Tetrahedron Vol. 46, No. 9, pp. 3173-3188, 1990
Printed in Great Britain

0040-4020/90 $3.00+.00
© 1990 Pergamon Press plc

STEREOSELECTIVE SYNTHESIS OF THE BRASSINOLIDE SIDE CHAIN: NOVEL SYNTHESES OF BRASSINOLIDE AND RELATED COMPOUNDS+

Wei-shan Zhou*[a], Biao Jiang[b] and Xin-fu Pan[b]

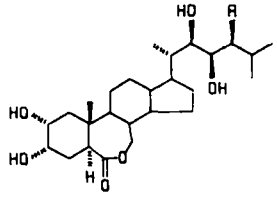
a. Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, 345 Lingling Lu, Shanghai 200032, China.

b. Department of Chemistry, Lanzhou University, Lanzhou 730000, China.

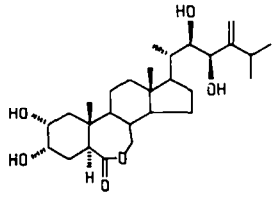
(*Received in Japan* 6 *November* 1989)

A stereoselective synthesis of the brassinolide side chain involves the lactonization of 2-**10** under acidic condition to give an α,β-unsaturated-δ-lactone **11** with the inversion of the configuration at C-22 of the epoxy steroid in quantitative yield. The 22R,23R,24S-γ-hydroxy-δ-lactone **14** was used as key intermediate for the syntheses of brassinolide(**1**), homobrassinolide(**2**), and typhasterol(**4**) as well as the side chain of the dolicholide(**3**).

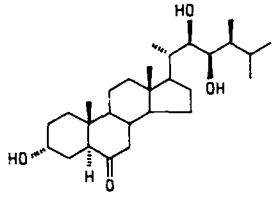
Brassinolide (**1**) isolated from the pollen of rape(Brassica napus), is a plant growth promoting steroid, having a seven-membered B-ring lactone and four successive chiral centers in the side chain[1]. Brassinolide promotes both cell elongation and cell division and possesses a broad spectrum of biological activities with the known plant hormones. Brassinolide may find practical application in agriculture. Since the discovery of brassinolide(**1**), a number of related compounds, e.g. homobrassinolide (**2**)[2], dolicholide(**3**)[3] and typhasterol(**4**)[4], have been isolated from higher plants and formed a new class of



1. R=CH_3 **2**. R=CH_2CH_3



3



4

+Dedicated to Professor Wang Yu on the occasion of his 80th birthday.

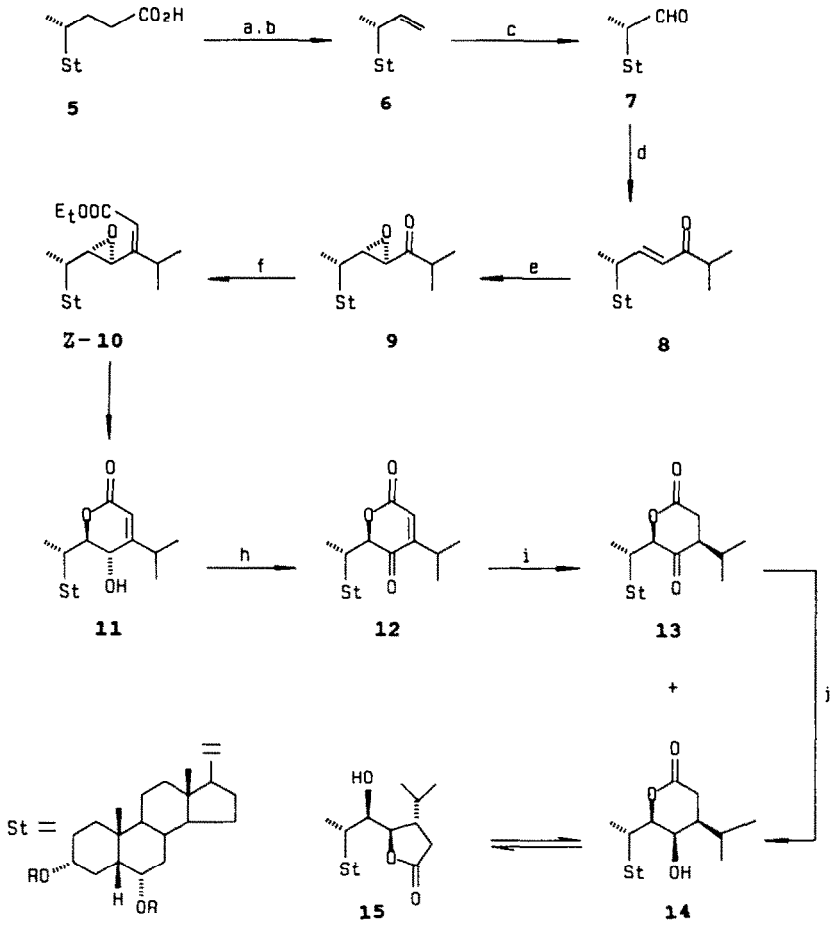
plant growth promoter. Their remarkable biological activities and novel chemical structure have led many laboratories to synthesize these natural products[5]. An important problem in the construction of the side chain is controlling the stereochemistry at C22, C23, C24. We report here a new method for constructing the brassinolide side chain, which is quite stereoselective and high in yield[6].

The 20-carbaldehyde **7** obtained from hyodeoxycholic acid (**5**) by the known procedure[7] was treated with isobutyl carbonyl arsonium ylide[8] to form α,β-unsaturated ketone **8** in 72% yield. Epoxidation of **8** with H_2O_2-NaOH afforded the α,β-epoxyketone **9** in 86% yield. The Wittig-Horner reaction of ethyl dimethylphosphonoacetate with **9** furnished a mixture of Z- and E-α,β-unsaturated-γ,δ-α-epoxy acid ester **Z-10** and **E-10** in 72% yield at a ratio of 10:1. **Z-10** was lactonized under acidic condition to give an α,β-unsaturated-δ-lactone **11** formed by the carboxylate-aided epoxide ring opening of this **Z-10** with the inversion of the configuration at C22 in quantitative yield. The 23S-configuration of **11** could be easily converted into a 23R configuration by successive oxidation and reduction. Thus, oxidation of **11** with PDC followed by hydrogenation over PtO_2 gave a mixture of 22R,23R,24S-γ-hydroxy-δ-lactone **14** and 22R-γ-keto-δ-lactone **13** in almost quantitative yield in a ratio of 88:12. Compound **13** could easily converted into **14** by KBH_4 in quantitative yield. Hydroxy-lactone **14** was partly isomerized into thermodynamically stable γ-lactone **15.** On successive treatment with alkali and acid, the hydroxy-δ-lactone **14** was quantitatively isomerized to **15**[9](Scheme 1). Both **14** and **15**[5d] could be used as key intermediate for syntheses of the three natural brassinosteroids **1**→**3** and the side chain of dolicholide(**4**).

