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

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A series of brassinosteroids with different alkyl or cycloalkyl substituents in place of the isopropyl group at C-24 of brassinolide (**1**) were prepared by the CuCN-catalyzed addition of Grignard reagents to (*threo*-2*R*,3*S*,5α,22*R*,23*R*,24*S*)-23,24-epoxy-6,6-(ethylenedioxy)-2,3-(isopropylidenedioxy)-26,27-dinorcholestan-22-ol (**9**), followed by deketalization and Baeyer−Villiger oxidation. Compound **9** was employed as part of a 70:30 threo/erythro mixture of epoxides **9** and **10**, from which the *erythro*-epoxide **10** was recovered intact after the Grignard additions. Thus, the corresponding *n*-dodecyl, *n*-hexyl, *n*-propyl, *tert*-butyl, cyclohexyl, cyclopentyl, cyclobutyl, and cyclopropyl analogues of brassinolide were obtained. A rearrangement byproduct was observed during the preparation of the cyclopropyl-substituted brassinosteroid when ether was used as the solvent in the Grignard reaction, but could be avoided by the use of THF. A method for recycling the undesired *erythro*-epoxide **10** was developed on the basis of deoxygenation with tellurium and lithium triethylborohydride. The rice leaf lamina inclination assay was then used to measure the bioactivity of the products. In general, increasing activity was observed as the length or ring size of the C-24 hydrocarbon substituent decreased. The novel cyclobutyl- and cyclopropyl-substituted analogues of brassinolide (**1**) were ca. 5−7 times as active as **1** and thus appear to be the most potent brassinosteroids reported to date. Further enhancement of the bioactivity of all of the above brassinosteroids, except that of the inactive *n*-dodecyl derivative, was observed when the brassinosteroid was applied together with an auxin, indole-3-acetic acid (IAA). The synergy between the brassinosteroids and IAA thus increased the bioactivity of the brassinosteroids, including the cyclopropyl and cyclobutyl derivatives, by ca. 1−2 orders of magnitude.

Brassinosteroids have attracted considerable interest since 1979, when Grove and co-workers[1] reported the isolation and structure determination of brassinolide (**1**) from the pollen of *Brassica napus*. Some brassinosteroids, including **1**, display powerful plant growth-regulating activity at doses as low as 1 ng per individual plant.[1] Brassinolide and some of its analogues have been reported to increase yield and stress resistance of a number of important crops when applied at doses as low as 50−100 mg per hectare.[2] Brassinosteroids occur naturally in a diverse range of plant species, but generally in such minute amounts that their production from natural sources is impractical. Numerous studies of the chemistry and biology of brassinosteroids, as well as of their potential commercial applications, have been reported during the past 20 years.[2,3]

Structure−activity investigations[2,3a,d,e,i,j,4] of brassinosteroids have revealed that the 2α,3α vicinal diol moiety is important in the expression of biological activity.[3d,j] The B-ring tolerates considerable structural variation, although the unusual seven-membered 7-oxa-6-oxo lactone moiety that is present in brassinolide (**1**) generally results in higher bioactivity than other structural motifs.[3j,4d,5] The 5α-configuration (i.e., trans-A/B ring junction) has

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(1) Grove, M. D.; Spencer, G. F.; Rohwedder, W. K.; Mandava, N.; Worley, J. F.; Warthen, J. D., Jr.; Steffens, G. L.; Flippen-Anderson, J. L.; Cook, J. C., Jr. *Nature* **1979**, *281*, 216.

(2) For monographs on brassinosteroids, see: (a) *Brassinosteroids: Chemistry, Bioactivity and Applications*; Cutler, H. G., Yokota, T., Adam, G., Eds.; ACS Symposium Series 474; American Chemical Society: Washington, DC, 1991. (b) *Brassinosteroids: Steroidal Plant Hormones*; Sakurai, A., Yokota, T., Clouse, S. D., Eds.; Springer-Verlag: Tokyo, 1999. (c) Khripach, V. A.; Zhabinskii, V. N.; de Groot, A. E. *Brassinosteroids: A New Class of Plant Hormones*; Academic Press: San Diego, 1999.

(3) For reviews of brassinosteroids, see: (a) Adam, G.; Porzel, A.; Schmidt, J.; Schneider, B.; Voigt, B. In *Studies in Natural Products Chemistry*; Atta-ur-Rahman, Ed.; Elsevier: Amsterdam, 1996; Vol. 18, pp 495−549. (b) Back, T. G. In *Studies in Natural Products Chemistry*; Atta-ur-Rahman, Ed.; Elsevier: Amsterdam, 1995; Vol. 16, pp 321−364. (c) Arteca, R. N. In *Plant Hormones*; Davies, P. J., Ed.; Kluwer Academic: 1995; pp 206−213. (d) Mandava, N. B. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **1988**, *39*, 23. (e) Adam, G.; Marquardt V. *Phytochemistry* **1986**, *25*, 1787. (f) Fujioka, S.; Sakurai, A. *Nat. Prod. Rep.* **1997**, *14*, 1. (g) Clouse, S. D. *Plant J.* **1996**, *10*, 1. (h) Clouse, S. D.; Sasse, J. M. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **1998**, *49*, 427. (i) Brosa, C. In *Biochemistry and Function of Sterols*; Parish, E. J., Nes, W. D., Eds.; CRC Press: Boca Raton, FL, 1997; pp 201−220. (j) Yokota, T.; Mori, K. In *Molecular Structure and Biological Activity of Steroids*; Bohl, M., Duax, W. L., Eds.; CRC Press: Boca Raton, FL, 1992; pp 317−340.

