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SYNTHESIS AND BIOLOGICAL ACTIVITY OF BRASSINOLIDE ANALOGUES, 26,27-BISNORBRASSINOLIDE AND ITS 6-OXO ANALOGUE

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Key Word Index—Plant growth hormone; brassinolide; 26,27-bisnorbrassinolide; structure–activity relationship; synthesis; *Raphanus* test; the rice-lamina inclination test.

Abstract—Two hitherto unknown brassinolide analogues, (22*R*,23*R*)-2α,3α,22,23-tetrahydroxy-*B*-homo-7-oxa-24-nor-5α-cholestan-6-one (**9b**) and (22*R*,23*R*)-2α,3α,22,23-tetrahydroxy-24-nor-5α-cholestan-6-one (**8a**), were stereoselectively synthesized. In both the *Raphanus* and rice-lamina inclination tests, **9b** exhibited almost the same activity as brassinolide (**1**) and **8a** also showed *ca* 10–50% of the activity of **1**.

INTRODUCTION

Since the discovery of a new plant growth promoter named brassinolide (**1**), (22*R*,23*R*,24*S*)-2α,3α,22,23-tetrahydroxy-*B*-homo-7-oxa-5α-ergostan-6-one, from the pollen of rape (*Brassica napus*) [1], much effort has been made to evaluate its biological activities using a number of bioassay systems for auxin, gibberellin and cytokinin [2–6]. We have already reported the syntheses of **1** and many of its analogues [7–14] and their plant growth-promoting activities [15–17]. The structural requirements for the activity was also clarified by us [15–17] and by U.S.D.A. scientists [18, 19].

During the course of our investigation into the structure–activity relationship, we synthesized a hitherto unknown highly active analogue, namely, (22*R*,23*R*)-2α,3α,22,23-tetrahydroxy-*B*-homo-7-oxa-24-nor-5α-cholestan-6-one (26,27-bisnorbrassinolide) (**9b**), which was found to have almost the same activity as **1** in both the *Raphanus* and rice-lamina inclination tests. In this paper, we describe the synthesis of 26,27-bisnorbrassinolide (**9b**) and its synthetic precursor, (22*R*,23*R*)-2α,3α,22,23-tetrahydroxy-24-nor-5α-cholestan-6-one (**8a**), and their plant growth-promoting activities.

RESULTS AND DISCUSSION

Synthesis

We have already developed a new method [10, 11] for the stereoselective introduction of (22*R*,23*R*)-vicinal diol in the steroidal side chain using the chelation-controlled Grignard reaction, which was also used to synthesize 26,27-bisnorbrassinolide (**9b**). The previously described (22*R*)-23-aldehyde **3** [14] was reacted with isopropylmagnesium bromide in THF at −78° to give the mixture of the (22*R*,23*R*)-23-ol (**4a**) and its (22*R*,23*S*)-isomer in 65% yield with a ratio of *ca* 3:1. These isomers were easily separated by recrystallization or column chromatography. Acetylation of **4a**, followed by treatment with 6 M hydrochloric acid and then with 5% potassium hydroxide–methanol provided the (22*R*,23*R*)-triol **4c**, mp 219–221°, in 81% yield. The stereochemical assignment of **4c** was based on the precedents in this system [10, 11] and also on the close similarity of the coupling constant of *H*-22 ($J_{22-23} = 0$, $J_{22-23} = 8$ Hz) in the ^{1}H NMR (200 MHz) spectrum of **9b** with that of **1**.