Reduction of lactone **14** with DIBAH afforded a hemiacetal and the compound was treated with 2,2-dimethoxypropane to give a 22,23-acetonide which was decarbonylated with tris(triphenylphosphine)rhodium chloride to give the known 24S-methyl derivative **16**[5g]. These three-step reations were performed in 76% overall yield. The overall yield for the synthesis of the side chain, staring from 20-carbaldehyde **7**, was 32%. This is one of the best methods for the construction of the side chain of brassinolide and related compounds[5]. Brassinolide was prepared from **16** in five sequential steps: (1) oxidation of **16** with PDC followed by acid treatment afforded **17** in 91% yield. Compound

17 was also readily obtained from δ-hydroxy lactone compound **14** by the sequence of reaction: 1.$LiAlH_4$, 2.$(MeO)_2CMe_2$/p-TsOH, 3.CrO_3, 4.$(Ph_3P)_3RhCl$ and 5. 5%HCl in 58% overall yield in five steps. (2) **17** was then subjected to a reductive elimination by treatment with TMSCl and zinc amalgam[10] to give Δ^2-6-keto compound **18** which on osmylation with OsO_4-NMMNO followed by Baeyer-Villiger oxidation afforded brassinolide mp. 273-275°C(lit.[5a], mp. 273-274°C) in 34% overall yield in three steps (Scheme 2).

Scheme 1

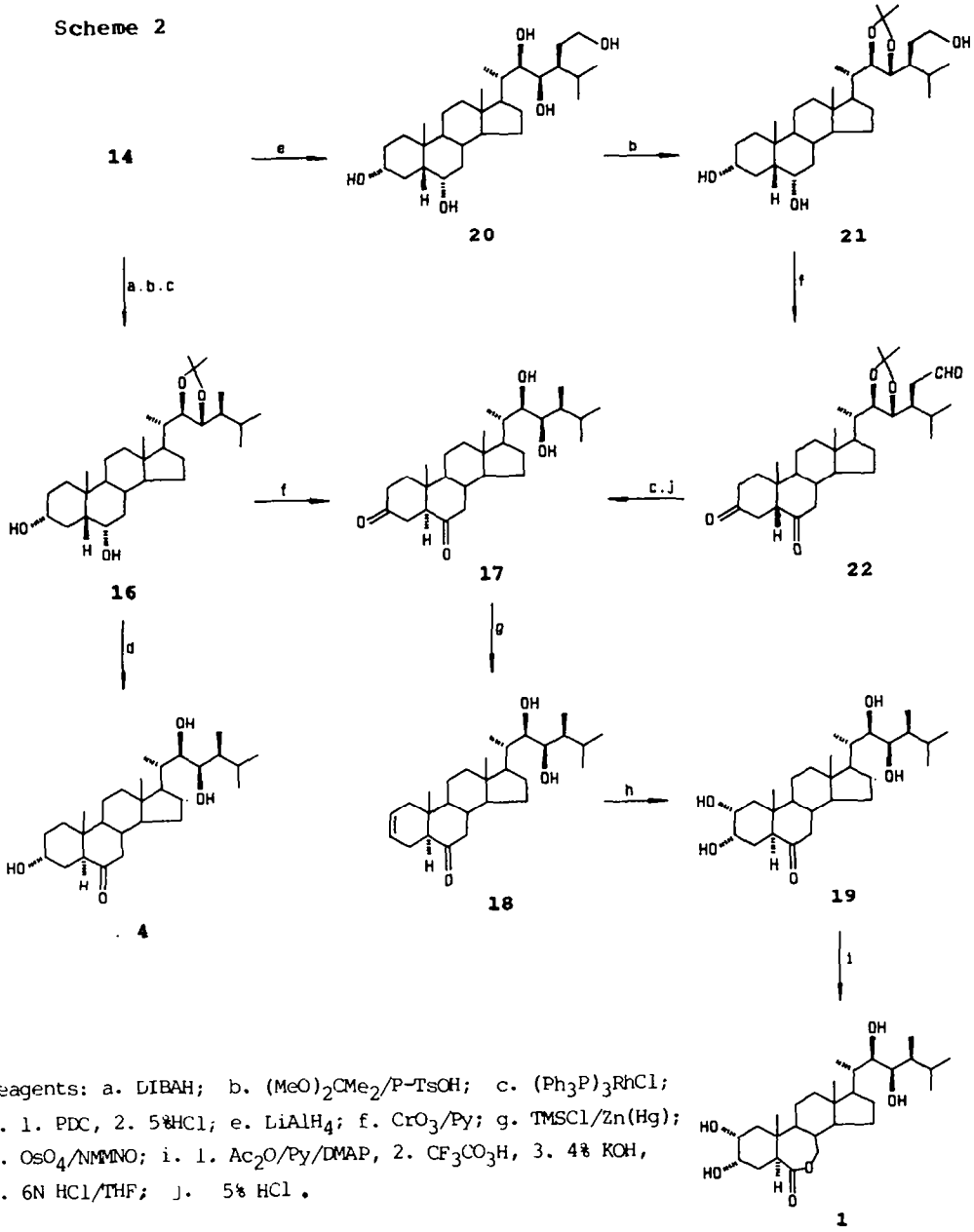


Reagents: a. 1. Ac_2O/p-TsOH; 2. 80% Py-H_2O; b. $Pd(OAc)_4/Cu(OAc)_2$/Py; c. O_3, d. $Ph_3As{=}CHCOCHMe_2$; e. 4N NaOH/30%H_2O_2; Ac_2O/Py; f. 1. NaH/$(MeO)_2P(O)CH_2CO_2Et$, 2. Ac_2O/Py, g. 30% $HClO_4$/MeOH; h. PDC; i. H_2/PtO_2 j. KBH_4.

Conversion of **16** to typhasterol (**4**) mp. 230-231°C (lit.[4] mp.227-230°C), was achieved in 56% yield in two steps by oxidation with PDC and the acid treatment with simultaneous epimerization of C5 (Scheme 2).