(4) (a) Brosa, C.; Capdevila, J. M.; Zamora, I. *Tetrahedron* **1996**, *52*, 2435. (b) Takatsuto, S.; Yazawa, N.; Ikekawa, N.; Takematsu, T.; Takeuchi, Y.; Koguchi, M. *Phytochemistry* **1983**, *22*, 2437. (c) Thompson, M. J.; Meudt, W. J.; Mandava, N. B.; Dutky, S. R.; Lusby, W. R.; Spaulding, D. W. *Steroids* **1982**, *39*, 89. (d) Takatsuto, S.; Ikekawa, N.; Morishita, T.; Abe, H. *Chem. Pharm. Bull.* **1987**, *35*, 211. (e) A very recent report has questioned the validity of the earlier results in ref 4a concerning the bioactivity of 5β-brassinosteroids: Seto, H.; Fujioka, S.; Koshino, H.; Suenaga, T.; Yoshida, S.; Watanabe, T.; Takatsuto, S. *Phytochemistry* **1999**, *52*, 815.

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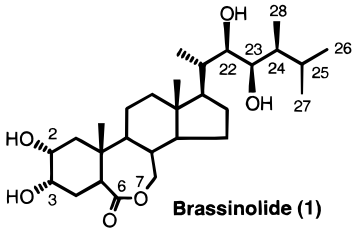
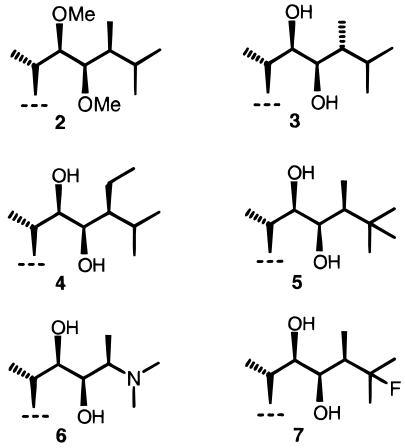
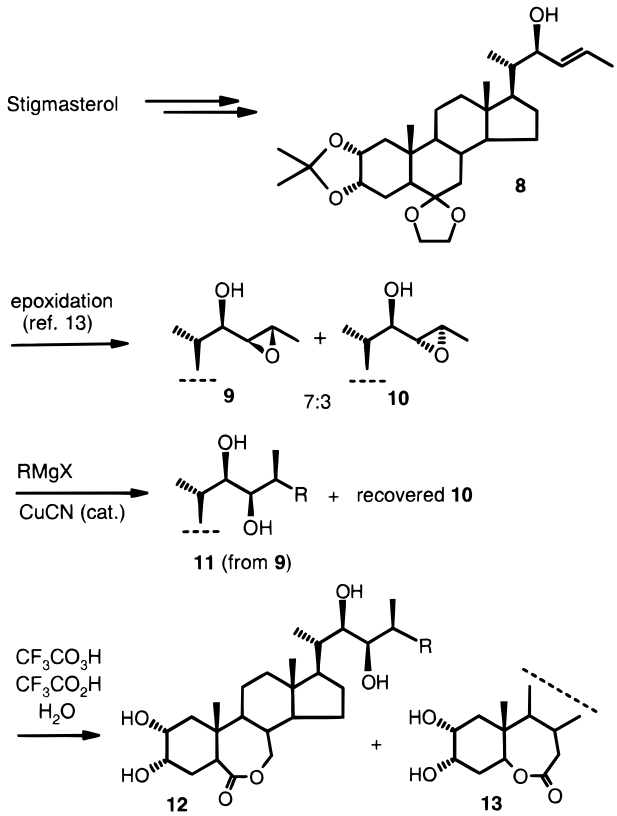
generally been considered necessary,[3d] although some 5β-analogues have recently been reported to be significantly active.[4a,e] The side chain also appears to play a major role in determining the biological activity of brassinosteroids.[3d,e,j,4b,c] While the (*22R,23R*)-vicinal diol moiety confers optimum bioactivity, free hydroxyl groups are not essential, since the corresponding methyl ether derivative **2** is also strongly active.[6] Moreover, 24-epibrassinolide (**3**) and 28-homobrassinolide (**4**) are reported to display relatively high bioactivity and have been the subjects of several field trials because they are more easily synthesized than is **1**.[2] The synthetic analogue 25-homobrassinolide (**5**) has been reported by Mori and Takeuchi[7] to be more active than **1**, while surprisingly, the 25-aza- and 25-fluoro analogues (**6** and **7**, respectively) are completely inactive, even at high doses.[8] The relative bioactivity of brassinosteroids depends to some extent on the dose and type of bioassay used,[3i,j] but **1** is generally considered to be the most active of the naturally occurring brassinosteroids across a wide range of doses. The intriguing report[7] that the bioactivity of **5** exceeds that of **1** therefore prompted us to study other variations in the structure of the hydrocarbon portion of the brassinosteroid side chain (C-24 to C-28) in order to determine more precisely the structure−activity relationships associated with this portion of the molecule. In particular, we wished to investigate the relative biological activity of analogues of **1** where the isopropyl moiety (C-25 to C-27) is replaced by other alkyl or cycloalkyl groups.





