Pergamon

PII: S0031-9422(97)00881-9

Phytochemistry, Vol. 49, No. 3, pp. 637–642, 1998

Printed in Great Britain
0031-9422/98/$—see front matter

BIOACTIVITY OF BRASSINOLIDE METHYL ETHERS

WEIDE LUO, LOEKE JANZEN,† RICHARD P. PHARIS,† and THOMAS G. BACK*

Department of Chemistry and † Department of Biological Sciences, University of Calgary, Calgary, AB, Canada, T2N 1N4

(*Received* 14 *July* 1997; *accepted* 20 *September* 1997)

Key Word Index—rice leaf lamina inclination assays; brassinosteroids; brassinolide; brassinolide methyl ethers; indole-3-acetic acid.

Abstract—Four new derivatives of brassinolide (BR) were prepared by single or multiple methylation of the hydroxyl groups at C-2, C-3, C-22 and or C-23 to afford (22*R*,23*R*,24*S*)-2α,3α,23-trihydroxy-22-methoxy-7-oxa-7a-homo-5α-ergostan-6-one (22-MeBR), (22*R*,23*R*,24*S*)-2α,3α,22-trihydroxy-23-methoxy-7-oxa-7a-homo-5α-ergostan-6-one (23-MeBR), (22*R*,23*R*,24*S*)-2α,3α-dihydroxy-22,23-dimethoxy-7-oxa-7a-homo-5α-ergostan-6-one (diMeBR), and (22*R*,23*R*,24*S*)-2α,3α,22,23-tetramethoxy-7-oxa-7a-homo-5α-ergostan-6-one (tetraMeBR). The formation of methyl ethers is expected to block glycosylation of the free hydroxyl groups, which should prevent potential metabolic deactivation of these compounds by plants when they are applied exogenously. The above BR derivatives were subjected to the rice leaf lamina inclination assay, in comparison with BR, to test for brassinosteroid activity. Whereas tetraMeBR and 23-MeBR showed negligible and very low activity, respectively, even at the high dosage of 1000 ng/plant, 22-MeBR showed weak activity relative to BR at low doses (1–10 ng/plant), and appreciable activity at high doses (1000 ng/plant). Of the four new compounds, diMeBR was the most active, comparable to 24-epibrassinolide (24-epiBR) at low to medium doses, and comparable to BR at doses of 1000 ng/plant. Thus, diMeBR is a good candidate for further investigation of persistence. As is the case for BR, a strong synergistic effect was observed when 22-MeBR or diMeBR were administered together with the auxin, indole-3-acetic acid.

INTRODUCTION

Brassinolide (BR) was first isolated by Grove *et al.* in 1979 [1], who determined that it possesses a steroidal structure (Fig. 1), and that it promotes growth in certain plant species with doses as low as 1 ng per individual plant. This discovery prompted intense investigations into its biological properties and potential practical applications [2–6], synthesis [7–12], biosynthesis and metabolism [13, 14] and, more recently, aspects of its molecular biology [15–19].

The low abundance of BR in natural sources as well as its generally long and expensive syntheses has forced most workers to employ more readily available, but less active analogues such as 24-epibrassinolide (24-epiBR) (Fig. 1) in field trials [4, 9, 20]. Structure-activity studies by several groups have been reported [2, 5, 21–24], and it is generally accepted that (*inter alia*) the (22*R*,23*R*)- and (2α,3α)-vicinal diol moieties are required for biological activity of BR and analogues. Moreover, Adam *et al.* [13, 25] and Yokota *et al.* [14], have reported that glucosylation at C-2, C-3 and C-23, as well as oxidation and glucosylation at C-25 and C-26 of various brassinosteroids, can occur in higher plants. Although hydroxylation at C-25 has been shown to give a highly active metabolite from 24-epiBR, glucosylation appears to decrease bioactivity [13].

It was therefore of interest to prepare novel analogues of BR in which one or more hydroxyl groups are converted into the corresponding methyl ethers in order to test the degree of biological activity retained. Since ether formation would prevent glucosylation from taking place at the blocked sites, it is possible that such methylated derivatives might retain their activity for longer durations and thus prove more useful for field application. We now report the preparation of four novel brassinosteroids, (22*R*,23*R*,24*S*)-2α,3α,23-trihydroxy-22-methoxy-7-oxa-7a-homo-5α-ergostan-6-one (22-MeBR), (22*R*,23*R*,24*S*)-2α,3α,22-trihydroxy-23-methoxy-7-oxa-7a-homo-5α-ergostan-6-one (23-MeBR), (22*R*,23*R*,24*S*)-2α,3α-dihydroxy-22,23-dimethoxy-7-oxa-7a-homo-5α-ergostan-6-one (diMeBR), and (22*R*,23*R*,24*S*)-2α,3α,22,23-tetramethoxy-7-oxa-7a-homo-5α-ergostan-6-one (tetraMeBR) (Fig. 1). We also report their biological activity on the rice leaf lamina inclination

* Author to whom correspondence should be addressed.