Functionalization of the triol **4c** at rings A and B was achieved according to our procedure used for the synthesis of brassinolide (**1**) [7, 13] as follows. The triol **4c** was submitted to acetonide formation and methanesulphonation to yield the mesylate **5b**. Hydroboration of **5b** with BH_3–THF complex, followed by treatment with alkaline hydrogen peroxide and the subsequent oxidation with pyridinium chlorochromate provided the 6-oxo steroid **6**. Treatment of **6** with lithium bromide in dimethylformamide under reflux gave the 2-ene **7**, mp 176–178°, in 63.5% overall yield from **4c**. Stereospecific α-face hydroxylation of the 2-ene **7** was carried out with a catalytic amount of osmium tetroxide and *N*-methylmorpholine *N*-oxide in tert. butanol–THF–water (10:8:1). Removal of the protecting group of the resulting 2α,3α-diol with acetic acid–water under reflux and recrystallization gave the tetrahydroxy-6-oxosteroid **8a** mp 290–295°, in 82.4% yield. The tetraol **8b** was acetylated in the usual manner to give the tetraacetate **8b**, which was submitted to Baeyer–Villiger oxidation [20]. Oxidation of **8b** with trifluoroperacetic acid in dichloromethane in the presence of disodium hydrogen phosphate at 0° provided, after chromatographic purification, the desired 7-oxalactone **9a** [^{1}H NMR ($CDCl_3$): δ2.98 (1H, *dd*, *J* = 13 and 5 Hz, H-5α), 4.05 (2H, *m*, H-7)] in 81.5% yield. Saponification of **9a** and relactonization with conc. hydrochloric acid provided (22*R*,23*R*)-26,27-bisnorbrassinolide (**9b**), mp 280–281°, in 93.7% yield. The overall yield of **9b** from **3** was *ca* 16%.

Biological activity

Plant growth-promoting activity of the synthetic brassinolide analogues **9b** and **8a** was examined by the *Raphanus* [17, 21] and the rice-lamina inclination tests [6, 15, 16], which have recently been found to be useful to evaluate the activity of brassinosteroids. The bioassays were carried out according to published methods [15, 17]. The results are summarized in Table 1.