We recently reported a relatively concise synthetic route to brassinolide that utilizes the epoxidation of allylic alcohol **8**, in turn obtained from stigmasterol in nine steps, followed by epoxidation and stereo- and

Scheme 1



regioselective ring-opening of the *threo*-epoxide **9** with isopropylmagnesium chloride in the presence of a catalytic amount of copper(I) cyanide.[9] The corresponding *erythro*-epoxide **10** that is formed together with **9** from **8** is considerably less reactive and can be recovered intact after the Grignard reaction. This is followed by simultaneous deprotection and Baeyer−Villiger oxidation of diol **11** (Scheme 1, R = isopropyl). We now report the preparation of a series of new alkyl and cycloalkyl analogues of **1** by this general approach, and their bioactivity in the rice leaf lamina inclination bioassay.[10] These biological assays show a correlation between chain length or ring size of the added group and activity and have resulted in the discovery of two new cycloalkyl side chain analogues of **1** that have significantly higher activity than **1** over a wide dosage range.

Results and Discussion

By use of the appropriate Grignard reagents in Scheme 1, the protected brassinosteroids **11a**−**h** were prepared from the mixture of epoxides **9** and **10**, as shown in Table 1. The yields of diols **11** are based on the amount of the *threo*-epoxide **9** in the original mixture of epoxide stereoisomers. The unreacted *erythro*-epoxide **10** was typically recovered in ca. 90% yield. One-pot deketalization and Baeyer−Villiger oxidation of **11a**−**h** then produced the lactones **12a**−**h**, respectively. The preparation of compounds **12a** and **12e** has been reported previously,[9] but their bioactivity had not yet been measured and so they are included here for comparison. Similarly, the known compound **5** (i.e., **12**, entry d in Table 1), which had been

(5) Baron, D. L.; Luo, W.; Janzen, L.; Pharis, R. P.; Back, T. G. *Phytochemistry* **1998**, *49*, 1849.
(6) Luo, W.; Janzen, L.; Pharis, R. P.; Back, T. G. *Phytochemistry* **1998**, *49*, 637.
(7) Mori, K.; Takeuchi, T. *Liebigs Ann. Chem.* **1988**, 815.
(8) Back, T. G.; Janzen, L.; Nakajima, S. K.; Pharis, R. P. *J. Org. Chem.* **1999**, *64*, 5494.
(9) Back, T. G.; Baron, D. L.; Luo, W.; Nakajima, S. K. *J. Org. Chem.* **1997**, *62*, 1179.
(10) Takeno, K.; Pharis, R. P. *Plant Cell Physiol.* **1982**, *23*, 1275.

Table 1. Preparation of Side-Chain Analogues of Brassinolide

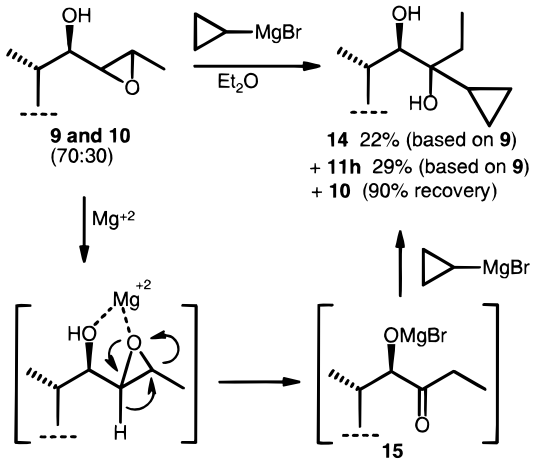
entry	RMgX[a]	yield of **11** (%)	combined yield and ratio[b] of **12** and **13**
a	*n*-$C_{12}H_{25}MgCl$		ref 9
b	*n*-$C_6H_{13}MgBr$	67	60 (8:1)
c	*n*-C_3H_7MgBr	65	66 (8.5:1.5)
d	*t*-C_4H_8MgCl	25	68 (9:1)
e	cyclo-$C_6H_{11}MgCl$		ref 9
f	cyclo-C_5H_9MgCl	81	65 (10:1)
g	cyclo-C_4H_7MgCl	78	62 (9:1)
h	cyclo-C_3H_5MgBr	76	67 (9:1)

[a] Reactions were performed in ether, except in entries g and h, where THF was used. [b] Isolated yields are reported; the ratio of **12** and **13** was measured by NMR integration; pure **12** was obtained by recrystallization of the mixture of **12** and **13**.

previously synthesized by Mori and Takeuchi[7] by a different route, was prepared by the method of Scheme 1 and is also included here for comparison (Table 1, entry d). In each case, the desired product **12** was initially obtained as a mixture containing a small amount of its lactone regioisomer **13** (Scheme 1), from which it was easily separated by crystallization.

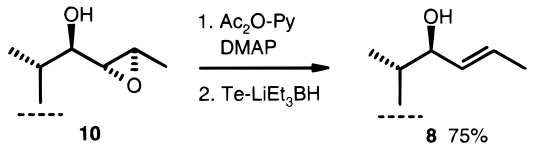
In general, the Grignard additions were performed in diethyl ether as the solvent, affording clean results and relatively high yields, as shown in entries a−f in Table 1. However, when ether was the solvent with cyclopropylmagnesium bromide (entry h), a poor yield of **11h** was obtained, along with the rearranged product **14** and recovered epoxide **10**, as shown in Scheme 2. The formation of **14** probably arises from the Lewis acid-catalyzed rearrangement of the epoxide,[11] followed by addition of the Grignard reagent to the carbonyl group of **15**. The Lewis acid is presumably magnesium bromide that is originally present in the Grignard reagent solution or produced in situ by disproportionation of the Grignard reagent to form dicylopropylmagnesium and magnesium bromide.[12] When THF was chosen as the solvent in entry h, a much cleaner reaction ensued, affording the yield of **12h** indicated in the Table. THF was similarly used in the preparation of **12g**.