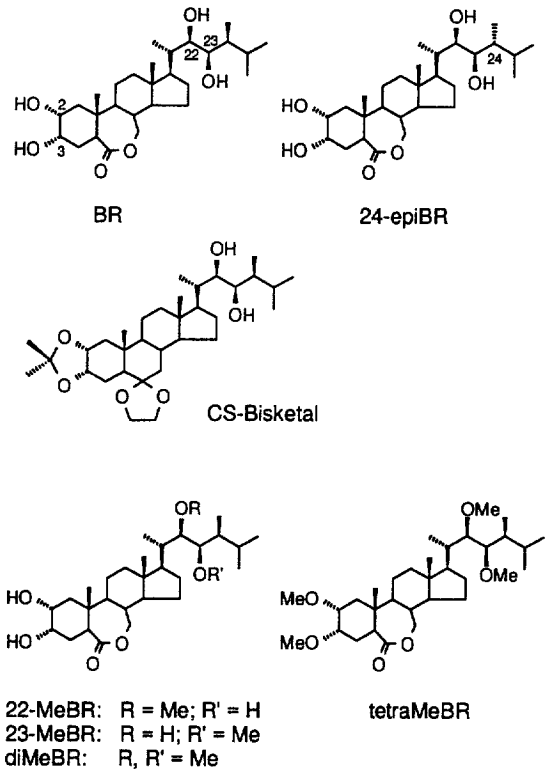

Fig. 1. Structures of Brassinosteroids.

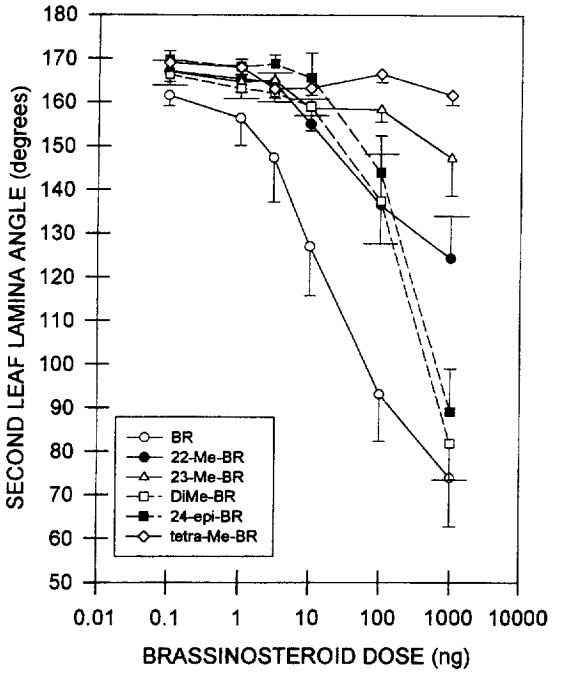

Fig. 2. Rice Leaf Lamina Bending Assay of BR, 24-epiBR and BR Methyl Ethers.

assay, a particularly rapid, sensitive and convenient procedure for assessing brassinosteroid activity [26]. Synergistic effects of brassinosteroids with auxins and other plant growth regulators have been reported previously [26, 27]. Thus, we also investigated the effects of simultaneous application of O-methylated BR derivatives together with the auxin, indole-3-acetic acid (IAA).

RESULTS AND DISCUSSION

The four O-methylated BR derivatives were prepared by means of Williamson ether syntheses, followed by deprotection and Baeyer-Villiger oxidation under previously reported conditions [11, 28]. It was possible to distinguish between the isomeric 22-MeBR and 23-MeBR products by means of their mass spectra, since cleavage of the C_{22}—C_{23} bond is a principal fragmentation pathway [1] and results in the loss of fragments of mass 101 ($C_6H_{13}O$) and 114 ($C_7H_{14}O$) in the case of 23-hydroxy and 23-methoxy derivatives, respectively.

