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SYNTHESIS OF (24*R*)-28-HOMOBRASSINOLIDE ANALOGUES AND STRUCTURE–ACTIVITY RELATIONSHIPS OF BRASSINOSTEROIDS IN THE RICE-LAMINA INCLINATION TEST

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Key Word Index—Brassinolide; plant hormones; brassinosteroids; structure–activity relationships; analogue synthesis; rice-lamina inclination test.

Abstract—In order to investigate the biological importance of the alkyl group at the C-24 position of brassinolide, four (24*R*)-28-homobrassinolide analogues were synthesized from poriferasterol. The plant growth-promoting activity of brassinosteroids with a 24-methyl, -ethyl, or -hydrogen was examined by the rice-lamina inclination test. Structure–activity relationships of brassinosteroids with respect to the stereochemistry of the side chain were also investigated in this bioassay system. The results indicate that an *S*-configuration of the alkyl group at C-24, a *cis*-configuration of the C-22, C-23 vicinal diol, and the A,B-ring functionalities of brassinolide are structural requirements for significant plant growth-promoting activity.

INTRODUCTION

Brassinolide (**1**), (22*R*,23*R*,24*S*)-2α,3α,22,23-tetrahydroxy-24-methyl-*B*-homo-7-oxa-5α-cholestan-6-one, isolated from the pollen of rape (*Brassica napus*), is a plant growth-promoting steroid having a seven-membered B-ring lactone and four successive asymmetric centers in the side chain [1]. Brassinolide promotes both cell elongation and cell division [1] and possesses a broad spectrum of biological activities compared with the known plant hormones [2, 3]. Therefore, brassinolide may find practical applications in agriculture [4]. The remarkable biological activities and novel structural features made brassinolide an attractive model for the synthesis of analogues. Following the synthesis of brassinolide [5–7], several analogues have been synthesized and their bio-

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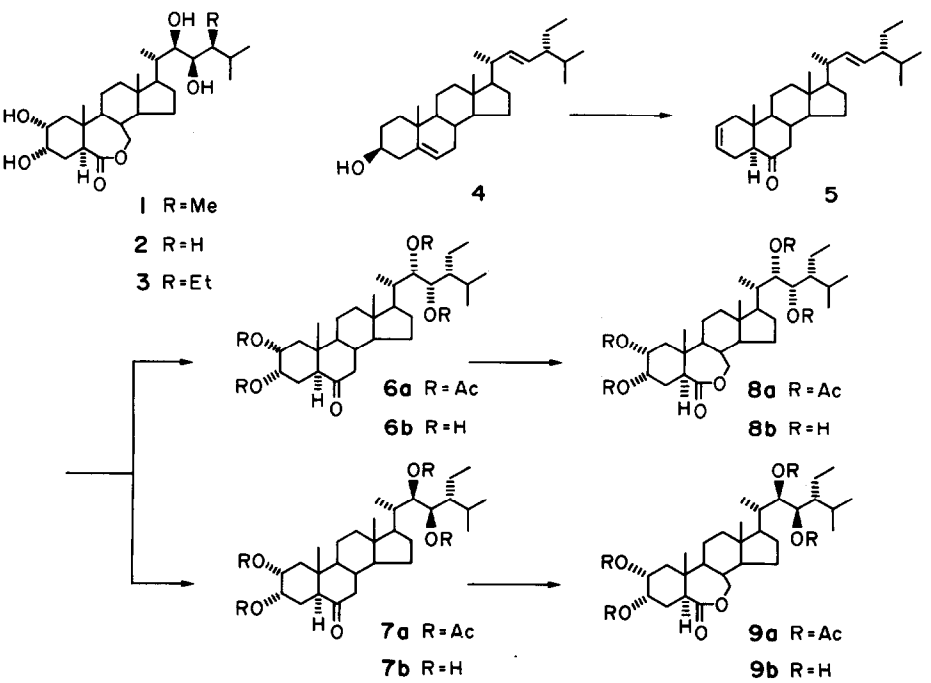


logical activities investigated in the bean internode bioassay systems [8, 9].