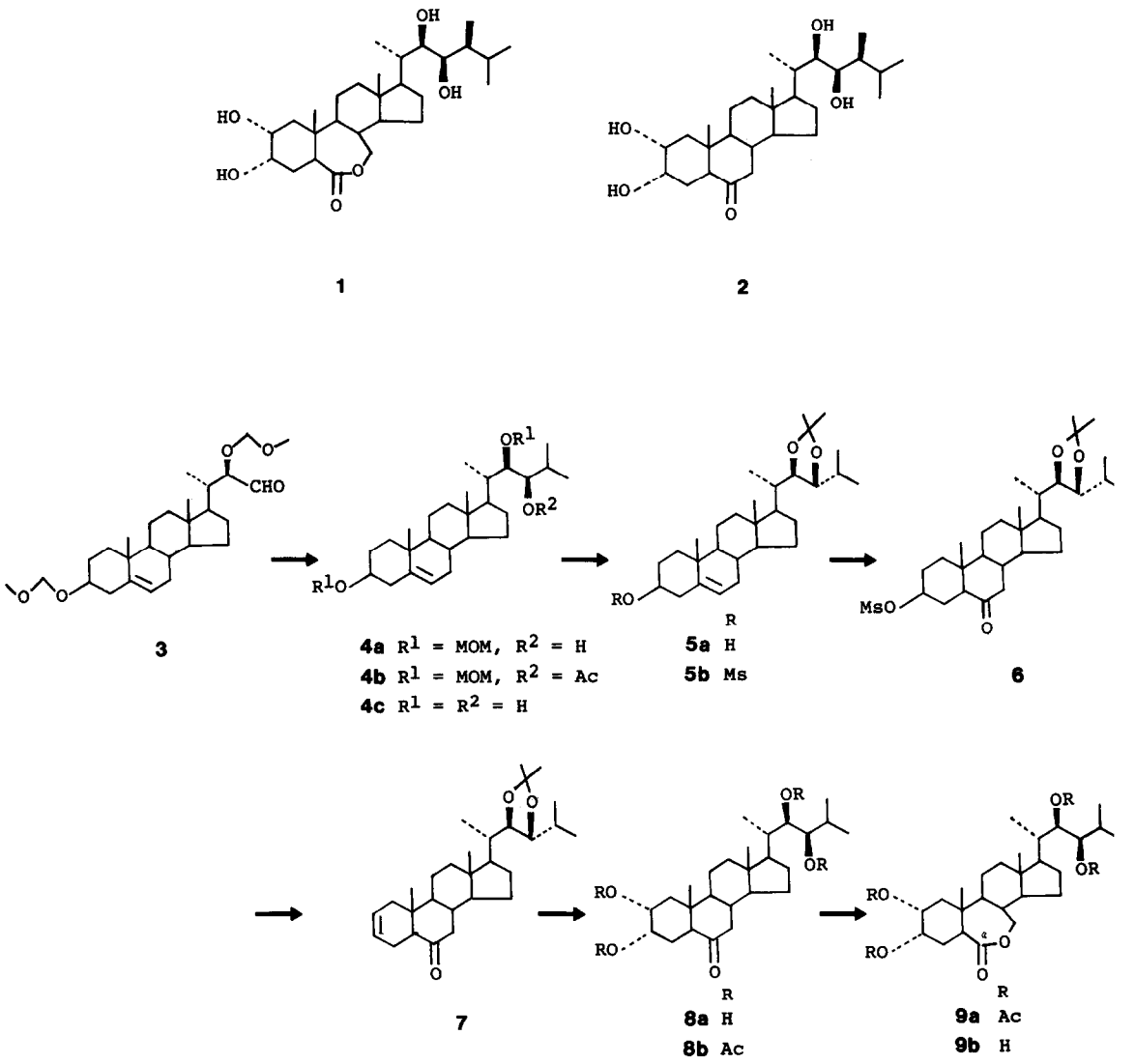

Table 1. Biological activities of brassinosteroids in the *Raphanus* and rice-lamina inclination tests

Concn (ppm)	Brassinolide (1)			Bisnor lactone (9b)			Bisnor ketone (8a)		
	Raphanus			*Raphanus*			*Raphanus*		
	H†	CP†	Lamina*	H†	CP†	Lamina	H†	CP†	Lamina
10	137‡	170‡		127‡	174‡		126‡	132‡	
1	129	140	180§	126	150	177§	125	118	180§
0.1	129	127	180	122	123	159	114	113	180
0.01	118	117	142	115	117	137	110	109	125
0.001	112	117	129	114	114	95			102
0.0001			110			103			104
control	111	110	102						

*(22*R*,23*R*,24*S*)-28-Homobrassinolide possessing the same activity as brassinolide (1) in this bioassay [15] was used as a reference compound.

†H, hypocotyl, CP, cotyledon petiole.

‡Elongation percentages (mean values).

§Angle degrees between laminae and sheaths (mean values).

In the *Raphanus* test, the lactone **9b** promoted significantly elongations of the cotyledon petiole and hypocotyl of young radish (*Raphanus sativus* cv. Tokinashi) at a range of 10–0.01 ppm concentrations, which were almost the same as those induced by **1** itself at the same range of concentrations. Morphogenetic responses (curvature) of the specific organs of young radish were also observed even at a concentration of 0.03 ppm. The 6-oxo compound **8a** showed *ca* one-tenth of the activity of **1**.

In the rice (*Oryza sativa L.* cv. Arborio J1)-lamina inclination test, 26,27-bisnorbrassinolide (**9b**) gave almost the same bending angles between laminae and sheaths at concentrations from 10 to 0.001 ppm. Judging from the concentrations giving *ca* 140° of the bending angles, the 26,27-bisnor analogue **9b** was found to be equally active as **1**. The 6-oxo analogue **8a** was slightly less active than **9b** and possessed *ca* 50% of the activity of **1** as in the case of castasterone (**2**) [15]. The results of the two bioassays indicate for the first time that the terminal methyl groups (C-26, C-27) of **1** are not important for activity.

In conclusion, we synthesized the highly active brassinolide analogues, 26,27-bisnorbrassinolide (**9b**), which was prepared by the present work from the commercially available 22,23-bisnorcholenic acid or stigmasterol in shorter steps and with better overall yield than **1**.

EXPERIMENTAL

Mps are uncorr. ^{1}H NMR spectra were recorded at 60 MHz in $CDCl_3$ soln with TMS as int. standard unless otherwise stated. Kiesel gel 60 F_{254} (Merck) was used for analytical TLC. CC was effected with Kieselgel 60 (70–230 mesh, Merck). Usual work-up refers to dilution with H_2O, extraction with an organic solvent, washing to neutrality, drying ($MgSO_4$) and removal of solvent under red. pres.