The four methylated compounds were tested in the rice leaf lamina assay under identical conditions against a standard of authentic BR. Linear plots of the second leaf lamina angle vs the logarithm of the amount of BR, 24-epiBR or the O-methylated BR derivatives applied to each rice plant, are shown in Fig. 2. The typical response to BR was a significant change in the downward inclination of the leaf lamina from its base of value of ca 160–165° at doses in the range of 3–10 ng/plant. The maximum inclination reached an angle of ca 75° at a dose of 1000 ng/plant. In contrast, 23-MeBR had little effect on the leaf lamina angle even at 1000 ng/plant. The 22-MeBR analogue displayed more pronounced activity, noticeable at 10 ng/plant and reaching 130° at 1000 ng/plant. Methylation of both side chain hydroxyl groups in diMeBR resulted in substantially stronger activity, again noticeable at 10 ng/plant, and producing a leaf lamina angle comparable to that obtained with BR at the highest dose of 1000 ng/plant. At intermediate dosage levels, diMeBr showed ca 10% of the potency of BR (i.e. it required a ca 10-fold increase in dose compared to BR to elicit a comparable leaf lamina bending response). The behaviour of diMeBR was thus very similar to that of 24-epiBR. Finally, the assay of tetraMeBR showed no significant activity, even at the highest doses.

The effects of adding IAA (at 1000 ng/plant) together with the methylated BR derivatives, are shown in Figs 3–6. For 22-MeBR, a very striking enhancement of activity by IAA was observed (Fig. 3), with 22-MeBR plus IAA being more potent than BR alone. However, BR plus IAA is still the most effective treatment, with significant leaf lamina bending occurring even at the lowest dose of BR (0.1 ng/plant). On the other hand, the use of IAA as a potential synergist with either the relatively inactive 23-MeBR or with tetraMeBr had little or no effect (Figs 4 and 6, respectively). Interestingly, IAA synergized the effect of diMeBR at mid-range doses (i.e. 10 or 100 ng), but suppressed activity at 1000 ng (Fig. 5).

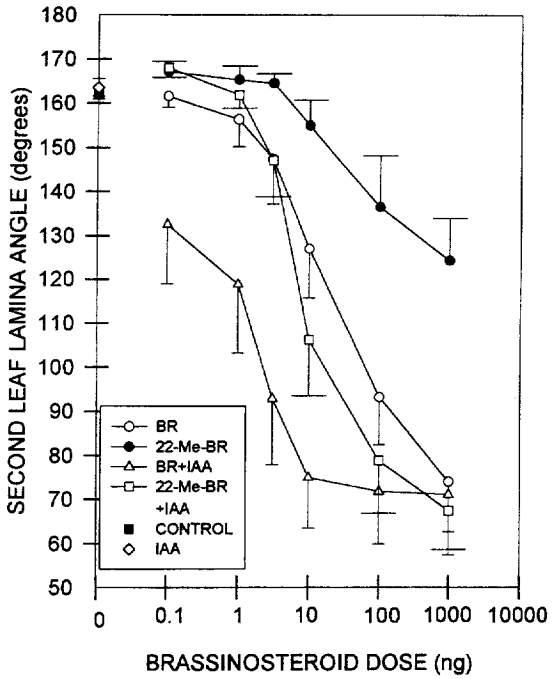

Fig. 3. Rice Leaf Lamina Bending Assay of BR and 22-MeBR with and without Preapplication of IAA.

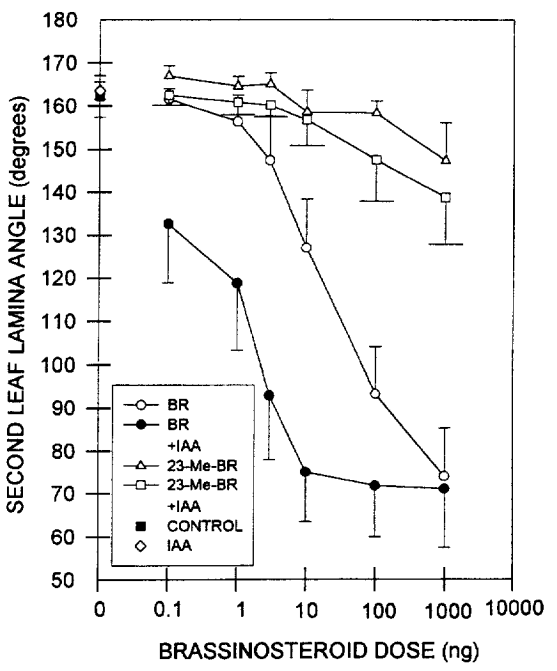

Fig. 4. Rice Leaf Lamina Bending Assay of BR and 23-MeBR with and without Preapplication of IAA.