Homobrassinolide (**2**) was prepared from **14** in nine sequential steps: (1) reduction of **14** with $LiAlH_4$ followed by treatment with 2,2-dimethoxy

Scheme 2



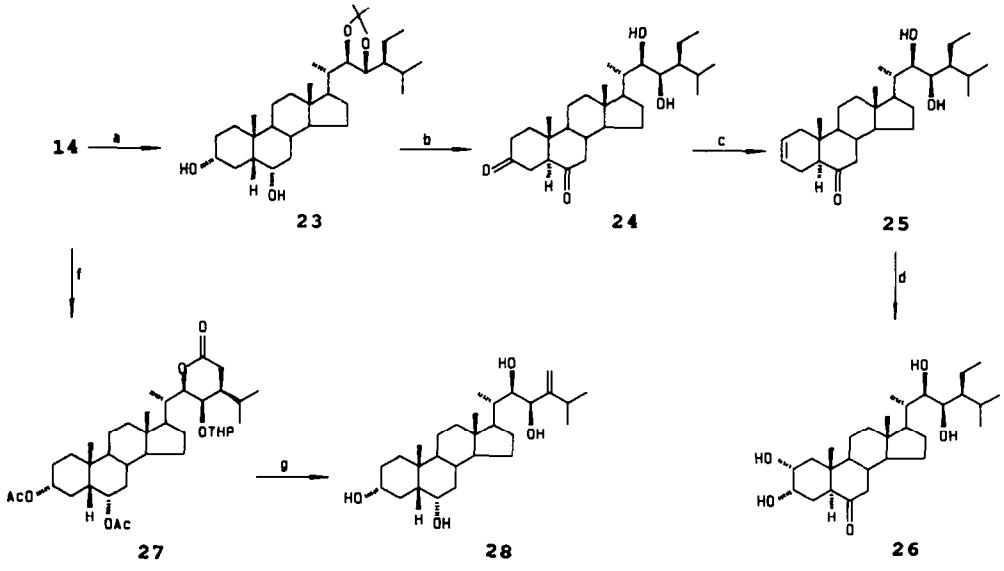
Reagents: a. DIBAH; b. $(MeO)_2CMe_2$/P-TsOH; c. $(Ph_3P)_3RhCl$; d. 1. PDC, 2. 5%HCl; e. $LiAlH_4$; f. CrO_3/Py; g. TMSCl/Zn(Hg); h. OsO_4/NMMNO; i. 1. Ac_2O/Py/DMAP, 2. CF_3CO_3H, 3. 4% KOH, 4. 6N HCl/THF; j. 5% HCl.

propane gave 24-hydroxy-ethyl-22,23-acetonide, which was then mesylated with methanesulphonyl chloride followed by reduction with $LiAlH_4$ to gave 24-ethyl-22,23-acetonide **23** and this compound was oxidized with PDC followed by treatment with acid to give **24** in 59% overall yield; (2) **24** was converted into homobrassinolide (**2**) mp. 269-271°C (lit.[2], 268-271°C) by using a procedure similar to that described for brassinolide(Scheme 3).

compound **14** could be readily converted to $\Delta^{24(28)}$-compound which makes available dolicholide (**3**)[3] as shown in Scheme 3. The key step is the oxidative decarboxylation effected with iodobenzenediacetate. Thus, compound **14** was first protected with dihydropyran to give the compound **27** and this compound was treated with 4% KOH/MeOH followed by acetylation and decarboxylation with iodobenzenediacetate in the presence of $Cu(OAc)_2$ to give $\Delta^{24(28)}$-compound **28** in 80% yield.

Thus, the new key intermadiate **14** could be used for the syntheses of natural plant growth promoting steroids brassinolide (1), homobrassinolide (2), typhaserol (**4**) and the side chain of dolicholide (3).

Scheme 3



Reagents: a. 1. $LiAlH_4$, 2. $(MeO)_2CMe_2$/p-TsOH, 3. CH_3SO_2Cl/Et_3N, 4. $LiAlH_4$ b. 1. PDC, 2. 5%HCl; c. TMSCl/Zn(Hg); d. OsO_4/NMMNO; e. 1. Ac_2O/Py/DMAP, 2. CF_3CO_3H, 3. 4%KOH/MeOH, 4. 6N HCl/THF, f. DHP/PPTS; g. 1. 4% KOH, 2. Ac_2O/Py, 3. IDBA/$Cu(OAc)_2$; 4. 4%KOH.

EXPERIMENTAL

All mps were uncorrected. IR spectra were recorded on Shimadzu 440 spectrometer. ^1H NMR spectra were determined with Varian XL-200 spectrometer, using $CDCl_3$ as solvent and TMS as an internal standard. The unit of δ is ppm. Mass spectra were run on JMS-01U spectrometer. The optical rotation was measured on Autpol III polarimeter. The work up as usual way meant that the extracts were washed by 10% HCl, saturated $NaHCO_3$, brine and dried over Na_2SO_4; the solvent was removed under reduced pressure. The silica gel H(10-40μ) was used for flash chromatography. Elemental analyses were performed by Analytical Department of this Institute.

(22E)-3α,6α-Diacetoxy-5β-cholesten-22-en-24-one (8):

A mixture of **7** (10 g, 23.1 mmol) and $Ph_3AsCHCOCH(CH_3)_2$(11 g, 28.2 mmol) in THF (90 ml) was stirred overnight at room temperature. After removal of solvent, the residue was chromatographed on silica gel to give **8** (8.5 g) in yield of 70%; mp. 129-131°C; $[\alpha]_D^{16}$ 20.2 (c, 1.29, $CHCl_3$); νmax: 1710 (α,β-unsaturated C=O), 1720 (CH_3COO), 1680, 1620 (α,β-unsturated C=C) cm^{-1}. δ_H: 0.64 (3H, s, 18-H), 0.99 (3H, s, 19-H), 1.02 (3H, d, J=6Hz, 21-H), 1.12, 1.14 (6H, 2d, J=6Hz, 26, 27-H), 4.66, 5.18 (2H, m, 3 and 6-H), 6.20 (1H, d, J=16Hz, 23-H), 6.71(1H, dd, J=16Hz, 8Hz, 22-H); m/z: 500 (M^+), 440 (M^+-CH_3COOH), 380 (M^+-2CH_3COOH); $C_{31}H_{48}O_5$ calc. C, 74.36, H, 9.66; found C, 74.03, H, 9.65.

