Characterization of Brassinazole, a Triazole-Type Brassinosteroid Biosynthesis Inhibitor[1]

Tadao Asami*, Yong Ki Min, Noriko Nagata, Kazutoshi Yamagishi, Suguru Takatsuto, Shozo Fujioka, Noboru Murofushi, Isomaro Yamaguchi, and Shigeo Yoshida

The Institute of Physical and Chemical Research (RIKEN), Hirosawa 2–1, Wako-shi, Saitama 351–0198, Japan (T.A., Y.K.M., N.N., K.Y., S.F., S.Y.); Department of Applied Biological Chemistry, The University of Tokyo, Bunkyo-Ku, Tokyo 113, Japan (Y.K.M., N.M., I.Y.); and Department of Chemistry, Joetsu University of Education, Joetsu-shi, Niigata 943–8512, Japan (S.T.)

Screening for brassinosteroid (BR) biosynthesis inhibitors was performed to find chemicals that induce dwarfism in Arabidopsis, mutants that resembled BR biosynthesis mutants that can be rescued by BR. Through this screening experiment, the compound brassinazole was selected as the most potent chemical. In dark-grown Arabidopsis, brassinazole-induced morphological changes were nearly restored to those of wild type by treatment with brassinolide. The structure of brassinazole is similar to pacrobutrazol, a gibberellin biosynthesis inhibitor. However, in assays with cress (*Lepidium sativum*) plants, brassinazole-treated plants did not show recovery after the addition of gibberellin but showed good recovery after the addition of brassinolide. These data demonstrate that brassinazole is a specific BR biosynthesis inhibitor. Brassinazole-treated cress also showed dwarfism, with altered leaf morphology, including the downward curling and dark green color typical of Arabidopsis BR-deficient mutants, and this dwarfism was reversed by the application of 10 nM brassinolide. This result suggests that BRs are essential for plant growth, and that brassinazole can be used to clarify the function of BRs in plants as a complement to BR-deficient mutants. The brassinazole action site was also investigated by feeding BR biosynthesis intermediates to cress grown in the light.

The application of many biologically active brassinosteroid (BR) homologs has been shown to cause remarkable growth responses in plants, including stem elongation, pollen tube growth, leaf bending, leaf unrolling, root inhibition, proton pump activation (Mandava, 1988), promotion of ethylene production (Schlagnhaufer and Arteca, 1991), tracheary element differentiation (Iwasaki and Shibaoka, 1991; Yamamoto et al., 1997), and cell elongation (Azpiroz et al., 1998). The functions of endogenous BRs have been revealed by identifying several BR-deficient Arabidopsis mutants, such as *dwf1* (Choe et al., 1999a), *cbb1* (Kauschmann et al., 1996), *dwf4* (Choe et al., 1998), *ste1/dwf7* (Gachotte et al., 1996; Choe et al., 1999b), *cpd* (Szekeres et al., 1996), and *det2* (Li et al., 1996; Fujioka et al., 1997). Recently, dwarf mutants of pea (Nomura et al., 1997) and tomato (Bishop et al., 1999) have also been characterized as BR deficient. The above findings indicate that the use of BR-deficient mutants has been invaluable in investigating an essential role of BRs in plant growth and development, and, consequently, BRs have recently been recognized as a new class of phytohormones (Yokota, 1997; Clouse and Sasse, 1998).

The use of specific biosynthesis inhibitors is an alternative way for the determination of physiological functions of endogenous substances. As shown in mode-of-action studies on gibberellins (GAs), GA-deficient mutants and GA biosynthesis inhibitors are both quite effective (Rademacher, 1989; Kamiya and Hedden, 1997). Similarly, a specific inhibitor of BR biosynthesis can provide a new and complementary approach to understanding the functions of BRs (Yokota, 1999). KM-01 is the first reported selective BR inhibitor, but appears to be of limited use for probing the role of BRs in plants due to its very low activity when applied alone (Kim et al., 1995). Other than KM-01, there have been no BR inhibitors, but Yokota et al. (1991) observed a slight reduction in the concentration of endogenous castasterone when plants were treated with uniconazole, and Iwasaki and Shibaoka (1991) reported that this compound inhibited brassinolide-induced tracheary element differentiation. These observations imply that brassinolide biosynthesis is also affected, since uniconazole is known to block GA biosynthesis.

Various triazole compounds, including uniconazole, are known to inhibit many cytochrome P450s, a large and ubiquitous group of enzymes that catalyze oxidative processes in life systems (Rademacher, 1991), but inhibition of particular enzymes can be strictly controlled by specific inhibitors. This indicates that every enzyme has its own characteristic three-dimensional inhibitor binding site structure. Furthermore, many steps of BR biosynthesis are

[1] This work was partly supported by a Grant-in-Aid for Scientific Research (no. 09660121) from the Ministry of Education, Science, Sports and Culture of Japan.

* Corresponding author; e-mail tasami@postman.riken.go.jp; fax 81–48–462–4674.