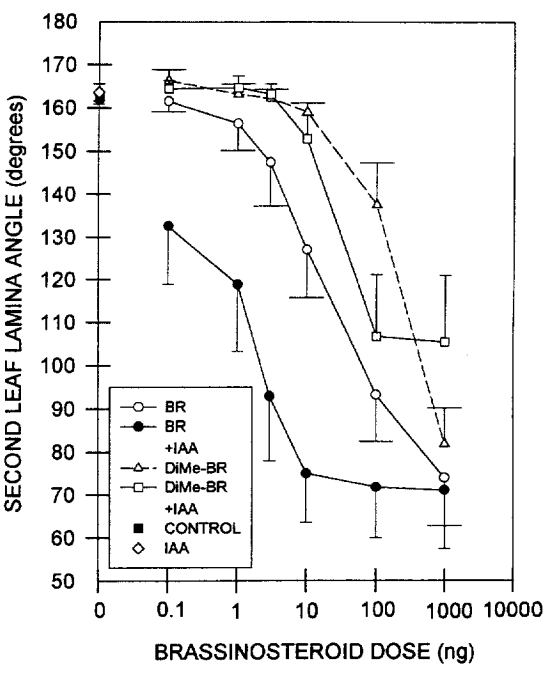

Fig. 5. Rice Leaf Lamina Bending Assay of BR and diMeBR with and without Preapplication of IAA.

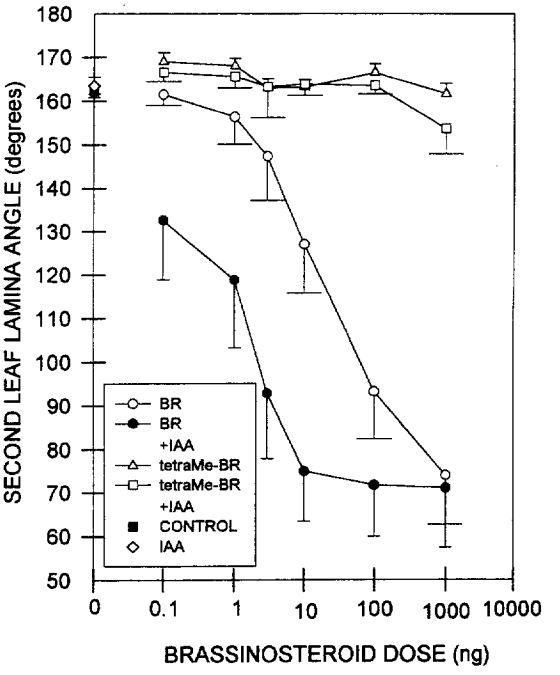

Fig. 6. Rice Leaf Lamina Bending Assay of BR and tetra-MeBR with and without Preapplication of IAA.

All four of the novel methylated BR derivatives are less active than BR itself when tested alone (without IAA) in the rice leaf lamina inclination assay. Although methylation of all four hydroxyl groups essentially resulted in complete loss of activity, partial methylation afforded products that retained low to substantial activity. Methylation of the C-22 hydroxyl group had a less detrimental effect than methylation at C-23. Surprisingly, methylation of both the C-22 and C-23 hydroxyl functions afforded a product (diMeBR) retaining greater activity than either of the monomethyl derivatives. Even so, diMeBR was still

ca one order of magnitude less potent than BR, and is thus comparable in activity to 24-epiBR, a product that has been employed extensively in field trials (see Introduction). Since glucosylation of the C-23 alcohol group is effectively precluded in diMeBR, metabolic deactivation by this route should be blocked, possibly conferring more persistent activity to this product than obtained with BR or 24-epiBR. Further investigation of this possibility is in progress.