We have focused our attention on the biological importance of the stereochemistry of the side chain. For this study, 28-norbrassinolide (**2**), 28-homobrassinolide (**3**) and their corresponding 6-ketone analogues, previously synthesized by us [10, 11], were available. In this paper we describe the synthesis of (22*S*,23*S*,24*R*)-28-homobrassinolide (**8b**) and (22*R*,23*R*,24*R*)-28-homobrassinolide (**9b**) and of two 6-keto compounds, (22*S*,23*S*,24*R*)-brassinosteroid (**6a**) and (22*R*,23*R*,24*R*)-brassinosteroid (**7a**), from poriferasterol (**4**). We also report the structure–activity relationships of brassinosteroids with respect to the role of the side chain stereochemistry. We employed the bioassay by the rice-lamina inclination test, which has recently been found to be highly sensitive and specific for brassinolide and related compounds [12]. The plant growth-promoting activity of brassinosteroids with a 6-ketone group instead of the 7-oxalactone functionality was also examined by this bioassay.

RESULTS AND DISCUSSION

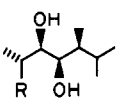
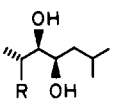
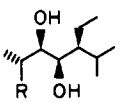
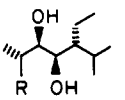
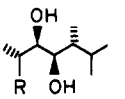
Synthesis

Poriferasterol (**4**), mp 156–157°, obtained by the method of ref. [13], was converted to (24*R*)-24-ethyl-5α-cholesta-2,22-dien-6-one (**5**), mp 105–106°, in seven steps by our method [11] in an overall 53% yield. Oxidation of the diene **5**, with a catalytic amount of osmium tetroxide in aqueous tetrahydrofuran in the presence of *N*-methylmorpholine *N*-oxide and subsequent acetylation at 60° overnight gave, after chromatographic separation, the less polar (22*S*,23*S*)-tetra-acetate (**6a**) and the more polar (22*R*,23*R*)-tetra-acetate (**7a**) in a 2:1 ratio in 89% yield. The assignments were deduced from the stereochemistry of the osmium tetroxide oxidation of the C-22 double bond. Examples are reported elsewhere [8, 10, 12]. In every case, the (22*S*,23*S*)-compounds are less polar than (22*R*,23*R*)-isomers in TLC. Saponification of the acetates **6a** and **7a** with 5% potassium hydroxide–methanol under reflux afforded (22*S*,23*S*,24*R*)-2α,3α,22,23-tetrahydroxy-24-ethyl-5α-cholestan-6-one (**6b**), mp 114–115° and (22*R*,23*R*,24*R*)-2α,3α,22,23-tetrahydroxy-24-ethyl-5α-cholestan-6-one (**7b**), mp 249–251°, respectively, in *ca* 90% yield. Baeyer–Villiger oxidation of the ketones (**6a** and **7a**) with trifluoroperacetic acid in dichloromethane in the presence of disodium hydrogen phosphate at 0°, followed by chromatographic purification, provided the (22*S*,23*S*)-7-oxalactone (**8b**) and (22*R*,23*R*)-7-oxalactone (**9b**), respectively, in *ca* 80% yield. The acetates **8a** and **9a** were saponified with 5% potassium hydroxide–methanol under reflux and, subsequently, acidified with conc. hydrochloric acid to afford (22*S*,23*S*,24*R*)-2α,3α,22,23-tetrahydroxy-24-ethyl-*B*-homo-7-oxa-5α-cholestan-6-one (**8b**), mp 182–184°, and (22*R*,23*R*,24*R*)-2α-3α,22,23-tetrahydroxy-24-ethyl-*B*-homo 7-oxa-5α-cholestan-6-one (**9b**), mp 266–267°, respectively, in *ca* 90% yield. Synthesis of the 28-norbrassinosteroids **13**–**15**, **19** and **20** will be reported in detail elsewhere.