(22S,23R)-3α,6α-diacetoxy-22,23-epoxy-5β-cholestan-24-one (9):

A solution of 4N NaOH (15 ml) was added dropwise to a solution of **8** (6.7g, 19.4 mmol) and 30%H_2O_2 (34 ml) in ethyl alcohol (340 ml) with stiring at 35°C for 4 h. The solvent was removed under reduced pressure. The residue was extracted with ethyl acetate. The extracts were worked up as usual to give crude epoxide compound which was treated with pyridine (20 ml) and acetic anhydride (15 ml) at room temperature overnight. The mixture was poured into cracked ice (100 g) and extracted with ethyl acetate. After working up as usual the crude product was chromatographed on silica gel to afford **9** (5.95 g) in 86% yield; mp. 108-110°C; $[\alpha]_D^{16}$ 31°(c, 1.24, $CHCl_3$); νmax: 1740, 1720 (CH_3COO, C=O) cm^{-1}, δ_H: 0.70 (3H, s, 18-H), 0.97 (3H, s, 19-H), 1.01 (3H, d, J=6Hz, 21

-H), 1.20 (6H, d, J=6.4Hz, 27 and 26-H), 2.00 (6H, s, $2CH_3COO$), 2.80 (1H, m, 22-H), 3.20 (1H, m, 23-H), 4.70, 5.18 (2H, 2m, 3 and 6-H); m/z: 517(M^++1), 475 (M^+-CH_3CO), 414 (M^+-CH_3CO-CH_3CO_2H); $C_{31}H_{48}O_6$ calc. C, 72.06, H, 9.36, found C, 72.10, H, 9.35.

(22S,23S, 24Z)-3α,6α-Diacetoxy-22,23-epoxy-5β-cholesten-24-carboxyethylene ethyl ester (Z-10):

A solution of $(CH_3O)_2P(O)CH_2CO_2Et$ (8.2g, 41.7mmol) in THF (30 ml) was added to a suspension of NaH (50%, 2 g, 41.7 mmol) in THF (150 ml) at room temperature over 30 min under N_2. Then a solution of **9** (3 g, 5.85 mmol) in THF (15 ml) was added. The mixture was continued stirring for 2 h. The reaction was quenched by slow addition of ethyl acetate (30 ml). When the solvent was removed under reduced pressure, the residue was poured into ice water and extracted with ethyl acetate. The extracts were worked up as usual way. The residue was acetylated by usual way. The residue was purified by flash chromatography to afford **Z-10** (2.44 g) in 72% yield; mp. 106-108°C, $[\alpha]_D^{16}$ 109° (c, 0.40, $CHCl_3$); νmax: 1740, 1720 (brs, C=O), 1680 (C=C) cm^{-1}; δ_H: 0.60 (3H, s, 18-H), 0.99 (3H, s, 19-H), 1.02(3H, d, J=6Hz, 21-H), 1.13, 1.15 (6H, 2d, J=8Hz, 26, 27-H), 1.20 (3H, t, J=8Hz, CH_3CH_2O), 2.01, 2.02(6H, 2s, $2CH_3CO$), 2.60 (1H, m, 22-H), 4.25(2H, q, J=8Hz, CH_3CH_2), 4.41 (1H, m, 23-H), 4.70, 5.18 (2H, 2m, 3 and 6-H), 5.83(1H, s, 28-H); m/z: 587 (M^++1), 569 (M^+-H_2O), 527 (M^+-CH_3CO_2H), 469 (M^+-$2CH_3COOH$); $C_{35}H_{54}O_7$ calc. C, 71.64, H, 9.28, found C, 71,68, H, 9.48.

$\Delta^{24(28)}$-(22R, 23S)-3α,6α-Diacetoxy-23-hydroxy-24-carboxyethylene-5β-cholesten-22(29)-lactone (11):

30% $HClO_4$ in MeOH was added to a solution of **Z-10** (2 g, 3.41 mmol) in MeOH (20 ml) at 0°C for 10 min. The reaction mixture was neutralized with 6N NaOH. After removal of solvent, the residue was extracted with ethyl acetate and worked up as usual way to afford **11** in quantitative yield; mp. 206-208°C; $[\alpha]_D^{26}$ 20° (c, 1.36, $CHCl_3$), νmax: 3400 (-OH), 1740, 1720 (C=O), 1620 (C=C) cm^{-1}; δ_H: 0.71 (3H, s, 18-H), 0.97 (3H, s, 19-H), 1.01 (3H, d, J=6.4Hz, 21-H), 1.14, 1.16 (6H, 2d, J=6Hz, 26, 27-H), 4.10 (1H, d, J=12Hz, 23-H), 4.28(1H, d,

J=12Hz, 22-H), 4.72, 5.14 (2H, 2m, 3 and 6-H), 5.84 (1H, s, 28-H); m/z: 558 (M^+), 498 (M^+-CH_3COOH), 456(M^+-CH_3COOH-CH_3CO), 438 (M^+-$2CH_3COOH$), 423(M^+-$2CH_3COOH$-H_2O); $C_{33}H_{50}O_7$ calc. C, 70.90, H, 9.02, found C, 71.02, H, 9.07.

$\Delta^{24(28)}$-(22R)-3α,6α-Diacetoxy-24-carboxyethylene-23-oxo-5β-cholesten-22(29)-lactone (12):

A solution of **11** (5 g, 8.9 mmol) in CH_2Cl_2 (20 ml) was treated with PDC (10 g) at room temperature for 5 h. Then dry ethyl ether (50 ml) was added to dilute the mixture. The solid was filtered. After concentration, the residue was recrystallized from ethyl ether to afford **12** (4.91 g) in yield of 95.5%, mp. 178-180°C, $[\alpha]_D^{26}$ 98.7° (c, 0.65, $CHCl_3$); νmax: 1740, 1720 (C=O), 1660 (C=C) cm^{-1}; δ_H: 0.69 (3H, s, 18-H), 0.97 (3H, s, 19-H), 1.01 (3H, d, J=8Hz, 21-H), 1.14, 1.16 (6H, 2d, J=4Hz, 26 and 27-H), 2.04, 2.06 (2H, 2s, $2CH_3CO$), 4.72, 5.14 (2H, 2m, 3 and 6-H), 4.91 (1H, d, J=2Hz, 22-H), 6.68 (1H, d, J=1Hz, 28-H); m/z: 556 (M^+), 454 (M^+-CH_3COOH-CH_3CO), 436 (M^+-$2CH_3$-COOH), 421 (M^+-$2CH_3COOH$-H_2O); $C_{33}H_{48}O_7$ calc. C, 71.19, H, 8.69, found C, 70.93, H, 8.68.