In an attempt to explain the greater bioactivity of 22-MeBR compared to 23-MeBR, and the even higher activity of diMeBR, we considered the possibility that methylation results in substantial conformational changes to the brassinolide side chain, which in turn could lead to different binding affinities of these compounds for the active site of a putative BR receptor in plants. Molecular modeling of BR, 22-MeBR, 23-MeBR and diMeBR was therefore carried out and, using MacroModel Version 4.5 and the MM2 force field for initial energy minimization, followed by a Monte Carlo search of 1000 substructures to determine the global energy minimum for each brassinosteroid. However, the side chain conformations showed relatively small differences and showed no correlation with the corresponding bioactivities. For example, the $O-C_{22}-C_{23}-O$ dihedral angles for these four compounds were determined to be $-58.8°$, $-57.6°$, $-59.6°$ and $-68.1°$, respectively. The slightly larger angle of $-68.1°$ for diMeBR reflects the increased gauche interaction caused by the replacement of both side chain OH groups by bulkier OMe groups. The significantly different bioactivities of the four O-methylated BR's therefore lack a clear explanation at this time.

The synergy between IAA and either BR, 22-MeBR or diMeBR is also noteworthy, although the suppressive effect observed with IAA plus diMeBR at the 1000 ng dose is anomalous and lacks a clear explanation. For example, 22-MeBR plus IAA is more potent than BR alone, and very much more potent than 24-epiBR. Hence, field trials of the 22-MeBR derivative in combination with an agriculturally effective auxin appear warranted. In fact, the generally observed enhancement of brassinosteroid activity by the relatively inexpensive auxin indicates that it may be feasible to use appreciably lower doses of brassinosteroids when applied with an effective and agriculturally acceptable auxin.

Our results also clearly show that free side chain hydroxyl groups are not essential for brassinosteroid activity in the leaf lamina bending assay. This in turn suggests that the interactions of BR and its analogues with a putative receptor do not require hydrogen bond donation from the side chain hydroxyl groups. On the other hand, hydrogen bond donation from the A-ring diol moiety at C-2 and C-3 may be of importance for binding to a receptor, since methylation at these positions results in the complete destruction of activity.

EXPERIMENTAL

BR and castasterone bisketal (CS-bisketal, Fig. 1) were prepared by methods reported previously [11, 28]. 24-EpiBR was obtained from Sigma. ^1H and ^{13}C NMR spectra were recorded using $CDCl_3$ as the solvent and residual chloroform as the internal standard unless otherwise indicated. Mass spectra were obtained by electron impact (direct probe) at 70 eV.

Preparation of 22-MeBR, 23-MeBR, diMeBR and tetraMeBR

Sodium hydride (100 mg of a 50% dispersion in mineral oil, 2.08 mmol) and methyl iodide (0.10 ml, 1.6 mmol) were added to CS-bisketal (446 mg, 0.812 mmol) in THF (5 ml) and the mixture was stirred for 3 h. After addition of brine (25 ml), the mixture was extracted with ether (3×20 ml). The combined extracts were dried (Na_2SO_4), concentrated and separated by flash chromatography on silica-el (elution with 30–80% ether–hexane) to afford the corresponding 22-O-methyl (140 mg, 31%) and 23-O-methyl (96 mg, 21%) derivatives, along with starting material (126 mg, 28%) and a trace of the 22,23-di-O-methyl analogue. The latter compound was obtained in 71% yield when CS-bisketal was treated similarly with an excess of sodium hydride and methyl iodide.

The three preceding methyl ethers were subjected to deketalization and Baeyer-Villiger oxidation by the same procedure described previously [11, 28] for the preparation of BR from CS-bisketal. Overall yields of the products after recrystallization from methanol were 17%, 36% and 32%, respectively. Substantial amounts of the products remained in the mother liquors, but second crops were contaminated with the 6-oxa-7-oxo regioisomers from the Baeyer-Villiger oxidation and were not used in biological testing.

DiMeBR was methylated with excess sodium hydride and methyl iodide by the same procedure used in the dimethylation of CS-bisketal, to afford tetraMeBR in 42% yield after recrystallization from methanol.

The products had the following properties:

22-MeBR. Mp 216–219°C; ^1H NMR (200 MHz) δ 4.08 (*m*, 2 H, H-7a), 4.01 (*m*, 1 H, H-3), 3.70 (*m*, 2 H, H-2, H-23), 3.55 (*s*, 3 H, OMe), 3.12 (*m*, 2 H, H-22, H-5), 0.95 (*d*, $J = 6.8$ Hz, 3 H), 0.92 (*d*, $J = 6.6$ Hz, 3H), 0.92 (*s*, 3 H, H-19), 0.90 (*d*, $J = 7.4$ Hz, 3 H), 0.87 (*d*, $J = 6.7$ Hz, 3 H), 0.71 (*s*, 3 H, H-18); ^{13}C NMR (50 MHz) δ 176.2 (C-6), 85.1 (C-22), 73.0, 70.4, 68.1, 68.0, 61.3, 58.2, 52.9, 51.4, 42.7, 41.5, 41.2, 40.9, 39.6, 39.2, 38.4, 38.3, 31.0, 30.8, 28.5, 24.9, 22.2, 20.9, 20.2, 15.4, 12.8, 11.4, 10.0. MS. *m/z* (relative intensity, %) 423 (3), 393 (60), 375 (19), 361 (42), 343 (29), 43 (100). HRMS, *m/z* 423.2742, 393.2609; calcd for $C_{24}H_{39}O_6$ ($M^+ - 71$): 423.2747; calcd for $C_{23}H_{37}O_5$ ($M^+ - 101$): 393.2641.

