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SYNTHESIS AND BIOLOGICAL ACTIVITY OF 28-HOMOBRASSINOLIDE AND ANALOGUES

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Key Word Index—Brassinosteroids; 28-homobrassinolide; stigmasterol; soybean epicotyl elongation; side chain conformation.

Abstract—28-Homobrassinolide has been synthesized from stigmasterol in an overall yield of 21%. The biological activity of 28-homobrassinolide, brassinolide, 24-epibrassinolide, 22*S*,23*S*,24-epibrassinolide, and 22*S*,23*S*,28-homobrassinolide have been compared in the soybean epicotyl elongation assay. All the steroids except 22*S*,23*S*,28-homobrassinolide exhibited a similar biological activity, but the latter compound was substantially less active. There appears to be a correlation between biological activity and overall dimensions of the steroidal side chain.

INTRODUCTION

Brassinosteroids, a group of polyhydroxy steroidal lactones, are now well-established as a class of plant growth regulators [1]. Brassinolide (**1**), the prototype compound first isolated from rape pollen (*Brassica napus* L), elicits a broad spectrum of responses in plants including enhanced cell elongation, cell division and differentiation [2]. 28-Homobrassinolide (**2**) has been detected in seeds and sheaths of *Brassica campestris* var. pekinensis [3]. It is reported to be as active as brassinolide in the rice lamina inclination bioassay but less active in certain other assays, e.g. the bean first internode assay [4].

Synthesis of 28-homobrassinolide has been reported [5]. The starting material was the readily available stigmasterol, which by well-known reactions, was converted to the target compound, but in low overall yield. We have improved the synthesis by employing the method of asymmetric hydroxylation reported recently [6] to prepare the 22*R*,23*R*-diol structure. Thus, stigmasterol can be transformed in six steps to homobrassinolide in an overall yield of 21%.

With this steroid in hand together with its 22*S*,23*S*-isomer (**3**), brassinolide, 24-epibrassinolide (**4**) and its 22*S*,23*S*-isomer (**5**) from our previously reported syntheses [7, 8], we have now made a comparative study of their biological activity in the soybean epicotyl elongation assay.

RESULTS AND DISCUSSION

Stigmasterol was converted to the mesylate and the product transformed to (22*E*,24*S*)-24-ethyl-3α,5-cyclo-5α-cholest-22-en-6-ol. Oxidation of the latter with Jones reagent gave the corresponding ketone which was heated with pyridinium chloride and lithium bromide in dimethylacetamide at 160° to furnish the 2,22-diene (**6**) in 80% yield.

A mixture of this diene, $K_3Fe(CN)_6$, K_2CO_3, methanesulphonamide, the chiral ligand dihydroquinidine 4-chlorobenzoate, and OsO_4 in *t*-butanol–H_2O (1:1), was stirred at room temperature for six days. Workup and chromatography yielded 2α,3α,22*S*,23*S*-tetrahydroxy-24*S*-ethyl-5α-cholestan-6-one (19%) and the desired 22*R*,23*R* isomer, 28-homocastasterone (**7**, 27.5%) as well as the 2α,3α-dihydroxysterol (45%). Use of the newly reported phthalazine ligand $(DHQD)_2$-PHAL gave a slightly improved ratio (33.5:15.7) of the 22*R*,23*R* to 22*S*,23*S*-isomers [6].

Recovered 2α,3α-dihydroxysterol was subjected to further hydroxylation to yield tetrahydroxy products thereby increasing the overall yield of **7** to 53%. It should be noted that previously reported hydroxylation with OsO_4 and N-methyl morpholine N-oxide of an intermediate with the stigmasterol side chain, yielded the 22*S*,23*S* and 22*R*,23*R*-isomers in a ratio of 96:4 [5]. Direct conversion of the isomer **7** to 28-homobrassinolide (**2**) was accomplished in 80% yield with trifluoroperoxyacetic acid. The 22*S*,23*S*-isomer of **2** was obtained in the same way. Essentially the same method has been employed by two groups in recently reported syntheses of 28-homobrassinolide [9, 10].

Many bioassays have been reported for brassinolide and its analogues. These bioassays include bean second internode, maize mesocotyl, pea, Adzuki bean and mung bean epicotyl, wheat coleoptile and rice lamina inclination [2, 11]. Several of the assays were first developed for auxins and it has been suggested that the effects of brassinolide are mediated through auxin or that brassinolide increases tissue sensitivity to endogenous auxins [2].