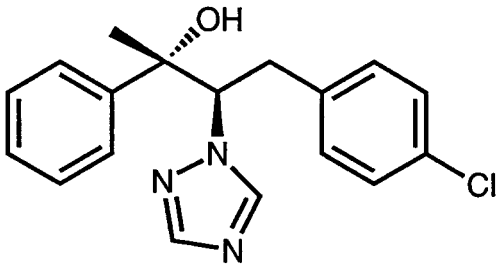

Figure 1. Structure of brassinazole. In this study, brassinazole was used as a racemic mixture.

thought to be performed by cytochrome P450 enzymes, for example, conversion from campestanol to 6α-hydroxycampestanol, 6-oxocampestanol to cathasterone, cathasterone to teasterone, typhasterol to castasterone, and castasterone to brassinolide (Sakurai and Fujioka, 1997). In this context, it would be beneficial to screen for a specific inhibitor of BR biosynthesis among triazole compounds. Eventually, we found some triazole derivatives to be good lead compounds for BR biosynthesis inhibitors (Min et al., 1999). Intensive study on structure-activity relationships of such lead compounds led us to the finding of a potent inhibitor, brassinazole, (Figure 1) (Asami and Yoshida, 1999). Brassinazole was synthesized on the basis of known methods (Buschmann et al., 1987), and is unique in that it has a tertiary hydroxy group on the carbon adjacent to the carbon where a triazole ring is attached, whereas other known triazolic PGRs have a secondary hydroxyl group at this position.

We report the characterization of brassinazole as a BR biosynthesis inhibitor and examine the putative target sites of this chemical.

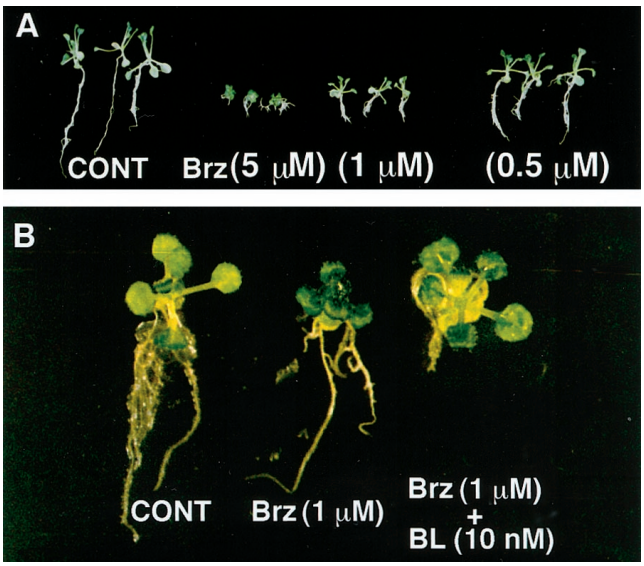

Figure 2. Effect of brassinazole (Brz) on Arabidopsis seedlings grown in the light. A, Brassinazole (5, 1, and 0.5 μM)-treated Arabidopsis (14-d-old) show dwarfism in a concentration-dependent manner. B, Brassinazole (1 μM)-treated Arabidopsis (14-d-old) show a BR-deficient mutant-like phenotype, which is rescued by the application of brassinolide (BL) (10 nM). CONT, Control.

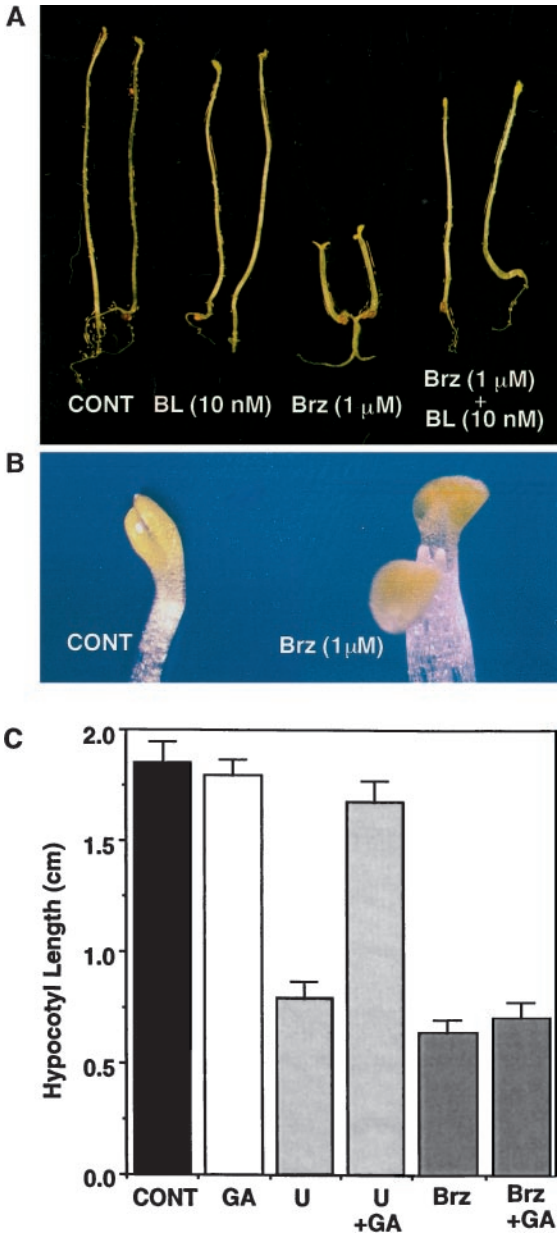

Figure 3. Effect of brassinazole (Brz) on Arabidopsis seedlings grown in the dark. Brassinazole (1 μM)-treated Arabidopsis (7-d-old) shows BR-deficient mutant-like phenotype. A, Brassinazole-induced short hypocotyl and open cotyledon, which are rescued by brassinolide (BL) treatment (10 nM). B, Open cotyledon. C, Both uniconazole (U) (0.1 μM) and brassinazole (1 μM) induced short hypocotyl. GA_3 (1 μM) treatment rescued uniconazole-induced short hypocotyl, but not the brassinazole-induced one. CONT, Control.