Biological activity

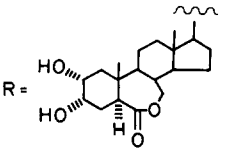
Plant growth-promoting activity of the synthetic brassinosteroids (**6b**, **7b**, **8b** and **9b**) relative to brassinolide (**1**) were investigated by the rice-lamina inclination test, originally developed as an auxin bioassay system [14] and recently found to be highly sensitive and specific for brassinolide and its analogues [12]. First, the effect of alkyl groups at C-24 of (22*R*,23*R*)-brassinosteroids on plant growth-promoting activity was examined by this bioassay (Table 1). The activity of 28-homobrassinolide (**3**) with a 24*S*-ethyl instead of 24*S*-methyl group was

Table 1. Effect of brassinolide and its 24-alkylated analogues on the lamina inclination of rice seedlings

			Angle (degrees) between laminae and sheaths (±s.e.)				
							
Concn. (μg/ml)	Control	IAA*	1	2	3	9b	10
—	93±8	—	—	—	—	—	—
50	—	128±12	—	—	—	—	—
10	—	104± 9	—	—	—	—	—
1	—	—	158±10	172±10	—	168±12	167±13
0.1	—	—	155± 9	152±18	158±10	164±17	157± 9
0.01	—	—	148± 7	137±15	162± 9	148±17	143±11
0.001	—	—	143±13	102±12	152±15	110±19	122± 5
0.0001	—	—	129±13	—	128± 9	—	—
0.00001	—	—	93± 2	—	92± 6	—	—

*IAA, indole-3-acetic acid.

Even at a concentration of 0.0001 μg/ml of brassinolide (**1**), the angle between laminae and sheaths reached *ca* 130°, while IAA, the typical plant growth-promoting hormone, was only slightly active at a concentration of 10 μg/ml. This means that the activity of brassinolide (**1**) is *ca* 10^5 times higher than that of IAA. Other analogues are also very active compared with IAA.

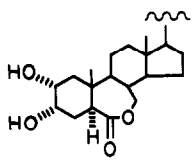
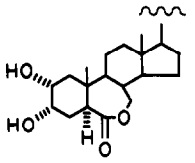
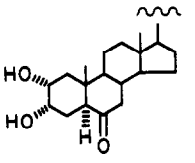
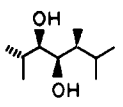
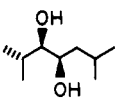
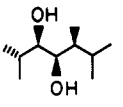
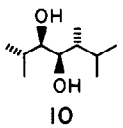
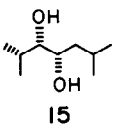
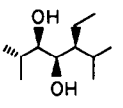
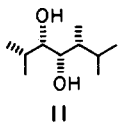
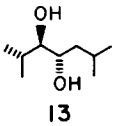
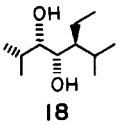
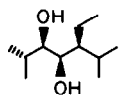
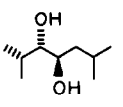
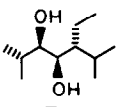
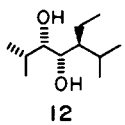
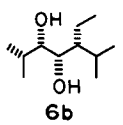
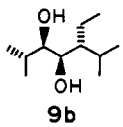
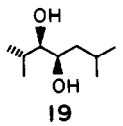
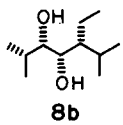
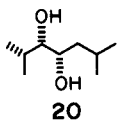


almost equal to that of brassinolide (**1**). The 24-epi-brassinolide (**10**) and its 28-homologue (**9b**) were less active than the corresponding 24*S*-isomers (**1** and **3**), respectively. The 28-norbrassinolide (**2**), lacking an alkyl group at C-24, was less active than the 24-alkylated brassinosteroids (**1**, **3**, **9b** and **10**). This result indicates that a 24-alkyl group is important for activity.

The structure–activity relationships of brassinolide analogues with respect to the side chain are summarized in Table 2. As far as the configurations of the hydroxy groups at C-22 and C-23 are concerned, brassinosteroids with the 22*R*,23*R*-configurations were more active than the corresponding 22*S*,23*S*-isomers, and brassinosteroids with 22*R*,23*S*- or 22*S*,23*R*-configurations were much less active. Surprisingly, the (22*S*,23*S*,24*S*)-28-homo-brassinolide (**12**) was highly active and more active than 28-nor-brassinolide (**2**), 24-epi-28-homobrassinolide (**9b**) or 24-epibrassinolide (**10**). This suggests that the configuration of an alkyl group at C-24 is more important than that of the 22,23-vicinal diol.