(22R,23R)-3β,22,23-*Trihydroxy*-24-*norcholest*-5-*ene* 3,22-*bismethoxymethyl ether* (**4a**). The 23-aldehyde **3** [14] (5.59 g, 12.48 mmol) in THF (60 ml) was reacted with isopropylmagnesium bromide (3 equiv) at −78° for 1 hr. Usual work-up (Et_2O for extraction) and chromatography on silica gel (100 g) eluting with C_6H_6–EtOAc (30:1) gave an epimeric mixture of 23-alcohols (4 g, 65%). Recrystallization from MeOH gave the more polar, major (22*R*,23*R*)-23-ol **4a** (819 mg), mp 123–125° (MeOH); ^{1}H NMR (60 MHz): δ0.68 (3H, *s*, H-18), 1.00 (3H, *s*, H-19), 3.35 (3H, *s*, OMe), 3.41 (3H, *s*, OMe), 4.67 (4H, *s*, –O–CH_2–O–), 5.33 (1H, *m*, H-6). (Found: C, 73.10; H, 10.69. Calc. for $C_{30}H_{52}O_5$: C, 73.12; H, 10.64%.) The mother liquor was concd to dryness to give the mixture of 23-alcohols (3.18 g).

(22R,23R)-3β,22,23-*Trihydroxy*-24-*norcholest*-5-*ene* (**4c**). The purified (22*R*,23*R*)-23-ol **4a** (777 mg, 1.58 mmol) was acetylated with Ac_2O (2 ml) and pyridine (5 ml) at room temp. for 16 hr. Usual work-up (EtOAc for extraction) gave the acetate **4b** (840 mg); ^{1}H NMR (60 MHz): δ0.69 (3H, *s*, H-18), 1.01 (3H, *s*, H-19), 2.05 (3H, *s*, Ac), 3.35 (6H, *s*, 2 × OMe), 3.52 (1H, *d*, *J* = 9 Hz, H-22), 4.65 (4H, *m*, –O–CH_2–O–), 5.00 (1H, *m*, H-23), 5.32 (1H, *m*, H-6). This was dissolved in MeOH–THF (1:1, 12 ml) and 6 M HCl (1 ml) was added. The mixture was refluxed for 1 hr. To the reaction mixture 5% KOH–MeOH (20 ml) was added. The mixture was further refluxed for 1 hr. Usual work-up (EtOAc for extraction) gave the (22*R*,23*R*)-triol **4c** (520 mg, 81%), mp 219–221° (MeOH–EtOAc), R_f = 0.11 (C_6H_6–EtOAc, 3:1). (Found: C, 77.18; H, 10.94. Calc. for $C_{26}H_{44}O_3$: C, 77.17; H, 10.96%.) The epimeric mixture of 23-alcohols (3.18 g, 6.46 mmol) was deprotected, as described above, to give the less polar (22*R*,23*S*)-triol (550 mg, 21%), mp 247–248° ($CHCl_3$–MeOH); R_f = 0.20 (C_6H_6–EtOAc, 3:1) (Found: C, 77.43; H, 10.94. Calc. for $C_{26}H_{44}O_3$: C, 77.17; H, 10.96%), and the more polar (22*R*,23*R*)-triol **4c** (1395 mg, 54%). Thus, the total amount of **4c** was 1.91 g.

(22R,23R)-22,23-*Isopropylidenedioxy*-3β-*methanesulphonyloxy*-24-*norcholest*-5-*ene* (**5b**). The triol **4c** (1.15 g, 2.84 mmol) was treated with *p*-toluenesulphonic acid (10 mg) and Me_2CO (100 ml) at room temp. for 14 hr. Usual work-up (Et_2O for extraction) gave the acetonide **5a** (1.26 g); ^{1}H NMR (60 MHz): δ0.67 (3H, *s*, H-18), 1.01 (3H, *s*, H-19), 1.33 (3H, *s*, acetonide), 1.36 (3H, *s*, acetonide), 3.43 (1H, *dd*, *J* = 8 and 8 Hz, H-23), 3.45 (1H, *m*, H-3), 3.90 (1H, *d*, *J* = 8 Hz, H-22), 5.32 (1H, *m*, H-6). The 3β-ol **5a** (1.26 g) was treated with methanesulphonyl chloride (1 ml) and pyridine (10 ml) at room temp. for 1 hr. Usual work-up (EtOAc for extraction) gave the mesylate **5b** (1.48 g); ^{1}H NMR (60 MHz): δ0.67 (3H, *s*, H-18), 1.01 (3H, *s*, H-19), 1.33 (3H, *s*, acetonide), 1.36 (3H, *s*, acetonide), 3.00 (3H, *s*, mesyl), 3.43 (1H, *dd*, *J* = 8 and 8 Hz, H-23), 3.90 (1H, *d*, *J* = 8 Hz, H-22), 4.45 (1H, *m*, H-3), 5.32 (1H, *m*, H-6).