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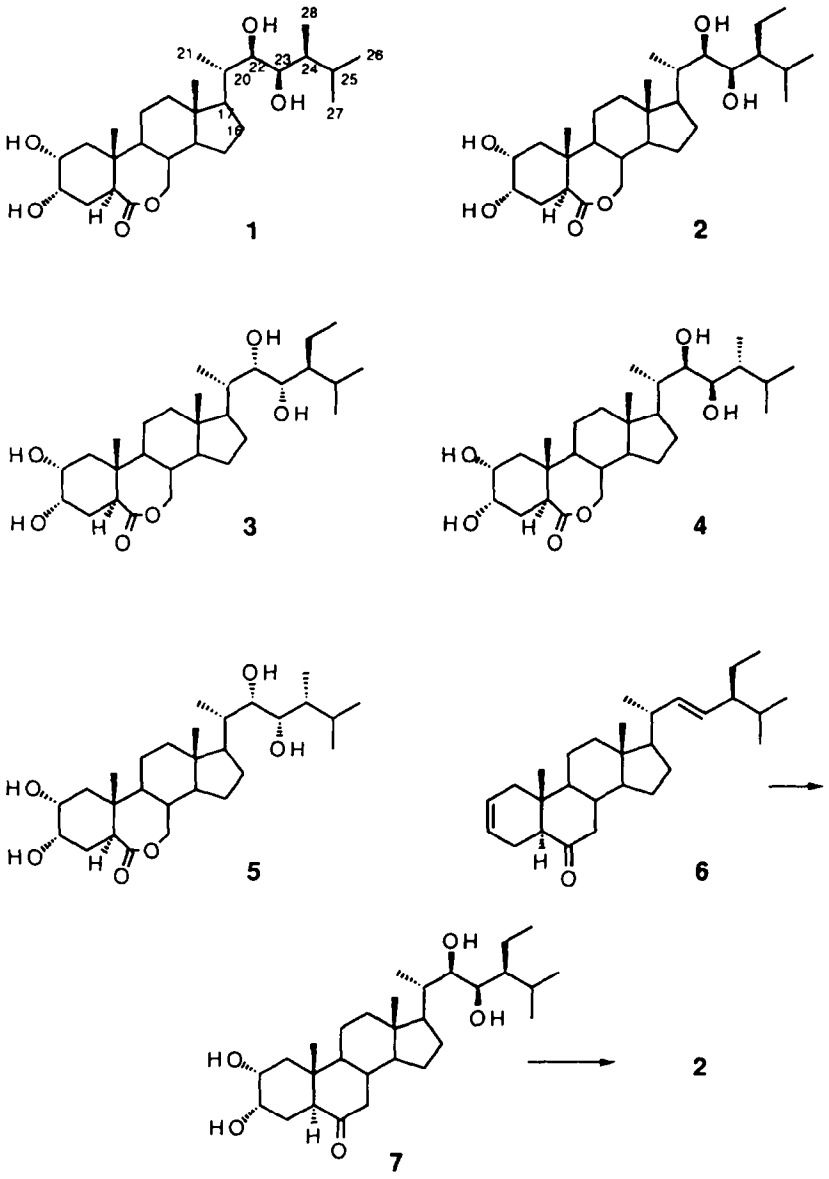

However, we have recently obtained molecular and genetic evidence of a possible independent mechanism for auxin and brassinolide action [12, 13]. We also showed that nanomolar concentrations of brassinolide altered the abundance of specific mRNA transcripts in elongating soybean epicotyls and *Arabidopsis thaliana* stems [12, 13]. This suggests that brassinolide, like other plant and animal hormones, acts in part by regulating gene expression.

Furthermore, there may exist in plants protein receptors for brassinosteroids similar to those found in vertebrates. This would be consistent with dose–response effects of brassinosteroids and with reported stringent structural requirements for high biological activity. They are: (22*R*,23*R*)-vicinal diol moiety, (24*S*)-methyl or ethyl group, 7-oxalactone or 6-oxo functionality in the B-ring, 3α-hydroxyl group, 2α,3α-vicinal diol or 3α,4α-vicinal diol and A/B-*trans*-fused ring junction [14].

Our objective in examining the biological activity of these steroids is to further refine structural requirements for high biological activity. Brassinolide (BR, **1**) and 28-homobrassinolide (HOMO BR, **2**) were first tested and were found to elicit similar activity over a range of concentrations as illustrated in Fig. 1, although BR was more effective than HOMO BR at lower concentrations. Four brassinosteroid analogues were then compared. Elongation assays were again carried out over a range of concentrations. Three of the steroids, **2**, **4** and **5**, showed similar biological activity. However, isomer **3** was substantially less active at all concentrations tested (Fig. 2).

