PERGAMON

Phytochemistry 55 (2000) 121–126

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

Synthesis and bioactivity of 6α- and 6β-hydroxy analogues of castasterone

Gabriel C.Y. Sung [a], Loeke Janzen [b], Richard P. Pharis [b,1], Thomas G. Back [a,*]

[a] *Department of Chemistry, University of Calgary, Calgary, AB, Canada, T2N 1N4*
[b] *Department of Biological Sciences, University of Calgary, Calgary, AB, Canada, T2N 1N4*

Received 12 April 2000; received in revised form 19 June 2000

Abstract

The reduction of castasterone with sodium in ethanol produced chiefly the known 6α-hydroxy stereoisomer, whereas reduction with sodium borohydride in methanol afforded mainly the novel 6β-epimer. Both compounds showed variable bioactivity through four separate assays via the rice leaf lamina inclination bioassay. However, when treated with an appropriate statistical program to remove outliers, the averaged results clearly indicated that the two 6-hydroxy epimers possess comparable and significant bioactivity, which is, however, lower than that of castasterone or brassinolide. When applied together with 1000 ng of the auxin, indole-3-acetic acid (IAA), the activity of both the 6α and 6β hydroxy epimers was enhanced by ca. one order of magnitude across a wide range of doses.

Keywords: Rice leaf lamina inclination assay; Brassinosteroids; Castasterone; 6-Hydroxycastasterone; Indole-3-acetic acid

1. Introduction

Brassinolide (**1**) is a powerful plant growth-regulator that was first discovered by Grove et al. (1979). Since then, numerous other naturally-occurring and synthetic brassinosteroids have been reported and some of these compounds are bioactive at doses as low as 1 ng per individual plant. There is considerable interest in their synthesis, biosynthesis and metabolism, field applications and molecular biology, and several reviews of various aspects of brassinosteroids have recently appeared (Adam and Marquardt, 1986; Mandava, 1988; Cutler et al., 1991; Yokota and Mori, 1992; Arteca, 1995; Back, 1995; Adam et al., 1996; Clouse, 1996; Brosa, 1997; Fujioka and Sakurai, 1997; Clouse and Sasse, 1998; Khripach et al., 1999; Sakurai et al., 1999). Although **1** is generally considered to be the most potent of the naturally-occurring brassinosteroids, related compounds such as castasterone (**2**) and 24-epibrassinolide (**3**) have also come under scrutiny (see Fig. 1 for structures **1**–**3**). Thus, it has been demonstrated that **2** is the biosynthetic precursor of **1** in some plant species such as *Catharanthus roseus*, but has phytohormonal activity per se in others such as mung bean and rice (Yokota et al., 1990, 1991, 1992). However, the 24-epi derivative **3** is more easily prepared than **1** or **2**, and has, therefore, been widely used in field trials (Ikekawa and Zhao, 1991).

A number of structure-activity studies of brassinosteroids have been reported (Thompson et al., 1982; Takatsuto et al., 1983; Adam and Marquardt, 1986; Mandava, 1988; Cutler et al., 1991; Yokota and Mori, 1992; Adam et al., 1996; Brosa et al., 1996; Brosa, 1997; Khripach et al., 1999; Sakurai et al., 1999). These have shown that the two vicinal diol moieties, with attendant stereochemistry, play an important role in biological activity, although conversion of the side-chain hydroxyl groups to the corresponding methyl ethers results in only a slight loss of activity (Luo et al., 1998). The structure of the hydrocarbon fragment at C-24 and the nature of substituents at C-25 are also important (Back et al., 1997, 1999, 2000). The stereochemistry at C-24 is less critical, since the 24-epi analogue **3** displays generally high bioactivity (Ikekawa and Zhao, 1991). While the most

* Corresponding author. Tel.: +1-403-220-6256; fax: +1-403-289-9488.

E-mail address: tgback@ucalgary.ca (T.G. Back).

[1] Also corresponding author.