(22R,23R)-22,23-*Isopropylidenedioxy*-3β-*methanesulphonyloxy*-24-*nor*-5α-*cholestan*-6-*one* (**6**). The mesylate **5b** (1.48 g) in THF (10 ml) was treated with BH_3–THF complex (4.5 ml, 4.5 mmol) at room temp. for 3 hr. To the reaction mixture H_2O was carefully added to destroy excess reagent. Then, 2 N NaOH (3 ml) and 30% H_2O_2 (5 ml) were added at 0° to the soln. The mixture was stirred at room temp. for 30 min. Usual work-up (Et_2O for extraction) gave a crude product (1.53 g), which was then dissolved in CH_2Cl_2 (20 ml). To the soln pyridinium chlorochromate (2.0 g, 9.28 mmol) was added. The mixture was stirred at room temp. for 3 hr. To this Et_2O (100 ml) was added. Filtration through a column of Florisil, elution with Et_2O and removal of solvent under red. pres. gave the 6-oxo steroid **6** (1.50 g); ^{1}H NMR (60 MHz): δ0.67 (3H, *s*, H-18), 1.33 (3H, *s*, acetonide), 1.36 (3H, *s*, acetonide), 3.00 (3H, *s*, mesyl), 3.42 (1H, *dd*, *J* = 8 and 8 Hz, H-23), 3.86 (1H, *d*, *J* = 8 Hz, H-22), 4.45 (1H, *m*, H-3).

(22R,23R)-22,23-*Isopropylidenedioxy*-24-*nor*-5α-*cholest*-2-*en*-6-*one* (**7**). The mixture of the 6-oxo steroid **6** (1.5 g), LiBr (500 mg, 5.75 mmol), and DMF (10 ml) was refluxed for 1 hr. Usual work-up (EtOAc for extraction) gave a crude product (1.23 g), which was applied to a column of silica gel (80 g) Elution with C_6H_6 gave the 2-ene **7** (806 mg, 63.5% from the triol **4c**); mp 176–178° (MeOH); ^{1}H NMR (60 MHz): δ0.67 (3H, *s*, H-18), 0.70 (3H, *s*, H-19), 1.33 (3H, *s*, acetonide), 1.36 (3H, *s*, acetonide), 3.42 (1H, *dd*, *J* = 8 and 8 Hz, H-23), 3.86 (1H, *d*, *J* = 8 Hz, H-22), 5.60 (2H, *m*, H-2 and H-3). (Found: H, 78.64; H, 10.42. Calc. for $C_{29}H_{46}O_3$: C, 78.68; H, 10.47%.)

(22R,23R)-2α,3α,22,23-*Tetrahydroxy*-24-*nor*-5α-*cholestan*-6-*one* (**8a**). The 2-ene **7** (446 mg, 1.01 mmol) in *t*-BuOH–THF–H_2O (10:8:1, 19 ml) was treated with OsO_4 (20 mg) in the presence of *N*-methylmorpholine *N*-oxide (400 mg, 2.96 mmol) at room temp. for 4 hr. To the reaction mixture satd $NaHSO_3$ soln (30 ml) was added. The mixture was stirred at room temp. for 1 hr. Usual work-up (EtOAc for extraction) gave a crude product (480 mg). This was refluxed with 70% HOAc (20 ml) for 3 hr. Removal of solvent under red. pres. and recrystallization from EtOH gave the tetraol **8a** (349 mg, 82.4% from the 2-ene **7**), mp 290–295° (EtOH); ^{1}H NMR (200 MHz, C_5D_5N–$CDCl_3$, 1:1): δ0.69 (3H, *s*, H-18), 0.79 (3H, *s*, H-19), 1.00 (3H, *d*, *J* = 7 Hz, H-21), 1.08 (3H, *d*, *J* = 7 Hz, H-26), 1.11 (3H, *d*, *J* = 7 Hz, H-27), 2.33 (1H, *dd*, *J* = 13 and 5 Hz, H-7β), 2.93 (1H, *dd*, *J* = 13 and 4 Hz, H-5α), 3.59 (1H, *dd*, *J* = 8 and 3 Hz, H-23), 3.76 (1H, *d*, *J* = 8 Hz, H-22), 3.90 (1H, *m*, $W_{1/2}$ = 20 Hz, H-2β), 4.25 (1H, *m*, $W_{1/2}$ = 8 Hz, H-3β). (Found: H, 71.69; H, 10.07. Calc. for $C_{26}H_{44}O_5$: C, 71.52; H, 10.16%.)