RESULTS

Arabidopsis mutants such as *det2* and *cpd* show strong dwarfism with curly dark green leaves in the light and a de-etiolated phenotype with short hypocotyls and open cotyledons in the dark, which are characteristic of light-grown plants. This phenotype was rescued by the application of brassinolide, but the other plant hormones, such as auxin and GA, had

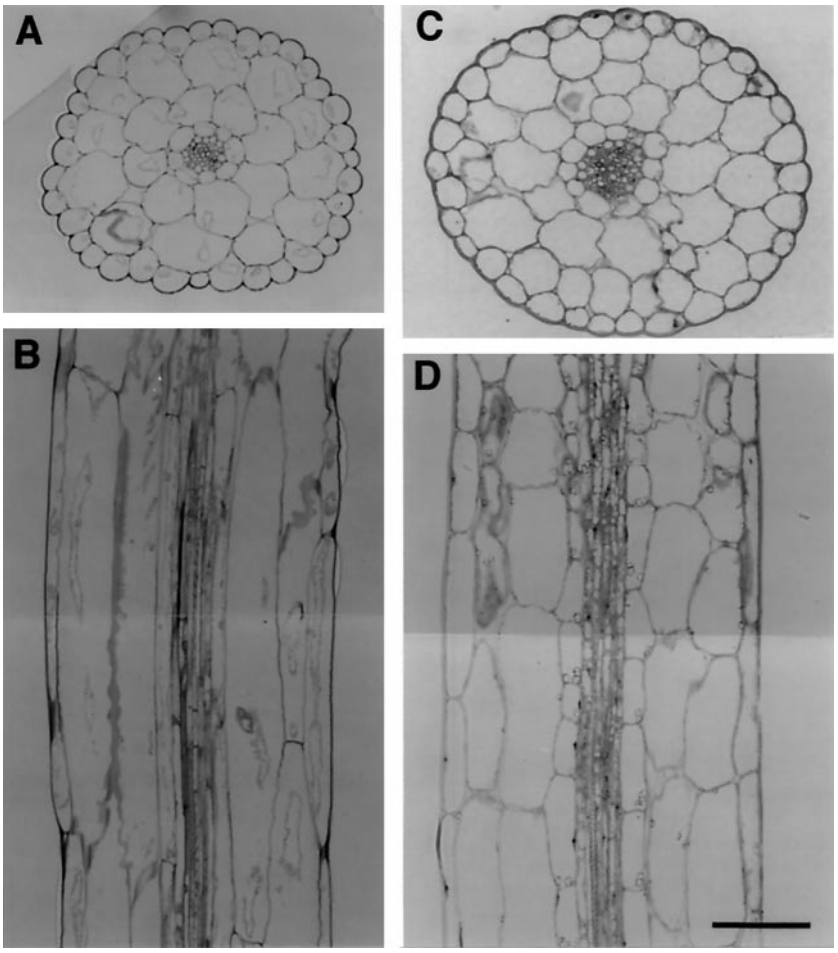

Figure 4. Light microscopy of brassinazole-treated and non-treated Arabidopsis stems sectioned longitudinally and transversally. A, Cross-section of non-treated plant. B, Longitudinal section of non-treated plant. C, Cross-section of brassinazole-treated plant. Cell size is increased in different tissues. D, Longitudinal section of brassinazole-treated plant. Cell size is reduced drastically in many different tissues. Arabidopsis seedlings were grown for 10 d in the dark. Bar = 100 μm.

no effect (Clouse and Sasse, 1998). Based on these facts, we tested brassinazole in the Arabidopsis seedling assay. Brassinazole markedly caused malformation of seedlings, which became morphologically similar to BR-deficient mutants (Fig. 2A). At a concentration higher than 1 μM, the phenotype became very similar to that of BR-deficient mutants. These brassinazole-induced phenotypes were rescued by co-application of 10 nM brassinolide (Fig. 2B). In the dark, brassinazole induced a de-etiolated phenotype with a short hypocotyl (Fig. 3A) and open cotyledons (Fig. 3B), similar to BR-deficient mutants. These phenotypes were rescued by the application of 10 nM brassinolide (Fig. 3A). Uniconazole, a potent GA biosynthesis inhibitor (Izumi et al., 1984), also induced short hypocotyls in Arabidopsis, which were returned to wild-type length by the application of 1 μM GA_3. However, brassinazole-induced short hypocotyls could not be rescued by GA_3 (Fig. 3C).