0031-9422/00/$ - see front matter
PII: S0031-9422(00)00259-4

active brassinosteroids possess the 5α-configuration (*trans*-fused A- and B-rings), some *cis*-fused analogues have also been reported to display significant activity (Brosa et al., 1996), although the validity of this observation has been questioned (Seto et al., 1999). Brassinosteroids tolerate considerable variation to the B-ring lactone functionality. Thus, ketone **2** (vide supra), as well as lactam **4**, thialactone **5**, cyclic ether **6** and the ketone homologue **7** all show significant bioactivity (although lower than that of **1**) (Baron et al., 1998). On the other hand, the carbocyclic analogues **8** and **9** are completely inactive (Baron et al., 1998) (see Fig. 2 for structures **4–9**). This suggests that a polar functional group is necessary in the B-ring, although its precise nature is not crucial. Very recently, Fujioka et al. (1998) reported that the 6α-hydroxy analogue (**10**) of castasterone has activity roughly intermediate between castasterone and its 6-deoxy derivative. Fujioka et al. (2000) also demonstrated that ^{2}H-labelled **10** is a biosynthetic precursor of castasterone (**2**) in *C. roseus*, and therefore also of brassinolide (**1**) (vide supra). We now report the preparation of the novel 6β-hydroxy epimer **11**, along with a comparison of the bioactivities of **10** and **11**, as determined by means of the rice leaf lamina inclination bioassay.

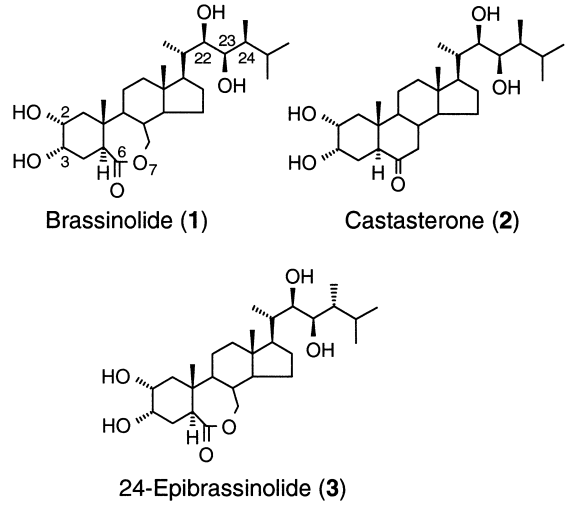

Fig. 1. Structures of some naturally-occurring brassinosteroids.

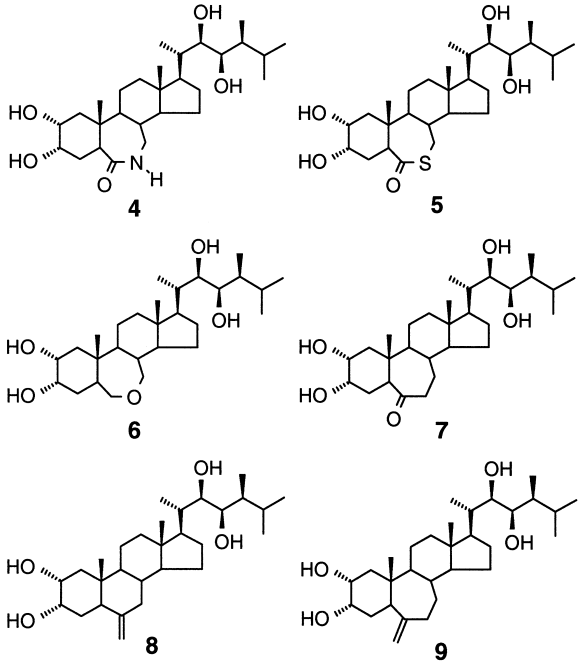

Fig. 2. Brassinosteroid B-ring analogues.

2. Results and discussion

Compounds **10** and **11** were readily prepared by appropriate stereoselective reductions of castasterone (**2**) (Fig. 3), which was in turn obtained by a literature method (Back et al., 1993, 1997). Thus, reduction of **2** by a similar method to the one employed by Fujioka et al. (1998), with sodium in ethanol, afforded the thermodynamically more stable equatorial 6α-alcohol **10** as the principal product, along with minor amounts of its epimer **11** and recovered starting material in yields of 53, 10 and 27%, respectively. On the other hand, reduction of **2** occurred preferentially from the more accessible equatorial direction with sodium borohydride