Scheme 2



During the course of this work, we accumulated a substantial amount of the undesired *erythro*-epoxide **10**. The deoxygenation of **10**[13] was therefore investigated, with the objective of regenerating the original allylic alcohol **8** for subsequent reoxidation. Of several procedures that were attempted, the best results were realized by using the tellurium-based method of Dittmer,[14] as shown in Scheme 3. Thus, **10** was first converted into the corresponding epoxy acetate, followed by treatment with tellurium and lithium triethylborohydride to effect simultaneous deacetylation and deoxygenation of the epoxide, affording **8** in 75% overall yield. The allylic alcohol **8** could then be recycled back to the original 70:30 mixture of *threo*- and *erythro*-epoxides **9** and **10** as described previously.[9]

Scheme 3



Bioactivity of products **12a**−**h** (Table 1) was assessed using the rice leaf lamina inclination assay.[10] This is a highly sensitive, convenient and reproducible bioassay that permits the rapid measurement of brassinosteroid activity. The leaf lamina angle in untreated rice seedlings typically assumes a control value of ca. 160−170° (i.e. nearly upright). However, when an active brassinosteroid is applied, the angle decreases dramatically and can be conveniently plotted vs the logarithm of the brassinosteroid dose in ng. The auxin indole-3-acetic acid (IAA) synergizes the effect of brassinosteroids, thereby making the assay exceptionally sensitive[10] (vide infra). Bioassay results for the alkyl-substituted products **12a**−**c** and **5** in Table 1 are shown in Figure 1, along with that of brassinolide (**1**) as the standard. The products with long normal chains at C-24 (R = *n*-hexyl and *n*-dodecyl, **12a** and **12b**, respectively) were either devoid of activity or showed only slight activity at the highest doses of 1000 and 10 000 ng in the case of **12b**. Bioactivity increased

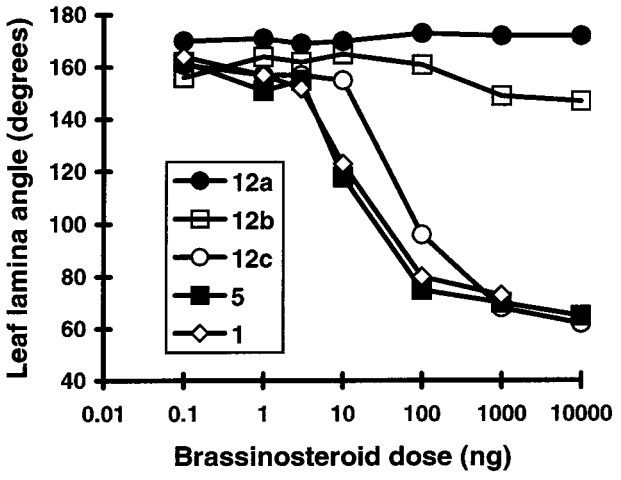


Figure 1. Rice leaf lamina inclination assay of compounds **12a**−**c** relative to 25-homobrassinolide (**5**) and brassinolide (**1**).

(11) For an example of a similar epoxide rearrangement promoted by $MgBr_2$ or other Lewis acids, see: Schauder, J. R.; Krief, A. *Tetrahedron Lett.* **1982**, *23*, 4389.

(12) Ashby, E. C.; Becker, W. E. *J. Am. Chem. Soc.* **1963**, *85*, 118.

(13) For a compilation of methods for the deoxygenation of epoxides to alkenes, see: Larock, R. C. In *Comprehensive Organic Transformations*; VCH: New York, 1989; pp 140−142.

(14) Dittmer, D. C.; Zhang, Y.; Discordia, R. P. *J. Org. Chem.* **1994**, *59*, 1004.

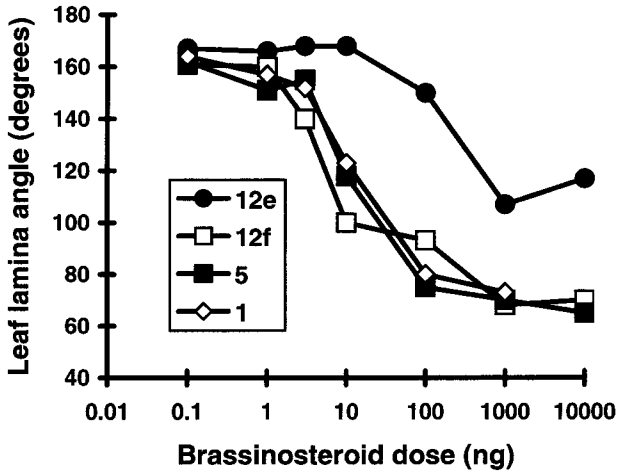


Figure 2. Rice leaf lamina inclination assay of compounds **12e** and **12f** relative to 25-homobrassinolide (**5**) and brassinolide (**1**).