23-MeBR. mp 306–310°C; ^1H NMR (200 MHz) δ 4.09 (*m*, 2 H, H-7a), 4.04 (*m*, 1 H, H-3), 3.70 (*m*, 1 H,

H-2), 3.54 (*s*, 3 H, OMe), 3.51 (*m*, 1 H, H-22), 3.25 (*dd*, $J = 8.5$, 1.6 Hz, 1 H, H-23), 3.13 (*dd*, $J = 11.9$, 4.9 Hz, 1 H, H-5), 1.00 (*d*, $J = 6.6$ Hz, 3 H), 0.94 (*d*, $J = 6.6$ Hz, 3 H), 0.94 (*s*, 3 H, H-19), 0.93 (*d*, $J = 6.5$ Hz, 3 H), 0.87 (*d*, $J = 6.9$ Hz, 3 H), 0.71 (*s*, 3 H, H-18); ^{13}C NMR (50 MHz, Py-d_5) δ 176.6 (C-6), 84.9 (C-23), 74.8, 70.3, 68.7, 68.4, 61.2, 58.4, 52.7, 51.5, 42.7, 42.6, 41.7, 41.4, 40.1, 39.7, 38.5, 38.1, 31.7, 31.1, 28.0, 24.9, 22.8, 21.6, 20.8, 15.8, 14.2, 12.6, 11.2. MS, *m/z*: (relative intensity, %) 423 (1), 380 (44), 115 (100). HRMS, *m/z* 423.2717, 380.2502; calcd for $C_{24}H_{39}O_6$ ($M^+ - 71$): 423.2747; calcd for $C_{22}H_{36}O_5$ ($M^+ - 114$): 380.2562.

DiMeBR. mp 113–115°C; ^{1}H NMR (200 MHz) δ 4.09 (*m*, 2 H, H-7a), 4.02 (*m*, 1 H, H-3), 3.65 (*m*, 1 H, H-2), 3.55 (*s*, 3 H, OMe), 3.52 (*s*, 3 H, OMe), 3.38 (*d*, $J = 8.5$ Hz, 1 H, H-23), 3.20 (*d*, $J = 8.5$ Hz, 1 H, H-22), 3.12 (*dd*, $J = 12.0$, 4.8 Hz, 1 H, H-5), 0.97 (*d*, $J = 6.4$ Hz, 3 H), 0.92 (*d*, $J = 6.7$ Hz, 3 H), 0.92 (*s*, 3 H, H-19), 0.87 (*d*, $J = 6.6$ Hz, 3 H), 0.82 (*d*, $J = 6.8$ Hz, 3H), 0.70 (*s*, 3 H, H-18); ^{13}C NMR (50 MHz) δ 176.2 (C-6), 86.0 (C-22 or C-23), 84.8 (C-22 or C-23), 70.4, 68.1, 68.0, 61.1, 60.6, 58.2, 52.1, 51.4, 42.5, 41.5, 41.2, 40.9, 39.6, 39.2, 38.3, 38.0, 31.1, 30.5, 28.5, 24.8, 22.2, 21.3, 20.5, 15.4, 12.3, 11.6, 10.5. MS, *m/z* (relative intensity, %) 394 (34), 393 (23), 361 (23), 343 (12), 321 (30), 115 (100). HRMS, *m/z*: 394.2699; calcd for $C_{23}H_{38}O_5$ ($M^+ - 114$): 394.2719.

