dd, *J* = 11.5, 4.6 Hz, 5-H), 4.91–4.98 (1 H, m, 2-H), 5.15 (1 H, dd, *J* = 9, 0.6 Hz, 22-H), 5.33 (1 H, dd, *J* = 9, 1.3 Hz, 23-H), 5.38 (1 H, br d, *J* = 2.3 Hz, 3-H); MS, *m/z* 632 (M$^+$), 572 (M$^+$ − CH$_3$CO$_2$H). The spectral data were identical with those reported.[8]

(20*R*,22*R*,23*R*,24*S*)-2α,3α,22,23-Tetrahydroxy-5α-ergostan-6-one (20-Epicastasterone) (10). The same procedure as for 28-hydroxybrassinolide (**2**) was applied to the tetraacetate **46** (30 mg, 0.0474 mmol) to afford 20-epicastasterone (**10**) (22 mg, 100%) as a colorless amorphous powder: $[\alpha]^{22}_D$ −2.74° (*c* 1.13, CHCl$_3$); IR (CHCl$_3$) 3400, 1700 cm^{-1}; ^{1}H NMR (400 MHz) δ 0.65 (3 H, s, 18-H$_3$), 0.76 (3 H, s, 19-H$_3$), 0.89 (3 H, d, *J* = 7 Hz, Me), 0.90 (3 H, d, *J* = 7 Hz, Me), 0.95 (3 H, d, *J* = 7 Hz, Me), 0.97 (3 H, d, *J* = 7 Hz, Me), 2.69 (1 H, dd, *J* = 12, 2.8 Hz, 5-H), 3.43 (1 H, dd, *J* = 4.3, 4.3 Hz, 22-H), 3.67 (1 H, dd, *J* = 4.3, 4.3 Hz, 23-H), 3.72–3.80 (1 H, m, 2-H), 4.05 (1 H, br d, *J* = 2.3 Hz, 3-H); MS, *m/z* 464 (M$^+$), 446 (M$^+$ − H$_2$O), 364 (M$^+$ − 101, C$_{22}$–C$_{23}$ cleavage); exact mass calcd for $C_{28}H_{48}O_5$ 464.3502, found 464.3512.

(20*R*,22*R*,23*R*,24*S*)-2α,3α,22,23-Tetraacetoxy-*B*-homo-7-oxa-5α-ergostan-6-one (47). The same procedure as for the pentaacetate **30** was applied to the tetraacetate **46** (35 mg, 0.0553 mmol) to afford the lactone **47** (30 mg, 84%) as a colorless amorphous powder: $[\alpha]^{22}_D$ +21.54° (*c* 1.19, CHCl$_3$); IR (CHCl$_3$) 1725 cm^{-1}; ^{1}H NMR (400 MHz) δ 0.68 (3 H, s, 18-H$_3$), 0.86 (3 H, d, *J* = 7 Hz, Me), 0.90 (3 H, d, *J* = 7 Hz, Me), 0.91 (3 H, d, *J* = 7 Hz, Me), 0.92 (3 H, d, *J* = 7 Hz, Me), 0.99 (3 H, s, 19-H$_3$), 2.01 (3 H, s, acetyl), 2.04 (3 H, s, acetyl), 2.05 (3 H, s, acetyl), 2.13 (3 H, s, acetyl), 3.00 (1 H, dd, *J* = 12.5, 4 Hz, 5-H), 4.02–4.16 (2 H, m, 7-H$_2$), 4.83–4.93 (1 H, m, 2-H), 5.03 (1 H, dd, *J* = 6.2, 2.8 Hz, 22-H), 5.26 (1 H, dd, *J* = 6.2, 3.7 Hz, 23-H), 5.37 (1 H, br s, $W_{1/2}$ = 9.5 Hz, 3-H); MS, *m/z* 648 (M$^+$), 588 (M$^+$ − CH$_3$CO$_2$H), 528 (M$^+$ − 2CH$_3$CO$_2$H); exact mass calcd for $C_{36}H_{56}O_{10}$ 648.3873, found 648.3874.

(20*R*,22*R*,23*R*,24*S*)-2α,3α,22,23-Tetrahydroxy-*B*-homo-7-oxa-5α-ergostan-6-one (20-Epibrassinolide) (5). The same procedure as for 28-hydroxybrassinolide (**2**) was applied for the lactone **47** (20 mg, 0.0308 mmol) to afford 20-epibrassinolide (**5**) (14 mg, 95%) as a colorless amorphous powder: $[\alpha]^{23}_D$ +21.25° (*c* 1.00, CHCl$_3$); IR (CHCl$_3$) 3400, 1720 cm^{-1}; ^{1}H NMR (400 MHz) δ 0.67 (3 H, s, 18-H$_3$), 0.88 (3 H, d, *J* = 7 Hz, Me), 0.89 (3 H, d, *J* = 7 Hz, Me), 0.93 (3 H, s, 19-H$_3$), 0.95 (3 H, d, *J* = 7 Hz, Me), 0.96 (3 H, d, *J* = 7 Hz, Me), 3.11 (1 H, dd, *J* = 12, 4 Hz, 5-H), 3.40 (1 H, dd, *J* = 4.3, 4.3 Hz, 22-H), 3.64 (1 H, dd, *J* = 4.3, 4.3 Hz, 23-H), 3.68–3.74 (1 H, m, 2-H), 4.02 (1 H, br s, $W_{1/2}$ = 8.5 Hz, 3-H), 4.05–4.13 (2 H, m, 7-H$_2$); MS, *m/z* 465 (M$^+$ − CH$_3$), 380 (M$^+$ − 100, C$_{22}$–C$_{23}$ cleavage); exact mass calcd for $C_{22}H_{36}O_5$ 380.2563, found 380.2564.