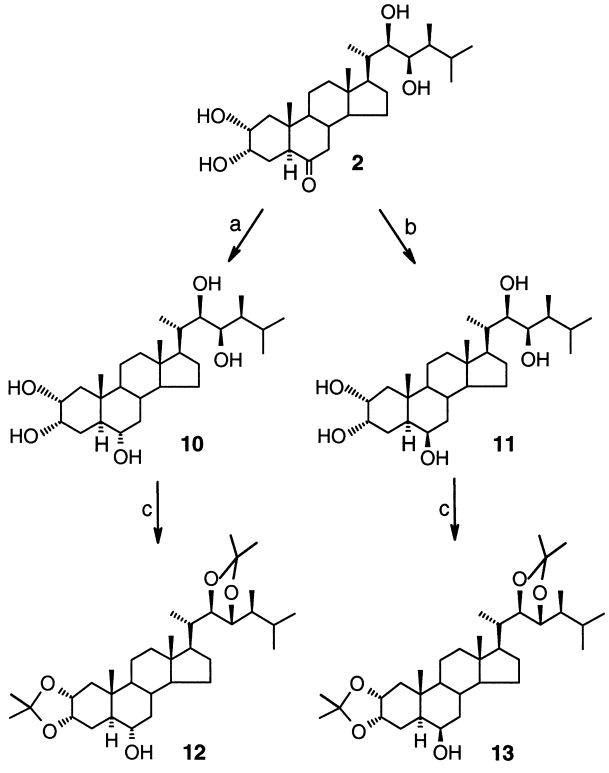

Fig. 3. Synthesis of alcohols **10** and **11** and their bisketals **12** and **13**. Reagents: (a) Na-EtOH; (b) $NaBH_4$-MeOH; (c) $(MeO)_2CMe_2$, TsOH.

in methanol to produce 77% of the axial 6β-hydroxy derivative **11** and 19% of the 6α-epimer **10**. Both epimers were characterized by ^{1}H- and ^{13}C-NMR spectroscopy, but their highly polar and nonvolatile nature necessitated prior conversion into the bisketals **12** and **13** for the purpose of further characterization by mass spectrometry. The spectroscopic properties of the novel 6β-epimer are given in the experimental section. The stereochemistry of the two complementary reduction methods is precedented with other 6-keto steroids (Kirk and Hartshorn, 1968).

Both **10** and **11** were then subjected to the rice leaf lamina inclination bioassay (Takeno and Pharis, 1982), using **1** and **2** as standards for comparison. Since synergy has been previously noted between the biological activity of brassinosteroids and auxins (Takeno and Pharis, 1982; Mandava, 1988; Sasse, 1991), the bioassays were also repeated in the presence of an applied auxin, indole-3-acetic acid (IAA). The results are shown in Figs. 4–6.

Biological activity of compounds **1**, **2**, **10** and **11** is shown for a single bioassay in Fig. 4, which indicates the enhanced efficacy of brassinolide (**1**) relative to castasterone (**2**) or its 6-hydroxy epimers, as well as a remarkably high degree of variation in leaf lamina inclination

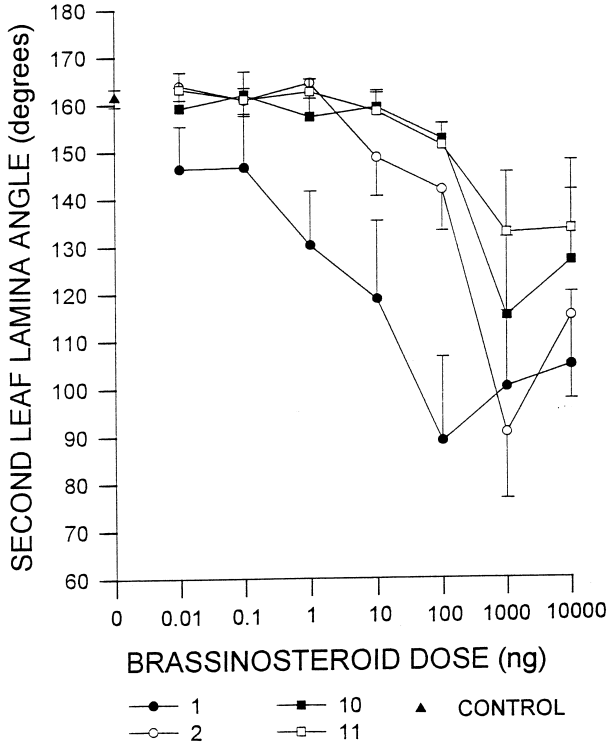

Fig. 4. Rice leaf lamina inclination assay of alcohols **10** and **11** from assay of 4 December 1998 for brassinolide (**1**) and castasterone (**2**), relative to the 6α- and 6β-hydroxylated derivatives (**10** and **11**). Brassinolide is appreciably more active than castasterone except at very high doses (see Baron et al., 1998, where brassinolide and castasterone have been compared across a range of doses).