(22R,23R,24S)-3α,6α-Diacetoxy-22,23-hydroxy-24-carboxyethyl-5β-cholest-22(29)-lactone (14) and (22R,24S)-3α,6α-Diacetoxy-24-carboxyethyl-5β-cholest-22(29)-lactone-23-one(13):

Compound **12** (2.1 g, 3.8 mmol) in anhydrous ethanol (25 ml) and ethyl acetate (25 ml) was hydrogenated over PtO_2 (300 mg) at room temperature for 3 h. The catalyst was filtered and solvent was removed under reduced pressure. The residue was chromatographed on silica gel to give **13** (200 mg) and **14** (1.8 g) in yield of 12% and 88% respectively; **13**: mp. 198-200°C; $[\alpha]_D^{16}$ 70.5° (c, 0.54, $CHCl_3$); νmax: 1740, 1710 (C=O) cm^{-1}; δ_H: 0.70 (3H, s, 18-H), 0.90 (3H, s, 19-H), 0.99 (3H, d, J=6Hz, 21-H), 1.01, 1.02 (6H, 2d, J=6.4Hz,26 and 27-H), 2.02, 2.04 (6H, 2s, CH_3CO), 4.68 (1H, d, J=2Hz, 22-H), 4.72, 5.16 (2H, 2m, 3 and 6-H); m/z: 558 (M^+), 492 (M^+-CH_3COOH), 456 (M^+-CH_3COOH-CH_3CO), 438 (M^+-$2CH_3COOH$), 423 (M^+-$2CH_3COOH$-CH_3); $C_{33}H_{50}O_7$ calc C, 70.94, H, 9.02, found C, 70.84, H, 9.05. **14**: mp. 210-212°C, $[\alpha]_D^{16}$ 19.5° (c, 1.07, $CHCl_3$), νmax: 3400 (OH), 1740, 1710 (C=O) cm^{-1}; δ_H: 0.70 (3H, s,

18-H), 0.88 (6H, 2d, J=6Hz, 26 and 27-H), 0.99(3H, s, 19-H), 1.01 (3H, d, J=6.4Hz, 21-H), 2.02, 2.04 (6H, 2s, $2CH_3CO$), 4.04 (1H, s, 22-H), 4.21 (1H, s, 23-H), 4.72, 5.14 (2H, 2m, 3 and 6-H); m/z: 561 (M^++1), 501 (M^+-CH_3COOH), 483 (M^+-CH_3COOH-H_2O), 458 (M^+-CH_3COOH-CH_3CO), 440 (M^+-$2CH_3COOH$); $C_{33}H_{52}O_7$ calc C, 70.68, H, 9.35, found C, 70.88, H,9.34.

Compound **13** (100 mg, 0.18 mmol) in CH_2Cl_2 (2 ml) and MeOH (2 ml) was reduced with KBH_4 (15mg) with stirring at room temperature for 1 h. The mixture was worked up as usual way to afford **14** in quantitative yield.

(22R,23R,24S)-3α, 6α, 22, 23-Tetrahydroxy-24-carboxymethyl-5β-cholest-23(29) -lactone (15):

Compound **14** (120 mg) in 4% KOH/MeOH (3 ml) was allowed to stand at room temperature for 6 h. The mixture was extracted with ethyl acetate and the extract was worked up as usual way to yield quantitatively **15** ; mp. 251-252°C. $[\alpha]_D^{22}$ 28°(c, 0.45, MeOH); νmax: 3450 (OH), 1780(C=O)cm^{-1}; δ_H: 0.72 (3H, s, 18-H), 0.90 (3H, s, 19-H), 0.93, 0.96 (6H, 2d, J=6.2Hz, 26 and 27 -H), 1.02 (3H, d, J=8Hz, 21-H), 3.64(1H, d, J=5Hz, 22-H), 3.65, 4.00(2H, 2m, 3 and 6-H); 4.24(1H, t, J=5Hz, 23-H); m/z: 477 (M^++1), 457(M^+-H_2O); $C_{29}H_{48}O_5$ calc. C, 73.07, H, 10.15, found C,73.20, H, 9.96.

(22R,23R,24S)-3α,6α-Dihydroxy-22,23-isopropylidenedioxy-24-methyl-5β,- cholestane(16):

A solution of **14** (100 mg, 0.178 mmol) in dry toluene (10 ml) was treated with DIBAH (0.4 ml, 1M) at -78°C for 1 h. The mixture was quenched with ethyl acetate (1 ml) at -78°C. The mixture was poured into saturated NH_4Cl solution (2 ml), and extracted with $CHCl_3$. The extracts were dried over Na_2SO_4. After removal of solvent, the residue dissolved in acetone(1 ml) was treated with 2,2-dimethoxypropane and p-TsOH (5 mg) at room temperature for 1 h. This protected compound was refluxed with $[Ph_3P]_3RhCl$ (100 mg) under N_2 for 4 h. The solid was filtered. The solvent was removed. The crude product was chromatographed on silica gel to afford **16** (65 mg) in yield of 74%; mp.167-168°C; νmax: 3450 (OH) cm^{-1}; δ_H: 0.67 (3H, s, 18-H), 0.80, 0.84 (6H, 2d, J=8Hz, 26 and 27-H), 0.94 (3H, d, J=6.8Hz, 28-H), 0.98 (3H, s,

19-H), 1.06 (3H, d, J=6Hz, 21-H), 1.34, 1.36 (6H, 2s, $(CH_3)_2C$), 3.68, 3.80 (2H, 2m, 6 and 3-H), 3.82 (1H, dd, J=9Hz, 4Hz, 23-H), 3.98 (1H, d, J=9Hz, 22-H); m/z: 491 (M^++1), 475(M^+-CH_3), 472(M^+-H_2O); $C_{31}H_{48}O_4$ calc. C, 75.87, H, 11.09, found C, 75.77, H, 11.01

Typhasterol(4):

A solution of **16** (40 mg) in CH_2Cl_2 (5 ml) was treated with PDC (40 mg) at room temperature for 3 h. The mixture was diluted with ether (10 ml) and the solid was filtered. After removal of solvent, the residue dissolved in 5% HCl /MeOH (2 ml) was allowed to stand overnight. The reaction mixture was worked up as usual. The crude product was purified by preparative thin layer chromatography to afford **4** (20 mg) in yield of 56%; mp. 230-231°C (Lit.[4] mp. 227-230°C), ν max: 3400(OH), 1730 (C=O) cm^{-1}; δ_H: 0.80 (3H, s, 18-H), 0.82, 0.86 (6H, 2d, J=8Hz, 26 and 27-H), 0.94 (3H, d, J=6.8Hz, 28-H), 0.98(3H, s, 19-H), 1.06(3H, d, J=6Hz, 21-H), 3.70 (1H, s,23-H), 3.90 (1H, s, 22-H), 4.00 (1H, m, 3-H); m/z: 448 (M^+), 430(M^+-H_2O); $C_{28}H_{48}O_4$ calc. C, 74.95, H, 10.78, found C, 74.66, H, 10.80.