One of the common characteristics found in BR-biosynthesis-deficient mutants is a reduction in longitudinal growth, which could be due to either a reduced number of cells or a failure in cell elongation (Szekeres et al., 1996; Azpiroz et al., 1998; Choe et al., 1999). As shown in Figure 4, the dwarfism in brassinazole-treated Arabidopsis was due to the reduction in longitudinal growth. In contrast to non-treated Arabidopsis, hypocotyl cell length was reduced and the thickness of cell walls increased in the brassinazole-treated plants, whereas no differences were detected in the number of cells along the length of either organ between the brassinazole-treated and non-treated plants.

BR-deficient mutants accumulate light-regulated genes in the dark (Chory et al., 1991; Li et al., 1996; Szekeres et al., 1996). Hybridization of steady-state RNAs prepared from brassinazole-treated seedlings of Arabidopsis grown in the dark clearly showed that photomorphogenesis in brassinazole-treated Arabidopsis was accompanied by an increase in the expression of light-regulated genes coding for the small subunit of ribulose 1,5-diphosphate carboxylase (*rbcS*), chlorophyll *a/b* binding protein (*cab*), and the 32-kD QB-binding protein of photosystem II (*psb*A; Fig. 5). These levels were significantly higher than those in dark-grown non-treated seedlings, but in the light there was no difference between brassinazole-treated and non-treated seedlings. The dose-response test of brassinazole in the dark also demonstrated that the effect of brassinazole on reducing hypocotyl length became saturated at 3 μM or higher concentrations (Fig. 6).

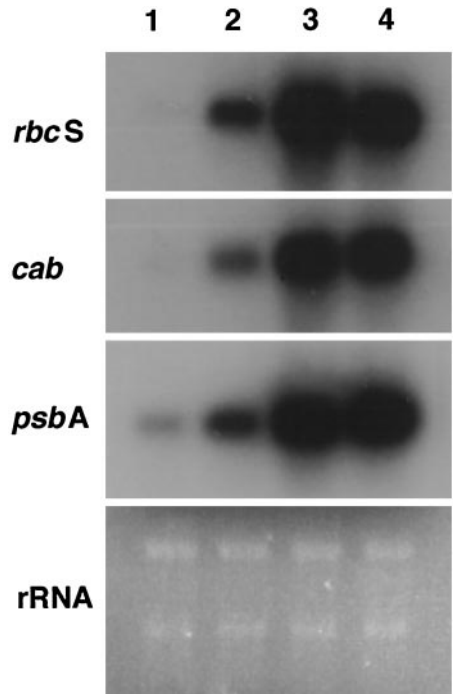

Figure 5. Accumulation of mRNAs for *rbc*S, *cab*, and *psb*A in light-grown and dark-grown brassinazole-treated and non-treated plants. Lane 1, Dark-grown non-treated plants; lane 2, dark-grown brassinazole-treated plants; lane 3, light-grown non-treated plants; lane 4, light- grown brassinazole-treated plants. Two micrograms of total RNA was loaded per lane. Bottom panel, Stained gel showing rRNAs.

To examine whether brassinazole can cause a BR-deficient-mutant-like phenotype in plants other than Arabidopsis, brassinazole was also applied to cress, a plant that has been used previously to investigate the effects of brassinolide (Yopp et al., 1981; Jones-Held et al., 1996). The hypocotyl length of cress seedlings treated with brassinazole at 1 μM and higher was about 40% of the control (Fig. 7). This effect of 1 μM brassinazole on retarding the hypocotyl elongation of cress seedling was negated by the application of 10 nM brassinolide, but not by 1 μM GA. The hypocotyl length of cress seedlings treated with uniconazole at 1 μM or higher was also about 40% of the control, and this effect was negated by the application of GA but not brassinolide.

Brassinazole is a triazole derivative, a number of which have been shown to inhibit cytochrome P450s. Therefore, brassinazole may also block a cytochrome P450, but specifically in the BR biosynthesis pathway. A BR biosynthesis pathway has been proposed that includes many steps likely to be catalyzed by a cytochrome P450 (Yokota, 1997). To investigate the biosynthetic step(s) affected by brassinazole, we examined the effect of biosynthetic intermediates downstream of cathasterone on hypocotyl elongation of both brassinazole-treated and non-treated etiolated Arabidopsis seedlings. As shown in Figure 8, 1 μM cathasterone and teasterone, 100 nM castasterone, and 10 nM brassinolide had no effect on non-treated seedlings.

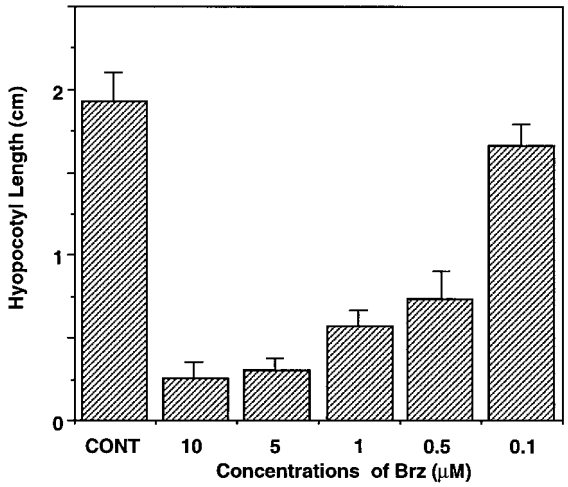

Figure 6. Dark-grown 10-d-old seedlings of Arabidopsis treated with different concentrations of brassinazole (Brz) from 0 to 10 μM. Hypocotyl length decreased as the concentration of brassinazole increased. The change in length occurred between 0.1 and 0.5 μM. Data are the means ± SE obtained from 20 seedlings. CONT, Control.