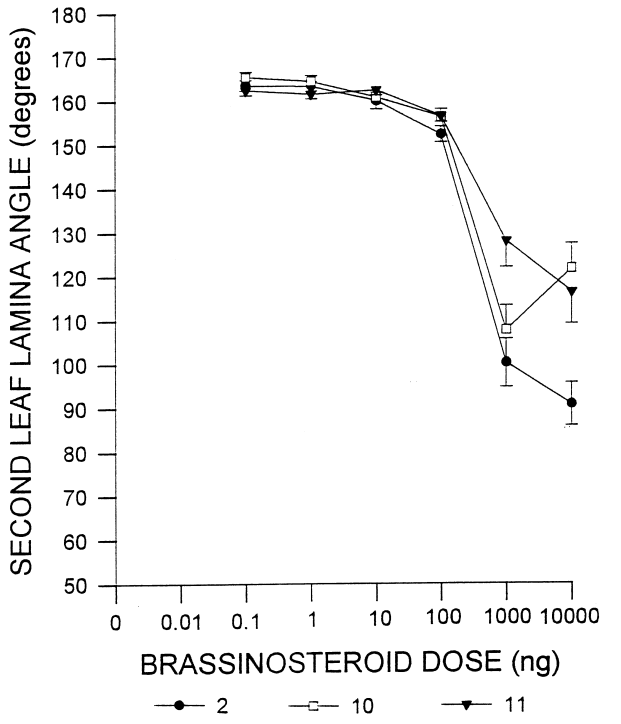

Fig. 5. Rice leaf lamina inclination assay across 4 replicate bioassays (4 December 1998–3 December 1999) for castasterone (**2**) relative to the 6α- and 6β-hydroxylated derivatives (**10** and **11**). Error bars represent 95% confidence limits.

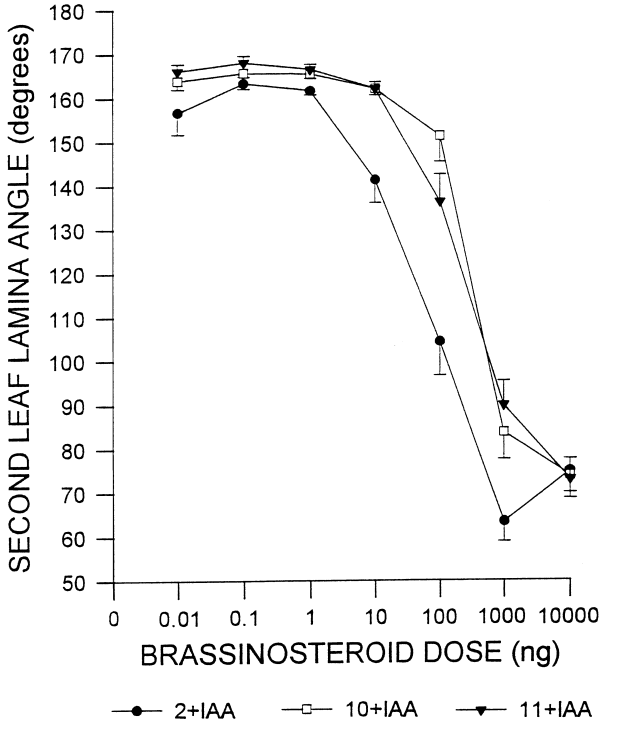

Fig. 6. Rice leaf lamina inclination assay across 4 replicate bioassays (4 December 1998–3 December 1999) for castasterone (**2**) + IAA, relative to the 6α- and 6β-hydroxylated derivatives **10** and **11**, also + IAA. Error bars represent 95% confidence limits.

across dose for **10** and **11**, and even for **2**. This high degree of variation, for **10** and **11** especially, was consistent from bioassay to bioassay, and it led us to perform four separate replicate bioassays across the course of a year, each comparing **10** and **11** with **2**, both with and without IAA. This variation in leaf lamina bending response was caused by a relatively large number of "poor responders", especially at high doses.

What might cause such extreme plant-to-plant variation? It is possible that both of the 6-hydroxy epimers **10** and **11** are precursors of **2**, as was recently reported for 6α-hydroxycastasterone (**10**) (Fujioka et al., 2000). If so, and if these metabolic conversions take place only in the rice root (Yokota et al., 1992), it is possible that plant-to-plant variations in movement of the applied brassinosteroid to the root, and re-translocation of **1** and/or **2** back to the shoot, (where per se function must occur) could explain the observed fluctuations.