TetraMeBR. mp 220–223°C; ^{1}H NMR (200 MHz) δ 4.09 (*m*, 2 H, H-7a), 3.73 (*m*, 1 H, H-3), 3.55 (*s*, 3 H, OMe), 3.52 (*s*, 3 H, OMe), 3.42 (*s*, 3 H, OMe), 3.38 (*s*, 3 H, OMe), 3.40 (*m*, 1H, H-2 or H-23), 3.22 (*m*, 1 H, H-2 or H-23), 3.21 (*m*, 1 H, H-22), 3.00 (*dd*, $J = 11.9$, 4.7 Hz, 1 H, H-5), 0.97 (*d*, $J = 6.7$ Hz, 3 H), 0.93 (*s*, 3 H, H-19), 0.92 (*d*, $J = 5.5$ Hz, 3 H), 0.87 (*d*, $J = 6.6$ Hz, 3 H), 0.82 (*d*, $J = 6.8$ Hz, 3 H), 0.70 (*s*, 3 H, H-18); ^{13}C NMR (100 MHz) δ 176.5 (C-6), 86.3 (C-22 or C-23), 85.0 (C-22 or C-23), 77.9 (C-2 or C-3), 74.0 (C-2 or C-3), 70.8, 61.4, 60.9, 58.4, 57.5, 56.3, 52.3, 51.6, 42.7, 42.0, 41.4, 39.8, 39.7, 39.4, 38.3, 38.2, 30.7, 28.8, 28.5, 25.1, 22.4, 21.6, 20.8, 15.9, 12.6, 11.8, 10.8. MS, *m/z* (relative intensity, %) 536 (0.6, M^+), 422 (75), 421 (58), 407 (35), 389 (64), 357 (32), 349 (44), 317 (34), 285 (31), 169 (52), 115 (100). HRMS, *m/z*: 422.3011; calcd for $C_{25}H_{42}O_5$ ($M^+ - 114$): 422.3032.

Bioassays. 22-MeBR, 23-MeBR, diMeBR, tetraMeBR, 24-epiBR and BR were tested by means of the rice leaf lamina assay, using cv. Tan-ginbozu, as described by Takeno and Pharis [26]. The compounds were dissolved in 95% ethanol and applied as 0.5 μl microdrops to the rice plant 48 h after planting onto 0.8% water agar. Where IAA was a co-treatment, 1000 ng of IAA was similarly applied per plant ca 2 h prior to the application of BR or its derivatives. Thus, in Figs 2–6, BR or its derivatives were applied at 0.1 to 1000 ng/plant in microdrops, and the leaf lamina angle was measured 60–65 h later. 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 ng doses. Parallel applications of IAA alone, BR alone and BR plus IAA were also carried out. One group of plants received only IAA at 1000 ng, and the Control group of plants received only a microdrop of 95% ethanol. In each of Figs 2–6, error bars represent 95% confidence limits.

Acknowledgements—This research was supported by a Natural Sciences and Engineering Research Council of Canada Strategic Grant (No. STR0167111). 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

1. Grove, M. D., Spencer, G. F., Rohwedder, W. K., Mandava, N., Worley, J. F., Warthen Jr., J.D., Steffens, G. L., Flippen-Anderson, J. L. and Cook Jr., J. C., *Nature*, 1979, **281**, 216.
2. Adam, G. and Marquardt, V., *Phytochemistry*, 1986, **25**, 1787.
3. Arteca, R. N., in *Plant Hormones*, ed P. J. Davies, Kluwer Academic, 1995, pp. 206–213.
4. Cutler, H. G., in *Brassintosteroids: Chemistry, Bioactivity and Applications*, ed. H. G. Cutler, T. Yokota and G. Adam. ACS Symposium Series 474, American Chemical Society, Washington, D.C., U.S.A., 1991, pp. 334–345.
5. Mandava, N. B., *Annual Reviews of Plant Physiology and Plant Molecular Biology*, 1988, **39**, 23.
6. Sakurai, A., Fujioka, S., *Plant Growth Regulation*, 1993, **13**, 147.
7. Adam, G., Marquardt, V., Vorbrodt, H. M., Hörhold, C., Andreas, W. and Gartz, J., in *Brassinosteroids: Chemistry, Bioactivity and Applications*, ed. H. G. Cutler, T. Yokota, and G. Adam. ACS Symposium Series 474, American Chemical Society, Washington, D.C., U.S.A., 1991, pp. 74–85.
8. Back, T. G., in *Studies in Natural Products Chemistry*, Vol. 16, ed. Atta-ur-Rahman. Elsevier, Amsterdam, 1995, pp. 321–364.
9. Khripach, V. A., Zhabinskii, V. N. and Litvinovskaya, R. P., in *Brassinosteroids: Chemistry, Bioactivity and Applications*, ed. H. G. Cutler, T. Yokota and G. Adam. ACS Symposium Series 474, American Chemical Society, Washington, D.C., U.S.A., 1991, pp. 43–55.
10. McMorris, T. C., Donaubauer, J. R., Silveira, M. H. and Molinski, T. F., in *Brassinosteroids: Chemistry, Bioactivity and Applications*, ed. H. G. Cutler, T. Yokota and G. Adam. ACS Symposium Series 474, American Chemical Society, Washington, D.C., U.S.A., 1991, pp. 36–42
11. Back, T. G., Baron, D. L., Luo, W. and Nakajima, S. K., *Journal of Organic Chemistry*, 1997, **62**, 1179.
12. McMorris, T. C., Chavez, R. G. and Patil, P. A.,