In the case of tetrahydroxy-6-ketones, the brassinosteroids with 22*R*-OH,23*R*-OH orientation were more active than the corresponding isomers with 22*S*-OH,23*S*-OH orientation. The synthetic precursor tetrahydroxy-6-ketone (**16**) of brassinolide was very active; it had about 50% of the activity of brassinolide (**1**). The activity of the tetrahydroxy-6-ketone (**17**) with a 24*S*-ethyl was almost equal to that of the ketone (**16**) with a 24*S*-methyl. However, the 22*S*,23*S*-isomer (**18**) of (22*R*,23*R*)-28-homo-6-oxobrassinosteroid (**17**) was much less active. The 28-nor-6-oxobrassinosteroid (**19**) was as active as the corresponding 28-nor-brassinolide (**2**), but the activity of brassinosteroids with the 7-oxalactone functionality was generally higher than that of the corresponding 6-ketone analogues. This suggests that the (22*R*,23*R*)-tetrahydroxy-6-ketones (**16**, **17** and **19**) are either active

Table 2. Relative activity of brassinolide and its analogues bioassayed by the rice-lamina inclination test

Compounds	Rel. act.*	Compounds	Rel. act.*	Compounds	Rel. act.*
					
 **1**	100	 **2**	5	 **16**	50
 **10**	10	 **15**	1	 **17**	50
 **11**	5–10	 **13**	0.1	 **18**	0.5
 **3**	100	 **14**	0.05	 **7b**	1
 **12**	50	—	—	 **6b**	0.5
 **9b**	10	—	—	 **19**	5
 **8b**	5	—	—	 **20**	0.1

*Activity of the analogues expressed as a percentage of the activity of brassinolide (**1**).

themselves or converted to the corresponding 7-oxalactones (**1**, **3** and **2**) by the rice plant segments if the 7-oxalactones are the active forms. These interesting questions remain to be answered.

We conclude that the following structural requirements are important for the plant growth-promoting activity comparable to that of brassinolide (**1**): (1) *S*-configuration of an alkyl group (methyl or ethyl) at C-24; (2) *cis*-configuration of the C-22,C-23 vicinal diol; and (3) the A,B-ring functionalities of brassinolide. Thompson *et al.* [8] have recently reported the similar structural requirements, obtained by the bean internode bioassays. However, the relative activities of synthetic brassinosteroids were different in the bioassay systems used, particularly in comparing the bioassay with plant segments and the bioassay with intact plants.

Using our newly established microanalytical technique [15], the new brassinolide analogues, 28-nor-brassinolide (**2**) and its 6-oxo analogue (**19**), the 6-oxo analogues (**16** and **17**) of brassinolide (**1**) and 28-homobrassinolide (**3**), have recently been identified in higher plants [16].

EXPERIMENTAL

Mp were determined with a hot-stage microscope and are uncorr. ^{1}H NMR spectra were taken with $CDCl_3$ as solvent and tetramethylsilane as int. reference.

CC was effected with Merck Kieselgel 60 (Si gel, 70–230 mesh). Organic extracts were dried over $MgSO_4$. The "usual work-up" refers to dilution with H_2O, extraction with an organic solvent, washing to neutrality, drying, filtration and evaporation under vacuum.

(24R)-24-*Ethyl*-5α-*cholest*-2,22-*dien*-6-*one* (**5**). Poriferasterol (**4**), mp 156–157°, (lit. [13] mp 154–156°), (850 mg, 2.07 mmol) was transformed into the diene **5** (445 mg, 53%), mp 105–107° (from MeOH), by our method [12]. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1710, ^{1}H NMR ($CDCl_3$): δ 0.70 (6H, *s*, H-18 and H-19), 5.06 (2H, *m*, H-22 and H-23), 5.55 (2H, *m*, H-2 and H-3). (Found: C, 84.71; H, 11.35. $C_{29}H_{46}O$ requires: C, 84.81; H, 11.29%.)