However, these concentrations of teasterone, castasterone, and brassinolide were effective in rescuing brassinazole-treated hypocotyl growth, respectively. Cathasterone had almost no effect on rescuing the brassinazole-treated hypocotyl growth. As in a similar experiment done by Fujioka et al. (1997), both cathasterone and teasterone rescued the defective hypocotyl growth of the dark-grown *det2* mutant. Our results suggest that at least one of the target sites of brassinazole in BR biosynthesis inhibition is the oxidation step of cathasterone to teasterone, which is catalyzed by *CPD* (Szekeres et al., 1996), a P450 cy-

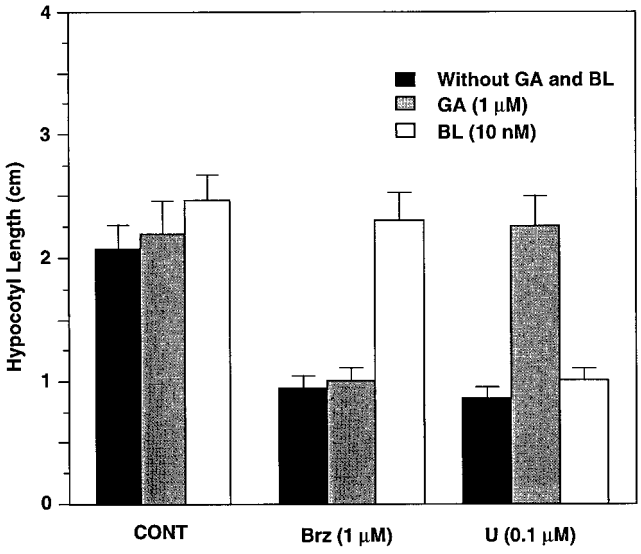

Figure 7. Retardation of cress seedling growth by brassinazole (Brz) or uniconazole (U) and rescue by brassinolide or GA_3. Brassinolide rescues plants treated with brassinazole but not uniconazole. GA_3 added to plants rescues those treated with uniconazole but not brassinazole. CONT, Control.

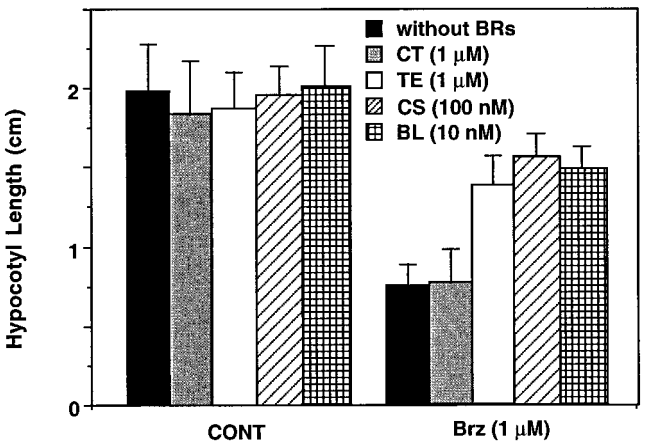

Figure 8. Brassinazole (Brz)-treated Arabidopsis hypocotyl elongation in the dark in response to applied cathasterone (CT), teasterone (TE), castasterone (CS), and brassinolide (BL). Data are the means ± SE obtained from 30 seedlings. CONT, Control.

tochrome. Therefore, brassinazole exhibits its effect by reducing the supply of brassinolide in the plant. In the reversion test, plants tended to be sensitive to growth conditions, maybe because of slow uptake and transport of brassinolide within cress and/or a non-specific effect(s) of brassinazole on other aspects of plant metabolism. This could be why brassinolide did not completely reverse the inhibition by brassinazole, as shown in Figure 8.

DISCUSSION

Both in the dark and in the light, brassinazole-induced morphological changes were nullified by the addition of brassinolide. In the dark, brassinazole-treated Arabidopsis develop as light-grown plants and express light-regulated genes, as do BR-deficient mutants. Light microscopic analysis of hypocotyls indicated that the brassinazole-induced dwarfism was due to a failure of cell elongation, as was also observed in the BR-deficient mutants *cpd*, *dwf4*, and *dwf7*. The *cpd* and *det2* mutations induce de-etiolation and expression of light-induced genes in the dark, which were also seen in brassinazole-treated Arabidopsis. These results strongly suggest that brassinazole treatment caused brassinolide deficiency in Arabidopsis.