This result suggests that if biological activity is mediated by a steroid–receptor complex, the first three steroids, as well as brassinolide, bind to the receptor with approximately the same affinity, whereas isomer **3** shows relatively weak binding.

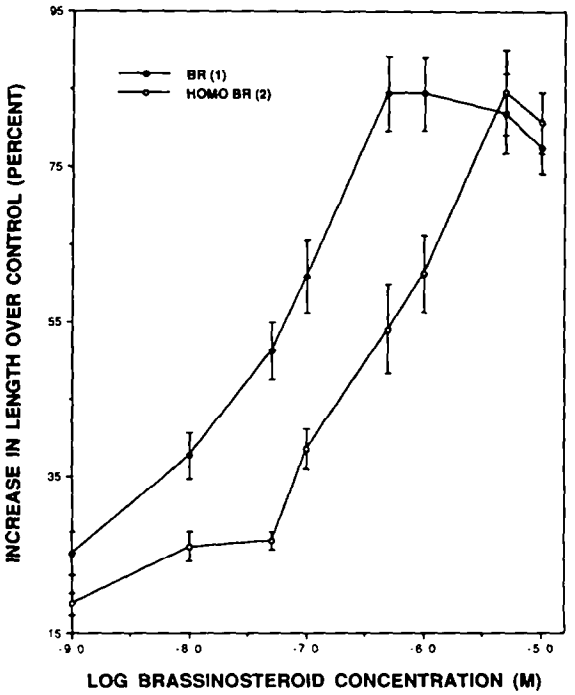

Fig. 1. Effect of brassinolide (BR) and homobrassinolide (HOMO BR) on soybean epicotyl elongation. Twenty replicate epicotyl sections (1.5 cm) were auxin-depleted in KPSC buffer for 2 hr followed by incubation in the indicated compound for 19 hr. Error bars are ±s.e.

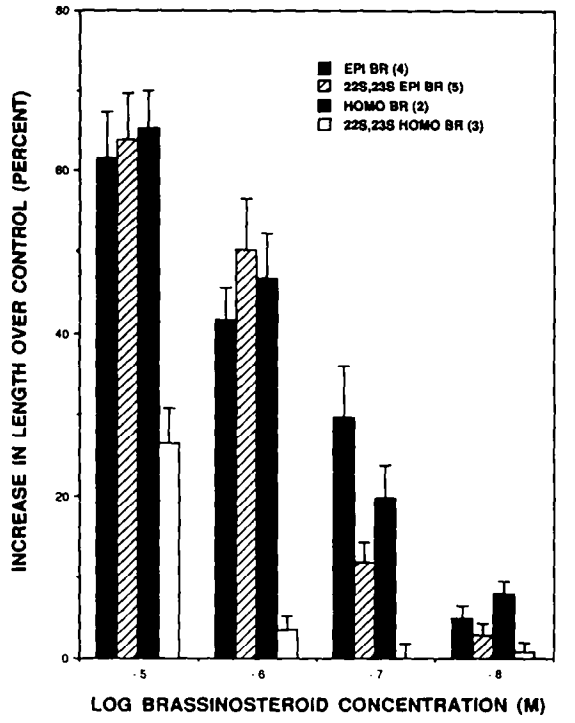

Fig. 2. Comparison of the effect of four brassinosteroids on soybean epicotyl elongation. Twenty replicate epicotyl sections (1.5 cm) for each compound were treated as described in Fig. 1 at the indicated concentrations. Error bars are ± s.e.

We have therefore been prompted to examine the molecular structures of the five steroids by comparing energy minimized conformations for differences in non-bonded distances between various atoms. PC Model molecular modelling software for the Silicon Graphics 4D series of workstations (Serena Software, Box 3076, Bloomington, IN 47402-3076) was used. A reference point, C-16, was selected on the rigid tetracyclic skeleton, and distances between C-16 and various atoms on the flexible side chains were determined. The results are shown in Table 1. It is seen that, apart from the C-16/C-22 and C-16/C-28 distances, all computed distance for isomer **3** differs substantially more from the corresponding distance for **1** than is the case with the other isomers. These differences may be reflected in the poorer fit of **3** into the active site of a receptor, compared to **1**, and hence to lower biological activity in the soybean elongation assay. In conclusion, we have found a correlation between biological activity and conformation as indicated by side chain dimensions of brassinosteroids.