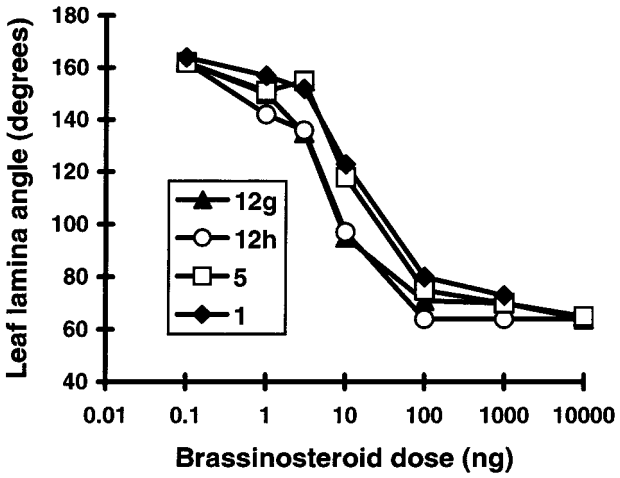


Figure 3. Rice leaf lamina inclination assay of compounds **12g** and **12h** relative to 25-homobrassinolide (**5**) and brassinolide (**1**).

significantly when the chain was shortened to *n*-propyl (**12c**), but was still lower than that of **1**. The known compound **5** (i.e., entry **12d** in Table 1) showed almost identical behavior to that of **1** at all doses studied. The results of the bioassay of cycloalkyl-substituted products **12e** and **12f** are shown in Figure 2, while those of **12 g** and **12h** are given in Figure 3. Again, compounds **1** and **5** are included as standards for comparison. An interesting correlation was evident between ring-size and activity, where the compound with the largest cycloalkyl substituent at C-24 (R = cyclohexyl, **12e**) showed the lowest activity, whereas the cyclopentyl analogue (**12f**) exceeded the activity of brassinolide at doses of 3 and 10 ng, but was less active at higher doses. Moreover, the small-ring compounds with R = cyclobutyl or cyclopropyl (**12g** and **12h**, respectively) showed remarkably high activity, significantly greater than that of either **1** or **5**, across a broad range of doses.[15] Indeed, the cyclopropyl derivative **12h** was ca. 5 to 7 times more potent than **1** at all dosage levels between 1 and 1000 ng, while the cyclobutyl analogue **12g** similarly exceeded the activity of **1** except at the 1 ng dose level.

(15) Back, T. G.; Pharis, R. P.; Nakajima, S. K. U.S. Patent Application 09/281,716, submitted March 30, 1999.

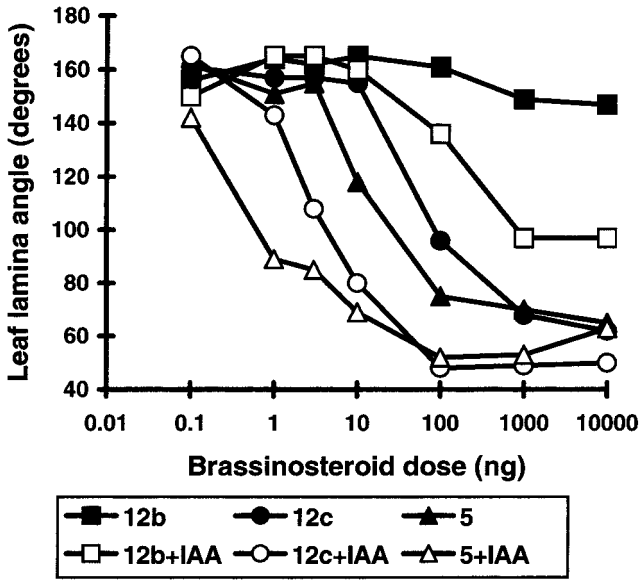


Figure 4. Rice leaf lamina inclination assay of compounds **12b** and **12c** relative to 25-homobrassinolide (**5**) in the presence and absence of 1000 ng of IAA.

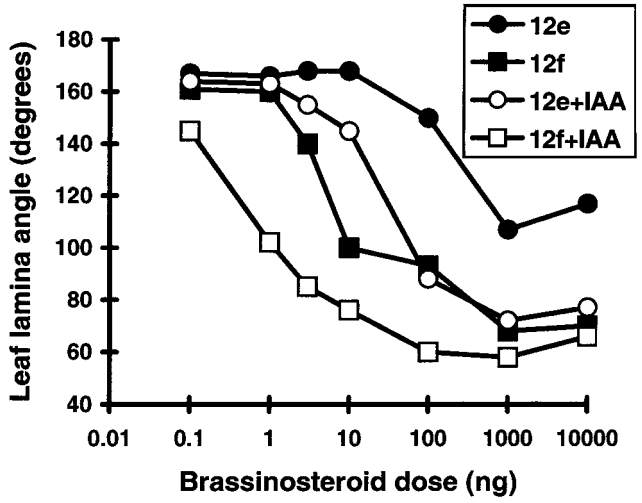


Figure 5. Rice leaf lamina inclination assay of compounds **12e** and **12f** in the presence and absence of 1000 ng of IAA.

Since auxins can synergize the effects of brassinosteroids[3d,10,16] by 1 or 2 orders of magnitude,[17] we also bioassayed compounds **12b**, **12c**, and **12e**−**h** with the simultaneous application of 1000 ng of IAA, which is essentially inactive by itself.[10] The inactive brassinosteroid **12a** remained so even with the coapplication of IAA. However, the synergy of IAA with **5**, **12b** and **12c**, **12e**−**f**, and **12g**−**h** is shown clearly in Figures 4−6, respectively. The results obtained with the small-ring cycloalkyl-substituted brassinosteroids **12g** and **12h** (Figure 6) are especially interesting, since the high bioactivity of these two exceptionally potent compounds can be further increased by 1−2 orders of magnitude over a broad range of doses by coapplication of the inexpensive auxin.