(22S,23S,24S)-2α, 3α,22,23-*Tetrahydroxy*-24-*ethyl*-5α-*cholestan*-6-*one* (**6b**) *and* (22R,23R,24R)-2α,3α,22,23-*tetrahydroxy*-24-*ethyl*-5α-*cholestan*-6-*one* (**7b**). The diene, **5** (440 mg, 1.08 mmol), was treated with OsO_4 (20 mg) and *N*-methylmorpholine *N*-oxide (1.0 g, 7.41 mmol) in tetrahydrofuran (10 ml) and H_2O (0.3 ml) at room temp. for 2 days. To this reaction mixture satd $NaHSO_4$ soln was added and stirring was continued for 1 hr. The usual work-up (EtOAc for extraction) gave a crude product (550 mg). This crude product was acetylated with Ac_2O (2 ml) and pyridine (4 ml) at 60° overnight. The usual work-up (EtOAc for extraction) and chromatography on Si gel (50 g), eluting with C_6H_6–EtOAc (20:1), afforded the less polar (22*S*,23*S*)-tetra-acetate (**6a**) (410 mg, 58%), oil, ^{1}H NMR ($CDCl_3$): δ 0.62 (3H, *s*, H-18), 0.76 (3H, *s*, H-19), 1.93 (3H, *s*, acetyl), 1.99 (3H, *s*, acetyl), 2.03 (6H, *s*, two acetyls), 4.65–5.40 (4H, *m*, H-2, H-3, H-22 and H-23), and the more polar (22*R*,23*R*)-tetra-acetate (**7a**) (220 mg, 31%), oil, ^{1}H NMR ($CDCl_3$): δ 0.65 (3H, *s*, H-18), 0.78 (3H, *s*, H-19), 1.94 (6H, *s*, two acetyls), 1.98 (3H, *s*, acetyl), 2.03 (3H, *s*, acetyl), 4.65–5.40 (4H, *m*, H-2, H-3, H-22 and H-23). The acetate **6a** (200 mg, 0.31 mmol) was treated with 5% KOH–MeOH (10 ml) under reflux for 1 hr. The usual work-up (EtOAc for extraction) yielded the (22*S*,23*S*)-tetraol (**6b**) (135 mg, 92%), mp 114–115° (from MeOH–H_2O), IR ν_{max}^{KBr} cm^{-1}: 3400, 1711, FDMS (*m/z*): 479 [M + 1]$^+$, 461 [M + 1–18]$^+$, 393 [M − 85, C_{23}–C_{24} fission]$^+$, 363 [M − 115, C_{22}–C_{23} fission]$^+$, 333 [M − 145, C_{20}–C_{22} fission]$^+$, 145, 115. The acetate **7a** (100 mg, 0.155 mmol) was treated as described above to afford the (22*R*,23*R*)-tetraol (**7b**) (70 mg, 94%), mp 249–251° (from EtOAc). IR ν_{max}^{KBr} cm^{-1}: 3420, 1710, FDMS (*m/z*): 479 [M + 1]$^+$, 461, 393, 363, 333, 145, 115.