EXPERIMENTAL

^{1}H NMR spectra were obtained at 300 MHz. Spectra were taken as solns in $CDCl_3$ with Me_4Si as int. standard. MPs were determined with a Kofler hot stage apparatus. CC was carried out with silica gel (Davisil 100–200 mesh and 230–425 mesh, Fisher Scientific). Analytical TLC was carried out on Whatman 4410 222 silica gel plates. Reactions were routinely monitored by TLC.

Stigmasta-2,22-dien-6-one (**6**). Mesylation of stigmasterol, followed by solvolysis with $KHCO_3$ in aq. Me_2CO under reflux gave (22*E*,24*S*)-24-ethyl-3α,5-cyclo-5α-cholest-22-en-6-ol [15], which was oxidized with Jones reagent to afford (22*E*, 24*S*)-24-ethyl-3α,5-cholest-22-en-6-one [16] in 60–65% overall yield. A mixture of the ketone (15.6 g, 38 mmol), pyridinium chloride (0.88 g, 7.6 mmol, 0.2 equiv), dry LiBr (1.65 g, 19 mmol, 0.5 equiv) and *N*,*N*-dimethylacetamide (150 ml) was heated at 160° in an argon atmosphere for 3 hr. The reaction mixture was allowed to come to room temp. and then poured on to crushed ice. The ppt was collected by filtration, washed well with H_2O, and dried under suction. Recrystallization from MeOH afforded **6** (12 g, 80%), mp 119–120°; (lit. 119–120° [17]); ^{1}H NMR ($CDCl_3$): δ0.69 (3H, *s*, H-18), 0.71 (3H, *s*, H-19), 0.77–0.86 (9H, *m*, 3 × Me), 1.02 (3H, *d*, J = 6.6 Hz, H-20), 0.9–1.82 (*m*), 1.98–2.07 (*m*), 2.2–2.38 (*m*), 5.02 (1H, *dd*, J = 15.3 and 8.4 Hz), 5.15 (1H, *dd*, J = 15.3 and 8.4 Hz), 5.53–5.61 (1H, *m*), 5.64–5.73 (1H, *m*).

28-*Homocastasterone* (**7**). A mixture of diene **6** (0.623 g, 1.52 mmol), $K_3Fe(CN)_6$ (3 g, 9.1 mmol, 6 equiv), K_2CO_3 (1.26 g, 9.1 mmol, 6 equiv), methanesulphonamide (0.29 g, 3.04 mmol, 2 equiv), dihydroquinidine 4-chlorobenzoate (0.14 g, 0.304 mmol, 0.2 equiv) and OsO_4 (0.015 g, 0.06 mmol, 0.04 equiv) in *t*-BuOH–H_2O (1:1, 30 ml) was stirred at room temp for 6 days (without methanesulphonamide, reaction was only 10% complete after 6 days). Solid Na_2SO_3 (1.2 g) was added and the mixture was stirred at room temp for 18 hr. *t*-BuOH was removed under red. pres. and the residue was extracted with EtOAc (× 6). Combined organic extracts were washed

Table 1. Interatomic distances between various atoms in brassinosteroids

	BR(1)	EPI BR(4)	Isomer 5	HOMOBR(2)	Isomer 3
C-16/C-22	3.268	3.330	3.376	3.280	3.293
C-16/C-23	3.254	3.241	3.253	3.254	3.465
C-16/C-25	5.181	5.268	5.272	5.060	5.818
C-16/C-28	5.781	5.591	5.796	5.756	5.809
C-16/O-22	4.569	4.436	4.247	4.581	3.701
C-16/O-23	3.333	3.109	3.220	3.404	3.030

with H_2O, 0.25 M H_2SO_4 (×3) to recover the ligand, then with brine, and dried and concentrated. The crude product was purified by flash chromatography on silica gel. Elution with $CHCl_3$–EtOH (9:1) provided the less polar 2α,3α-dihydroxy-24*S*-ethyl-5α-cholest-22*E*-en-6-one (0.3 g, 45%), mp 234–236° (dec.) (EtOH), (lit. 235–238° [16]), ^{1}H NMR ($CDCl_3$): δ0.67 (3H, *s*, H-18), 0.75 (3H, *s*, H-19), 0.76–0.86 (9H, *m*, 3 × Me), 1.02 (3H, *d*, *J* = 6.6 Hz, H-20), 1.05–2.1 (*m*), 2.29 (1H, *dd*, *J* = 13.0 and 4.3 Hz), 2.68 (1H, *dd*, *J* = 12.5 and 3.0 Hz, H-5), 3.71–3.82 (1H, *m*), 4.05 (1H, *brs*), 5.02 (1H, *dd*, *J* = 15.3 and 8.4 Hz), 5.14 (1H, *dd*, *J* = 15.3 and 8.4 Hz).