In conclusion, the nature of the hydrocarbon portion of the side chain of brassinosteroids attached at C-24

(16) (a) See: Sasse, J. M. in ref 2, Chapter 13. (b) Sakurai, A.; Fujioka, S. *Plant Growth Regul.* **1993**, *13*, 147.

(17) We have observed similar increases in the bioactivity of other novel brassinosteroids when they were synergized with IAA; see refs 5, 6, and 8.

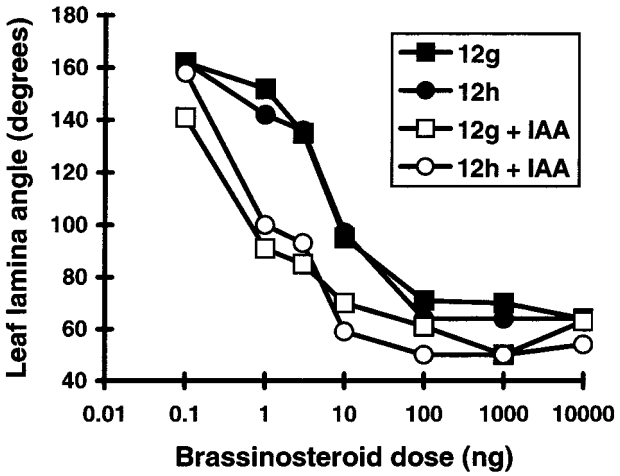


Figure 6. Rice leaf lamina inclination assay of compounds **12g** and **12h** in the presence and absence of 1000 ng of IAA.

strongly affects their bioactivity. When this substituent is a long normal chain (e.g., **12a** and **12b**), negligible activity is observed, perhaps because of impeded binding to a receptor molecule. However, bioactivity increases when a shorter normal chain (as in **12c**) is present, and is even greater still when a branched chain is incorporated at C-24 (as in **1** and **5**). Cycloalkyl groups at C-24 also affect bioactivity, which depends on the ring size. Thus, the presence of a cyclohexyl substituent, as in **12e**, yields relatively weak activity, while the cyclopentyl analogue **12f** is comparable to **1** (Figure 2). It is especially noteworthy that the cyclobutyl and cyclopropyl analogues **12g** and **12h**, respectively, both display significantly greater bioactivity than either brassinolide (**1**) or 25-homobrassinolide (**5**) across a broad range of doses (Figure 3). It therefore appears that **12g** and **12h** are the most potent brassinosteroids reported to date. The reason for this high biological activity is not known, but may be associated with restricted conformational mobility of the side chain. Finally, the synergy of active brassinosteroids with IAA enhances their bioactivity dramatically. The effect of the auxin further increases the already very high activity of the cyclobutyl and cyclopropyl analogues by ca. 10- to 100-fold (Figure 6), thereby dramatically decreasing the dose of these two brassinolide analogues required to elicit a given biological response.

Experimental Section

Brassinolide (**1**) and the mixture of epoxides **9** and **10** were prepared according to our previously reported procedure.[9] Grignard reagents were either purchased or prepared from magnesium powder and the corresponding alkyl halide, followed by titration with 1 M *sec*-butyl alcohol in xylene, using 1,10-phenanthroline as the indicator.[18] The rice leaf lamina assay was conducted by the same protocol as described earlier.[5,6,8] Typically, 36 rice plants were used for each data point in Figures 1−6, except at the highest doses of 1000 and 10 000 ng, where 24 plants were used. Variation was similar to bioassay results reported earlier.[5,6] The Baeyer−Villiger oxidation was performed under similar conditions to those used in the preparation of brassinolide.[9,19] NMR spectra were recorded in deuteriochloroform unless otherwise indicated. Flash chromatography was performed with 230−400 mesh silica gel.

(18) Watson, S. C.; Eastham, J. F. *J. Organomet. Chem.* **1967**, *9*, 165.

(19) McMorris, T. C.; Chavez, R. G.; Patil, P. A. *J. Chem. Soc., Perkin Trans. 1* **1996**, 295.

A typical procedure for the preparation of the compounds listed in Table 1 is given below for **12c**, along with characterization data for the final products **12b**,**f**−**h** and **5**. The preparation of products **12a** and **12e** has been reported previously.[9]

(2*R*,3*S*,5α,22*R*,23*R*,24*S*)-6,6-(Ethylenedioxy)-2,3-(isopropylidenedioxy)-26-homo-27-norergostane-22,23-diol (12c). *n*-Propylmagnesium chloride (5 mL of a 2 M solution in diethyl ether, 10 mmol) was added to a suspension of CuCN (63 mg, 0.70 mmol) in 10 mL of diethyl ether at −78 °C. The mixture was stirred for 1 h, followed by the slow addition of the 7:3 mixture of epoxides **9** and **10**, containing 240 mg (0.476 mmol) of the *threo*-isomer **9**, in 10 mL of ether. The reaction mixture was stirred for 1 h at −78 °C and for 4 h at 0 °C, followed by the addition of 20% aqueous NH_4Cl solution, and extraction with ether. The organic extracts were washed with $NaHCO_3$ solution and NaCl solution, dried ($MgSO_4$), and concentrated under vacuum. The crude product was chromatographed over silica gel (elution with 40−60% ether−hexanes) to afford 96% of recovered *erythro*-epoxide **10** and 170 mg (65%, based on *threo*-epoxide **9**) of the 22,23-diol **11c** as a colorless oil that was used directly in the next step.