(22R,23R)-2α,3α,22,23-*Tetraacetoxy*-24-*nor*-5α-*cholestan*-6-*one* (**8b**). The tetraol **8a** (320 mg, 0.671 mmol) was treated with

Ac_2O (7 ml) and pyridine (10 ml) in the presence of 4-dimethylaminopyridine (20 mg) at 60° for 16 hr. Usual work-up (EtOAc for extraction) gave a crude product (430 mg), which was applied to a column of silica gel (40 g). Elution with C_6H_6–EtOAc (5:1) provided the tetraacetate **8b** (402 mg, 99%), amorphous; ^{1}H NMR (60 MHz): δ0.67 (3H, *s*, H-18), 0.80 (3H, *s*, H-19), 1.97 (3H, *s*, Ac), 2.01 (6H, *s*, 2 × Ac), 2.05 (3H, *s*, Ac), 4.90 (1H, *m*, H-2), 5.09 (2H, *br s*, $W_{1/2}$ = 4 Hz, H-22 or H-23), 5.35 (1H, *m*, H-3).

(22R,23R)-2α,3α,22,23-*Tetraacetoxy*-B-*homo*-7-*oxa*-24-*nor*-5α-*cholestan*-6-*one* (**9a**). The tetraacetoxy-6-oxosteroid **8b** (251 mg, 0.415 mmol) in CH_2Cl_2 (8 ml) was treated with trifluoroperacetic acid (10 equiv) in the presence of Na_2HPO_4 (800 mg) at 0° for 2.5 hr. To the reaction mixture satd $NaHSO_3$ soln (20 ml) was added to destroy excess reagent. Usual work-up (EtOAc for extraction) and chromatography on silica gel (15 g) eluting with C_6H_6–EtOAc (5:1) provided the chromatographically pure 7-oxalactone **9a** (210 mg, 81.5%), amorphous; ^{1}H NMR (60 MHz): δ0.70 (3H, *s*, H-18), 0.95 (3H, *s*, H-19), 1.98 (3H, *s*, Ac), 2.01 (6H, *s*, 2 × Ac), 2.08 (3H, *s*, Ac), 2.98 (1H, *dd*, *J* = 13 and 5 Hz, H-5α), 4.05 (2H, *m*, H-7), 4.90 (1H, *m*, H-2), 4.08 (2H, *br s*, $W_{1/2}$ = 4 Hz, H-22 and H-23), 5.32 (1H, *m*, H-3).

(22R,23R)-2α,3α,22,23-*Tetrahydroxy*-B-*homo*-7-*oxa*-24-*nor*-5α-*cholestan*-6-*one*, 26,27-*bisnorbrassinolide* (**9b**). The tetraacetoxy-7-oxalactone **9a** (146 mg, 0.235 mmol) was refluxed with 5% KOH–MeOH (6 ml) for 1 hr. After the mixture had been cooled to room temp., 6 M HCl (10 ml) was added. The mixture was stirred at room temp. for 1 hr. Usual work-up (EtOAc for extraction) and recrystallization from MeOH–EtOAc gave 26,27-bisnorbrassinolide (**9b**) (99.7 mg, 93.7%), mp 280–281° (MeOH–EtOAc); ^{1}H NMR (200 MHz, C_5D_5N–$CDCl_3$, 1:1): δ0.70 (3H, *s*, H-18), 0.97 (3H, *s*, H-19), 1.00 (3H, *d*, *J* = 7 Hz, H-21), 1.06 (3H, *d*, *J* = 7 Hz, H-26), 1.12 (3H, *d*, *J* = 7 Hz, H-26), 3.37 (1H, *dd*, *J* = 13 and 5 Hz, H-5α), 3.58 (1H, *dd*, *J* = 8 and 3 Hz, H-23), 3.75 (1H, *d*, *J* = 8 Hz, H-22), 3.87 (1H, *m*, $W_{1/2}$ = 20 Hz, H-2β), 4.08 (2H, *d*, *J* = 6 Hz, H-7), 4.24 (1H, *m*, $W_{1/2}$ = 8 Hz, H-3β). (Found: H, 68.92; H, 9.69. Calc. for $C_{26}H_{44}O_6$: H, 68.99; H, 9.80%.)

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