(22R,23R,24S)-22,23-Dihydroxy-24-methyl-5α-cholestan-3,6-dione (17):

1). From **16:** A solution of **16** (100 mg) in CH_2Cl_2 (10 ml) was treated with PDC (200 mg) at room temperature for overnight. The mixture was diluted with ethyl ether (10 ml) and the solid was filtered. The filtrate was concentrated under reduced pressure, the residue dissolved in 5% HCl/MeOH (3 ml) was allowed to stand overnight and then worked up as usual. The crude product was recrystallized from acetone-ethyl ether to afford **17** (86 mg) in yield of 94%; mp. 201-202°C; νmax: 3450 (OH), 1720(C=O); δ_H : 0.71 (3H, s, 18-H), 0.84, 0.86 (6H, 2d, J=8Hz, 26 and 27-H), 0.94 (3H, d, J=6.4Hz, 28-H), 0.98 (3H, s, 19-H), 1.06 (3H, d, J=6Hz, 21-H), 3.80 (1H, d, J=9Hz, 22-H), 3.90 (1H, d, J=9Hz, 23-H), m/z: 447 (M^++1), 429 (M^+-H_2O); $C_{28}H_{46}O_4$ calc. C, 75.29, H, 10.37, found C, 75.30, H, 10.20.

2). From **22:** A solution of **22** (100 mg) in toluene (10 ml) was refluxed with $(Ph_3P)_3RhCl$ (100 mg) under N_2 for 2 h. The mixture was filtered over a short

column of celite. The solvent was removed under reduced pressure. The residue was treated with 5% HCl/MeOH (5 ml) overnight. The reaction mixture was worked up as usual way. The crude product was purified by flash chromatography to afford **17** (65 mg) in yield of 75%. mp. 199-201°C. The spectral date of this compound is same with that of **17** obtained from **16**.

Δ^2-(22R,23R,24S)-22,23-Dihydroxy-24-methyl-5α-cholesten-6-one (18):

A solution of **17** (40 mg) in dry THF (5 ml) was stirred with Zn(Hg) (200 mg) and TMSCl (0.5 ml) under N_2 at room temperature for 12 h. The catalyst was filtered and solvent was removed under reduced pressure. The residue was chromatographed on silica gel to afford **18** (20 mg) in 52% yield; mp. 132-134°C, νmax: 3450 (HO), 1730 (C=O), 1620, 890 (C=C) cm^{-1}; δ_H: 0.7 (3H, s, 18-H), 0.84, 0.86 (6H, 2d, J=8Hz, 26 and 27-H), 0.94 (3H, d, J=6.4Hz, 28-H), 0.98 (3H, s, 19-H), 1.02 (3H, d, J=6Hz, 21-H), 3.59 (1H, d, J=9Hz, 22-H), 3.72 (1H, d, J=9Hz, 23-H), 5.60 (2H, m, 2 and 3-H); m/z: 431 (M^++1), 412 (M^+-H_2O), $C_{28}H_{46}O_3$ calc. C, 78.09, H, 10.77; found C, 78.11, H, 10.63.

(22R,23R,24S)-2α,3α, 22,23-Tetrahydroxy-24-methyl-5α-cholestan-6-one (19):

Compound **18** (10 mg) in a mixed solvent (t-BuOH/THF/H_2O=10:3:1, 5 ml) was stirred with NMMNO(50 mg) and OsO_4(5 mg) at room temperature for 2 days. To the mixture was added a saturated $NaHSO_3$ solution (0.5 ml). The mixture was stirred for 30 min. After concentration under reduced pressure, the residue was extracted with $CHCl_3$. The extract was worked up as usual. The crude product was recrystallized from ethyl acetate to afford **19** (10.4 mg) in yield of 93%; mp. 258-259°C (lit.[5a], mp. 259-261°C); $[\alpha]_D^{25}$ -2° (c, 0.542, MeOH), νmax: 3450(OH), 1720 (C=O) cm^{-1}; δ_H: 0.68 (3H, s, 18-H), 0.97 (3H, s, 19-H), 0.85, 0.90 (6H, 2d, J=6.8Hz, 26, 27-H), 0.95 (3H, d, J=6.4Hz, 28-H), 1.01(3H, d, J=6.4Hz, 21-H), 2.72 (1H, dd, J=12Hz, 4Hz, 5-H), 3.55, 3.70, 4.09 (4H, 3m, 2, 3, 22 and 23-H); m/z: 465 (M^++1), 447 (M^+-H_2O), 429 (M^+-2H_2O), 411 (M^+-3H_2O), $C_{28}H_{48}O_5$ calc. C, 72.37, H, 10.41; found C, 72.40, H, 10.30.

Brassinolide (1):

Compound **19** (5 mg) dissolved in pyridine (0.5 ml) was treated with Ac_2O (0.4 ml) and DMAP (1 mg) at room temperature overnight. The solvent was removed under reduced pressure. The residue was dissolved in CH_2Cl_2 (1 ml) and the mixture was treated with CF_3CO_3H (60%,0.5 ml) at 0°C for 4 h. Then the solvent was removed to dryness under reduced pressure. The residue was refluxed with 4%KOH/MeOH (5 ml) for 1 h. After concentration in vacuum, the residue in THF (1 ml) was acidified with 6N HCl (1 ml) overnight. Then the solvent was removed. The crude product was purified with preparative thin layer chromatography to afford **1** (3.6 mg) in yield of 70%; mp. 273-275°C (lit.[5a], mp. 274-275°C); $[\alpha]_D^{20}$ +41.9°(c, 0.253, MeOH); νmax: 3450 (OH); 1725 (C=O) cm^{-1}; δ_H(C_6D_5N-$CDCl_3$): 0.72 (3H, s, 18-H), 1.04 (3H, d, J=6.8Hz, 28-H), 1.05 (3H, s, 19-H), 1.11, 1.14 (6H, 2d, J=6.8Hz, 26 and 27-H), 1.21 (3H, d, J=6.3Hz, 21-H), 2.32 (1H, dd, J=4.0 14.5Hz, 5-H), 3.99-4.10(4H, m, 2, 3, 22 and 23-H), 4.13, 4.43 (2H, 2m, 7-H); m/z: 481 (M^++1), 463 (M^+-H_2O), 427 (M^+-$3H_2O$); $C_{38}H_{48}O_6$ calc. C, 69.96, H, 10.06; found C, 70.10, H, 9.94.