Aqueous hydrogen peroxide (0.4 mL of a 30% solution, ca. 4 mmol) was slowly added to trifluoroacetic anhydride (3 mL, 20 mmol) at 0 °C, and the mixture was stirred for 30 min. In a separate vessel, trifluoroacetic acid (2.5 mL) was added to a solution of 148 mg (0.270 mmol) of **11c** in 5 mL of chloroform. The latter solution was stirred at room temperature for 40 min and was then added slowly to the pregenerated trifluoroperoxyacetic acid solution at 0 °C, followed by warming to room temperature and stirring for an additional 1.5 h. The mixture was diluted with chloroform, washed with water and 10% aqueous Na_2SO_3 solution, dried ($MgSO_4$), and concentrated under vacuum. Flash chromatography (elution with 5−10% methanol-chloroform) provided 86 mg (66%) of an 8.5:1 mixture (NMR integration) of **12c** and its 6-oxa-7-oxo regioisomer **13c**. Recrystallization from methanol afforded 40% of pure **12c**: mp 237−240 °C; IR (KBr) 3405, 1722 cm^{-1}; ^1H NMR (400 MHz) δ 4.12 (m, 2 H), 4.02 (m, 1 H), 3.72 (m, 1 H), 3.55 (br s, 2 H), 3.12 (dd, J = 12.2, 4.5 Hz, 1 H), 0.92 (t, J = 7.8 Hz, 3 H), 0.93 (s, 3 H), 0.92 (d, J = 6.5 Hz, 3 H), 0.85 (d, J = 6.8 Hz, 3 H), 0.73 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$−CD_3OD) δ 176.7, 74.9, 74.0, 70.5, 67.9, 58.1, 52.2, 51.2, 42.4, 41.3, 40.9, 39.6, 39.1, 38.2, 37.0, 36.6, 33.6, 31.0, 27.5, 24.7, 22.2, 20.3, 15.4, 14.2, 12.5, 11.9, 11.6. MS data were recorded for the corresponding tetraacetate: *m/z* (relative intensity) 589 (M^+ − AcOH, 1), 528 (M^+ − 2AcOH, 2), 463 (100). Exact mass: calcd for $C_{32}H_{48}O_6$ (M^+ − 2AcOH) 528.3451, found 528.3405.

The following products were prepared similarly (see Table 1):

12b: mp 228−230 °C (from methanol); IR (KBr) 3442, 1709 cm^{-1}; ^1H NMR (400 MHz) δ 4.11 (m, 2 H), 4.03 (m, 1 H), 3.72 (m, 1 H), 3.55 (br s, 2 H), 3.13 (dd, J = 12.1, 4.5 Hz, 1 H), 0.93 (s, 3 H), 0.91 (d, J = 6.5 Hz, 3 H), 0.89 (t, J = 6.8 Hz, 3 H), 0.84 (d, J = 6.7 Hz, 3 H), 0.72 (s, 3 H); ^{13}C NMR (100 MHz) δ 176.3, 75.2, 74.2, 70.4, 68.14, 68.12, 58.1, 52.3, 51.4, 42.4, 41.4, 40.9, 39.7, 39.2, 38.3, 37.1, 34.5, 33.9, 31.8, 31.1, 29.5, 27.6, 27.3, 24.7, 22.6, 22.2, 15.5, 14.1, 12.6, 12.0, 11.7. Anal. Calcd for $C_{31}H_{54}O_6$: C, 71.23; H, 10.41. Found: C, 71.49; H, 10.37.

12f: mp 283−286 °C (from methanol); IR (KBr) 3451, 1696 cm^{-1}; ^1H NMR (200 MHz) δ 4.10 (m, 2 H), 4.03 (m, 1 H), 3.72 (m, 2 H), 3.54 (br d, J = 8.6 Hz, 1 H), 3.13 (dd, J = 12.0, 4.5 Hz, 1 H), 0.93 (s, 3 H), 0.91 (d, J = 6.0 Hz, 3 H), 0.87 (d, J = 6.7 Hz, 3 H), 0.73 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$−CD_3OD) δ 177.0, 74.3, 74.1, 70.5, 67.8, 58.0, 52.1, 51.2, 43.5, 42.3, 41.1, 40.9, 39.8, 39.6, 39.0, 38.1, 36.5, 31.3, 31.0, 30.9, 27.4, 25.0, 24.6, 22.1, 20.9, 15.3, 11.7, 11.6, 11.3. Anal. Calcd for $C_{30}H_{50}O_6$: C, 71.11; H, 9.95. Found: C, 71.13; H, 10.37.

12g: mp 284−286 °C (from methanol); IR (KBr) 3415, 1714 cm^{-1}; ^1H NMR (400 MHz) δ 4.12 (m, 2 H), 4.03 (m, 1 H), 3.72 (m, 1 H), 3.53 (br s, 2 H), 3.13 (dd, J = 12.2, 4.5 Hz, 1 H), 0.94 (s, 3 H), 0.92 (d, J = 6.6 Hz, 3 H), 0.74 (s, 3 H), 0.73 (d, J = 6.8 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$−CD_3OD) δ 177.2, 73.8, 71.7, 70.5, 67.7, 58.0, 52.0, 51.1, 42.2, 41.03, 40.99, 40.8, 39.5, 39.0, 38.7, 38.1, 36.6, 31.0, 27.33, 27.25, 26.9, 24.5, 22.1,

17.3, 15.2, 11.5, 8.7. Anal. Calcd for $C_{29}H_{48}O_6$: C, 70.70; H, 9.82. Found: C, 70.88; H, 10.03.