Journal of the Chemical Society, Perkin Transactions 1, 1996, 295.
13. Adam, G., Porzel, A., Schmidt, J., Schneider, B. and Voigt, B., in *Studies in Natural Products Chemistry*, Vol. 18, ed. Atta-ur-Rahman. Elsevier, Amsterdam, 1995, pp. 495–549.
14. Yokota, T., Ogino, Y., Suzuki, H., Takahashi, N., Saimoto, H., Fujioka, S. and Sakurai, A., in *Brassinosteroids: Chemistry, Bioactivity and Applications*, ed. H. G. Cutler, T. Yokota and G. Adam. ACS Symposium Series 474, American Chemical Society, Washington, D.C., U.S.A., 1991, pp. 86–96.
15. Clouse, S. D., *The Plant Journal*, 1996, **10**, 1.
16. Clouse, S. D., Langford, M. and McMorris, T. C., *Plant Physiology*, 1996, **111**, 671.
17. Clouse, S. D. and Zurek, D., in *Brassinosteroids: Chemistry, Bioactivity and Applications*, ed. H. G. Cutler, T. Yokota and G. Adam. ACS Symposium Series 474, American Chemical Society, Washington, D.C., U.S.A., 1991, pp. 122–140.
18. Kulaeva, O. N., Burkhanova, E. A., Fedina, A. B., Khokhlova, V. A., Bokebayeva, G. A., Vorbrodt, H. M. and Adam, G., in *Brassinosteroids: Chemistry, Bioactivity and Applications*, ed. H. G. Cutler, T. Yokota and G. Adam. ACS Symposium Series 474, American Chemical Society, Washington, D.C., U.S.A., 1991, pp.141–155.
19. Li, J., Nagpal, P., Vitart, V., McMorris, T. C. and Chory, J., *Science*, 1996, **272**, 398.
20. Ikekawa, N. and Zhao, Y.-J., in *Brassinosteroids: Chemistry, Bioactivity and Applications*, ed. H. G. Cutler, T. Yokota and G. Adam. ACS Symposium Series 474, American Chemical Society, Washington, D.C., U.S.A., 1991, pp. 280–291.
21. Brosa, C., Capdevila, J. M. and Zamora, I., *Tetrahedron*, 1996, **52**, 2435.
22. Takatsuto, S., Yazawa, N., Ikekawa, N., Takematsu, T., Takeuchi, Y. and Koguchi, M., *Phytochemistry*, 1983, **22**, 2437.
23. Thompson, M. J., Meudt, W. J., Mandava, N. B., Dutky, S. R., Lusby, W. R. and Spaulding, D. W., *Steroids*, 1982, **39**, 89.
24. Yokota, T. and Mori, K., in *Molecular Structure and Biological Activity of Steroids*, ed. M. Bohl and W. L. Duaz, CRC Press, Boca Raton, Florida, 1992, pp. 317–340.
25. Schneider, B., Kolbe, A., Hai, T., Porzel, A. and Adam, G., *Proceedings of the 23rd Annual Meeting of the Plant Growth Regulation Society of America*, Calgary, Alberta, 1996, p. 43.
26. Takeno, K. and Pharis, R. P., *Plant and Cell Physiology*, 1982, **23**, 1275.
27. Sasse, J. M., in *Brassinosteroids: Chemistry, Bioactivity and Applications*, ed. H. G. Cutler, T. Yokota and G. Adam. ACS Symposium Series 474, American Chemical Society, Washington, D.C., U.S.A., 1991, pp. 158–166.
28. Back, T. G., Blazecka, P. G. and Krishna, M. V., *Canadian Journal of Chemistry*, 1993, **71**, 156.