(22R,23R,24S)-3α, 6α, 22, 23-Tetrahydroxyl-24-hydroxyethyl-5β-cholestane (20):

To a solution of **14** (360 mg, 0.64 mmol) in THF (60 ml) was added portion-wise $LiAlH_4$ (370 mg, 10 mmol) at room temperature for 4 h with stirring. The reaction was quenched by careful addition of ethyl acetate (10 ml) at 0°C. The mixture was poured into saturated NH_4Cl solution and extracted with $CHCl_3$. The extracts were worked up as usual. The crude product was recrystallized from methanol to afford **20** (340 mg) in yield of 96%; mp.236-238°C, $[\alpha]_D^{26}$ 87°(c, 0.42, MeOH), νmax: 3450 (OH) cm^{-1}; δ_H: 0.70 (3H, s, 18-H), 0.80 (3H, s, 19-H), 0.84, 0.90 (6H, 2d, J=8Hz, 26 and 27-H), 1.12 (3H, d, J=6Hz, 21-H), 3.7, 3.9, 4.10, (6H, m, 22, 23, 29, 3 and 6-H); m/z: 481 (M^++1), 445 (M^+-$2H_2O$), 409 (M^+-$4H_2O$). $C_{29}H_{52}O_5$ calc. C,72.47, H, 10.90; found C, 72.20, H, 10.40.

(22R,23R,24S)-22,23-Isopropylenedioxy-5β-cholestan-3,6-dione-24-carbaldehyde (22):

A solution of **20** (150 mg) in acetone (2 ml) was treated with 2,2-dimethoxy propane (1 ml) and p-TsOH (3 mg) at room temperature to stand for 20 min. The

solvent was removed under reduced pressure. The crude product **21** dissolved in pyridine (10 ml) was treated with CrO_3 (60 mg) with stirring at room temperature for 5 h. The reaction mixture was diluted with dry ethyl ether (10 ml). The solid was filtered. The filtrate was concentrated under reduced pressure. The crude product was chromatographed on silica gel to give **22** (120 mg) in yield of 81%; mp. 124-127°C. νmax: 1780(CO) cm^{-1}; δ_H: 0.70 (3H, s, 18-H), 1.21, 1.23 (6H, 2s, $(CH_3)_2C$); 3.72 (1H, d, J=9Hz, 22-H), 3.84 (1H, d, J=9Hz, 23-H), 9.78 (1H, s, 29-H); m/z: 515 (M^++1), 499 (M^+-CH_3).

(22R,23R,24S)-3α,6α,-Dihydroxy-22,23-isopylidenedioxy-24-ethyl-5β-cholestane (23):

A solution of **20** (96 mg) in acetone (2 ml) was treated with 2,2-dimethoxypropane and p-TsOH (5 mg) at room temperature for 10 min. The mixture was worked up as usual way to give the protected compound which dissolved in triethylamine (30 μl 0.22 mmol) in CH_2Cl_2 (2 ml) was treated with CH_3SO_2Cl (17 μl, 0.22 mmol) at 0°C for 10 min. Then $NaHCO_3$ (0.5 ml) was added to quench the reaction. After working up as usual, the resulting compound dissolved in THF (10 ml) was reduced with $LiAlH_4$ (80 mg) at room temperature for 4 h. The excess $LiAlH_4$ was destroyed with ethyl acetate (5 ml). The mixture was poured into saturated NH_4Cl, and extracted with $CHCl_3$. The extracts were worked up as usual way. The crude product was purified by flash chromatography to give **23** (50 mg) in 60% yield; mp.162-164°C; νmax: 3450 (OH) cm^{-1}; δ_H: 0.70 (3H, s, 18-H), 0.83 (3H, s, 19-H), 1.01 (3H, d, J=6.8Hz, 21-H), 1.34 (6H, s, acetonide), 3.75-3.99 (4H, br, 22, 23, 3, and 6-H); m/z: 505 (M^++1); $C_{32}H_{56}O_4$ calc. C, 69.04, H, 11.18, found C, 70.10, H, 11.42.

(22R,23R,24S)-22,23-Dihydroxy-24-ethyl-5α-cholestan-3,6-dione (24):

Compound **23** (90 mg) in CH_2Cl_2 (5 ml) was treated with PDC (200 mg) and worked up as described for **17** to afford **24** (70 mg) in 91% yield; mp. 197-199°C; $[\alpha]_D^{20}$ 30° (c, 0.510, $CHCl_3$); νmax: 3450 (OH), 1720 (C=O) cm^{-1}; δ_H: 0.70 (3H, s, 18-H), 0.84, 0.90 (6H, d, J=6Hz, 26 and 27-H), 0.99 (3H, s, 19-H), 1.01 (3H, d, J=6Hz, 21-H), 3.58 (1H, d, J=8Hz, 22-H), 3.72 (1H, d,

J=8Hz, 23-H); m/z: 460 (M^+), $C_{29}H_{48}O_4$, calc. C, 75.60, H, 10.50; found C, 75.30, H, 10.40.

(22R,23R,24S)-22,23-Dihydroxy-24-ethyl-5α-cholesten-2-en-6-one (25):

Compound **24** (70 mg) in THF (10 ml) was stirred with Zn(Hg) (0.4 g) and TMSCl (0.4 ml) under N_2 and worked up as described for **18** to afford **25** (35 mg) in yield of 52%; mp. 240-242°C; $[\alpha]_D^{21}$ 9.5 (c, 0.501, $CHCl_3$); νmax: 3450 (OH), 1720 (C=O), 1660 (C=C)cm^{-1}; δ_H: 0.67 (3H, s, 18-H), 0.70 (3H, s, 19-H), 0.83, 0.90 (6H, d, J=6.4Hz, 26, 27-H), 0.95 (3H, d, J=6.5Hz, 21-H), 3.60 (1H, d, J=9Hz, 22-H), 3.68(1H, d, J=9Hz, 23-H), 5.62(2H, m, 2 and 3-H); m/z: 445 (M^++1), 427 (M^+-H_2O), 409 (M^+-2H_2O); $C_{29}H_{48}O_3$ calc. C,78.33, H, 10.88; found C, 78.87, H, 10.78.

(22R,23R,24S)-2α,3α,22,23-Tetrahydroxy-24-ethyl-5α-cholestan-6-one (26):

Compound **25** (20 mg) in THF/t-BuOH/H_2O (10:3:1, 5 ml) was treated with NMMNO (50 mg) and OsO_4 (5 mg) and worked up as described for **19** to afford **25** (19.5 mg) in 91% yield; mp. 256-257°C (lit.[2] mp. 258-260°C); $[\alpha]_D^{25}$ 15° (c, 0.51, MeOH); νmax: 3450(OH), 1720 (C=O) cm^{-1}; δ_H: 0.70 (3H, s, 18-H), 0.86(3H, s, 19-H), 0.90, 0.92 (6H, 2d, J=8Hz, 26, 27-H), 0.99 (3H, d, J=6.8 Hz, 21-H), 3.56, 3.82 (2H, 2m, 22, 23-H), 3.76, 4.10 (2H, 2m, 2 and 3-H); m/z: 479 (M^++1), 461 (M^+-H_2O), 443 (M^+-2H_2O), 424 (M^+-3H_2O), 409(M^+-4H_2O); $C_{29}H_{50}O_5$ calc. C, 72.76, H, 10.53; found C, 72.80, H, 10.50