12h: mp 272−274 °C (from methanol); IR (KBr) 3418, 1711 cm^{-1}; ^1H NMR (200 MHz) δ 4.08 (m, 3 H) 3.72 (m, 2 H), 3.57 (br d, J = 9.1 Hz, 1 H), 3.13 (dd, J = 11.8, 4.8 Hz, 1 H), 0.97 (d, J = 6.4 Hz, 3 H), 0.93 (s, 3 H), 0.89 (d, J = 6.6 Hz, 3 H), 0.70 (s, 3 H), 0.52 (t, J = 6.7 Hz, 2 H), 0.13 (d, J = 4.6 Hz, 2 H); ^{13}C NMR (50 MHz, $CDCl_3$−CD_3OD) δ 177.2, 75.8, 73.4, 70.4, 67.7, 57.9, 52.0, 51.1, 42.2, 41.0, 40.8, 40.3, 39.5, 38.9, 38.0, 37.0, 31.0, 27.3, 24.5, 22.0, 15.5, 15.2, 12.3, 11.6, 11.5, 4.1, 3.9. Anal. Calcd for $C_{28}H_{46}O_6$: C, 70.26; H, 9.69. Found: C, 70.26; H, 9.60.

5 (entry **12d** in Table 1): mp 273−275 °C (from methanol) (lit.[7] mp 274−276 °C); ^1H NMR (400 MHz) δ 4.11 (m, 2 H), 4.03 (br s, 1 H), 3.82 (br d, J = 7.8 Hz, 1 H), 3.72 (m, 1 H), 3.47 (br d, J = 9.0 Hz, 1 H), 3.13 (dd, J = 12.2, 4.5 Hz, 1 H), 0.96 (s, 9 H), 0.94 (s, 3 H), 0.92 (d, J = 6.6 Hz, 3 H), 0.86 (d, J = 7.1 Hz, 3 H), 0.73 (s, 3 H).

Addition of Cyclopropylmagnesium Bromide to 9. The Grignard reagent was prepared in diethyl ether and was allowed to react with the 70:30 mixture of epoxides **9** and **10,** as in the typical procedure given for **12c** above. Flash chromatography afforded 90% of recovered epoxide **10**, 29% (based on **9**) of the 22,23-diol **11h** and 22% (based on **9**) of the rearranged product **14** as an oil: IR (film) 3480, 1228, 1054 cm^{-1}; ^1H NMR (400 MHz) δ 4.29 (m, 1 H), 4.10 (m, 1 H), 3.92 (m, 3 H), 3.76 (m, 1 H), 3.61 (br s, 1 H), 1.48 (s, 3 H), 1.33 (s, 3 H), 1.05 (d, J = 6.0 Hz, 3 H), 0.98 (t, J = 6.0 Hz, 3 H), 0.85 (s, 3 H), 0.69 (s, 3 H), 0.36 (m, 4 H); ^{13}C NMR (100 MHz) δ 109.7, 107.5, 77.7, 73.7, 72.9, 72.8, 65.5, 64.1, 56.0, 53.4, 52.9, 45.4, 42.7, 42.4, 40.9, 39.7, 38.0, 36.7, 32.9, 29.9, 28.6, 28.3, 26.5, 24.1, 21.9, 20.7, 17.3, 13.4, 13.3, 11.7, 8.1, −0.3, −0.8; mass spectrum, m/z (relative intensity) 546 (M^+, 1), 528 ($M^+ - H_2O$, 16), 55 (100); exact mass calcd for $C_{33}H_{54}O_6$ 546.3920, found 546.3903.

Deoxygenation of *erythro*-Epoxide 10. Acetic anhydride (8.70 mL, 92.2 mmol) and DMAP (0.845 mg, 6.91 mmol) were added to a solution of **10** (2.33 g, 4.61 mmol) in 125 mL of 20% pyridine−dichloromethane, and the mixture was stirred at room temperature for 1 h. It was then poured into ice-cold 10% HCl solution, followed by extraction with chloroform. The combined organic extracts were dried ($MgSO_4$), and evaporated. The crude product was chromatographed over silica gel (elution with 50% ethyl acetate−hexanes) to afford 2.39 g (95%) of the corresponding epoxy acetate as a colorless oil.

Elemental tellurium (1.68 g, 13.2 mmol) was stirred in 100 mL of dry THF. The solution was degassed with nitrogen for 5 min, followed by the addition of 1.0 M lithium triethylborohydride (24 mL, 24 mmol), and the mixture was stirred at room temperature for 30 min, resulting in a purple color. The above epoxy acetate (2.39 g, 4.39 mmol) in 25 mL of THF was degassed with argon for 5 min, and was slowly added to the purple solution, which was then refluxed for 20 h. Air was passed through the cooled mixture, it was filtered through a pad of Celite to remove tellurium, and the filtrate was evaporated to dryness. The product was triturated with water, extracted with chloroform, dried ($MgSO_4$), concentrated under vacuum and chromatographed over silica gel (elution with 5−10% acetonitrile-dichloromethane) to furnish 1.70 g (79%) of allylic alcohol **8** as a colorless oil, identical in all respects with an authentic sample.

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Supporting Information Available: The ^1H and ^{13}C NMR spectra of compounds **12b**,**c**,**f**−**h** and **14**. This material is available free of charge via the Internet at http://pubs.acs.org.

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