(22R,23R,24R)-2α,3α,22, 23-*Tetrahydroxy*-24-*ethyl*-B-*homo*-7-*oxa*-5α-*cholestan*-6-*one* (**9b**). The 6-oxotetra-acetate **7a** (120 mg, 0.186 mmol) in CH_2Cl_2 (5 ml) was treated with trifluoroperacetic acid (seven equivalents), prepared from 90% H_2O_2 and trifluoroacetic anhydride in dichloromethane, at 0° for 2 hr. To this reaction mixture satd $NaHSO_4$ soln was added. The usual work-up (EtOAc for extraction) and chromatography on Si gel (20 g), eluting with C_6H_6–EtOAc (20:1), afforded the chromatographically pure 7-oxalactone **9a** (102 mg, 83%), oil, ^{1}H NMR ($CDCl_3$): δ 0.69 (3H, *s*, H-18), 0.95 (3H, *s*, H-19), 1.97 (9H, *s*, three acetyls), 2.05 (3H, *s*, acetyl), 2.98 (1H, *dd*, *J* = 6 and 13 Hz, H-5), 4.09 (2H, *m*, H-7), 4.80 (1H, *m*, H-2), 5.00–5.40 (3H, *m*, H-3, H-22 and H-23). The acetate (**9a**) (100 mg, 0.154 mmol) was treated with 5% KOH–MeOH (7 ml) under reflux for 1 hr. After cooling to room temp., 6 M HCl (10 ml) was added and stirring was continued for 1 hr. The usual work-up (EtOAc for extraction) afforded the (22*R*,23*R*)-tetraol **9b** (69 mg, 91%), mp 266–267° (from EtOAc), IR ν_{max}^{KBr} cm^{-1}: 3452, 1702, FDMS (*m/z*): 495 [M + 1]$^+$, 477 [M + 1 − 18]$^+$, 379 [M − 115, C_{22}–C_{23} fission]$^+$, 349 [M − 145, C_{20}–C_{22} fission]$^+$, 145, 115.

(22S,23S,24R)-2α,3α,22, 23-*Tetrahydroxy*-24-*ethyl*-B-*homo*-7-*oxa*-5α-*cholestan*-6-*one* (**8b**). The 6-oxotetra-acetate (**8a**) (210 mg, 0.325 mmol) in CH_2Cl_2 (10 ml) was treated with trifluoroperacetic acid (seven equivalents) as described for **9a** to yield, after chromatographic purification, the 7-oxalactone **8a** (175 mg, 81%), oil, ^{1}H NMR ($CDCl_3$): δ 0.67 (3H, *s*, H-18), 0.94 (3H, *s*, H-19), 1.93 (3H, *s*, acetyl), 1.98 (3H, *s*, acetyl), 2.02 (3H, *s*, acetyl), 2.04 (3H, *s*, acetyl), 2.98 (1H, *dd*, *J* = 6 and 13 Hz, H-5), 4.10 (2H, *m*, H-7), 4.55–5.45 (4H, *m*, H-2, H-3, H-22 and H-23). Saponification of the acetate **8a** (175 mg, 0.264 mmol), followed by acidification as described for **9b**, provided the (22*S*,23*S*)-tetraol **8b** (118 mg, 90%), mp 182–184° (from MeOH–H_2O), IR ν_{max}^{KBr} cm^{-1}: 3437, 1701; FDMS (*m/z*): 495 [M + 1]$^+$, 477, 379, 349, 145, 115.

Brassinolide and its analogues. Brassinolide (**1**), (22*R*,23*R*)-28-norbrassinolide (**2**), (22*R*,23*R*)-28-homobrassinolide (**3**) and their corresponding 6-keto analogues were synthesized by our methods [6, 11, 12]. (22*S*,23*S*,24*R*)2α,3α,22,23-Tetrahydroxy-24-methyl-*B*-homo-7-oxa-5α-cholestan-6-one (**11**) and (22*R*,23*R*,24*R*)-2α,3α,22,23-tetrahydroxy-24-methyl-*B*-homo-7-oxa-5α-cholestan-6-one (**10**) were synthesized by the method of Thompson *et al.* [9]. Other brassinolide analogues were synthesized as will be described elsewhere.

Rice-lamina inclination test of brassinolide and its analogues. Etiolated seedlings of rice (*Oryza sativa* L. cv Arborio J1) were cultivated in H_2O at 28° in the dark for 6 days. The leaf segments, each consisting of the second lamina (length 1 cm), a lamina joint and a sheath (length 1 cm), were excised from the seedlings. After the leaf segments were floated in H_2O at 28° for 24 hr in the dark, they were transferred to 1 ml 2.5 mM potassium malate soln containing a known amount of the test sample. After incubation at 28° for 48 hr in the dark, the magnitudes of the induced angles between laminae and sheaths were measured.

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