Further elution with the same solvent gave the most polar 2α,3α,22*S*,23*S*-tetrahydroxy-24*S*-ethyl-5α-cholestan-6-one (0.137 g, 19.0%), mp 204–207° (EtOAc), (lit. mp 200–204° [5]); ^{1}H NMR ($CDCl_3$): δ0.7 (3H, *s*, H-18), 0.76 (3H, *s*, H-19), 0.88 (3H, *d*, *J* = 6.9 Hz), 0.92–1.0 (6H, *m*, 2 × Me), 1.05–2.15 (*m*), 2.22 (1H, *d*, *J* = 6.3 Hz), 2.3 (1H, *dd*, *J* = 13.0 and 4.3 Hz), 2.68 (1H, *dd*, *J* = 12.5 and 3.0 Hz, H-5), 3.55–3.65 (2H, *m*, H-22 and H-23), 3.72–3.83 (1H, *m*), 4.05 (1H, *br s*).

Further elution with the same solvent gave the most polar 2α,3α,22*R*,23*R*-tetrahydroxy-24*S*-ethyl-5α-cholestan-6-one (**7**) (0.2 g, 27.5%), mp 251–253° (EtOAc) (lit. mp 253–255° [5]); ^{1}H NMR ($CDCl_3$): δ0.68 (3H, *s*, H-18), 0.76 (3H, *s*, H-19), 0.88–0.98 (12H, *m*, 4 × Me), 1.0–2.2 (*m*), 2.3 (1H, *dd*, *J* = 13.0 and 4.3 Hz), 2.69 (1H, *dd*, *J* = 12.5 and 3.0 Hz, H-5), 3.59 (1H, *d*, *J* = 8.7 Hz), 3.66–3.82 (2H, *m*), 4.05 (1H, *br s*).

28-*Homobrassinolide* (**2**). A soln of homocastasterone (**7**) (0.175 g, 0.37 mmol) in $CHCl_3$ (10 ml) was added dropwise to a stirred soln of CF_3CO_3H (3.7 mmol, 10 equiv) [prepared from 30% aq. H_2O_2 (0.42 ml, 3.7 mmol) and $(CF_3CO)_2O$ (2.61 ml, 18.5 mmol, 5 equiv) in $CHCl_3$ (5 ml) at 0°]. The reaction mixture was stirred at room temp. for 2 hr, diluted with $CHCl_3$ (10 ml), and the resulting soln was washed with H_2O, aq. Na_2CO_3 (×2), aq. $NaHSO_3$ (×2), brine, and dried. Evaporation of the solvent gave a colourless solid which was recrystallized from EtOAc to give 2α,3α,22*R*,23*R*-tetrahydroxy-24*S*-ethyl-B-homo-7-oxa-5α-cholestan-6-one (**2**) (0.146 g, 80%), mp 268–270° (dec.) (EtOAc) (lit. mp 268–271° [5]), ^{1}H NMR ($CDCl_3$): δ0.71 (3H, *s*, H-18), 0.87–1.0 (15H, *m*, 5 × Me), 1.05–2.2 (*m*), 3.13 (1H, *dd*, *J* = 12 and 4.5 Hz), 3.58 (1H, *d*, *J* = 8.7 Hz), 3.72 (1H, *d*, *J* = 8.7 Hz), 4.03 (1H, *br s*), 4.06–4.14 (1H, *m*).

Epicotyl elongation assay. Soybean seedlings (glycine max cv Williams 82) were grown as previously described [12]. Epicotyl sections for the elongation assay were obtained by excision of the first 1.5 cm immediately below the plumule of 10–14-day-old soybean seedlings. Excised sections were floated in ice cold KPSC buffer (potassium phosphate, pH 6.0, 2% sucrose, 25 μg ml^{-1} chloramphenicol) until required for the assay. Twenty sections were placed in a 50 ml Erlenmeyer flask containing 10 ml of KPSC and rotated at 125 rpm in a 27° shaking incubator under continuous illumination (25 μ Einsteins m^{-2} sec^{-1}). After a 2 hr preincubation in KPSC, the buffer was removed and brassinosteroids were added in 10 ml of fresh KPSC. Epicotyl length was measured to the nearest 1 mm.

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