Not one of the four replicate bioassays provided a definitive answer with regard to the relative bioactivities of **10** and **11**. However, castasterone (**2**) was generally more active than either of the 6-hydroxy derivatives, especially at the higher doses. We thus performed an outlier programme analysis at 2 standard errors of the mean to eliminate the extreme outliers, and then averaged the mean leaf angle values at each dose across the four replicate bioassays (Figs. 5 and 6). Once done, it is apparent that neither of the 6-hydroxy epimers **10** and **11** is consistently more active than the other across a wide range of doses. However, both tend to be less active than castasterone, except at the highest dose (Figs. 5 and 6). Fujioka et al. (1998) found that **10** exhibited similar behaviour, with activity comparable to that of castasterone (**2**) only at the highest dose.

The removal of extreme outliers was similarly performed for each of **2**, **10** and **11** when IAA was co-applied (Fig. 6). Here, the enhanced synergistic effect of IAA on leaf lamina bending is quite pronounced, not only for castasterone (**2**), but also for the two 6-hydroxylated epimers **10** and **11**. Their biological activity was thus enhanced by ca. one order of magnitude (i.e. it required ca. 10 times the amount of brassinosteroid in the absence of IAA to elicit a comparable biological response to that observed in the presence of IAA) at both low and high doses (compare Fig. 6 with Fig. 5).

Unfortunately, the mechanism by which IAA synergizes the brassinosteroid response is not understood (Arteca, 1995), although the IAA effects on cell wall loosening and subsequent expansion have been extensively researched at both the physiological and molecular levels (Cleland, 1995). Whatever the mechanism, IAA certainly brings the bioactivity of both 6-hydroxy epimers closer to that of castasterone (**2**) at the highest dose. Hence, a promotive effect by IAA on substrate-induced brassinosteroid conversions and/or on possible transport of the metabolites of the 6-hydroxy epimers from the rice root to shoot cannot be ruled out (Yokota et al., 1992).

3. Experimental

Castasterone (**2**) was prepared as described previously (Back et al., 1993, 1997). ^{1}H- and ^{13}C-NMR spectra were recorded at 400 and 100 MHz, respectively, using residual chloroform as the internal standard unless otherwise indicated. Assignments of carbon signals as primary, secondary, tertiary or quaternary were made on the basis of DEPT experiments. Mass spectra were obtained by electron impact (direct probe) at 70 eV.

3.1. Reduction of castasterone with Na in ethanol

Sodium metal (960 mg, 0.042 g atom) was cut into small pieces and added over 2 h to a refluxing solution of castasterone (**2**) (69.7 mg, 0.15 mmol) in absolute ethanol (15 ml). The reaction mixture was then cautiously diluted with water (20 ml) and extracted with 10% isopropanol-chloroform (8 × 10 ml). The combined organic layers were washed twice with brine and the organic layer was dried ($MgSO_4$), filtered and concentrated to afford a crude mixture of products. The residue was flash chromatographed over silica-gel (elution with 0–10% methanol in chloroform) to afford 19.0 mg (27% recovery) of starting material, 6.9 mg (10%) of the minor epimer **11** (vide infra), and 36.9 mg (53%) of the known major epimer **10**.

The above product (15.2 mg, 0.0326 mmol), 2,2-dimethoxypropane (194 μl, 1.6 mmol) and a catalytic amount of *p*-toluenesulfonic acid were dissolved in dichloromethane (5 ml) and stirred overnight at room temperature. The reaction mixture was diluted with saturated K_2CO_3 solution (10 ml) and extracted with dichloromethane (8 × 5 ml). The combined organic layers were washed with brine (20 ml), dried ($MgSO_4$) and concentrated in vacuo. The crude residue was flash chromatographed over silica-gel (elution with 0–2% methanol in chloroform) to afford 12.1 mg (68%) of the bisketal **12** as a pale yellow oil: mass spectrum *m*/*z* (relative intensity%) 546 (M^+, 0.8), 531 (M^+-CH_3, 7), 271 (8), 229 (7), 171 (35), 142 (29), 59 (65), 43 (100). HRMS calculated for $C_{33}H_{55}O_5$ (M^+-CH_3): 531.4050. Found: 531.4009.