Feeding experiments demonstrated that brassinazole-induced retardation of hypocotyl growth was rescued by the addition of exogenous BR biosynthesis intermediates such as teasterone, but not by cathasterone, suggesting that the inhibition site of brassinazole may be the step from cathasterone to teasterone. The enzyme involved in this step mediates a hydroxylation at C-23 of the side chain of cathasterone and is known to be encoded by *CPD*, which has been proposed to be a novel cytochrome P450 (Szekeres et al., 1996). Considering that brassinazole is in a chemical class of triazole derivatives that exhibit their activities by inhibiting cytochrome P450s, it is likely that brassinazole activity is caused by this enzyme and possibly others as well. In some cases, triazole derivatives have multiple inhibition sites. For example, GA biosynthesis inhibitors blocking *ent*-kaurene oxidation can also affect other cytochrome P450 monooxygenases, although in most cases at a far lower degree of activity; for example, in the inhibition of sterol formation by the blocking of 14α-demethylation and the inhibition of the oxidative inactivation of abscisic acid (Rademacher, 1991). Therefore, secondary effects of brassinazole may also play a role in plants, but subsequent results indicated that brassinazole showed little effect on GA biosynthesis at 1 μM or lower concentrations. That is, uniconazole disrupts Arabidopsis germination due to the inhibition of GA biosynthesis, while brassinazole has no effect on germination. Morphological changes induced by brassinazole are rescued by the addition of exogenous brassinolide but not by GA.

The C-22 and C-23 positions of BRs are successively hydroxylated by cytochrome P450s encoded by *DWF4* and *CPD*, respectively. The enzymes catalyzing these two steps are different; however, not only are their functions similar but the DNA sequences are similar to each other (Choe et al., 1998). Taking the similar function of the enzyme active sites into consideration, it is possible to speculate that brassinazole inhibits both steps. But in this case, brassinazole-treated Arabidopsis does not show a response to cathasterone or to earlier intermediates of BR biosynthesis such as campestanol and 6-oxocampestanol. This low response makes it difficult to investigate the target sites of brassinazole upstream of cathasterone by feeding experiments. In BR biosynthesis, Fujioka and Sakurai (1997) have demonstrated that there are at least two branched biochemical pathways to the end product brassinolide: the early and the late C-6 oxidation pathways (Fujioka and Sakurai, 1997). In the late C-6 oxidation pathway, there are two hydroxylation steps of the side chains of campestanol to 6-deoxoteasterone via 6-deoxocathasterone. These steps in the late C-6 oxidation pathway are very similar to those in the early C-6 oxidation pathway. On the basis of these experiments, we cannot rule out that brassinazole may attack these sites as well as the step from cathasterone to teasterone. Further investigations will answer these questions.

In this report we demonstrate that brassinazole induces morphological changes in plants by interfering with the biosynthesis of BRs. At present, BR-deficient mutants are known only in Arabidopsis, tomato, and pea. This novel BR biosynthesis inhibitor will play an important role in investigations into the function of BRs not only in other plants, but also in tissues, organs, and biochemical processes. As shown in Figure 6, we varied the concentration of BRs in plants by varying the concentration of brassinazole. This may make it possible to titrate the minimum

concentration of BRs for the growth of plants by comparing the concentration of BRs in brassinazole-treated and non-treated plants. Moreover, the ability to select a group of new mutants using GA biosynthesis inhibitors (Jacobsen and Olszewski, 1993; Nambara et al., 1994) suggests that this inhibitor will provide a way to find a new BR pathway or other novel mutants. Other than its use in basic science, brassinazole can be developed as a new commercial plant growth regulator.

MATERIALS AND METHODS

Chemicals

Brassinolide and castasterone were purchased from CIDtech Research (Cambridge, Ontario, Canada). Other intermediates in the BR biosynthesis pathway used in this report were synthesized as described previously (Takatsuto et al., 1984; Takatsuto, 1986; Fujioka et al., 1995). Murashige and Skoog salt and vitamin mixture was purchased from Gibco-BRL (Grand Island, NY). Brassinazole was synthesized on the basis of known methods (Buschmann et al., 1987). Brassinazole exists as enantiomers, and the structure-activity relationships of brassinazole and other triazole derivatives will be reported elsewhere.

Plant Materials and Growth Conditions

Wild-type seeds of Arabidopsis (ecotype Columbia) were purchased from LEHLE Seeds (Round Rock, TX). Cress (*Lepidium sativum*) seeds were purchased locally. Wild-type and *det2* seeds were cold treated (4°C) for 2 d, then surface-sterilized in 1% (w/v) solution of NaOCl for 20 min and washed with sterile distilled water five times. Seeds were sown on 1% (w/v) agar-solidified medium containing 0.5× Murashige and Skoog salts and 1.5% (w/v) Suc in plastic plates with or without chemicals. Wild-type and *det2* plants were grown in 16-h light (240 μE m^{-2} s^{-1}) and 8-h dark conditions in a growth chamber (22°C). The plates were sealed with Parafilm (American National Can, Chicago) for the screening experiment. For the rescue experiment, which required a longer experimental period, seeds were sown on 1% (w/v) agar-solidified medium containing 0.5× Murashige and Skoog salts and 1.5% (w/v) Suc in Agripots (Kirin Brew., Tokyo). Plants were grown in 16-h light (240 μE m^{-2} s^{-1}) and 8-h dark conditions in a growth chamber (25°C). Cress seeds were sown on 1% (w/v) agar-solidified medium containing 0.5× Murashige and Skoog salts and 1.5% (w/v) Suc in Agripots with or without chemicals. Plants were grown in 16-h light (240 μE m^{-2} s^{-1}) and 8-h dark conditions in a growth chamber (25°C).