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25, 113599-35-2; **26**, 113599-36-3; **27**, 113599-37-4; **28**, 113599-38-5; **29**, 113599-39-6; **30**, 113599-40-9; **31**, 113599-41-0; **32**, 113599-42-1; **33**, 113599-43-2; **34**, 77027-48-6; **35**, 113599-44-3; **36**, 113599-45-4; **37**, 113599-46-5; **38**, 113599-47-6; **39**, 113599-48-7; **40**, 113599-49-8; **41**, 113599-50-1; **42**, 113599-51-2; **43**, 113599-52-3; **44**, 113599-53-4; **45**, 113599-54-5; **46**, 113666-81-2; **47**, 113666-82-3; 3-isopropyltetronic acid, 113599-55-6; (23*R*,22*R*)-6-(ethylenedioxy)-20,22,23-trihydroxy-2α,3α-(isopropylidenedioxy)-5α-23-ergosten-28-oic acid γ-lactone, 113599-56-7; (22*R*,23*R*,24*R*)-22,28-diacetoxy-2α,3α-23-trihydroxy-5α-ergostan-6-one, 113599-57-8; (22*R*,23*R*,24*S*)-22-acetoxy-2α,3α,23-trihydroxy-5α-ergostan-6-one, 113599-58-9.

Polarographic and Spectroscopic Examination of the Reaction of the Anabolic Steroid Oxymetholone with Methanol and Ethanol

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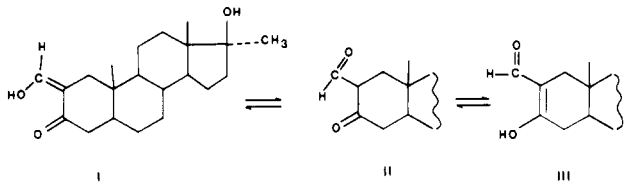
Studies employing electrochemical reduction at mercury electrodes, carbon-13 nuclear magnetic resonance spectroscopy, mass spectrometry, and other techniques have contributed to an understanding of the solution chemistry of the anabolic steroid oxymetholone (17-hydroxy-2-(hydroxymethylene)-17-methyl-5α,17β-androstan-3-one). The compound can exist in three tautomeric forms, I–III. In mixtures of aqueous buffer and acetonitrile, the geminal diol of form III is electrochemically reducible. In mixtures containing methanol or ethanol, a hemiacetal VI is formed, which is in slowly established equilibrium with the hydrate. Two separate peaks in differential pulse polarography show establishment of the equilibria between the hydrate and the hemiacetal, whereas UV spectra show overlapping peaks. An approximate value $K = 0.29$ has been found for the equilibrium constant K = [hemiacetal][H_2O]/[hydrate][CH_3OH]. Interaction of oxymetholone with alcohols was confirmed by mass spectra of reaction products. ^{13}C NMR spectra indicate that addition of methanol also occurs in chloroform solutions. Electrochemical studies enhance the observation of an unexpected chemical reaction.

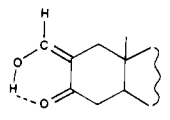
Introduction

Oxymetholone (17-hydroxy-2-(hydroxymethylene)-17-methyl-5α,17β-androstan-3-one) is a commonly prescribed anabolic steroid used in treating anemias. Clinical assessments of oxymetholone have been made to establish the degree of anabolism and androgenicity of this steroid,[1-6] and information on side effects has been documented. Despite the wide use of this steroid in pharmaceutical preparations and significant studies on side effects,[4-6] relatively little is known about the solution chemistry of this compound.

Oxymetholone[7] is a 1,3-dicarbonyl compound, which can exist in three tautomeric forms I–III. There is no quantitative information available regarding the positions of these equilibria and on solvent effects on them. Nevertheless, the structure of oxymetholone is usually presented as I, which in nonhydroxylic media is stabilized by formation of an intramolecular hydrogen bond (IV).



I II III



IV

The very broad relatively low intensity band in the infrared spectrum at 2500–3000 cm^{-1} has been attributed[8] to intramolecularly bonded enolic OH, a peak at 3350 cm^{-1} to the hydroxy group, and the strong peak at 1615 cm^{-1} to the C=O stretching in CO—C=C—OH. On the other hand, the proton NMR spectra have been interpreted[9] to favor, in some solvents, the formyl enol form III.

In hydroxylic solvents able to form intermolecular hydrogen bonds, the role of intramolecular hydrogen bonds is usually negligible[10] whereas covalent solvation, resulting in formation of hydrates and hemiacetals, may occur in nonhydroxylic solvents. As aldehydes form hydrates and hemiacetals more readily than ketones,[11] nucleophilic addition of the solvent will favor the formyl groups in structures II and III. As conjugation deactivates the carbonyl function toward hydrate and hemiacetal formation,[11] stronger hydration and reaction with alcohols is predicted to involve structure II rather than III. In this structure, the presence of the electron-withdrawing carbonyl groups in the β-position can be expected to enhance the reactivity of the formyl group toward nucleophilic addition. The only other reported[8] nucleophilic attack on oxymetholone, that of hydroxide ions, is also assumed to involve structure II. Resulting deformylation resembles that of other 3-formyl ketones.[12,13] On the other hand,

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