3.2. Reduction of castasterone with sodium borohydride.

Sodium borohydride (30 mg, 0.79 mmol) was added to castasterone (**2**) (33.6 mg, 0.0723 mmol) in dry methanol (15 ml). The mixture was stirred at room temperature for 4 h, at which time a second portion of 30 mg of sodium borohydride was added. The mixture was stirred overnight at room temperature and 10 ml of

1 N HCl solution was added. The reaction mixture was then extracted with 10% isopropanol-chloroform (8 × 10 ml), the combined organic layers were washed with saturated $NaHCO_3$ solution and brine, dried ($MgSO_4$) and concentrated in vacuo. The residue was flash chromatographed over silica-gel (elution with 0–10% methanol in chloroform) affording a trace amount of starting material, 6.3 mg (19%) of epimer **10** and 25.9 mg (77%) of **11** as a white crystalline powder: mp 213–216°C (from methanol); IR (KBr) 3416, 1457, 1378, 1059, 1028, 751 cm^{-1}; ^{1}H-NMR (CD_3OD) δ 3.96 (*br d*, $J=2.7$ Hz, 1H), 3.69 (m, 3 H), 3.51 (*br d*, $J=8.4$ Hz, 1H), 1.99 (*m*, 3H), 1.02 (*s*, 3H, 19-Me), 0.96 (*d*, $J=6.7$ Hz, 3H), 0.93 (*d*, $J=6.7$ Hz, 3H), 0.90 (*d*, $J=6.7$ Hz, 3H), 0.84 (*d*, $J=6.4$ Hz, 3H), 0.74 (*s*, 3H); ^{13}C-NMR ($CDCl_3$-CD_3OD) δ 77.4 (CH), 74.8 (CH), 73.4 (CH), 71.3 (CH), 69.2 (CH), 56.2 (CH), 54.2 (CH), 52.6 (CH), 42.7 (C), 42.4 (CH_2), 40.9 (CH), 40.3 (CH), 40.1 (CH_2), 39.7 (CH_2), 37.1 (CH), 36.8 (C), 30.9 (CH), 30.0 (CH), 27.9 (CH_2), 24.3 (CH_2), 21.7 (CH_2), 21.0 (CH_3), 20.9 (CH_2), 20.8 (CH_3), 16.1 (CH_3), 12.1 (CH_3), 12.0 (CH_3), 10.2 (CH_3).

The above product (9.3 mg, 0.020 mmol) was treated by the same procedure as in the preparation of **12** to afford 8.7 mg (80%) of **13** as pale yellow oil: mass spectrum, *m/z* (relative intensity%) 531 (M^+- CH_3, 70), 475 (23), 431 (36), 373 (24), 171 (86), 142 (87), 99 (88), 43 (100). HRMS calculated for $C_{33}H_{55}O_5$ (M^+- CH_3): 531.4050 Found: 531.4076.

3.3. Bioassays

Brassinolide (**1**), castasterone (**2**) and each of 6α-hydroxy- and 6β-hydoxycastasterone (**10** and **11**, respectively) were tested for biological activity by means of the rice leaf lamina assay, using a dwarf rice (*Oryza sativa*) cv., Tan-ginbozu, as described by Takeno and Pharis (1982). The compounds were dissolved in 95% ethanol and applied as 0.5 μl microdrops to the rice plants 48 h after planting the germinated seeds on 0.8% water agar. At high doses, several rounds of application of the 0.5 μl microdrops were required to attain the desired dose per plant. Where IAA was a co-treatment, 1000 ng of IAA was similarly applied per plant ca. 2 h prior to the application of **1**, **2**, **10** or **11**. Thus, the brassinosteroids were applied at doses of 0.01, 0.10, 1.00, 10.0, 100.0, 1000.0 and 10,000.0 ng/plant and the resultant leaf lamina angle was measured 60–65 h later. For an individual bioassay, each data point is the mean of the leaf angles from ca. 36 plants for doses up to 100 ng and from ca. 24 plants for the 1000 and 10,000 ng doses. Parallel applications of ethanol alone (control) and IAA alone (1000 ng) were always carried out (see Figs. 4–6). In all figures error bars represent 95% confidence limits.