The samples were fixed in 4% (w/v) paraformaldehyde buffered with 20 mM sodium cacodylate at pH 7.0 for 20 h at 4°C, dehydrated through an ethanol series, and then embedded in resin (Technovit 7100, Kulzer and Co., Wehrheim, Germany). The sections (2 μm thick) were cut with a glass knife on an ultramicrotome (ULTRACUT UCT, Leica, Wien, Austria), placed on cover slips, and dried. They were stained with 0.5% (w/v) toluidine blue O in 0.1 M phosphate-buffered saline (pH 7.0) for 30 s, and then washed in distilled water for 10 s. The samples were observed with a microscope (model IX70, Olympus, Tokyo).

RNA filter hybridizations were performed using standard molecular techniques (Sambrook et al., 1989) with slight modifications. For hybridization of RNA blots, the cDNA probes used were the same as described previously (Deng et al., 1991).

ACKNOWLEDGMENTS

We thank Drs. Yukihisa Shimada and Takeshi Nakano for valuable suggestions on this work.

Received November 1, 1999; accepted January 25, 2000.

LITERATURE CITED

Asami T, Yoshida S (1999) Brassinosteroid biosynthesis inhibitors. Trends Plant Sci **4:** 348–353

Azpiroz R, Wu Y, LoCascio JC, Feldmann KA (1998) An *Arabidopsis* brassinosteroid-dependent mutant is blocked in cell elongation. Plant Cell **10:** 219–230

Bishop GJ, Nomura T, Yokota T, Harrison K, Noguchi T, Fujioka S, Takatsuto S, Jones JDG, Kamiya Y (1999) The tomato DWARF enzyme catalyses C-6 oxidation in brassinosteroid biosynthesis. Proc Natl Acad Sci USA **96:** 1761–1766

Buschmann E, Zeeh B, Gotz N, Sproesser L, Walker N (1987) Chelat kontrollierte Grignard-Reaktionen mit α-(1H-1,2,4-Triazol-1-yl) ketonen. Liebigs Ann Chem 349–355

Choe S, Dilkes BP, Fujioka S, Takatsuto S, Sakurai A, Feldmann KA (1998) The *DWF4* gene of *Arabidopsis* encodes a cytochrome P450 that mediates multiple 22α-hydroxylation steps in brassinosteroid biosynthesis. Plant Cell **10:** 231–243

Choe S, Dilkes BP, Gregory BD, Ross AS, Yuan H, Noguchi T, Fujioka S, Takatsuto S, Tanaka A, Yoshida S, Tax FE, Feldmann KA (1999a) The *Arabidopsis dwarf1* mutant is defective in the conversion of 24-methylenecholesterol to campesterol in brassinosteroid biosynthesis. Plant Physiol **119:** 897–907

Choe S, Noguchi T, Fujioka S, Takatsuto S, Tissier CP, Gregory BD, Ross AS, Tanaka A, Yoshida S, Tax FE, Feldmann KA (1999b) The *Arabidopsis dwf7/ste1* mutant is defective in the Δ^7 sterol C-5 desaturation step leading to brassinosteroid biosynthesis. Plant Cell **11:** 207–221

Chory J, Nagpal P, Peto CA (1991) Phenotypic and genetic analysis of *det2*, a new mutant that affects light-regulated seedling development in *Arabidopsis*. Plant Cell **3:** 445–459

Clouse SD, Sasse JM (1998) Brassinosteroids: essential regulators of plant growth and development. Annu Rev Plant Physiol Plant Mol Biol **49:** 427–451

Deng X-W, Caspar T, Quail PH (1991) *cop1*: a regulatory locus involved in light-controlled development and gene expression in *Arabidopsis*. Genes Dev **5:** 1172–1182

Fujioka S, Inoue T, Takatsuto S, Yanagisawa T, Yokota T, Sakurai A (1995) Identification of new brassinosteroid,

cathasterone, in cultured cells of *Catharanthus roseus* as a biosynthetic precursor of teasterone. Biosci Biotech Biochem **59:** 1543–1547

Fujioka S, Li J, Choi YH, Seto H, Takatsuto S, Noguchi T, Watanabe T, Kuriyama H, Yokota T, Chory J, Sakurai A (1997) The *Arabidopsis deetiolated2* mutant is blocked early in brassinosteroid biosynthesis. Plant Cell **9:** 1951–1962

Fujioka S, Sakurai A (1997) Biosynthesis and metabolism of brassinosteroids. Physiol Plant **100:** 710–715

Gachotte D, Husselstein T, Bard M, Lacroute F, Benveniste P (1996) Isolation and characterization of an *Arabidopsis thaliana* cDNA encoding a Δ^7-sterol-C-5-desaturase by functional complementation of a defective yeast mutant. Plant J **9:** 391–398

Iwasaki T, Shibaoka H (1991) Brassinosteroids act as regulators of tracheary-element differentiation in isolated *Zinnia* mesophyll cells. Plant Cell Physiol **32:** 1007–1014