Homobrassinolide (2):

Compound **26** (10 mg) dissolved in pyridine (1 ml) was treated with Ac_2O (1 ml) and DMAP (2 mg) at room temperature overnight. The solvent was removed under reduced pressure. The residue was dissolved in CH_2Cl_2 (2 ml) and the mixture was treated with CF_3CO_3H (60%, 1 ml) at 0°C for 4 h. Then the solvent was removed to dryness under reduced pressure. The residue was refluxed with 4%KOH/MeOH (4 ml) for 1 h. After concentration in vacuum, the residue in THF (1 ml) was acidified with 6N HCl (1 ml) overnight. Then the solvent was removed. The crude product was purified by preparative thin layer chromotography to afford **2** (8.6 mg) in yield of 83%; mp. 269-270°C

(lit.[2] mp. 268-271°C); νmax: 3450 (OH), 1725 (C=O) cm^{-1}. $\delta_H(C_5D_5N\text{-}CDCl_3)$: 0.67 (3H, s, 18-H), 0.90 (3H, s, 19-H), 1.01, 1.04 (6H, 2d, J=6.8Hz, 26 and 27-H), 1.12 (3H, s, 21-H), 3.90-4.10 (4H, m, 2, 3 ,22 and 23-H), 4.12, 4.44 (2H, 2m, 7-H), m/z: 495 (M^++1), 477 (M^+-H_2O), 449 (M^+-H_2O-CO); $C_{29}H_{52}O_6$ calc. C,70.41, H, 10.19, found C, 70.32, H, 10.08.

(22R,23R,24S)-3α,6α-Diacetoxy-22-hydroxy-23-tetrahydropyranyloxy-24-carboxymethyl-5β-cholest-22(29)-lactone (27):

To solution of **14** (200 mg) in CH_2Cl_2 (2 ml) was treated with dihydropyran (0.4 ml) and PPTS (20 mg) at room temperature for 24 h. The mixture was extracted with ethyl acetate. After working up as usual, the crude product was chromatographed on silica gel to afford **27** (215 mg) in 93% yield; νmax: 1730 (CO) cm^{-1}; δ_H: 0.69 (3H, s, 18-H), 0.86 (6H, 2d, J=6Hz, 26, 27-H), 0.99 (3H, s, 19-H), 1.01 (3H, d, J=6.4Hz, 21-H), 2.02, 2.04(6H, 2s, $2CH_3CO$), 3.50, (2H, m, THP), 4.04 (1H, s, 22-H), 4.21 (2H, s, 23-H and THP), 4.72, 5.14(2H, m, 3-H, 6-H); m/z: 647 (M^++1), 563 ($M^+-C_5H_8O$).

(22R,23R)-3α,6α,22,23-Tetrahydroxy-24-methylene-5β-cholestane (28):

Compound **27** (180 mg) was treated with 4% KOH/MeOH (5 ml) at room temperature for 5 h. After the solvent was removed to dryness under reduced pressure, the residue was acetylated with Ac_2O/Py. The acetylated compound (100 mg), was dissolved in dry benzene (10ml) containing $Cu(OAc)_2$ (30 mg) and pyridine (0.6 ml) under N_2. The IDBA (50 mg) was added slowly with vigorously stirring under reflux for 8 h. The reaction mixture was washed with 10% HCl, saturated $NaHCO_3$ and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was hydrolysed with 4% KOH/MeOH (4 ml) at room temperature. After working up as usual, the crude product was purified by flash chromatography on silica gel to afford **28** (16mg) in 80% yield; mp. 241-243°C; $[\alpha]_D^{21}$ 17° (c, 0.811, $CHCl_3$); νmax: 3450 (OH), 1660 (C=C) cm^{-1}; δ_H: 0.72(3H, s, 18-H), 0.82 (3H, s, 19-H), 0.94 (3H, d J=6.8Hz, 21-H), 1.08, 1.10 (6H, 2d, J=8Hz, 26, 27-H), 3.65-4.01 (4H, m, 3, 6, 22 and 23-H), 5.02, 5.05 (2H, 2s, 28-H); m/z:

449 (M^++1), 430(M^+-H_2O); $C_{28}H_{48}O_4$ calc. C, 74.95, H, 10.78; found C, 75.05 H, 10.48.

This investigation was supported by the Science Found of the Chinese Academy of Sciences.

References

1. M. D. Grove, G. F. Spencer, W. K. Rohwedder. N. Mandava, J. F. Worley, J. D. Warthen, Jr., G. L. Steffens, J. L. Flippen-Anderson, J. C. Cook, Jr., Nature(London), 1979, **281**, 216.
2. S. Takatsuto, N. Ikekawa, Chem. Pharm. Bull. 1982, **30**, 4181.
3. T. Yokota, J. Baba, N. Takahashi, Tatrahedron Lett., 1982, **23**, 4965.
4. J. A. Schneider, K. Yoshihara, K. Nakanishi, N. Kato, Tetrahedron Lett., 1983, **24**, 3859.
5. a. S. Fung, J.B. Siddall, J. Am. Chem. Soc., 1980, **102**, 6580; b. S. Takatsuto, N. Ikekawa, J. Chem. Soc., Perkin Trans. 1, 1983, 2133; c. K. Mori, M. Sakakibara, K. Okada, Tetrahedron, 1984, **40**, 1767; d. J. R. Donaubauer, A. M. Greaves, T. C. McMorris, J. Org. Chem., 1984, **49**, 2833; e. M. Anastasia, P. Allevi, P. Ciuffreda, A. Fiecchi, A. Scalca, ibid., 1984, **49**, 4297, f. T. Kametani, T. Katoh, M. Tsubuki, T. Honda, J. Am. Chem. Soc., 1986, **108**, 7055; g. W. S. Zhou., W. S. Tian, Tetrahedron, 1987, **43**, 3705.
6. A preliminary account of this work has been published: W. S. Zhou, B. Jiang, X. F. Pan, J. Chem. Soc., Chem. Commun., 1989, 612.
7. W. S. Zhou, W. S. Tian, Acta Chemica Sinica, 1984, **42**, 1173.
8. Y. Z. Huang, L. L. Shi, S. W. Li, Synthesis, 1988, 975.
9. T. Kametani, M. Kigawa, M. Tsubuki, T. Honda, J. Chem. Soc., Perkin Trans. 1, 1988, 1503.
10. W. B. Motherwell, J. Chem. Soc., Chem. Commun., 1973, 935.