A total of four separate bioassays (replicates in time) were carried out for **2**, **10** and **11** over the course of 1 year. Two of the replicate bioassays also included brassinolide (**1**). The results obtained with **1** were consistent across a wide dose range. However, results obtained with the two 6-hydroxycastasterone epimers **10** and **11**, and to a lesser extent with **2**, were highly variable across the four bioassay dates, especially at the higher doses. This variability was caused by ''outliers'', e.g. individual plants within the treatment that showed very little leaf lamina bending response. Hence, for each of the four replicate bioassays we utilized an outlier program at 2.0 standard errors of the mean. The following statistics software was used for the removal of outliers: SPSS for Windows, standard version 9.0.0 (18 December 1998); outliers were removed outside 2 standard deviations via linear regression analysis with casewise diagnostics (for further information, see the SPSS home page: http://www.spss.com/). Use of the outlier program often removed 1–7 individual seedlings for higher doses of **10** and **11**, except in two replicates where 10 and 15 out of 36 seedlings were removed at doses of 1000 ng. No individual seedlings were removed by the outlier program for the bioassay of brassinolide (**1**). We then averaged the means across the four replicate bioassays for each of the seven doses, with outliers removed when present. These averaged values thus represent individual measurements from 75 to 120 plants.

Acknowledgements

This research was supported by the Environmental Science and Technology Alliance of Canada (ESTAC) and the Natural Sciences and Engineering Research Council of Canada (NSERC). We thank Mrs. S. Horácek for assistance with the rice leaf lamina assay, and Dr. R. Yamdagni, Ms. D. Fox and Ms. Q. Wu for mass spectra.

References

Adam, G., Marquardt, V., 1986. Brassinosteroids. Phytochemistry 25, 1787–1799.

Adam, G., Porzel, A., Schmidt, J., Schneider, B., Voigt, B., 1996. New developments in brassinosteroid research. In: Atta-ur-Rahman (Ed.), Studies in Natural Products Chemistry Vol. 18. Elsevier, Amsterdam, pp. 495–549.

Arteca, R.N., 1995. Brassinosteroids. In: Davies, P.J. (Ed.), Plant Hormones. Kluwer Academic, The Netherlands, pp. 206–213.

Back, T.G., 1995. Stereoselective synthesis of brassinosteroids. In: Atta-ur-Rahman (Ed.), Studies in Natural Products Chemistry, Vol. 16. Elsevier, Amsterdam, pp. 321–364.

Back, T.G., Baron, D.L., Luo, W., Nakajima, S.K., 1997. A concise, improved procedure for the synthesis of brassinolide and some vovel side chain analogues. Journal of Organic Chemistry 62, 1179–1182.

Back, T.G., Blazecka, P.G., Krishna, M.V., 1993. A new synthesis of castasterone and brassinolide from stigmasterol. A concise and stereoselective elaboration of the side chain from a C-22 aldehyde. Canadian Journal of Chemistry 71, 156–163.

Back, T.G., Janzen, L., Nakajima, S.K., Pharis, R.P., 1999. Synthesis and biological activity of 25-methoxy-, 25-fluoro- and 25-azabrassinolide, and 25-fluorocastasterone: surprising effects of heteroatom substituents at C-25. Journal of Organic Chemistry 64, 5494–5498.

Back, T.G., Janzen, L., Nakajima, S.K., Pharis, R.P., 2000. Effect of chain length and ring size of alkyl and cycloalkyl side chain substituents upon the biological activity of brassinosteroids. Preparation of novel analogues with activity exceeding that of brassinolide. Journal of Organic Chemistry 65, 3047–3052.

Baron, D.L., Luo, W., Janzen, L., Pharis, R.P., Back, T.G., 1998. Structure-activity studies of brassinolide B-ring analogues. Phytochemistry 49, 1849–1858.

Brosa, C., 1997. Biological effects of brassinosteroids. In: Parish, E.J., Nes, W.D. (Eds.), Biochemistry and Function of Sterols. CRC Press, Boca Raton, FL, pp. 201–220.

Brosa, C., Capdevila, J.M., Zamora, I., 1996. Brassinosteroids: a new way to define the structural requirements. Tetrahedron 52, 2435–2448.

Cleland, R.E., 1995. In: Davies, P. J. (Ed.), Plant Hormones, Kluwer Academic, The Netherlands, pp. 214–227.

Clouse, S.D., 1996. Molecular genetic studies confirm the role of brassinosteroids in plant frowth and development. The Plant Journal 10, 1–8.

Clouse, S.D., Sasse, J.M., 1998. Brassinosteroids: essential regulators of plant growth and development. Annual Review of Plant Physiology and Plant Molecular Biology 49, 427–451.

Cutler, H.G., Yokota, T., Adam, G. (Eds.), 1991. Brassinosteroids: Chemistry, Bioactivity and Applications. ACS Symposium Series 474, American Chemical Society, Washington, DC.

Fujioka, S., Noguchi, T., Takatsuto, S., Yoshida, S., 1998. Activity of brassinosteroids in the dwarf rice lamina inclination bioassay. Phytochemistry 49, 1841–1848.