Izumi K, Yamaguchi I, Wada A, Ohshio H, Takahashi N (1984) Effects of a new plant growth retardant (*E*)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-1-penten-3-ol (S-3307) on the growth and gibberellin content of rice plants. Plant Cell Physiol **25:** 611–617

Jacobsen SE, Olszewski NE (1993) Mutations at the *SPINDLY* locus of *Arabidopsis* alter gibberellin signal transduction. Plant Cell **5:** 887–896

Jones-Held S, VanDoren M, Lockwood T (1996) Brassinolide application to *Lepidium sativum* seeds and the effects on seedling growth. J Plant Growth Regul **15:** 63–67

Kamiya Y, Hedden P (1997) Gibberellin biosynthesis: enzymes, genes, and their regulation. Annu Rev Plant Physiol Plant Mol Biol **48:** 431–460

Kauschmann A, Jessop A, Koncz C, Szekeres M, Willmitzer L, Altmann T (1996) Genetic evidence for an essential role of brassinosteroids in plant development. Plant J **9:** 701–713

Kim SK, Asano T, Marumo S (1995) Biological activity of brassinosteroid inhibitor KM-01 produced by a fungus *Drechslera avenae*. Biosci Biotech Biochem **59:** 1394–1397

Li JM, Nagapal P, Vitart V, McMorris TC, Chory J (1996) A role for brassinosteroids in light-dependent development of *Arabidopsis*. Science **272:** 398–401

Mandava N (1988) Plant growth-promoting brassinosteroids. Annu Rev Plant Physiol Plant Mol Biol **39:** 23–52

Min YK, Asami T, Fujioka S, Murofushi N, Yamaguchi I, Yoshida S (1999) New lead compounds for brassinosteroid biosynthesis inhibitors. Bioorg Med Chem Lett **9:** 425–430

Nambara E, Keith K, McCourt P, Naito S (1994) Isolation of an internal deletion mutant of the *Arabidopsis thaliana ABI3* gene. Plant Cell Physiol **35:** 509–513

Nomura T, Nakayama M, Reid JB, Takeuchi Y, Yokota T (1997) Blockage of brassinosteroid biosynthesis and sensitivity causes dwarfism in *Pisum sativum*. Plant Physiol **113:** 31–37

Rademacher W (1989) Gibberellins: metabolic pathways and inhibitors of biosynthesis. *In* P Boeger, G Sandmann, eds, Target Sites for Herbicide Action. CRC Press, Boca Raton, FL, pp 127–145

Rademacher W (1991) Inhibitors of gibberellin biosynthesis: applications in agriculture and horticulture. *In* N Takahashi, BO Phinney, J MacMillan, eds, Gibberellins. Springer-Verlag, New York, pp 296–310

Sakurai A, Fujioka S (1997) Studies on biosynthesis of brassinosteroids. Biosci Biotech Biochem **61:** 757–762

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular Cloning: A Laboratory Manual, Ed 2. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY

Schlagnhaufer CD, Arteca RN (1991) The uptake and metabolism of brassinosteroid by tomato (*Lycopersicon esculentum*) plants. J Plant Physiol **138:** 191–194

Szekeres M, Nemeth K, Koncz-Kalman Z, Mathur J, Kauschmann A, Altmann T, Redei GP, Nagy F, Schell J, Koncz C (1996) Brassinosteroids rescue the deficiency of CYP90, a cytochrome P450, controlling cell elongation and deetiolation in Arabidopsis. Cell **85:** 171–182

Takatsuto S (1986) Synthesis of teasterone and typhasterol, brassinolide-related steroids with plant-growth-promoting activity. J Chem Soc Perkin Trans **1:** 1833–1836

Takatsuto S, Yazawa N, Ushiguro M, Morisaki M, Ikekawa N (1984) Stereo selective synthesis of plant growth-promoting steroids: brassinolide, castasterone, typhasterol, and their 28-nor analogues. J Chem Soc Perkin Trans **1:** 139–146

Yamamoto R, Demura T, Fukuda H (1997) Brassinosteroids induce entry into the final stage of tracheary element differentiation in cultured *Zinnia* cells. Plant Cell Physiol **38:** 980–983

Yokota T (1997) The structure, biosynthesis, and function of brassinosteroids. Trends Plant Sci **2:** 137–143

Yokota T (1999) Brassinosteroids. *In* PJJ Hooykaas, MA Hall, KR Libbenga, eds, Biochemistry and Molecular Biology of Plant Hormones. Elsevier Science Publishing, New York, pp 277–293

Yokota T, Nakamura Y, Takahashi N, Nonaka M, Sekimoto H, Oshio H, Takatsuto S (1991) Inconsistency between growth and endogenous levels of gibberellins, brassinosteroids, and sterols in *Pisum sativum* treated with uniconazole antipodes. *In* N Takahashi, BO Phinney, J MacMillan, eds, Gibberellins. Springer-Verlag, New York, pp 339–349

Yopp J, Mandava N, Sasse JM (1981) Brassinolide, a growth-promoting steroidal lactone: I. Activity in selected auxin bioassays. Physiol Plant **53:** 445–452