Fujioka, S., Noguchi, T., Watanabe, T., Takatsuto, S., Yoshida, S., 2000. Biosynthesis of brassinosteroids in cultured cells of *Catharanthus roseus*. Phytochemistry 53, 549–553.

Fujioka, S., Sakurai, A., 1997. Brassinosteroids. Natural Product Reports 14, 1–10.

Grove, M.D., Spencer, G.F., Rohwedder, W.K., Mandava, N., Worley, J.F., Warthen Jr., J.D. et al., 1979. Brassinolide, a plant growth-promoting steroid isolated from *Brassica napus* pollen. Nature 281, 216–217.

Ikekawa, N, Zhao, Y.-J., 1991. Application of 24-epibrassinolide in agriculture. In: Cutler, H.G., Yokota, T., Adam, G. (Eds.), Brassinosteroids: Chemistry, Bioactivity and Applications, ACS Symposium Series 474, American Chemical Society, Washington, DC, pp. 280–291.

Khripach, V.A., Zhabinskii, V.N., de Groot, A.E., 1999. Brassinosteroids: a new class of plant hormones. Academic Press, San Diego.

Kirk, D.N, Hartshorn, M.P., 1968. Steroid Reaction Mechanisms. Elsevier, Amsterdam, pp. 131–147.

Luo, W., Janzen, L., Pharis, R.P., Back, T.G., 1998. Bioactivity of brassinolide methyl ethers. Phytochemistry 49, 637–642.

Mandava, N.B., 1988. Plant growth-promoting brassinosteroids. Annual Review of Plant Physiology and Plant Molecular Biology 39, 23–52.

Sakurai, A., Yokota, T., Clouse, S.D. (Eds.), 1999. Brassinosteroids: Steroidal Plant Hormones. Springer-Verlag, Tokyo

Sasse, J.M., 1991. The case for brassinosteroids as endogenous plant hormones. In: Cutler, H.G., Yokota, T., Adam, G. (Eds.), Brassinosteroids: Chemistry, Bioactivity and Applications. ACS Symposium Series 474, American Chemical Society, Washington, DC, pp. 158–166.

Seto, H., Fujioka, S., Koshino, H., Suenaga, T., Yoshida, S., Watanabe, T., Takatsuto, S., 1999. 2,3,5-Tri-epi-brassinolide: preparation and biological activity in rice lamina inclination test. Phytochemistry 52, 815–818.

Takatsuto, S., Yazawa, N., Ikekawa, N., Takematsu, T., Takeuchi, Y., Koguchi, M., 1983. Structure-activity relationship of brassinosteroids. Phytochemistry 22, 2437–2441.

Takeno, K., Pharis, R.P., 1982. Brassinosteroid-induced bending of the leaf lamina of dwarf rice seedlings: an auxin-mediated phenomenon. Plant and Cell Physiology 23, 1275–1281.

Thompson, M.J., Meudt, W.J., Mandava, N.B., Dutky, S.R., Lusby, W.R., Spaulding, D.W., 1982. Synthesis of brassinosteroids and relationship of structure to plant growth-promoting effects. Steroids 39, 89–105.

Yokota, T., Higuchi, K., Kosaka, Y., Takahashi, N., 1992. Transport and metabolism of brassinosteroids in rice. In: Karssen, C.M., van Loon, L.C., Vreugdenhil, D. (Eds.), Progress in Plant Growth Regulation. Kluwer, Dordrecht, pp. 298–305.

Yokota, T., Mori, K., 1992. Molecular structure and biological activity of brassinolide and related brassinosteroids. In: Bohl, M., Duax, W.L. (Eds.), Molecular Structure and Biological Activity of Steroids. CRC Press, Boca Raton, FL, pp. 317–340.

Yokota, T., Ogino, Y., Suzuki, H., Takahashi, N., Saimoto, H., Fujioka, S. et al., 1991. Metabolism and Biosynthesis of Brassinosteroids. In: Cutler, H. G.; Yokota, T., Adam, G. (Eds.), *Brassinosteroids: Chemistry, Bioactivity and Applications*, ACS Symposium Series 474, American Chemical Society, Washington, DC, pp. 86–96.

Yokota, T., Ogino, Y., Takahashi, N., Saimoto, H., Fujioka, S., Sakurai, A., 1990. Brassinolide is biosynthesized from castasterone in *Catharanthus roseus* crown gall cells. Agricultural and Biological Chemistry 54, 1107.